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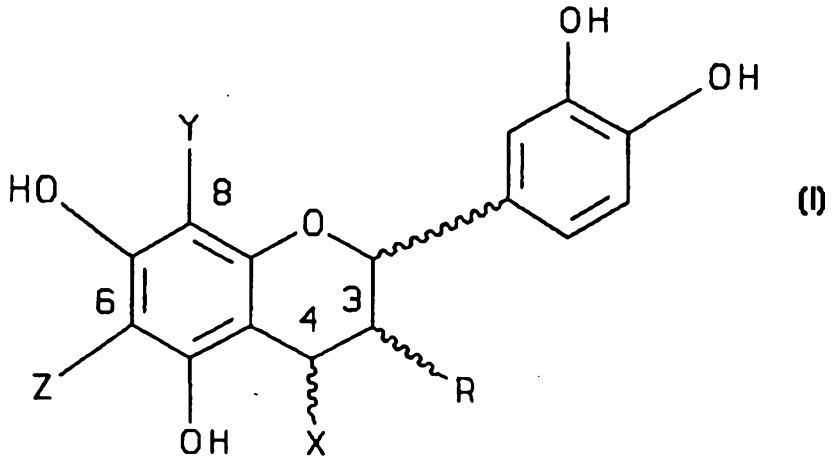
(54) Title: COCOA EXTRACT COMPOUNDS AND METHODS FOR MAKING AND USING THE SAME

(57) Abstract

Disclosed and claimed are cocoa extracts, compounds, combinations thereof and compositions containing the same, such as polyphenols or procyanidins, methods for preparing such extracts, compounds and compositions, as well as uses for them, especially a polymeric compound of the formula A_n, wherein A is a monomer of formula (I) wherein n is an integer from 2 to 18, such that there is at least one terminal monomeric unit A, and one or a plurality of additional monomeric units; R is 3-(α)-OH, 3-(β)-OH, 3-(α)-O-sugar ou 3-(β)-O-sugar; bonding between adjacent monomers takes place at positions 4, 6 or 8; a bond of an additional monomeric unit in position 4 has alpha or beta stereochemistry; X, Y and Z are selected from the group consisting of monomeric unit A, hydrogen, and a sugar, with the provisos that as to the at least one terminal monomeric unit, bonding of the additional monomeric unit thereto (the bonding of the additional monomeric unit adjacent to the terminal monomeric unit) is at position 4 and optionally Y = Z = hydrogen; the sugar is optionally substituted with a phenolic moiety, at any position on the sugar, for instance via an ester bond; and pharmaceutically acceptable salts or derivatives thereof (including oxidation products).



(I)

COCOA EXTRACT COMPOUNDS AND METHODS FOR MAKING AND USING THE SAME

REFERENCE TO RELATED APPLICATION

Reference is made to copending U.S. application Nos. 08/709,406, filed September 6, 1996, 08/631,661, filed April 2, 1996, and 08/317,226, filed October 3, 1994 (now U.S. Patent No. 5,554,645) and PCT/US96/04497, each of which is incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates to cocoa extracts and compounds therefrom such as polyphenols preferably polyphenols enriched with procyanidins. This invention also relates to methods for preparing such extracts and compounds, as well as to uses for them; for instance, as antineoplastic agents, antioxidants, DNA topoisomerase II enzyme inhibitors, cyclo-oxygenase and/or lipoxygenase modulators, NO (Nitric Oxide) or NO-synthase modulators, as non-steroidal antiinflammatory agents, apoptosis modulators, platelet aggregation modulators, blood or *in vivo* glucose modulators, antimicrobials, and inhibitors of oxidative DNA damage.

Documents are cited in this disclosure with a full citation for each appearing thereat or in a References section at the end of the specification, preceding the claims. These documents pertain to the field of this invention; and, each document cited herein is hereby incorporated herein by reference.

BACKGROUND OF THE INVENTION

Polyphenols are an incredibly diverse group of compounds (Ferreira et al., 1992) which widely occur in a variety of plants, some of which enter into the food chain. In some cases they represent an important class of compounds for the human diet. Although some of the polyphenols are considered to be nonnutrative, interest in these compounds has arisen because of their possible beneficial effects on health.

For instance, quercetin (a flavonoid) has been shown to possess anticarcinogenic activity in

experimental animal studies (Deshner et al., 1991 and Kato et al., 1983). (+)-Catechin and (-)-epicatechin (flavan-3-ols) have been shown to inhibit Leukemia virus reverse transcriptase activity (Chu et al., 1992). Nobotanin (an oligomeric hydrolyzable tannin) has also been shown to possess anti-tumor activity (Okuda et al., 1992). Statistical reports have also shown that stomach cancer mortality is significantly lower in the tea producing districts of Japan. Epigallocatechin gallate has been reported to be the pharmacologically active material in green tea that inhibits mouse skin tumors (Okuda et al., 1992). Ellagic acid has also been shown to possess anticarcinogen activity in various animal tumor models (Bukharta et al., 1992). Lastly, proanthocyanidin oligomers have been patented by the Kikkoman Corporation for use as antimutagens. Indeed, the area of phenolic compounds in foods and their modulation of tumor development in experimental animal models has been recently presented at the 202nd National Meeting of The American Chemical Society (Ho et al., 1992; Huang et al., 1992).

However, none of these reports teaches or suggests cocoa extracts or compounds therefrom, any methods for preparing such extracts or compounds therefrom, or, any uses for cocoa extracts or compounds therefrom, as platelet aggregation inhibitors or antimicrobials.

OBJECTS AND SUMMARY OF THE INVENTION

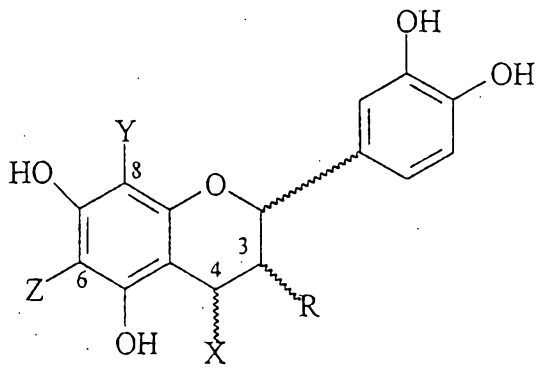
Since unfermented cocoa beans contain substantial levels levels of polyphenols, the present inventors considered it possible that similar activities of and uses for cocoa extracts, e.g. compounds within cocoa,

could be revealed by extracting such compounds from cocoa and screening the extracts for activity. The National Cancer Institute has screened various *Theobroma* and *Herrania* species for anti-cancer activity as part of their massive natural product selection program. Low levels of activity were reported in some extracts of cocoa tissues, and the work was not pursued. Thus, in the antineoplastic or anti-cancer art, cocoa and its extracts were not deemed to be useful; i.e., the teachings in the antineoplastic or anti-cancer art lead the skilled artisan away from employing cocoa and its extracts as cancer therapy.

Since a number of analytical procedures were developed to study the contributions of cocoa polyphenols to flavor development (Clapperton et al., 1992), the present inventors decided to apply analogous methods to prepare samples for anti-cancer screening, contrary to the knowledge in the antineoplastic or anti-cancer art. Surprisingly, and contrary to the knowledge in the art, e.g., the National Cancer Institute screening, the present inventors discovered that cocoa polyphenol extracts which contain procyanidins, have significant utility as anti-cancer or antineoplastic agents.

It was then surprisingly discovered that compounds extracted from cocoa have activity as antimicrobial agents and as platelet aggregation inhibitors.

Accordingly, the present invention provides a polymeric compound of the formula A_n in an amount to effect said treatment wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or

a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof;

and a pharmaceutically, veterinarily or food science acceptable carrier.

The invention also provides a method of inhibing platelet aggregation, comprising administering to a mammal in need of such treatment a composition comprising a polymeric compound of the formula A_n, as defined above, or a derivative or oxidation product thereof, and a pharmaceutically, veterinarily or food science acceptable carrier.

The invention additionally provides a method of treating, preventing or reducing atherosclerosis or restenosis in a mammal, comprising administration to said mammal a composition comprising a polymeric compound of the formula A_n, as defined above, or a derivative or oxidation product thereof, and a pharmaceutically, veterinarily or food science acceptable carrier.

The invention further provides a method of modulating thrombosis in a mammal, comprising administering to said mammal a composition comprising a polymeric compound of the formula A_n, as defined above, or a derivative or oxidation product thereof and a pharmaceutically, veterinarily or food science acceptable carrier.

In addition the invention provides a method of inhibiting bacterial growth in a mammal, comprising administering to said mammal a composition comprising a polymeric compound of the formula A_n, as defined above, or a derivative or oxidation product thereof, and a pharmaceutically, veterinarily or food science acceptable carrier.

The invention also provides a method for the identification of at least one gene induced or repressed by a polymeric compound of the formula A_n, as defined above, or a derivative or oxidation product thereof; said method comprising contacting said at least one gene or a gene product thereof with the polymeric compound using a gene expression assay.

The polymeric compound is preferably at least one cocoa procyanidin selected from (-) epicatechin, (+) catechin, procyanidin B-2, procyanidin oligomers 2 through 18, e.g. 3 through 18, such as 2 through 12 or 3 through 12, preferably 2 through 5 or 4 through 12, more preferably 3 through 12, and most preferably 5 through 12, procyanidin B-5, procyanidin A-2 and procyanidin C-1.

The compounds are preferably obtained by a process comprising reducing cocoa beans to powder, defatting the powder and, extracting and purifying active compound(s) from the powder.

The methods of the present invention as defined above comprise administering to the patient a composition comprising an effective quantity of a substantially pure cocoa extract or compound therefrom or synthetic cocoa polyphenol(s) or procyanidin(s) and a pharmaceutically, veterinary or food science acceptable carrier. The cocoa extract or compound therefrom can be cocoa procyanidin(s); and, is preferably obtained by reducing cooca beans to powder, defatting the powder and, extracting and purifying active compound(s) from the powder. The substantially pure cocoa extract or compounds therefrom or synthetic cocoa polyphenol(s) or procyanidin(s) may be provided in a kit with a suitable carrier, e.g. a pharmaceutically, veterinary or food science acceptable carrier, for admixture with the

extract or compound therefrom or synthetic polyphenol(s) or procyanidin(s).

The polymeric compounds A_n used in the present invention are as illustrated in Figs. 16A to 16P and 17A to 17AA; and linkages of 4→6 and 4→8 are presently preferred.

BRIEF DESCRIPTION OF THE DRAWINGS

The following Detailed Description will be better understood by reference to the accompanying drawings wherein:

Fig. 1 shows a representative gel permeation chromatogram from the fractionation of crude cocoa procyanidins;

Fig. 2A shows a representative reverse-phase HPLC chromatogram showing the separation (elution profile) of cocoa procyanidins extracted from unfermented cocoa;

Fig. 2B shows a representative normal phase HPLC separation of cocoa procyanidins extracted from unfermented cocoa;

Fig. 3 shows several representative procyanidin structures;

Figs. 4A-4E show representative HPLC chromatograms of five fractions employed in screening for anti-cancer or antineoplastic activity;

Fig 5A shows a representative semi-preparative reverse phase HPLC separation for combined cocoa procyanidin fractions D and E;

Fig 5B shows a representative normal phase semi-preparative HPLC separation of a crude cocoa polyphenol extract;

Fig 5C shows the effect of enzymatically oxidised cocoa procyanidins against Hela cells (dose response for polyphenol oxidase treated crude cocoa polyphenol; % control vs. concentration, µg/ml; darkened square is crude UIT-1 (with caffeine and theobromine), open circle crude UIT-1 (without caffeine and theobromine) and darkened circle crude UIT-1 (polyphenol oxidase catalysed);

Fig. 6 shows a normal phase HPLC separation of crude, enriched and purified pentamers from cocoa extract;

Figs. 7A., B and C show MALDI-TOF/MS of pentamer enriched procyanidins, and of Fractions A-C and of Fractions D-E, respectively;

Fig. 8 A shows an elution profile of oligomeric procyanidins purified by modified semi-preparative HPLC;

Fig. 8B shows an elution profile of a trimer procyanidin by modified semi-preparative HPLC;

Figs. 9A-D each show energy minimized structures of all (4-8) linked pentamers based on the structure of epicatechin;

Fig. 10A shows relative fluorescence of epicatechin upon thiolysis with benzylmercapten;

Fig. 10B shows relative fluorescence of catechin upon thiolysis with benzylmercapten;

Fig. 10C shows relative fluorescence of dimers (B2 and B5) upon thiolysis with benzylmercapten;

Fig. 11A shows relative fluorescence of dimer upon thiolysis;

Fig. 11B shows relative fluorescence of B5 dimer upon thiolysis of dimer and subsequent desulphurization;

Fig. 12 shows the elution profile from halogen-free analytical separation of acetone extract of procyanidins from cocoa extract;

Fig. 13 shows the effect of pore size of stationary phase for normal phase HPLC separation of procyanidins;

Fig. 14A shows the substrate utilization during fermentation of cocoa beans;

Fig. 14B shows the metabolite production during fermentation;

Fig. 14C shows the plate counts during fermentation of cocoa beans;

Fig. 14D shows the relative concentrations of each component in fermented solutions of cocoa beans;

Fig. 15 shows the purification scheme for the isolation of procyanidins from cocoa;

Fig. 16A to 16P shows the preferred structures of the pentamer;

Figs. 17A-AA show a library of stereoisomers of pentamers;

Figs. 18A-B show 70 minute gradients for normal phase HPLC separation of procyanidins, detected by UV and fluorescence, respectively;

Figs. 19A-B show 30 minute gradients for normal phase HPLC separation of procyanidins, detected by UV and fluorescence, respectively;

Fig. 20 shows a preparation normal phase HPLC separation of procyanidins;

Figs. 21A-G show CD (circular dichroism) spectra of procyanidin dimers, trimers, tetramers, pentamers, hexamers, heptamers and octamers, respectively;

Fig. 22A shows the structure and $^{1}H/^{13}C$ NMR data for epicatechin;

Figs. 22B-F show the APT, COSY, XHCORR, ^{1}H and ^{13}C NMR spectra for epicatechin;

Fig. 23A shows the structure and $^{1}H/^{13}C$ NMR data for catechin;

Figs. 23B-E show the ^{1}H, APT, XHCORR and COSY NMR spectra for catechin;

Fig. 24A shows the structure and $^{1}H/^{13}C$ NMR data for B2 dimer;

Figs. 24B-G show the ^{13}C, APT, ^{1}H, HMQC, COSY and HOHAHA NMR spectra for the B2 dimer;

Fig. 25A shows the structure and $^{1}H/^{13}C$ NMR data for B5 dimer;

Figs. 25B-G show the ^{1}H, ^{13}C, APT, COSY, HMQC and HOHAHA NMR spectra for B5 dimer;

Figs. 26A-D show the ^{1}H, COSY, HMQC and HOHAHA NMR spectra for epicatechin/catechin trimer;

Figs. 27A-D show the ^{1}H, COSY, HMQC and HOHAHA NMR spectra for epicatechin trimer;

Fig. 28 shows a micellar electrokinetic capillary chromatographic separation of cocoa procyanidin oligomers;

Fig. 29A-F show MALDI-TOF mass spectra for Cu^{+2}-, Zn^{+2}-, Fe^{+2}-, Fe^{+3}-, Ca^{+2}-, Mg^{+2}- ions, respectively, complexed to a trimer;

Fig 30 shows a MALDI-TOF mass spectrum of cocoa procyanidin oligomers (tetramers to octadecamers);

Fig 31 shows time-temperature effects on a hexamer hydroxylsis;

Fig 32 shows time-temperature effects on trimer formation.

Fig 33 shows the effect of cocoa procyanidin fractions on macrophage NO production; and

Fig 34 shows the effect of cocoa procyanidin fractions on LPS induced and gamma interferon primed macrophages.

DETAILED DESCRIPTION

COMPOUNDS USED IN THE INVENTION

As discussed above, it has now been surprisingly found that compounds derived from cocoa have antimicrobial and platelet aggregation inhibiting activities and can also be used to treat thrombosis, atherosclerosis and restenosis.

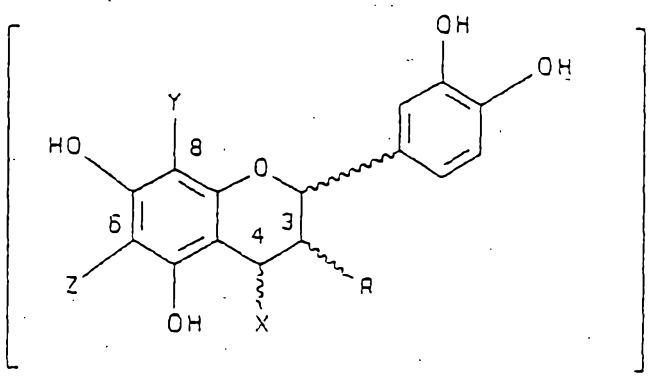
The compounds are generally prepared by reducing cocoa beans to a powder, defatting the powder, and extracting and purifying the active compound(s) from the defatted powder. The powder can be prepared by freeze-drying the cocoa beans and pulp, depulping and dehulling the freeze-dried cocoa beans and grinding the dehulled beans. The extraction of active compound(s) can be by solvent extraction techniques. The extracts comprising the active compounds can be purified, e.g., to be substantially pure, for instance, by gel permeation chromatography or by preparative High Performance Liquid Chromatography (HPLC) techniques or by a combination of such techniques.

With reference to the isolation and purification of the compounds of the invention derived from cocoa, it will be understood that any species of *Theobroma*, *Herrania* or inter- and intra-species crosses thereof may be employed. In this regard, reference is made to Schultes, "Synopsis of *Herrania*," Journal of the Arnold Arboretum, Vol. XXXIX, pp. 217 to 278, plus plates I to XVII (1985), Cuatrecasas, "Cocoa and Its Allies, A Taxonomic Revision of the Genus *Theobroma*," Bulletin of the United States National Museum, Vol. 35, part 6, pp. 379 to 613, plus plates 1 to 11 (Smithsonian Institution, 1964), and Addison, et al., "Observations on the Species of the Genus *Theobroma* Which Occurs in the Amazon," Bol. Tech. Inst. Agronomico de Nortes, 25(3) (1951).

Additionally, Example 18 lists the heretofore never reported concentrations of the inventive compounds found in *Theobroma* and *Herrania* species and their inter- and intra-species crosses; and Example 18 also describes methods of modulating the amounts of the inventive compounds which may be obtained from cocoa by manipulating cocoa fermentation conditions.

An outline of the purification protocol utilized in the isolation of substantially pure procyanidins is shown in Fig. 15. Steps 1 and 2 of the purification scheme are described in Examples 1 and 2; steps 3 and 4 are described in Examples 3, 7 and 16; step 5 is described in Examples 4 and 8; and step 6 is described in Examples 4, 8 and 10. The skilled artisan would appreciate and envision modifications in the purification scheme outlined in Figure 15 to obtain the active compounds without departing from the spirit or scope thereof and without undue experimentation.

The compound used in the methods of the present invention may be of the formula:

A = 

wherein:

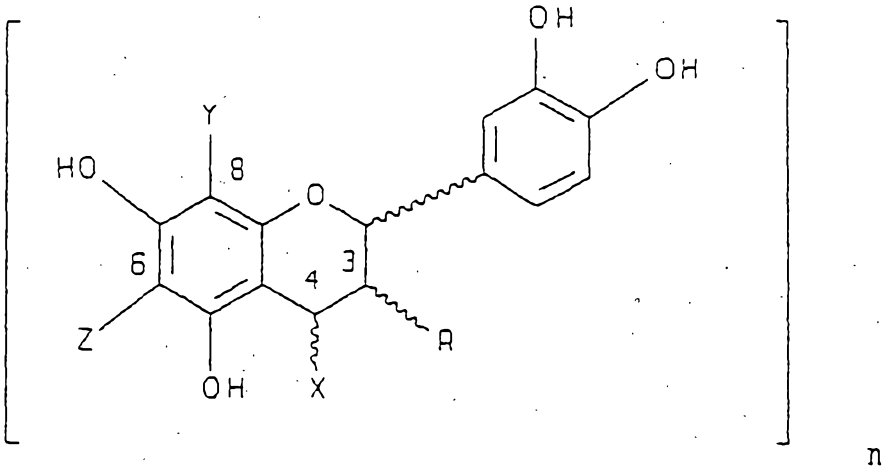
n is an integer from 2 to 18, e.g., 3 to 12, such that there is a first monomeric unit A, and a plurality of other monomeric units;

R is 3-(α)-OH, 3-(β)-OH, 3-(α)-O-sugar, or 3-(β)-O-sugar;

position 4 is alpha or beta stereochemistry;

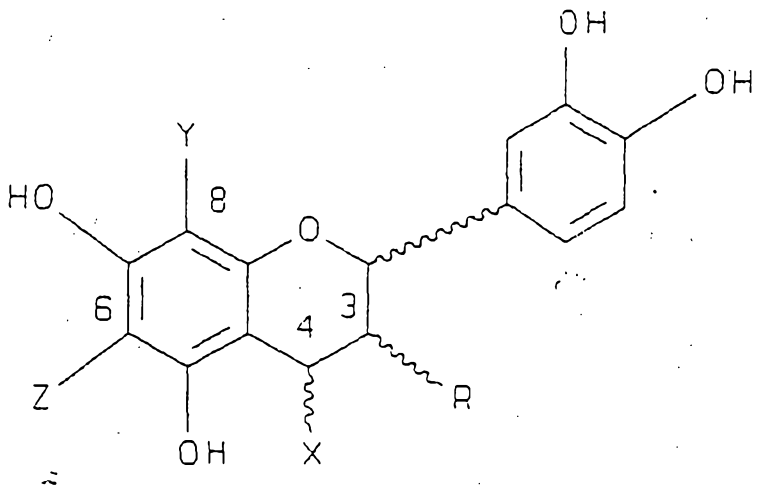
X, Y and Z represent positions for bonding between monomeric units, with the provisos that as to the first monomeric unit, bonding of another monomeric unit thereto is at position 4 and Y = Z = hydrogen, and, that when not for bonding monomeric units, X, Y and Z are hydrogen, or Z, Y are sugar and X is hydrogen, or X is alpha or beta sugar and Z, Y are hydrogen, or combinations thereof. The compound can have n as 5 to 12, and certain preferred compounds have n as 5. The sugar can be selected from the group consisting of glucose, galactose, xylose, rhamnose, and arabinose. The sugar of any or all of R, X, Y and Z can optionally be substituted with a phenolic moiety via an ester bond.

For instance, the compound may be of the formula:



wherein:

n is an integer from 2 to 18, e.g., 3 to 12, advantageously 5 to 12, and preferably n is 5, such that there is a first monomeric unit A,



and a plurality of other monomeric units of A;

R is 3-(α)-OH, 3-(β)-OH, 3-(α)-O-sugar, or 3-(β)-O-sugar;

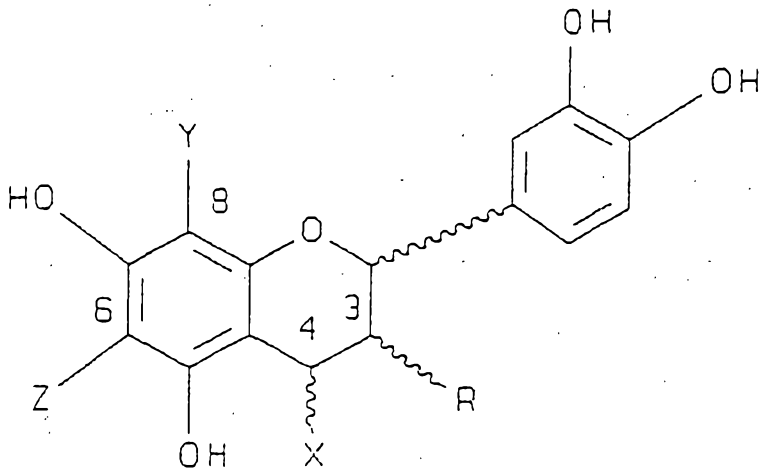
position 4 is alpha or beta stereochemistry;

X, Y and Z represent positions for bonding between monomeric units, with the provisos that as to the first monomeric unit, bonding of another monomeric unit thereto is at position 4 and Y = Z = hydrogen, and, that when not for bonding monomeric units, X, Y and Z are hydrogen or Z, Y are sugar and X is hydrogen, or X is

alpha or beta sugar and Z and Y are hydrogen, or combinations thereof; and

said sugar is optionally substituted with a phenolic moiety via an ester bond.

Accordingly, the compound used in the present invention is a polymeric compound of the formula A_n, wherein A is a monomer having the formula



wherein

n is an integer from 2 to 18, such that there is at least one terminal monomeric unit A, and at least one or a plurality of additional monomeric units;

R is 3-(α)-OH, 3-(ß)-OH, 3-(α)-O-sugar, or 3-(ß)-O-sugar;

bonding between adjacent monomers takes place at positions 4, 6 or 8;

a bond of an additional monomeric unit in position 4 has α or ß stereochemistry;

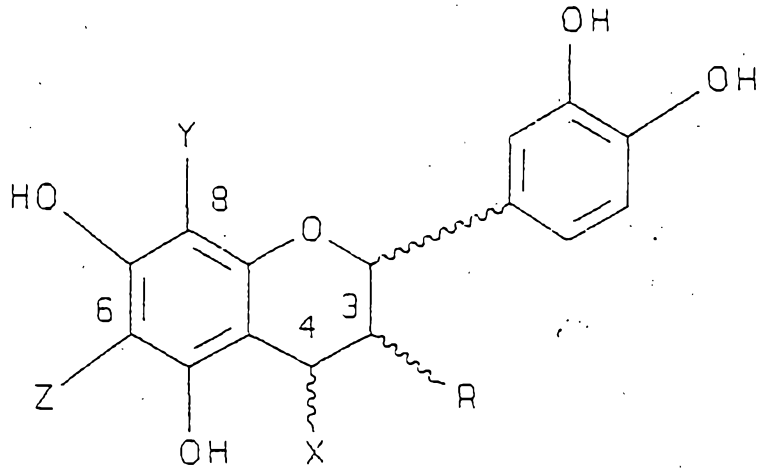
X, Y and Z are selected from the group consisting of monomeric unit A, hydrogen, and a sugar, with the provisos that as to the at least one terminal monomeric unit, bonding of the additional monomeric unit thereto (i.e., the bonding of the monomeric unit adjacent the terminal monomeric unit) is at position 4 and optionally, Y = Z = hydrogen; and

the sugar is optionally substituted with a phenolic moiety at any position, for instance via an

ester bond; or a pharmaceutically acceptable salt or derivative thereof (including oxidation products).

In preferred embodiments, n can be 3 to 18, 2 to 18, 3 to 12, e.g., 5 to 12; and, advantageously, n is 5. The sugar is selected from the group consisting of glucose, galactose, xylose, rhamnose and arabinose. The sugar of any or all of R, X, Y and Z can optionally be substituted at any position with a phenolic moiety via an ester bond. The phenolic moiety is selected from the group consisting of caffeic, cinnamic, coumaric, ferulic, gallic, hydroxybenzoic and sinapic acids.

Additionally, the polymeric compound may be of the formula A_n, wherein A is a monomer having the formula:



wherein

n is an integer from 2 to 18, e.g., 3 to 18, advantageously 3 to 12, e.g., 5 to 12, preferably n is 5;

R is 3-(α)-OH, 3-(ß)-OH, 3-(α)-O-sugar, or 3-(ß)-O-sugar;

adjacent monomers bind at position 4 by (4→6) or (4→8);

each of X, Y and Z is H, a sugar or an adjacent monomer, with the provisos that if X and Y are adjacent monomers, Z is H or sugar and if X and Z are adjacent monomers, Y is H or sugar, and that as to at least one of the two terminal monomers, bonding of the adjacent

monomer is at position 4 and optionally, Y = Z = hydrogen;

a bond at position 4 has α or β stereochemistry;

the sugar is optionally substituted with a phenolic moiety at any position, for instance, via an ester bond,

or a pharmaceutically acceptable salt or derivative thereof (including oxidation products).

With regard to the recitation of "at least one terminal monomeric unit A", it will be understood that the polymeric compounds have two terminal monomeric units, and that the two terminal monomeric unit A may be the same or different. Additionally, it will be understood that the recitation of "at least one terminal monomeric unit A" includes embodiments wherein the terminal monomeric unit A is referred to as a "first monomeric unit", with the recitation of "first monomeric unit" relating to that monomer to which other monomeric units are added, resulting in a polymeric compound of the formula A_n. Moreover, with regard to the at least one of the two terminal monomers, bonding of the adjacent monomer is at position 4 and optionally, Y = Z = hydrogen.

As to the recitation of the term "combinations thereof" it will be understood that one or more of the polymeric compounds of formula A_n may be used simultaneously, e.g., administered to a subject in need of treatment in a formulation comprising one or more of the polymeric compounds.

The compounds or combinations thereof display the utilities noted above for cocoa extracts; and throughout the disclosure, the term "cocoa extract" may be substituted by compounds of the invention or combinations thereof, such that it will be understood that the inventive compounds or combinations thereof can be cocoa extracts. As used herein the term "inventive compounds" therefore denotes polymeric compounds of formula A_n and their derivatives as defined above, and combinations thereof, whether extracted from cocoa or produced synthetically.

The term "oligomer", as used herein, refers to any compounds or combinations thereof of the formula presented above, wherein n is 2 through 18. When n is 2, the oligomer is termed a "dimer"; when n is 3, the oligomer is termed a "trimer"; when n is 4, the oligomer is termed a "tetramer"; when n is 5, the oligomer is termed a "pentamer"; and similar recitations may be designated for oligomers having n up to and including 18, such that when n is 18, the oligomer is termed an "octadecamer".

The inventive compounds or combinations thereof can be isolated, e.g., from a natural source such as any species of *Theobroma*, *Herrania* or inter- or intra-species crosses thereof; or, the inventive compounds or combinations thereof can be purified, e.g., compounds or combinations thereof can be substantially pure; for instance, purified to apparent homogeneity. Purity is a relative concept, and the numerous Examples demonstrate isolation of inventive compounds or combinations thereof, as well as purification thereof, such that by methods exemplified a skilled artisan can obtain a substantially pure inventive compound or combination thereof, or purify them to apparent homogeneity (e.g., purity by separate, distinct chromatographic peak). Considering the Examples, a substantially pure compound or combination of compounds is at least about 40% pure, e.g., at least about 50% pure, advantageously at least about 60% pure, e.g., at least about 70% pure, more advantageously at least about 75-80% pure, preferably, at least about 90% pure, more preferably greater than 90% pure, e.g., at least 90-95% pure, or even purer, such as greater than 95% pure, e.g., 95-98% pure.

Further, examples of the monomeric units comprising the oligomers used herein are (+)-catechin and (-)-epicatechin, abbreviated C and EC, respectively. The linkages between adjacent monomers are from position 4 to

position 6 or position 4 to position 8; and this linkage between position 4 of a monomer and position 6 and 8 of the adjacent monomeric units is designated herein as (4→6) or (4→8). There are four possible stereochemical linkages between position 4 of a monomer and position 6 and 8 of the adjacent monomer; and the stereochemical linkages between monomeric units is designated herein as (4α→6) or (4ß→6) or (4α→8) or (4ß→8). When C is linked to another C or EC, the linkages are designated herein as (4α→6) or (4α→8). When EC is linked to another C or EC, the linkages are designated herein as (4ß→6) or (4ß→8).

Examples of compounds eliciting the activities cited above include dimers, EC-(4ß→8)-EC and EC-(4ß→6)-EC, wherein EC-(4ß→8)-EC is preferred; trimers [EC-(4ß→8)]$_2$-EC, [EC-(4ß→8)]$_2$-C and [EC-(4ß→6)]$_2$-EC, wherein [EC-(4ß→8)]$_2$-EC is preferred; tetramers [EC-(4ß→8)]$_3$-EC, [EC-(4ß→8)]$_3$-C and [EC-(4ß→8)]$_2$-EC-(4ß→6)-C, wherein [EC-(4ß→8)]$_3$-EC is preferred; and pentamers [EC-(4ß→8)]$_4$-EC, [EC-(4ß→8)]$_3$-EC-(4ß→6)-EC, [EC-(4ß→8)]$_3$-EC-(4ß→8)-C and [EC-(4ß→8)]$_3$-EC-(4ß→6)-C, wherein the 3-position of the pentamer terminal monomeric unit is optionally derivatized with a gallate or ß-D-glucose; [EC-(4ß→8)]$_4$-EC is preferred.

Additionally, compounds which elicit the activities cited above also include hexamers to dodecamers, examples of which are listed below:

A hexamer, wherein one monomer (C or EC) having linkages to another monomer (4ß→8) or (4ß→6) for EC linked to another EC or C, and (4α→8) or (4α→6) for C linked to another C or EC; followed by a (4ß→8) linkage to a pentamer compound listed above, e.g., [EC-(4ß→8)]$_5$-EC, [EC-(4ß→8)]$_4$-EC-(4ß→6)-EC, [EC-(4ß→8)]$_4$-EC-(4ß→8)-C, and [EC-(4ß→8)]$_4$-EC-(4ß→6)-C, wherein the 3-position of the hexamer terminal monomeric unit is optionally derivatized with a gallate or a ß-D-glucose; in a preferred embodiment, the hexamer is [EC-(4ß→8)]$_5$-EC;

A heptamer, wherein any combination of two monomers (C and/or EC) having linkages to one another (4ß→8) or (4ß→6) for EC linked to another EC or C, and (4α→8) or (4α→6) for C linked to another C or EC; followed by a (4ß→8) linkage to a pentamer compound listed above, e.g., $[EC-(4ß→8)]_6$-EC, $[EC-(4ß→8)]_5$-EC-(4ß→6)-EC, $[EC-(4ß→8)]_5$-EC-(4ß→8)-C, and $[EC-(4ß→8)]_5$-EC-(4ß→6)-C, wherein the 3-position of the heptamer terminal monomeric unit is optionally derivatized with a gallate or a ß-D-glucose; in a preferred embodiment, the heptamer is $[EC-(4ß→8)]_6$-EC;

An octamer, wherein any combination of three monomers (C and/or EC) having linkages to one another (4ß→8) or (4ß→6) for EC linked to another EC or C, and (4α→8) or (4α→6) for C linked to another C or EC; followed by a (4ß→8) linkage to a pentamer compound listed above, e.g., $[EC-(4ß→8)]_7$-EC, $[EC-(4ß→8)]_6$-EC-(4ß→6)-EC, $[EC-(4ß→8)]_6$-EC-(4ß→8)-C, and $[EC-(4ß→8)]_6$-EC-(4ß→6)-C, wherein the 3-position of the octamer terminal monomeric unit is optionally derivatized with a gallate or a ß-D-glucose; in a preferred embodiment, the octamer is $[EC-(4ß→8)]_7$-EC;

A nonamer, wherein any combination of four monomers (C and/or EC) having linkages to one another (4ß→8) or (4ß→6) for EC linked to another EC or C, and (4α→8) or (4α→6) for C linked to another C or EC; followed by a (4ß→8) linkage to a pentamer compound listed above, e.g., $[EC-(4ß→8)]_8$-EC, $[EC-(4ß→8)]_7$-EC-(4ß→6)-EC, $[EC-(4ß→8)]_7$-EC-(4ß→8)-C, and $[EC-(4ß→8)]_7$-EC-(4ß→6)-C, wherein the 3-position of the nonamer terminal monomeric unit is optionally derivatized with a gallate or a ß-D-glucose; in a preferred embodiment, the nonamer is $[EC-(4ß→8)]_8$-EC;

A decamer, wherein any combination of five monomers (C and/or EC) having linkages to one another (4ß→8) or (4ß→6) for EC linked to another EC or C, and (4α→8) or (4α→6) for C linked to another C or EC;

followed by a (4ß→8) linkage to a pentamer compound listed above, e.g., [EC-(4ß→8)]$_9$-EC, [EC-(4ß→8)]$_8$-EC-(4ß→6)-EC, [EC-(4ß→8)]$_8$-EC-(4ß→8)-C, and [EC-(4ß→8)]$_8$-EC-(4ß→6)-C, wherein the 3-position of the decamer terminal monomeric unit is optionally derivatized with a gallate or a ß-D-glucose; in a preferred embodiment, the decamer is [EC-(4ß→8)]$_9$-EC;

An undecamer, wherein any combination of six monomers (C and/or EC) having linkages to one another (4ß→8) or (4ß→6) for EC linked to another EC or C, and (4α→8) or (4α→6) for C linked to another C or EC; followed by a (4ß→8) linkage to a pentamer compound listed above, e.g., [EC-(4ß→8)]$_{10}$-EC, [EC-(4ß→8)]$_9$-EC-(4ß→6)-EC, [EC-(4ß→8)]$_9$-EC-(4ß→8)-C, and [EC-(4ß→8)]$_9$-EC-(4ß→6)-C, wherein the 3-position of the undecamer terminal monomeric unit is optionally derivatized with a gallate or a ß-D-glucose; in a preferred embodiment, the undecamer is [EC-(4ß→8)]$_{10}$-EC; and

A dodecamer, wherein any combination of seven monomers (C and/or EC) having linkages to one another (4ß→8) or (4ß→6) for EC linked to another EC or C, and (4α→8) or (4α→6) for C linked to another C or EC; followed by a (4ß→8) linkage to a pentamer compound listed above, e.g., [EC-(4ß→8)]$_{11}$-EC, [EC-(4ß→8)]$_{10}$-EC-(4ß→6)-EC, [EC-(4ß→8)]$_{10}$-EC-(4ß→8)-C, and [EC-(4ß→8)]$_{10}$-EC-(4ß→6)-C, wherein the 3-position of the dodecamer terminal monomeric unit is optionally derivatized with a gallate or a ß-D-glucose; in a preferred embodiment, the dodecamer is [EC-(4ß→8)]$_{11}$-EC.

It will be understood from the detailed description that the aforementioned list is exemplary and provided as an illustrative source of several non-limiting examples of compounds of the invention, which is by no means an exhaustive list of the inventive compounds encompassed by the present invention.

Examples 3A, 3B, 4, 8, 16, 17, 20 and 21 describe methods to separate the compounds of the

invention. Examples 7, 8A-D and 10 describe methods to purify the compounds of the invention. Examples 5, 9, 12, 13, 14 and 19 describe methods to identify compounds of the invention. Figures 16A-P and 17A-AA illustrate a stereochemical library for representative pentamers of the invention. Example 11 describe a method to molecular model the compounds of the invention. Example 23 provides evidence for higher oligomers in cocoa, wherein n is 13 to 18.

Furthermore, while the invention is described with respect to cocoa extracts preferably comprising cocoa procyanidins, from this disclosure the skilled organic chemist will appreciate and envision synthetic routes to obtain and/or prepare the active compounds (see e.g. Example 6). Accordingly, the invention comprehends the use of synthetic cocoa polyphenols or procyanidines or their derivatives and/or their synthetic precursors which include, but are not limited to glycosides, gallates, esters, etc. and the like. That is, the inventive compounds can be prepared from isolation from cocoa or from any species within the *Theobroma* or *Herrania* genera, as well as from synthetic routes; and derivatives and synthetic precursors of the inventive compounds such as glycosides, gallates, esters, etc. are included in the inventive compounds. Derivatives can also include compounds of the above formulae wherein a sugar or gallate moiety is on the terminal monomer at positions Y or Z, or a substituted sugar or gallate moiety is on the terminal monomer at Y or Z.

Reference Example 1 describes the use of cocoa enzymes to oxidatively modify the compounds of the invention or combinations thereof to elicit improved cytotoxicity (see Fig. 5C) against certain cancer cell lines. The invention therefore includes the ability to enzymatically modify (e.g., cleavage or addition of a chemically significant moiety) the compounds of the invention, e.g., enzymatically with polyphenol oxidase,

peroxidase, catalase combinations, and/or enzymes such as hydrolases, esterases, reductases, transferases, and the like and in any combination, taking into account kinetic and thermodynamic factors (see also Example 27 regarding hydrolysis).

With regard to the synthesis of the inventive compounds, the skilled artisan will be able to envision additional routes of synthesis, based on this disclosure and the knowledge in the art, without undue experimentation. For example, based upon a careful retrosynthetic analysis of the polymeric compounds, as well as the monomers. For instance, given the phenolic character of the inventive compounds, the skilled artisan can utilize various methods of selective protection/deprotection, coupled with organometallic additions, phenolic couplings and photochemical reactions, e.g., in a convergent, linear or biomimetic approach, or combinations thereof, together with standard reactions known to those well-versed in the art of synthetic organic chemistry, as additional synthetic methods for preparing the inventive compounds, without undue experimentation. In this regard, reference is made to W. Carruthers, Some Modern Methods of Organic Synthesis, 3rd ed., Cambridge University Press, 1986, and J. March, Advanced Organic Chemistry, 3rd ed., John Wiley & Sons, 1985, van Rensburg et al., Chem. Comm., 24: 2705-2706 (Dec. 21, 1996), Ballenegger et al., (Zyma SA) European Patent 0096 007 B1, and documents in the References section below, all of which are hereby incorporated herein by reference.

UTILITIES OF COMPOUNDS OF THE INVENTION

With regard to the inventive compounds, it has been surprisingly found that the inventive compounds have discrete activities, and as such, the inventive compounds have broad applicability to the treatment of a variety of disease conditions, discussed hereinbelow.

Atherosclerosis, the most prevalent of cardiovascular diseases, is the principle cause of heart attack, stroke and vascular circulation problems. Atherosclerosis is a complex disease which involves many cell types, biochemical events and molecular factors. There are several aspects of this disease, its disease states and disease progression which are distinguished by the interdependent consequences of Low Density Lipoprotein (LDL) oxidation, cyclo-oxygenase (COX)/lipoxygenase (LOX) biochemistry and Nitric Oxide (NO) biochemistry.

Clinical studies have firmly established that the elevated plasma concentrations of LDL are associated with accelerated atherogenesis. The cholesterol that accumulates in atherosclerotic lesions originate primarily in plasma lipoproteins, including LDL. The oxidation of LDL is a critical event in the initiation of atheroma formation and is associated with the enhanced production of superoxide anion radical ($O_2\bullet-$). Oxidation of LDL by $O_2\bullet-$ or other reactive species (e.g., $\bullet OH$, $ONOO\bullet-$, lipid peroxy radical, copper ion, and iron based proteins) reduces the affinity of LDL for uptake in cells via receptor mediated endocytosis. Oxidatively modified LDLs are then rapidly taken up by macrophages which subsequently transform into cells closely resembling the "foam cells" observed in early atherosclerotic lesions.

Oxidized lipoproteins can also promote vascular injury through the formation of lipid hydroperoxides within the LDL particle. This event initiates radical chain oxidation reactions of unsaturated LDL lipids, thus producing more oxidized LDL for macrophage incorporation.

The collective accumulation of foam cells engorged with oxidized LDL from these processes results in early "fatty streak" lesions, which eventually progress to the more advanced complex lesions of atherosclerosis leading to coronary disease.

As discussed generally by Jean Marx at page 320 of Science, Vol. 265 (July 15, 1994), each year about 330,000 patients in the United States undergo coronary and/or peripheral angioplasty, a procedure designed to open up blood vessels, e.g., coronary arteries, clogged by dangerous atherosclerotic plaques (atherosclerosis) and thereby restore normal blood flow. For a majority of these patients, the operation works as intended. Nearly 33% of these patients (and maybe more by some accounts), however, develop restenosis, wherein the treated arteries become quickly clogged again. These patients are no better off, and sometimes worse off, than they were before angioplasty. Excessive proliferation of smooth muscle cells (SMCs) in blood vessel walls contributes to restenosis. Increased accumulation of oxidized LDL within lesion SMCs might contribute to an atherogenic-related process like restenosis. Zhou et al., "Association Between Prior Cytomegalovirus Infection And The Risk Of Restenosis After Coronary Atherectomy," August 29, 1996, New England Journal of Medicine, 335:624-630, and documents cited therein, all incorporated herein by reference. Accordingly, utility of the present invention with respect to atherosclerosis can apply to restenosis.

The inventive compounds have utility in the treatment of conditions involving platelet aggregation.

Nitric oxide (NO) is known to inhibit platelet aggregation, monocyte adhesion and chemotaxis, and proliferation of vascular smooth muscle tissue which are critically involved in the process of atherogenesis. Evidence supports the view that NO is reduced in atherosclerotic tissues due to its reaction with oxygen free radicals. The loss of NO due to these reactions leads to increased platelet and inflammatory cell adhesion to vessel walls to further impair NO mechanisms of relaxation. In this manner, the loss of NO promotes atherogenic processes, leading to progressive disease states.

When the normal level of NO is not produced, either because production is blocked by administration of an NOS inhibitor or possibly, in pathological states, such as atherosclerosis, the vascular muscles do not relax to the appropriate degree. The resulting vasoconstriction increases blood pressure and may be responsible for some forms of hypertension.

Although the inventive compounds inhibit the oxidation of LDL, the more comprehensive effects of these compounds is their multidimensional effects on atherosclerosis via NO. NO modulation by the inventive compounds brings about a collage of beneficial effects, including inhibiting platelet aggregation, which is involved with the progression of atherosclerosis.

The role of NO in the immune system is different from its function in blood vessels. Macrophages contain a form of NOS that is inducible, rather than constitutive, referred to as iNOS.

Transcription of the iNOS gene is controlled both positively and negatively by a number of biological response modifiers called cytokines. The most important inducers are gamma-interferon, tumor necrosis factor, interleukin-1, interleukin-2 and lipopolysaccharide (LPS), which is a component of the cell walls of gram negative bacteria. Stimulated macrophages produce enough NO to inhibit ribonuclease reductase, the enzyme that converts ribonucleotides to the deoxyribonucleotides necessary for DNA synthesis. Inhibition of DNA synthesis may be an important way in which macrophages and other tissues possessing iNOS can inhibit the growth of rapidly dividing tumor cells or infectious bacteria.

With regard to the effects of NO and infectious bacteria, microorganisms play a significant role in infectious processes which reflect body contact and injury, habits, profession, environment of the individual, as well as food borne diseases brought about by improper storage, handling and contamination.

Example 35 provides evidence for the formation of Cu^{+2}-, Fe^{+2}- and Fe^{+3}-oligomer complexes detected by MALDI/TOF/MS. These results indicate that the inventive compounds can complex with copper and/or iron ions to minimize their effects on LDL oxidation.

Moreover, the inventive compounds have useful anti-microbial activities for the treatment of infections and for the prevention of food spoilage. Examples 15 and 20 describe the antimicrobial activity of the inventive compounds against several representative microbiota having clinical and food significance, as outlined below.

MICROORGANISM	TYPE	CLINICAL/FOOD RELEVANCE
Helicobacter pylori	gram negative	gastritis, ulcers, gastric cancer

Bacillus species	gram positive	food poisoning, wound infections, bovine mastitis, septicemia
Salmonella species	gram negative	food poisoning, diarrhea
Staphylococcus aureus	gram positive	boils, carbuncles, wound infection, septicemia, breast abscesses
Escherichia coli	gram negative	infant diarrhea, urinary tract infection
Pseudomonas species	gram negative	urinary tract infections, wound infections, "swimmer's ear"
Saccharomyces cervisea	yeast	food spoilage
Acetobacter pasteurianus	gram negative	food spoilage

Example 20A describes the effects of the inventive compounds on macrophage NO production. In this example, the results demonstrate that the inventive compounds induce monocyte/macrophage NO production, both independent and dependent of stimulation by lipopolysaccharide (LPS) or cytokines. Macrophages producing NO can inhibit the growth of infectious bacteria.

Compounds of the invention eliciting antimicrobial activity are oligomers, where n is 2 to 18,

and preferably, are oligomers where n is 2, 4, 5, 6, 8 and 10.

Examples of compounds eliciting the antimicrobial activity with respect to NO cited above include dimers, tetramers, pentamers, hexamers, octamers and decamers, discussed above.

FORMULATIONS AND METHODS

Therefore, collectively, the inventive compounds, combinations thereof and compositions comprising the same have exhibited a wide array of activities against several aspects of atherosclerosis. cardiovascular disease, infectious agents and food spoilage.

Hence, the compounds of the invention, combinations thereof and compositions containing the same are COX inhibitors which affect platelet aggregation by inhibiting thromboxane A_2 formation, thus reducing the risk for thrombosis. Further, the inhibition of COX

leads to decreased platelet and inflammatory cell adhesion to vessel walls to allow for improved NO mechanisms of relaxation. These results, coupled with the inhibition of COX at concentrations similar to a known NSAID, indomethacin, indicates antithrombotic efficacy.

Moreover, the compounds of the invention, combinations thereof and compositions containing the same are antioxidants which suppress the oxidation of LDL by reducing the levels of superoxide radical anion and lipoxygenase mediated lipid peroxy radicals. The inhibition of LDL oxidation at this stage slows macrophage activation and retards foam cell formation to interrupt further progression of atherosclerosis. The inhibition of LDL oxidation can also slow the progression of restenosis. Thus, compounds of the invention or combinations thereof or compositions containing compounds of the invention or combinations thereof can be used for prevention and/or treatment of atherosclerosis and/or restenosis. And thus, the inventive compounds can be administered before or after angioplasty or similar procedures to prevent or treat restenosis in patients susceptible thereto.

For treatment or prevention of restenosis and/or atherosclerosis, an inventive compound or compounds or a composition comprising an inventive compound or compounds, alone or with other treatment, may be administered as desired by the skilled medical practitioner, from this disclosure and knowledge in the art, e.g., at the first signs or symptoms of restenosis and/or atherosclerosis, immediately prior to, concomitant with or after angioplasty, or as soon thereafter as desired by the skilled medical practitioner, without any undue experimentation required; and the administration of the inventive compound or compounds or a composition thereof, alone or with other treatment, may be continued as a regimen, e.g., monthly, bi-monthly, biannually,

annually, or in some other regimen, by the skilled medical practitioner for such time as is necessary, without any undue experimentation required.

Formulations of the inventive compounds, combinations thereof and compositions comprising the same can be prepared with standard techniques well known to those skilled in the pharmaceutical, food science, medical and veterinary arts, in the form of a liquid, suspension, tablet, capsule, injectable solution or suppository, for immediate or slow-release of the active compounds.

The carrier may also be a polymeric delayed release system. Synthetic polymers are particularly useful in the formulation of a composition having controlled release. An early example of this was the polymerization of methyl methacrylate into spheres having diameters less than one micron to form so-called nano particles, reported by Kreuter, J., Microcapsules and Nanoparticles in Medicine and Pharmacology, M. Donbrow (Ed). CRC Press, p. 125-148.

A frequent choice of a carrier for pharmaceuticals and more recently for antigens is poly (d,l-lactide-co-glycolide) (PLGA). This is a biodegradable polyester that has a long history of medical use in erodible sutures, bone plates and other temporary prostheses where it has not exhibited any toxicity. A wide variety of pharmaceuticals have been formulated into PLGA microcapsules. A body of data has accumulated on the adaption of PLGA for controlled, for example, as reviewed by Eldridge, J.H., et al. Current

Topics in Microbiology and Immunology, 1989, 146:59-66. The entrapment in PLGA microspheres of 1 to 10 microns in diameter can have an effect when administered orally. The PLGA microencapsulation process uses a phase separation of a water-in-oil emulsion. The inventive compound or compounds is or are prepared as an aqueous solution and the PLGA is dissolved in a suitable organic solvents such as methylene chloride and ethyl acetate. These two immiscible solutions are co-emulsified by high-speed stirring. A non-solvent for the polymer is then added, causing precipitation of the polymer around the aqueous droplets to form embryonic microcapsules. The microcapsules are collected, and stabilized with one of an assortment of agents (polyvinyl alcohol (PVA), gelatin, alginates, methyl cellulose) and the solvent removed by either drying *in vacuo* or solvent extraction.

Additionally, with regard to the preparation of slow-release formulations, reference is made to U.S. Patent Nos. 5,024,843, 5,091,190, 5,082,668, 4,612,008 and 4,327,725, hereby incorporated herein by reference.

Additionally, selective processing coupled with the identification of cocoa genotypes of interest could be used to prepare Standard-of-Identity (SOI) and non-SOI chocolate products as vehicles to deliver the active compounds to a patient in need of treatment for the disease conditions described above, as well as a means for the delivery of conserved levels of the inventive compounds.

In this regard, reference is made to copending U.S. Application Serial No. 08/709,406, filed September 6, 1996, hereby incorporated herein by reference. USSN 08/709,406 relates to a method of producing cocoa butter and/or cocoa solids having conserved levels of polyphenols from cocoa beans using a unique combination of processing steps which does not require separate bean roasting or liquor milling equipment, allowing for the option of processing cocoa beans without exposure to

severe thermal treatment for extended periods of time and/or the use of solvent extraction of fat. The benefit of this process lies in the enhanced conservation of polyphenols in contrast to that found in traditional cocoa processing, such that the ratio of the initial amount of polyphenol found in the unprocessed bean to that obtainable after processing is less than or equal to 2.

Compositions used in the invention include one or more of the above noted compounds in a formulation having a pharmaceutically, veterinarily or food science acceptable carrier or excipient.

Such compositions can be administered to a subject or patient in need of such administration in dosages and by techniques well known to those skilled in the medical, nutritional or veterinary arts taking into consideration the data herein, and such factors as the age, sex, weight, genetics and condition of the particular subject or patient, and the route of administration, relative concentration of particular oligomers, and toxicity (e.g., LD_{50}).

The compositions can be co-administered or sequentially administered with other

platelet aggregation inhibiting agents and/or with agents which reduce or alleviate ill effects of platelet aggregation inhibiting agents; again, taking into consideration such factors as the age, sex, weight, genetics and condition of the particular subject or patient, and, the route of administration.

Examples of compositions of the invention for human or veterinary use include edible compositions for oral administration, such solid or liquid formulations, for instance, capsules, tablets, pills and the like, as well as chewable solid or beverage formulations, to which the present invention may be well-suited since it is from an edible source (e.g., cocoa or chocolate flavored solid or liquid compositions); liquid preparations for orifice, e.g., oral, nasal, anal, vaginal etc., administration such as suspensions, syrups or elixirs (including cocoa or chocolate flavored compositions); and, preparations for parental, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration) such as sterile suspensions or emulsions. However, the active ingredient in the compositions may complex with proteins such that when administered into the bloodstream, clotting may occur due to precipitation of blood proteins; and, the skilled artisan should take this into account. In such compositions the active cocoa extract may be in admixture with a suitable carrier, diluent, or excipient such as sterile water,

this into account. In such compositions the polymeric compound of formula A_n may be in admixture with a suitable carrier, diluent, or excipients such as sterile water.

physiological saline, glucose, DMSO, ethanol, or the like. The active cocoa extract of the invention can be provided in lyophilized form for reconstituting, for instance, in isotonic aqueous, saline, glucose or DMSO buffer. In certain saline solutions, some precipitation has been observed; and, this observation may be employed as a means to isolate inventive compounds, e.g., by a "salting out" procedure.

Example 24 describes the preparation of the inventive compounds in a tablet formulation for application in the pharmaceutical, supplement and food areas. Further, Example 25 describes the preparation of the inventive compounds in capsule formulations for similar applications. Still further, Example 26 describes the formulation of Standard of Identity (SOI) and non-SOI chocolates containing the compounds of the invention or cocoa solids obtained from methods described in copending U.S. Application Serial No. 08/709,406, hereby incorporated herein by reference.

The polymeric compound of formula A_n may be provided in a kit. The kit can include a separate container containing a suitable carrier, diluent or excipient. The kit can also include an additional antimicrobial, or platelet aggregation inhibiting agent and/or an agent which reduces or alleviates ill effects of antimicrobial or platelet aggregation inhibiting agents

for co- or sequential-administration. The additional agent(s) can be provided in separate container(s) or in admixture with the active cocoa extract. Additionally, the kit can include instructions for mixing or combining ingredients and/or administration.

IDENTIFICATION OF GENES

A further embodiment of the invention comprehends the modulation of genes expressed as a result of intimate cellular contact by the inventive compounds or a combination of compounds. As such, the present invention comprehends methods for the identification of genes induced or repressed by the polymeric compounds of formula A_n or a combination of compounds which are associated with several diseases, including but not limited to atherosclerosis, hypertension, cancer, cardiovascular disease, and inflammation. Specifically, genes which are differentially expressed in these disease states, relative to their expression in "normal" nondisease states are identified and described before and after contact by the inventive compounds or a combination of compounds.

As mentioned in the previous discussion, these diseases and disease states are based in part on free radical interactions with a diversity of biomolecules. A central theme in these diseases is that many of the free radical reactions involve reactive oxygen species, which in turn induce physiological conditions involved in disease progression. For instance, reactive oxygen species have been implicated in the regulation of transcription factors such as nuclear factor (NF)-κB. The target genes for NF-κB comprise a list of genes linked to coordinated inflammatory response. These include genes encoding tumor necrosis factor (TNF)-α, interleukin (IL)-I, IL-6, IL-8, inducible NOS, Major Histocompatabilty Complex (MHC) class I antigens, and others. Also, genes that modulate the activity of transcription factors may in turn be induced by oxidative

stress. Oxidative stress is the imbalance between radical scavenging and radical generating systems. Several known examples (Winyard and Blake, 1997) of these conditions include gadd153 (a gene induced by growth arrest and DNA damage), the product of which has been shown to bind NF-IL6 and form a heterodimer that cannot bind to DNA. NF-IL6 upregulates the expression of several genes, including those encoding interleukins 6 and 8. Another example of oxidative stress inducible genes are gadd45 which regulates the effects of the transcription factor p53 in growth arrest. p53 codes for the p53 protein which can halt cell division and induce abnormal cells (e.g. cancer) to undergo apoptosis.

Given the full panoply of unexpected, nonobvious and novel utilities for the inventive compounds or combination of compounds for utility in a diverse array of diseases based in part by free radical mechanisms, the invention further comprehends strategies to determine the temporal effects on gene(s) or gene product(s) expression by the inventive compounds in animal *in vitro* and/or *in vivo* models of specific disease or disease states using gene expression assays. These assays include, but are not limited to Differential Display, sequencing of cDNA libraries, Serial Analysis of Gene Expression (SAGE), expression monitoring by hybridization to high density oligonucleotide arrays and various reverse transcriptase-polymerization chain reaction (RT-PCR) based protocols or their combinations (Lockhart et al., 1996).

The comprehensive physiological effects of the inventive compounds or combination of compounds embodied in the invention, coupled to a genetic evaluation process permits the discovery of genes and gene products, whether known or novel, induced or repressed. For instance, the invention comprehends the *in vitro* and *in vivo* induction and/or repression of cytokines (e.g. IL-1, IL-2, IL-6, IL-8, IL-12, and TNF-α) in lymphocytes using RT-PCR.

Similarly, the invention comprehends the application of Differential Display to ascertain the induction and/or repression of select genes; for the cardiovascular area (e.g. superoxide dismutase, heme oxidase, COX I and 2, and other oxidant defense genes) under stimulated and/or oxidant stimulated conditions (e.g. TNF-α or H_2O_2) conditions. For the cancer area, the invention comprehends the application of Differential Display to ascertain the induction and/or repression of genes or gene products such as CuZn-superoxide dismutase, Mn-superoxide dismutase, catalase, etc., in control and oxidant stressed cells.

The following non-limiting Examples are given by way of illustration only and are not to be considered a limitation of this invention, many apparent variations of which are possible without departing from the spirit or scope thereof.

EXAMPLES

Example 1: Cocoa Source and Method of Preparation

Several *Theobroma cacao* genotypes which represent the three recognized horticultural races of cocoa (Enriquez, 1967; Engels, 1981) were obtained from the three major cocoa producing origins of the world. A list of those genotypes used in this study are shown in Table 1. Harvested cocoa pods were opened and the beans with pulp were removed for freeze drying. The pulp was manually removed from the freeze dried mass and the beans were subjected to analysis as follows. The unfermented, freeze dried cocoa beans were first manually dehulled, and ground to a fine powdery mass with a TEKMAR Mill. The resultant mass was then defatted overnight by Soxhlet extraction using redistilled hexane as the solvent. Residual solvent was removed from the defatted mass by vacuum at ambient temperature.

Table 1: Description of *Theobroma cacao* Source Material

GENOTYPE	ORIGIN	HORTICULTURAL RACE

UIT-1	Malaysia	Trinitario
Unknown	West Africa	Forastero
ICS-100	Brazil	Trinitario (Nicaraguan Criollo ancestor)
ICS-39	Brazil	Trinitario (Nicaraguan Criollo ancestor)
UF-613	Brazil	Trinitario
EEG-48	Brazil	Forastero
UF-12	Brazil	Trinitario
NA-33	Brazil	Forastero

Example 2: Procyanidin Extraction Procedures

A. Method 1

Procyanidins were extracted from the defatted, unfermented, freeze dried cocoa beans of Example 1 using a modification of the method described by Jalal and Collin (1977). Procyanidins were extracted from 50 gram batches of the defatted cocoa mass with 2X 400 mL 70% acetone/deionized water followed by 400mL 70% methanol/deionized water. The extracts were pooled and the solvents removed by evaporation at 45°C with a rotary evaporator held under partial vacuum. The resultant aqueous phase was diluted to 1L with deionized water and extracted 2X with 400mL $CHCl_3$. The solvent phase was discarded. The aqueous phase was then extracted 4X with 500mL ethyl acetate. Any resultant emulsions were broken by centrifugation on a Sorvall RC 28S centrifuge operated at 2,000 xg for 30 min. at 10°C. To the combined ethyl

acetate extracts, 100-200mL deionized water was added. The solvent was removed by evaporation at 45°C with a rotary evaporator held under partial vacuum. The resultant aqueous phase was frozen in liquid N_2 followed by freeze drying on a LABCONCO Freeze Dry System. The yields of crude procyanidins that were obtained from the different cocoa genotypes are listed in Table 2.

Table 2: Crude Procyanidin Yields

GENOTYPE	ORIGIN	YIELDS (g)
UIT-1	Malaysia	3.81
Unknown	West Africa	2.55
ICS-100	Brazil	3.42
ICS-39	Brazil	3.45
UF-613	Brazil	2.98
EEG-48	Brazil	3.15
UF-12	Brazil	1.21
NA-33	Brazil	2.23

B. Method 2

Alternatively, procyanidins are extracted from defatted, unfermented, freeze dried cocoa beans of Example 1 with 70% aqueous acetone. Ten grams of defatted material was slurried with 100 mL solvent for 5-10 min. The slurry was centrifuged for 15 min. at 4°C at 3000 xg and the supernatant passed through glass wool. The filtrate was subjected to distillation under partial vacuum and the resultant aqueous phase frozen in liquid N_2, followed by freeze drying on a LABCONCO Freeze Dry System. The yields of crude procyanidins ranged from 15-20%.

Without wishing to be bound by any particular theory, it is believed that the differences in crude

yields reflected variations encountered with different genotypes, geographical origin, horticultural race, and method of preparation.

Example 3: Partial Purification of Cocoa Procyanidins

A. Gel Permeation Chromatography

Procyanidins obtained from Example 2 were partially purified by liquid chromatography on Sephadex LH-20 (28 x 2.5 cm). Separations were aided by a step gradient from deionized water into methanol. The initial gradient composition started with 15% methanol in deionized water which was followed step wise every 30 min. with 25% methanol in deionized water, 35% methanol in deionized water, 70% methanol in deionized water, and finally 100% methanol. The effluent following the elution of the xanthine alkaloids (caffeine and theobromine) was collected as a single fraction. The fraction yielded a xanthine alkaloid free subfraction which was submitted to further subfractionation to yield five subfractions designated MM2A through MM2E. The solvent was removed from each subfraction by evaporation at 45°C with a rotary evaporator held under partial vacuum. The resultant aqueous phase was frozen in liquid N_2 and freeze dried overnight on a LABCONCO Freeze Dry System. A representative gel permeation chromatogram showing the fractionation is shown in Figure 1. Approximately, 100mg of material was subfractionated in this manner.

Chromatographic Conditions: Column; 28 x 2.5 cm Sephadex LH-20, Mobile Phase: Methanol/Water Step Gradient, 15:85, 25:75, 35:65, 70:30, 100:0 Stepped at 1/2 Hour Intervals, Flow Rate; 1.5mL/min, Detector; UV at λ_1 = 254 nm and λ_2 = 365 nm, Chart Speed: 0.5mm/min, Column Load; 120mg.

B. Semi-preparative High Performance Liquid Chromatography (HPLC)

Method 1. Reverse Phase Separation

Procyanidins obtained from Example 2 and/or 3A were partially purified by semi-preparative HPLC. A Hewlett Packard 1050 HPLC System equipped with a variable wavelength detector, Rheodyne 7010 injection valve with 1mL injection loop was assembled with a Pharmacia FRAC-100 Fraction Collector. Separations were effected on a Phenomenex Ultracarb™ 10 μ ODS column (250 x 22.5mm) connected with a Phenomenex 10 μ ODS Ultracarb™ (60 x 10 mm) guard column. The mobile phase composition was A = water; B = methanol used under the following linear gradient conditions: [Time, %A]; (0,85), (60,50), (90,0), and (110,0) at a flow rate of 5mL/min. Compounds were detected by UV at 254nm

A representative Semi-preparative HPLC trace is shown in Figure 5A for the separation of procyanidins present in fraction D + E. Individual peaks or select chromatographic regions were collected on timed intervals or manually by fraction collection for further purification and subsequent evaluation. Injection loads ranged from 25-100mg of material.

Method 2. Normal Phase Separation

Procyanidin extracts obtained from Examples 2 and/or 3A were partially purified by semi-preparative HPLC. A Hewlett Packard 1050 HPLC system, Millipore-Waters Model 480 LC detector set at 254nm was assembled with a Pharmacia Frac-100 Fraction Collector set in peak mode. Separations were effected on a Supelco 5μm Supelcosil LC-Si column (250 x 10mm) connected with a Supelco 5μm Supelguard LC-Si guard column (20 x 4.6mm). Procyanidins were eluted by a linear gradient under the following conditions: (Time, %A, %B); (0,82,14), (30, 67.6, 28.4), (60, 46, 50), (65, 10, 86), (70, 10, 86) followed by a 10 min. re-equilibration. Mobile phase composition was A = dichloromethane; B = methanol; and C = acetic acid: water (1:1). A flow rate of 3mL/min was used. Components were detected by UV at 254nm, and

recorded on a Kipp & Zonan BD41 recorder. Injection volumes ranged from 100-250μL of 10mg of procyanidin extracts dissolved in 0.25mL 70% aqueous acetone. A representative semi-preparative HPLC trace is shown in Figure 5B. Individual peaks or select chromatographic regions were collected on timed intervals or manually by fraction collection for further purification and subsequent evaluation.

HPLC Conditions:

250 x 10mm Supelco Supelcosil LC-Si (5μm) Semipreparative Column
20 x 4.6mm Supelco Supelcosil LC-Si (5μm) Guard Column
Detector: Waters LC Spectrophotometer Model 480 @ 254nm
Flow rate: 3mL/min,
Column Temperature: ambient,
Injection: 250μL of 70% aqueous acetone extract.

Gradient: Time (min)	CH_2Cl_2	Methanol	Acetic Acid:H_2O (1:1)
0	82	14	4
30	67.6	28.4	4
60	46	50	4
65	10	86	4
70	10	86	4

The fractions obtained were as follows:

FRACTION	TYPE
1	dimers
2	trimers
3	tetramers
4	pentamers
5	hexamers
6	heptamers
7	octamers
8	nonamers
9	decamers
10	undecamers
11	dodecamers
12	higher oligomers

Example 4: Analytical HPLC Analysis of Procyanidin Extracts

Method 1. Reverse Phase Separation

Procyanidin extracts obtained from Example 3 were filtered through a 0.45μ filter and analyzed by a Hewlett Packard 1090 ternary HPLC system equipped with a Diode Array detector and a HP model 1046A Programmable Fluorescence Detector. Separations were effected at 45°C on a Hewlett-Packard 5μ Hypersil ODS column (200 x 2.1mm). The flavanols and procyanidins were eluted with a linear gradient of 60% B into A followed by a column wash with B at a flow rate of 0.3mL/min. The mobile phase composition was B = 0.5% acetic acid in methanol and A = 0.5% acetic acid in nanopure water. Acetic acid

levels in A and B mobile phases can be increased to 2%. Components were detected by fluorescence, where λ_{ex} = 276nm and λ_{ex} = 316nm and by UV at 280nm. Concentrations of (+)-catechin and (-)-epicatechin were determined relative to reference standard solutions. Procyanidin levels were estimated by using the response factor for (-)-epicatechin. A representative HPLC chromatogram showing the separation of the various components is shown in Figure 2A for one cocoa genotype. Similar HPLC profiles were obtained from the other cocoa genotypes.

HPLC Conditions: Column: 200 x 2.1mm Hewlett Packard Hypersil ODS (5μ)

Guard column: 20 x 2.1mm Hewlett Packard Hypersil ODS (5μ)

Detectors: Diode Array @ 280nm

Fluorescence λ_{ex} = 276nm;

λ_{em} = 316nm.

Flow rate: 0.3mL/min.

Column Temperature: 45°C

Gradient: Time (min)	0.5% Acetic Acid in nanopure water	0.5% Acetic acid in methanol
0	100	0
50	40	60
60	0	100

Method 2. Normal Phase Separation

Procyanidin extracts obtained from Examples 2 and/or 3 were filtered through a 0.45μ filter and analyzed by a Hewlett Packard 1090 Series II HPLC system equipped with a HP model 1046A Programmable Fluorescence detector and Diode Array detector. Separations were effected at 37°C on a 5μ Phenomenex Lichrosphere® Silica 100 column (250 x 3.2mm) connected to a Supelco Supelguard LC-Si 5μ guard column (20 x 4.6mm). Procyanidins were eluted by linear gradient under the following conditions: (Time, %A, %B); (0, 82, 14), (30, 67.6, 28.4), (60, 46, 50), (65, 10, 86), (70, 10, 86) followed by an 8 min. re-equilibration. Mobile phase composition was A=dichloromethane, B=methanol, and C=acetic acid: water at a volume ratio of 1:1. A flow rate of 0.5 mL/min. was used. Components were detected by fluorescence, where λ_{ex} = 276nm and λ_{em} = 316nm or by UV at 280 nm. A representative HPLC chromatogram showing the separation of the various procyanidins is shown in Figure 2B for one genotype. Similar HPLC profiles were obtained from other cocoa genotypes.

HPLC Conditions:

250 x 3.2mm Phenomenex Lichrosphere® Silica 100 column (5μ) 20 x 4.6mm
Supelco Supelguard LC-Si (5μ) guard column

Detectors: Photodiode Array @ 280nm
Fluorescence λ_{ex} = 276nm; λ_{em} = 316nm.

Flow rate: 0.5 mL/min.

Column Temperature: 37°C

Gradient: Time (min.)	CH_2-Cl_2	Methanol	Acetic Acid/Water (1:1)
0	82	14	4
30	67.6	28.4	4
60	46	50	4
65	10	86	4
70	10	86	4

Example 5: Identification of Procyanidins

Procyanidins were purified by liquid chromatography on Sephadex LH-20 (28 x 2.5cm) columns followed by semi-preparative HPLC using a 10μ Bondapak C18 (100 x 8mm) column or by semi-preparative HPLC using a 5μ Supelcosil LC-Si (250 x 10mm) column.

Partially purified isolates were analyzed by Fast Atom Bombardment - Mass Spectrometry (FAB-MS) on a VG ZAB-T high resolution MS system using a Liquid Secondary Ion Mass Spectrometry (LSIMS) technique in positive and negative ion modes. A cesium ion gun was used as the ionizing source at 30kV and a "Magic Bullet Matrix" (1:1 dithiothreitol/dithioerythritol) was used as the proton donor.

Analytical investigations of these fractions by LSIMS revealed the presence of a number of flavan-3-ol oligomers as shown in Table

Table 3: LSIMS (Positive Ion) Data from Cocoa Procyanidin Fractions

Oligomer	$(M + 1)^+$ *m/z*	$(M + Na)^+$ *m/z*	Mol. Wt.
Monomers (catechins)	291	313	290
Dimer(s)	577/579	599/601	576/578
Trimer(s)	865/867	887/889	864/866
Tetramer(s)	1155	1177	1154
Pentamer(s)	1443	1465	1442
Hexamer(s)	1731	1753	1730
Heptamer(s)	---	2041	2018
Octamer(s)	---	2329	2306
Nonamer(s)	---	2617	2594
Decamer(s)	---	2905	2882
Undecamer(s)	---	---	3170
Dodecamer(s)	---	---	3458

The major mass fragment ions were consistent with work previously reported for both positive and negative ion FAB-MS analysis of procyanidins (Self et al., 1986 and Porter et al., 1991). The ion corresponding to *m/z* 577 $(M+H)^+$ and its sodium adduct at *m/z* 599 $(M+Na)^+$ suggested the presence of doubly linked procyanidin dimers in the isolates. It was interesting to note that the higher oligomers were more likely to form sodium adducts $(M+Na)^+$ than their protonated molecular ions $(M+H)^+$. The procyanidin isomers B-2, B-5 and C-1 were tentatively identified based on the work reported by Revilla et al. (1991), Self et al. (1986) and Porter et al. (1991). Procyanidins up to both the octamer and decamer were verified by FAB-MS in the

partially purified fractions. Additionally, evidence for procyanidins up to the dodecamer were observed from normal phase HPLC analysis (see Figure 2B). Table 4 lists the relative concentrations of the procyanidins found in xanthine alkaloid free isolates based on reverse phase HPLC analysis. Table 5 lists the relative concentrations of the procyanidins based on normal phase HPLC analysis.

Table 4: Relative Concentrations of Procyanidins in the Xanthine Alkaloid Free Isolates

Component	Amount
(+)-catechin	1.6%
(-)-epicatechin	38.2%
B-2 Dimer	11.0%
B-5 Dimer	5.3%
C-1 Trimer	9.3%
Doubly linked dimers	3.0%
Tetramer(s)	4.5%
Pentamer-Octamer	24.5%
Unknowns and higher oligomers	2.6%

Table 5: Relative Concentrations of Procyanidins in Aqueous Acetone Extracts

Component	Amount
(+)-catechin and (-) -epicatechin	41.9%
B-2 and B-5 Dimers	13.9%
Trimers	11.3%
Tetramers	9.9%
Pentamers	7.8%
Hexamers	5.1%
Heptamers	4.2%
Octamers	2.8%
Nonamers	1.6%
Decamers	0.7%
Undecamers	0.2%
Dodecamers	<0.1%

Figure 3 shows several procyanidin structures and Figures 4A-4E show the representative HPLC chromatograms of the five fractions employed in the following screening for anti-cancer or antineoplastic activity. The HPLC conditions for Figs. 4A-4E were as follows:

HPLC Conditions: Hewlett Packard 1090 ternary HPLC System equipped with HP Model 1046A Programmable Fluorescence Detector.

Column: Hewlett Packard 5μ Hypersil ODS (200 x 2.1mm) Linear Gradient of 60% B into A at a flow rate of 0.3mL/min. B = 0.5% acetic acid in methanol; A = 0.5% acetic acid in deionized water. λ_{ex} = 280nm; λ_{em} = 316nm.

Reference Example 1: Effect of oxidative modification

It is known (Lehrian and Patterson, 1983) that polyphenol oxidase (PPO) will oxidize polyphenols during the fermentation stage. To determine what effect enzymatically oxidized polyphenols would have on activity, another experiment was performed. Crude PPO was prepared by extracting finely ground, unfermented, freeze dried, defatted Brazilian cocoa beans with acetone at a ratio of 1gm powder to 10mL acetone. The slurry was centrifuged at 3,000 rpm for 15 min. This was repeated three times, discarding the supernatant each time with the fourth extraction being poured through a Buchner filtering funnel. The acetone powder was allowed to air dry, followed by assay according to the procedures described by McLord and Kilara, (1983). To a solution of crude polyphenols (100mg/10mL Citrate-Phosphate buffer, 0.02M, pH 5.5) 100mg of acetone powder (4,000 units activity/mg protein) was added and allowed to stir for 30 min. with a stream of air bubbled through the slurry. The sample was centrifuged at 5,000xg for 15 min. and the supernatant extracted 3X with 20mL ethyl acetate. The ethyl acetate extracts were combined, taken to dryness by distillation under partial vacuum and 5mL water added, followed by lyophilization. The material was then assayed against Hela cells and the dose-response compared to crude polyphenol extracts that were not enzymatically treated. The results (Figure 5C) showed a significant shift in the dose-response curve for the enzymatically oxidized extract, showing that the oxidized products were more inhibitory than their native forms.

Example 6: Synthesis of Procyanidins

The synthesis of procyanidins was performed according to the procedures developed by Delcour et al. (1983), with modification. In addition to condensing (+)-catechin with dihydroquercetin under reducing conditions, (-)-epicatechin was also used to reflect the high concentrations of (-)-epicatechin that naturally occur in unfermented cocoa beans. The synthesis products were isolated, purified, analyzed, and identified by the procedures described in Examples 3, 4 and 5. In this manner, the biflavanoids, triflavanoids and tetraflavanoids are prepared and used as analytical standards and, in the manner described above with respect to cocoa extracts.

Example 7: HPLC Purification Methods

Method A. GPC Purification

Procyanidins obtained as in Example 2 were partially purified by liquid chromatography on Sephadex LH 20 (72.5 x 2.5cm), using 100% methanol as the eluting solvent, at a flow rate of 3.5mL/min. Fractions of the eluent were collected after the first 1.5 hours, and the fractions were concentrated by a rotary evaporator, redissolved in water and freeze dried. These fractions were referred to as pentamer enriched fractions. Approximately 2.00g of the extract obtained from Example

2 was subfractionated in this manner. Results are shown in Table 6.

Table 6: Composition of Fractions Obtained:

Fraction (Time)	Monomer (% Area)	Dimer (% Area)	Trimer (% Area)	Tetramer (% Area)	Pentamer (% Area)	Hexamer (% Area)	Heptamer (% Area)	Octamer (% Area)	Nonamer (% Area)	Decamer (% Area)	Undecamer (% Area)	Others (% Area)
1:15	73	8	16	3	ND	ND	ND	ND	ND	ND	ND	ND
1:44	67	19	10	3	1	tr	tr	tr	tr	tr	tr	tr
2:13	30	29	24	11	4	1	tr	tr	tr	tr	tr	tr
2:42	2	16	31	28	15	6	2	tr	tr	tr	tr	tr
3:11	1	12	17	25	22	13	7	2	1	tr	tr	tr
3:40	tr	18	13	18	20	15	10	5	2	tr	tr	tr
4:09	tr	6	8	17	21	19	14	8	4	2	tr	tr

ND = not detected
tr = trace amount

Method B. Normal Phase Separation

Procyanidins obtained as Example 2 were separated purified by normal phase chromatography on Supelcosil LC-Si, 100Å, 5μm (250 x 4.6mm), at a flow rate of 1.0mL/min, or, in the alternative, Lichrosphere® Silica 100, 100Å, 5μm (235 x 3.2mm), at a flow rate of 0.5mL/min. Separations were aided by a step gradient under the following conditions: (Time, %A, %B); (0, 82, 14), (30, 67.6, 28.4), (60, 46, 50), (65, 10, 86), (70, 10, 86). Mobile phase composition was A = dichloromethane; B = methanol; and C = acetic acid:water (1:1). Components were detected by fluorescence where λ_{ex} = 276nm and λ_{em} = 316nm, and by UV at 280nm. The injection volume was 5.0μL (20mg/mL) of the procyanidins obtained from Example 2. These results are shown in Fig. 18A and 18B.

In the alternative, separations were aided by a step gradient under the following conditions: (Time, %A, %B); (0, 76, 20); (25, 46, 50); (30, 10, 86). Mobile phase composition was A = dichloromethane; B = methanol; and C = acetic acid : water (1:1). The results are shown in Fig. 19A and 19B.

Method C. Reverse - Phase Separation

Procyanidins obtained as in Example 2 were separated purified by reverse phase chromatography on Hewlett Packard Hypersil ODS 5μm. (200 x 2.1mm), and a Hewlett Packard Hypersil ODS 5μm guard column (20 x 2.1mm). The procyanidins were eluted with a linear gradient of 20% B into A in 20 minutes, followed by a column wash with 100% B at a flow rate of 0.3mL/min. The mobile phase composition was a degassed mixture of B = 1.0% acetic acid in methanol and A = 2.0% acetic acid in nanopure water. Components were detected by UV at 280nm, and fluorescence where λ_{ex} = 276nm and λ_{em} = 316nm; and the injection volume was 2.0μL (20mg/mL).

Example 8: HPLC Separation of Pentamer Enriched Fractions

Method A. Semi-Preparative Normal Phase HPLC

The pentamer enriched fractions were further purified by semi-preparative normal phase HPLC by a Hewlett Packard 1050 HPLC system equipped with a Millipore - Waters model 480 LC detector set at 254nm, which was assembled with a Pharmacia Frac-100 Fraction Collector set to peak mode. Separations were effected on a Supelco 5μm Supelcosel LC-Si, 100Å column (250 x 10mm) connected with a Supelco 5μ Supelguard LC-Si guard column (20 x 4.6mm). Procyanidins were eluted by a linear gradient under the following conditions: (Time, %A, %B); (0, 82, 14), (30, 67.6, 28.4), (60, 46, 50), (65, 10, 86), (70, 10, 86) followed by a 10 minute re-equilibration. Mobile phase composition was A = dichloromethane; B = methanol; and C = acetic acid:water (1:1). A flow rate of 3mL/min was used. Components were detected by UV at 254nm; and recorded on a Kipp & Zonan BD41 recorder. Injection volumes ranged from 100-250μl of 10mg of procyanidin extracts dissolved in 0.25mL 70% aqueous acetone. Individual peaks or select chromatographic regions were collected on timed intervals or manually by fraction collection for further purification and subsequent evaluation.

HPLC conditions: 250 x 100mm Supelco Supelcosil LC-Si (5μm) Semipreparative Column
20 x 4.6mm Supelco Supelcosil LC-Si (5μm) Guard Column
Detector: Waters LC Spectrophotometer Model 480 @ 254nm
Flow rate: 3mL/min.,
Column Temperature: ambient,
Injection: 250μL of pentamer enriched extract

Gradient:	CH_2Cl_2	methanol	acetic acid: water (1:1)

0	82	14	4
30	67.6	28.4	4
60	46	50	4
65	10	86	4
70	10	86	4

Method B. Reverse Phase Separation

Procyanidin extracts obtained as in Example 7 were filtered through a 0.45μ nylon filter and analyzed by a Hewlett Packard 1090 ternary phase HPLC system equipped with a Diode Array detector and a HP model 1046A Programmable Fluorescence Detector. Separations were effected at 45°C on a Hewlett Packard 5μ Hypersil ODS column (200 x 2.1mm). The procyanidins were eluted with a linear gradient of 60% B into A followed by a column wash with B at a flow rate of 0.3mL/min. The mobile phase composition was a de-gassed mixture of B = 0.5% acetic acid in methanol and A = 0.5% acetic acid in nanopure water. Acetic acid levels in A and B mobile phases can be increased to 2%. Components were detected by fluorescence, where λ_{ex} = 276nm and λ_{em} = 316nm, and by UV at 280nm. Concentrations of (+)-catechin and (-)-epicatechin were determined relative to reference standard solutions. Procyanidin levels were estimated by using the response factor for (-)-epicatechin.

Method C. Normal Phase Separation

Pentamer enriched procyanidin extracts obtained as in Example 7 were filtered through a 0.45μ nylon filter and analyzed by a Hewlett Packard 1090 Series II HPLC system equipped with a HP Model 1046A Programmable Fluorescence detector and Diode Array detector. Separations were effected at 37°C on a 5μ Phenomenex Lichrosphere® Silica 100 column (250 x 3.2mm) connected to a Supelco Supelguard LC-Si 5μ guard column (20 x 4.6mm). Procyanidins were eluted by linear gradient under the following conditions: (time, %A, %B); (0, 82,

14), (30, 67.6, 28.4), (60, 46, 50), (65, 10, 86), (70, 10, 86), followed by an 8 minute re-equilibration. Mobile phase composition was A = dichloromethane, B = methanol, and C = acetic acid:water at a volume ratio of 1:1. A flow rate of 0.5mL/min was used. Components were detected by fluorescence, where λ_{ex} = 276nm and λ_{em} = 316nm or by UV at 280nm. A representative HPLC chromatogram showing the separation of the various procyanidins is shown in Figure 2 for one genotype. Similar HPLC profiles were obtained from other *Theobroma*, *Herrania* and/or their inter or intra specific crosses.

HPLC conditions:

250 x 3.2mm Phenomenex Lichrosphere® Silica 100 column (5μ) 20 x 4.6mm Supelco Supelguard LC-Si (5μ) guard column

Detectors: Photodiode Array @ 280nm

Fluorescence λ_{ex} = 276nm; λ_{em} = 316nm

Flow rate: 0.5 mL/min.

Column temperature: 37°C

acetic acid:

Gradient:	CH_2Cl_2	methanol	water (1:1)
0	82	14	4
30	67.6	28.4	4
60	46	50	4
65	10	86	4
70	10	86	4

Method D. Preparative Normal Phase Separation

The pentamer enriched fractions obtained as in Example 7 were further purified by preparative normal phase chromatography by modifying the method of Rigaud et al., (1993) J. Chrom. 654, 255-260.

Separations were affected at ambient temperature on a 5μ Supelcosil LC-Si 100Å column (50 x

2cm), with an appropriate guard column. Procyanidins were eluted by a linear gradient under the following conditions: (time, %A, %B, flow rate); (0, 92.5, 7.5, 10); (10, 92.5, 7.5, 40); (30, 91.5, 18.5, 40); (145, 88, 22, 40); (150, 24, 86, 40); (155, 24, 86, 50); (180, 0, 100, 50). Prior to use, the mobile phase components were mixed by the following protocol:

Solvent A preparation (82% CH_2Cl_2, 14% methanol, 2% acetic acid, 2% water):

1. Measure 80mL of water and dispense into a 4L bottle.

2. Measure 80mL of acetic acid and dispense into the same 4L bottle.

3. Measure 560mL of methanol and dispense into the same 4L bottle.

4. Measure 3280mL of methylene chloride and dispense into the 4L bottle.

5. Cap the bottle and mix well.

6. Purge the mixture with high purity Helium for 5-10 minutes to degas.

Repeat steps 1-6 two times to yield 8 volumes of solvent A.

Solvent B preparation (96% methanol, 2% acetic acid, 2% water):

1. Measure 80mL of water and dispense into a 4L bottle.

2. Measure 80mL of acetic acid and dispense into the same 4L bottle.

3. Measure 3840mL of methanol and dispense 3840mL of methanol and dispense into the same 4L bottle.

4. Cap the bottle and mix well.

5. Purge the mixture with high purity Helium for 5-10 minutes to degas.

Repeat steps 1-5 to yield 4 volumes of solvent B. Mobile phase composition was A = methylene chloride with 2% acetic acid and 2% water; B = methanol with 2% acetic acid and 2% water. The column load was 0.7g in 7mL. components were detected by UV at 254nm. A typical preparative normal phase HPLC separation of cocoa procyanidins is shown in Figure 42.

HPLC Conditions:

Column: 50 x 2cm 5μ Supelcosil LC-Si run @ ambient temperature.

Mobile Phase: A = Methylene Chloride with 2% Acetic Acid and 2% Water.

B = Methanol with 2% Acetic Acid and 2% Water.

Gradient/Flow Profile:

TIME (MIN)	%A	%B	FLOW RATE (mL/min)
0	92.5	7.5	10
10	92.5	7.5	40
30	91.5	8.5	40
145	88.0	22.0	40
150	24.0	86.0	40
155	24.0	86.0	50
180	0.0	100.0	50

Example 9: Identification of Procyanidins

Procyanidins obtained as in Example 8, method D were analyzed by Matrix Assisted Laser Desorption Ionization-Time of Flight/Mass Spectrometry (MALDI-TOF/MS) using a HP G2025A MALDI-TOF/MS system equipped with a Lecroy 9350 500 MHz Oscilloscope. The instrument was calibrated in accordance with the manufacturer's instructions with a low molecular weight peptide standard (HP Part No. G2051A) or peptide standard (HP Part No. G2052A) with 2,5-dihydroxybenzoic acid (DHB)(HP Part No. G2056A) as the sample matrix. One (1.0) mg of sample was dissolved in 500μL of 70/30 methanol/water, and the sample was then mixed with DHB matrix, at a ratio of 1:1, 1:10 or 1:50 (sample:matrix) and dried on a mesa under vacuum. The samples were analyzed in the positive ion mode with the detector voltage set at 4.75kV and the laser power set between 1.5 and 8μJ. Data was collected as the sum of a number of single shots and displayed as units of molecular weight and time of flight. A representative MALDI-TOF/MS is shown in Figure 7A.

Figures 7A, B and C show MALDI-TOF/MS spectra obtained from partially purified procyanidines prepared as described in Example 3, Method A. The inventive compounds described herein were predominantly found in fractions D-E, but not A-C.

The spectra were obtained as follows:

The purified D-E fraction was subjected to MALDI-TOF/MS as described above, with the exception that the fraction was initially purified by SEP-PACK® C-18 cartridge. Five (5) mg of fraction D-E in 1 mL nanopure water was loaded onto a pre-equilibrated SEP-PACK® cartridge. The column was washed with 5mL nanopure water to eliminate contaminants, and procyanidins were eluted with 1mL 20% methanol. Fractions A-C were used directly, as they were isolated in Example 3, Method A, without further purification.

These results confirmed and extended earlier results (see Example 5, Table 3) and indicate that the inventive compounds have utility as sequestrants of cations. Separate results conclusively indicated that procyanidin oligomers of n = 5 and higher were strongly associated with anti-cancer activity with the HeLa and SKBR-3 cancer cell line model. Oligomers of n = 4 or less were ineffective with these models. The pentamer structure apparently has a structural motif which is present in it and in higher oligomers which provides the activity. Additionally, it was observed that the MALDI-TOF/MS data showed strong M^+ ions of Na^+, 2 Na^+, K^+, 2 K^+, Ca^{++}, demonstrating the utility as cation sequestrants.

Example 10: Purification of Oligomeric Fractions Method A. Purification by Semi-Preparative Reverse Phase HPLC

Procyanidins obtained from Example 8, Method A and B and D were further separated to obtain experimental quantities of like oligomers for further structural identification and elucidation (e.g., Example 9, 12, 13, and 14). A Hewlett Packard 1050 HPLC system equipped with a variable wavelength detector, Rheodyne 7010 injection valve with 1mL injection loop was assembled with a Pharmacia FRAC-100 Fraction Collector. Separations were effected on a Phenomenex Ultracarb® 10μ ODS column (250 x 22.5mm) connected with a Phenomenex 10μ ODS Ultracarb® (60 x 10mm) guard column. The mobile phase composition was A = water; B = methanol used under the following linear gradient conditions: (time, %A); (0,85), (60,50), (90,0 and (110,0) at a flow rate of 5 mL/min. Individual peaks or select chromatographic regions were collected on timed intervals or manually by fraction collection for further evaluation by MALDI-TOF/MS and NMR. Injection loads ranged from 25-100mg of material. A representative elution profile is shown in Fig. 8B.

Method B. Modified Semi-Preparative HPLC

Procyanidins obtained from Example 8, Method A and B and D were further separated to obtain experimental quantities of like oligomers for further structural identification and elucidation (e.g., Example 9, 12, 13, and 14). Supelcosil LC-Si 5μ column (250 x 10mm) with a Supelcosil LC-Si 5μ (20 x 2mm) guard column. The separations were effected at a flow rate of 3.0mL/min, at ambient temperature. The mobile phase composition was A = dichloromethane; B = methanol; and C = acetic acid:water (1:1); used under the following linear gradient conditions: (time, %A, %B); (0, 82, 14); (22, 74, 21); (32, 74, 21); (60, 74, 50, 4); (61, 82, 14), followed by column re-equilibration for 7 minutes. Injection volumes were 60μL containing 12mg of enriched pentamer. Components were detected by UV at 280nm. A representative elution profile is shown in Figure 8A.

Example 11: Molecular Modeling of Pentamers

Energy minimized structures were determined by molecular modeling using Desktop Molecular Modeller, version 3.0, Oxford University Press, 1994. Four representative views of $[EC(4 \rightarrow 8)]_4$-EC (EC = epicatechin) pentamers based on the structure of epicatechin are shown in Figures 9 A-D. A helical structure is suggested. In general when epicatechin is the first monomer and the bonding is 4→8, a beta configuration results, when the first monomer is catechin and the bonding is 4→8, an alpha configuration results; and, these results are obtained regardless of whether the second monomer is epicatechin or catechin (an exception is ent-EC(4 → 8)ent-EC). Figures 16A - 16P show preferred pentamers, and, Figures 17A to 17P show a library of stereoisomers up to and including the pentamer, from which other compounds within the scope of the invention can be prepared, without undue experimentation.

Example 12: NMR Evaluation of Pyrocyanidins

^{13}C NMR spectroscopy was deemed a generally useful technique for the study of procyanidins, especially as the phenols usually provide good quality spectra, whereas proton NMR spectra are considerably broadened. The ^{13}C NMR spectra of oligomers yielded useful information for A or B ring substitution patterns, the relative stereochemistry of the C ring and in certain cases, the position of the interflavanoid linkages. Nonetheless, ^{1}H NMR spectra yielded useful information.

Further, HOHAHA, makes use of the pulse technique to transfer magnetization of a first hydrogen to a second in a sequence to obtain cross peaks corresponding to alpha, beta, gamma or delta protons. COSY is a 2D-Fourier transform NMR technique wherein vertical and horizontal axes provide ^{1}H chemical shift and 1D spectra; and a point of intersection provides a correlation between protons, whereby spin-spin couplings

can be determined. HMQC spectra enhances the sensitivity of NMR spectra of nuclei; other than protons and can reveal cross peaks from secondary and tertiary carbons to the respective protons. APT is a ^{13}C technique used in determining the number of hydrogens present at a carbon. An even number of protons at a carbon will result in a positive signal, while an odd number of protons at a carbon will result in a negative signal.

Thus ^{13}C NMR, ^{1}H NMR, HOHAHA (homonuclear Hartmann-Hahn), HMQC (heteronuclear multiple quantum coherence), COSY (Homonuclear correlation spectroscopy), APT (attached proton test), and XHCORR (a variation on HMQC) spectroscopy were used to elucidate the structures of the inventive compounds.

Method A. Monomer

All spectra were taken in deuterated methanol, at room temperature, at an approximate sample concentration of 10mg/mL. Spectra were taken on a Bruker 500 MHZ NMR, using methanol as an internal standard.

Figures 22A-E represent the NMR spectra which were used to characterize the structure of the epicatechin monomer. Figure 22A shows the ^{1}H and ^{13}C chemical shifts, in tabular form. Figures 22 B-E show ^{1}H, APT, XHCORR and COSY spectra for epicatechin.

Similarly, Figures 23A-F represent the NMR spectra which were used to characterize the structure of the catechin monomer. Figure 23A shows the ^{1}H and ^{13}C chemical shifts, in tabular form. Figures 22 B-F show ^{1}H, ^{13}C, APT, XHCORR and COSY spectra for catechin.

Method B. Dimers

All spectra were taken in 75% deuterated acetone in D_2O, using acetone as an internal standard, and an approximate sample concentration of 10mg/mL.

Figures 24A-G represent the spectra which were used to characterize the structure of the B2 dimer. Fig. 24A shows ^{1}H and ^{13}C chemical shifts, in tabular form.

The terms T and B indicate the top half of the dimer and the bottom half of the dimer.

Figures 24B and C show the ^{13}C and APT spectra, respectively, taken on a Bruker 500 MHZ NMR, at room temperature.

Figures 24D-G show the ^{1}H, HMQC, COSY and HOHAHA, respectively, which were taken on AMZ-360 MHZ NMR at a -7°C. The COSY spectrum was taken using a gradient pulse.

Figures 25A-G represent the spectra which were used to characterize the structure of the B5 dimer. Figure 47A shows the ^{13}C and ^{1}H chemical shifts, in tabular form.

Figures 25B-D show the ^{1}H, ^{13}C and APT, respectively, which were taken on a Bruker 500 MHZ NMR, at room temperature.

Figure 25E shows the COSY spectrum, taken on an AMX-360, at room temperature, using a gradient pulse.

Figures 25F and G show the HMQC and HOHAHA, respectively, taken on an AMX-360 MHZ NMR, at room temperature.

<u>Method C. Trimer - Epicatechin/Catechin</u>

All spectra were taken in 75% deuterated acetone in D_2O, at -3°C using acetone as an internal standard, on an AMX-360 MHZ NMR, and an appropriate sample concentration of 10mg/mL.

Figures 26A-D represent the spectra which were used to characterize the structure of the epicatechin/catechin trimer. These figures show ^{1}H, COSY, HMQC and HOHAHA, respectively. The COSY spectrum was taken using a gradient pulse.

<u>Method D. Trimer -All Epicatechin</u>

All spectra were taken in 70% deuterated acetone in D_2O, at -1.8°C, using acetone as an internal standard, on an AMX-360 MHZ NMR, and an appropriate sample concentration of 10mg/mL.

Figures 27A-D represent the spectra which were used to characterize the structure of all epicatechin trimer. These figures show ^1H, COSY, HMQC and HOHAHA, respectively. The COSY spectrum was taken using a gradient pulse.

Example 13: Thiolysis of Procyanidins

In an effort to characterize the structure of procyanidins, benzyl mercaptan (BM) was reacted with catechin, epicatechin or dimers B2 and B5. Benzyl mercaptan, as well as phloroglucinol and thiophenol, can be utilized in the hydrolysis (thiolysis) of procyanidins in an alcohol/acetic acid environment. Catechin, epicatechin or dimer (1:1 mixture of B2 and B5 dimers)(2.5mg) was dissolved in 1.5mL ethanol, 100μL BM and 50μL acetic acid, and the vessel (Beckman amino acid analysis vessel) was evacuated and purged with nitrogen repeatedly until a final purge with nitrogen was followed by sealing the reaction vessel. The reaction vessel was placed in a heat block at 95°C, and aliquots of the reaction were taken at 30, 60, 120 and 240 minutes. The relative fluorescence of each aliquot is shown in Figures 10A-C, representing epicatechin, catechin and dimers, respectively. Higher oligomers are similarly thiolyzed.

Example 14: Thiolysis and Desulfurization of Dimers

Dimers B2 and B5 were hydrolyzed with benzylmercaptan by dissolving dimer (B2 or B5; 1.0 mg) in 600μl ethanol, 40μL BM and 20μL acetic acid. The mixture was heated at 95°C for 4 hours under nitrogen in a Beckman Amino Acid Analysis vessel. Aliquots were removed for analysis by reverse-phase HPLC, and 75μL of each of ethanol Raney Nickel and gallic acid (10mg/mL) were added to the remaining reaction medium in a 2mL hypovial. The vessel was purged under hydrogen, and occasionally shaken for 1 hour. The product was filtered through a 0.45μ filter and analyzed by reverse-phase HPLC. Representative elution profiles are shown in Figures 11 A and B. Higher oligomers are similarly

desulfurized. This data suggests polymerization of epicatechin or catechin and therefore represents a synthetic route for preparation of inventive compounds.

Example 15: **Antimicrobial Activity of Cocoa Extracts**

Method A:

A study was conducted to evaluate the antimicrobial activity of crude procyanidin extracts from cocoa beans against a variety of microorganisms important in food spoilage or pathogenesis. The cocoa extracts from Example 2, method A were used in the study. An agar medium appropriate for the growth of each test culture (99mL) was seeded with 1 mL of each cell culture suspension in 0.45% saline (final population 10^2 - 10^4 cfu/mL), and poured into petri dishes. Wells were cut into hardened agar with a #2 cork borer (5mm diameter). The plates were refrigerated at 4°C overnight, to allow for diffusion of the extract into the agar, and subsequently incubated at an appropriate growth temperature for the text organism. The results were as follows:

Sample Zone of Inhibition (mm)

Extract Concentration (mg/mL)	*B. sphericus*	*B. cereus*	*S. aureus*	*P. aeruginosa*	*B. subtilis*
0	NI	NI	NI	NI	NI
25	NI	12	NI	11	NI
250	12	20	19	19	11
500	14	21	21	21	13

NI = no inhibition

Antimicrobial activity of purified procyanidin extracts from cocoa beans was demonstrated in another study using the well diffusion assay described above (in Method A) with *Staphylococcus aureus* as the text culture.

The results were as follows:

cocoa extracts: 10mg/100 μL decaffeinated/ detheobrominated acetone extract as in Example 7, method A

10mg/100 μL dimer (99% pure) as in Example 14, method D

10mg/100 μL tetramer (95% pure) as in Example 14, method D

10mg/100 μL hexamer (88% pure) as in Example 8, method D

10mg/100 μL octamer/nonamer (92% pure) as in Example 8, method D

10mg/100 μL nonamer & higher (87% pure) as in Example 8, method D

Sample	Zone of Inhibition (mm)
0.45% saline	0
Dimer	33
Tetramer	27
Hexamer	24
0.45% saline	0
Octamer	22
Nonamer	20
Decaff./detheo.	26

Method B:

Crude procyanidin extract as in Example 2, method 2 was added in varying concentrations to TSB (Trypticase Soy Broth) with phenol red (0.08g/L), The TSB were inoculated with cultures of *Salmonella enteritidis* or *S. newport* (10^5 cfu/mL), and were incubated for 18 hours at 35°C. The results were as follows:

	S. enteritidis	*S. Newport*
0mg/mL	+	+
50	+	+
100	+	+
250	+	-
500	-	-
750	-	-

where + = outgrowth, and - = no growth, as evidenced by the change in broth culture from red to yellow with acid production. Confirmation of inhibition was made by plating from TSB tubes onto XLD plates.

This Example demonstrates that the inventive compounds are useful in food preparation and preservation.

This Example further demonstrates that gram negative and gram positive bacterial growth can be inhibited by the inventive compounds. From this, the inventive compounds can be used to inhibit *Helicobacter pylori*. *Helicobacter pylori* has been implicated in causing gastric ulcers and stomach cancer. Accordingly, the inventive compounds can be used to treat or prevent these and other maladies of bacterial origin. Suitable routes of administration, dosages, and formulations can be determined without undue experimentation considering factors well known in the art such as the malady, and the age, weight, sex, general health of the subject.

Example 16: Halogen-free Analytical Separation of Extract

Procyanidins obtained from Example 2 were partially purified by Analytical Separation by Halogen-free Normal Phase Chromatography on 100Å Supelcosil LC-Si 5μm (250 x 4.6mm), at a flow rate of 1.0mL/min, and a column temperature of 37°C. Separations were aided by a linear gradient under the following conditions: (time, %A, %B); (0, 82, 14); (30, 67.6, 28.4); (60, 46, 50).

Mobile phase composition was A = 30/70 % diethyl ether/Toluene; B = Methanol; and C = acetic acid/water (1:1). Components were detected by UV at 280nm. A representative elution profile is shown in Figure 12.

Example 17: Effect of Pore Size of Stationary Phase for Normal Phase HPLC Separation of Procyanidins

To improve the separation of procyanidins, the use of a larger pore size of the silica stationary phase was investigated. Separations were effected on Silica-300, 5μm, 300Å (250 x 2.0mm), or, in the alternative, on Silica-1000, 5μm, 1000Å (250 x 2.0mm). A linear gradient was employed as mobile phase composition was: A = Dichloromethane; B = Methanol; and C = acetic acid/water (1:1). Components were detected by fluorescence, wherein λ_{ex} = 276nm and λ_{em} = 316nm, by UV detector at 280nm. The flow rate was 1.0mL/min, and the oven temperature was 37°C. A representative chromatogram from three different columns (100Å pore size, from Example 7, Method D) is shown in Figure 13. This shows effective pore size for separation of procyanidins.

Example 18: Obtaining Desired Procyanidins Via Manipulating Fermentation

Microbial strains representative of the succession associated with cocoa fermentation were selected from the M&M/Mars cocoa culture collection. The following isolates were used:

Acetobacter aceti ATCC 15973
Lactobacillus sp. (BH 42)
Candida cruzii (BA 15)
Saccharomyces cerevisiae (BA 13)
Bacillus cereus (BE 35)
Bacillus sphaericus (ME 12)

Each strain was transferred from stock culture to fresh media. The yeasts and *Acetobacter* were incubated 72 hours at 26°C and the bacilli and *Lactobacillus* were incubated 48 hours at 37°C. The slants were harvested with 5mL phosphate buffer prior to use.

Cocoa beans were harvested from fresh pods and the pulp and testa removed. The beans were sterilized with hydrogen peroxide (35%) for 20 seconds, followed by treatment with catalase until cessation of bubbling. The beans were rinsed twice with sterile water and the process repeated. The beans were divided into glass jars and processed according to the regimens detailed in the following Table:

Water	Ethanol/acid	Fermentation infusate	Model Fermentation
daily transfer to fresh water	daily transfer to solutions of alcohol and acid correspondin g to levels determined at each stage of a model pulp fermentation	daily transfer to fermented pulp pasteurized on each successive day of fermentation	bench scale model fermentation in sterile pulp coinoculated with test strains

The bench scale fermentation was performed in duplicate. All treatments were incubated as indicated below:

Day 1: 26°C

Day 2: 26°C to 50°C

Day 3: 50°C

Day 4: 45°C

Day 5: 40°C

The model fermentation was monitored over the duration of the study by plate counts to assess the microbial population and HPLC analysis of the fermentation medium for the production of microbial metabolites. After treatment, the beans were dried under a laminar flow hood to a water activity of 0.64 and were roasted at 66°C for 15 min. Samples were prepared for procyanidin analysis. Three beans per treatment were ground and defatted with hexane, followed by extraction

with an acetone:water:acetic acid (70:29.5:0.5%) solution. The acetone solution extract was filtered into vials and polyphenol levels were quantified by normal phase HPLC as in Example 7, method B. The remaining beans were ground and tasted. The cultural and analytical profiles of the model bench-top fermentation process is shown in Figures 14A-C. The procyanidin profiles of cocoa beans subjected to various fermentation treatments is shown in Figure 14D.

This Example demonstrates that the invention need not be limited to any particular cocoa genotype; and, that by manipulating fermentation, the levels of procyanidins produced by a particular *Theobroma* or *Herrania* species or their inter or intra species specific crosses thereof can be modulated, e.g., enhanced.

The following Table shows procyanidin levels determined in specimens which are representative of the *Theobroma* genus and their inter and intra species specific crosses. Samples were prepared as in Examples 1 and 2 (methods 1 and 2), and analyzed as in Examples 7, method B. This data illustrates that the extracts containing the inventive compounds are found in *Theobroma and Herrania* species, and their intra and inter species specific crosses.

Theobroma and Herrania Species Procyanidin Levels

ppm (µg/g) in defatted powder

	Oligomer											
SAMPLE	Monomer	Dimer	Trimer	Tetramer	Pentamer	Hexamer	Heptamer	Octamer	Nonamer	Decamer	Undecamer	Total
T. grandiflorum x T. obovatum 1[1]	3822	3442	5384	4074	3146	2080	850	421	348	198	tr*	23,765
T. grandiflorum x T. obovatum 2[1]	3003	4098	5411	3983	2931	1914	1090	577	356	198	tr	23,561
T. grandiflorum x T. obovatum 3A[1]	4990	4980	7556	5341	4008	2576	1075	598	301	144	tr	31,569
T. grandiflorum x T. obovatum 3B[1]	3880	4498	6488	4930	3706	2560	1208	593	323	174	tr	28,360
T. grandiflorum x T. obovatum 4[1]	2647	3591	5328	4240	3304	2380	1506	815	506	249	tr	24,566
T. grandiflorum x T. obovatum 6[1]	2754	3855	5299	3872	2994	1990	1158	629	359	196	88	23,194
T. grandiflorum x T. obovatum SIN[1]	3212	4134	7608	4736	3590	2274	936	446	278	126	ND*	23,750
T. obovatum 1[1]	3662	5683	9512	5358	3858	2454	1207	640	302	144	ND	32,820
T. grandiflorum TEFFE[2]	2608	2178	3090	2704	2241	1586	900	484	301	148	tr	16,240
T. grandiflorum TEFFE x T. grandiflorum[2]	4773	4096	5289	4748	3804	2444	998	737	335	156	tr	27,380
T. grandiflorum x T. subincanum[1]	4752	3336	4916	3900	3064	2039	782	435	380	228	ND	23,832
T. obovatum x T. subincanum[1]	3379	3802	5836	3940	2868	1807	814	427	271	136	tr	23,280
T. speciosum x T. sylvestris[1]	902	346	1350	217	152	120	60	tr	tr	ND	ND	3,147
T. microcarpum[2]	5694	3250	2766	1490	822	356	141	tr	ND	ND	ND	14,519
T. cacao, SIAL 659, t0	21,929	10,072	10,106	7788	5311	3242	1311	626	422	146	tr	60,753
T. cacao, SIAL 659, t24	21,088	9762	9119	7094	4774	2906	1364	608	361	176	tr	57,252
T. cacao, SIAL 659, t48	20,887	9892	9474	7337	4906	2929	1334	692	412	302	tr	58,165
T. cacao, SIAL 659, t96	9552	5780	5062	3360	2140	1160	464	254	138	tr	ND	27,910
T. cacao, SIAL 659, t120	8581	4665	4070	2527	1628	888	326	166	123	tr	ND	22,974
Pod Rec. 10/96, Herrania mariae	869	1295	545	347	175	97	tr	*ND	ND			3329
Sample Rec. prior to 10/96, Herrania mariae	130	354	151	131	116	51	tr	ND	ND			933

ND = none detected [illegible] sample designated CPATU [illegible] * sample designated ERJOH

Reference Example 2: Procyanidin levels

The data presented in Tables 12 and 13 illustrates the fact that extracts of the invention pertaining to cocoa raw materials and commercial chocolates, and inventive compounds contained therein can be used as a vehicle for pharmaceutical, veterinary and food science preparations and applications.

Table 7: Procyanidin Levels in Commercial Chocolates
µg/g

Sample	Monomers	Dimers	Trimers	Tetramers	Pentamers	Hexamers	Heptamers and Higher	Total
Brand 1	366	166	113	59	56	23	18	801
Brand 2	344	163	111	45	48	ND*	ND	711
Brand 3	316	181	100	41	40	7	ND	685
Brand 4	310	122	71	27	28	5	ND	563
Brand 5	259	135	90	46	29	ND	ND	559
Brand 6	308	139	91	57	47	14	ND	656
Brand 7	196	98	81	58	54	19	ND	506
Brand 8	716	472	302	170	117	18	ND	1,795
Brand 9	1,185	951	633	298	173	25	21	3,286
Brand 10	1,798	1,081	590	342	307	93	ND	4,211
Brand 11	1,101	746	646	372	347	130	75	3,417
Brand 12	787	335	160	20	10	8	ND	1,320

ND* = None detected.

Table 8: Procyanidin Levels in Cocoa Raw Materials

µg/g

Sample	Monomers	Dimers	Trimers	Tetramers	Pentamers	Hexamers	Heptamers and Higher	Total
Unfermented	13,440	6,425	6,401	5,292	4,236	3,203	5,913	44,910
Fermented	2,695	1,538	1,362	740	470	301	277	7,383
Roasted	2,656	1,597	921	337	164	ND*	ND	5,675
Choc. Liquor	2,805	1,446	881	442	184	108	ND	5,866
Cocoa Hulls	114	53	14	ND	ND	ND	ND	181
Cocoa Powder 1% Fat	506	287	112	ND	ND	ND	ND	915
Cocoa Powder 11% Fat	1,523	1,224	680	46	ND	ND	ND	3,473
Red Dutch Cocoa Powder, pH 7.4, 11% fat	1,222	483	103	ND	ND	ND	ND	1,808
Red Dutch Cocoa Powder, pH 8.2, 23% fat	168	144	60	ND	ND	ND	ND	372

ND* = None detected.

Example 19: **Circular Dichroism/Study of Procyanidins**

CD studies were undertaken in an effort to elucidate the structure of purified procyanidins as in Example 8, Method D. The spectra were collected at 25°C using CD spectrum software AVIV 60DS V4.1f.

Samples were scanned from 300nm to 185nm, every 1.00nm, at 1.50nm bandwidth. Representative CD spectra are shown in Figures 21A through G, which show the CD spectra of dimer through octamer.

These results are indicative of the helical nature of the inventive compounds.

Example 20: **Inhibitory Effects of Cocoa Procyanidins on *Helicobacter pylori* and *Staphylococcus aureus***

A study was conducted to evaluate the antimicrobial activity of procyanidin oligomers against *Helicobacter pylori* and *Staphylococcus aureus*. Pentamer enriched material was prepared as described in Example 7, Method A and analyzed as described in Example 8, Method C, where 89% was pentamer, and 11% was higher oligomers (n is 6 to 12). Purified pentamer (96.3%) was prepared as described in Example 8, Method D.

Helicobacter pylori and *Staphylococcus aureus* were obtained from the American Type Culture Collection

(ATCC). For *H. pylori*, the vial was rehydrated with 0.5 mL Trypticase Soy broth and the suspension transferred to a slant of fresh TSA containing 5% defibrinated sheep blood. The slant was incubated at 37°C for 3 to 5 days under microaerophilic conditions in anaerobic jars (5 to 10% carbon dioxide; CampyPakPlus, BBL). When good growth was established in the pool of broth at the bottom of the slant, the broth was used to inoculate additional slants of TSA with sheep blood. Because viability decreased with continued subculturing, the broth harvested from the slants was pooled and stored at -80°C. Cultures for assay were used directly from the frozen vials. The *S. aureus* culture was maintained on TSA slants and transferred to fresh slants 24 h prior to use.

A cell suspension of each culture was prepared (*H. pylori*, 10^8 to 10^9 cfu/mL; *S. aureus* 10^6 to 10^7 cfu/mL) and 0.5 mL spread onto TSA plates with 5% sheep blood. Standard assay disks (Difco) were dipped into filter sterilized, serial dilutions of pentamer (23mg/mL into sterile water). The test disks and the blank control disks (sterile water) were placed on the inoculated plates. Control disks containing 80ug metronidazole (inhibitory to *H. pylori*) or 30ug vancomycin (inhibitory to *S. aureus*) (BBL Sensidiscs) were also placed on the appropriate set of plates. The *H. pylori* inoculated plates were incubated under microaerophilic conditions. The *S. aureus* set was incubated aerobically. Zones of inhibition were measured following outgrowth.

Table 9: Bioassays with pentamer against *Helicobacter pylori* and *Staphylococcus aureus*

Pentamer Enriched Fraction (mg/ml)	*S. aureus* Inhibition (mm)	*H. pylori* Inhibition (mm)
0	NI	NI
15	0	10
31	10	10
62	11	11
125	13	13
250	15	13
Vancomycin standard	15	--
Metronidazole standard	--	11
96% pure pentamer	15	11

NI = no inhibition

Example 20A Effect of Cocoa Procyanidin Fractions on Macrophage NO Production

Fresh, human heparinized blood (70 mL) was added with an equal volume of phosphate buffer saline

(PBS) at room temperature. A Ficoll-Hypaque solution was layered underneath the blood-PBS mixture using a 3mL Ficoll-Hypaque to 10mL blood-PBS dilution ratio. The tubes were centrifuged for 30 minutes at 2,000 rpm at 18-20°C. The upper layer containing plasma and platelets was discarded. The mononuclear cell layer was transferred to another centrifuge tube and the cells were washed 2X in Hanks balanced saline solution. The mononuclear cells were resuspended in complete RPMI 1640 supplemented with 10% fetal calf serum, counted and the viability determined by the trypan blue exclusion method. The cell pellet was resuspended in complete RPMI 1640 supplemented with 20% fetal calf serum to a final concentration of 1 x 10^6 cells/mL. Aliquots of the cell suspension were plated into a 96 well culture plate and rinsed 3X with RPMI 1640 supplemented with 10% fetal calf serum and the nonadherent cells (lymphocytes) were discarded.

These cells were incubated for 48 hours in the presence or absence of the following five procyanidin fractions:

Fraction A: Represents a preparative HPLC fraction comprised of monomers-tetramers. HPLC analysis revealed the following composition:

Monomers	47.2%
Dimers	23.7
Trimers	18.7
Tetramers	10.3

Fraction B: Represents a preparative HPLC fraction comprised of pentamers-decamers. HPLC analysis revealed the following composition:

Pentamers	64.3%
Hexamers	21.4
Heptamers	7.4
Octamers	1.9
Nonamers	0.9
Decamers	0.2

Fraction C: Represents an enriched cocoa procyanidin fraction used in the preparation of Fractions A and B (above). HPLC analysis revealed the following composition:

Monomers	34.3%
Dimers	17.6
Trimers	16.2
Tetramers	12.6
Pentamers	8.5
Hexamers	5.2
Heptamers	3.1
Octamers	1.4
Nonamers	0.7
Decamers	0.3

Fraction D: Represents a procyanidin extract prepared from a milk chocolate. HPLC analysis revealed a composition similar to that listed in the Table 12 for Brand 8. Additionally, caffeine 10% and theobromine 6.3% were present.

Fraction E: Represents a procyanidin extract prepared from a dark chocolate prepared with alkalized liquor. HPLC analysis revealed a composition similar to that listed in the Table 12 for Brand 12. Additionally, caffeine 16.0% and theobromine 5.8% were present.

At the end of the incubation period, the culture media were collected, centrifuged and cell free supernatants were stored frozen for nitrate assay determinations.

Macrophage NO production was determined by measuring nitrite concentrations by the Greiss reaction. Greiss reagent was 1% sulfanilamide, 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride. Briefly, 50μL aliquots were removed from the supernatants in quadruplicate and incubated with 150μL of the Greiss reagent. The absorbency at 540 nm was determined in a multiscan (Labsystems Multiskans MCC/340) apparatus. Sodium nitrite was used at defined concentrations to establish standard curves. The absorbency of the medium without cells (blank) was subtracted from the value obtained with the cell containing supernatants.

In a separate experiment, macrophages were primed for 12 hours in the presence of 5U/mL gamma-interferon and then stimulated with 10μg/mL LPS for the next 36 hours in the presence or absence of 100μg/mL of the five procyanidin fractions.

Figure 33 indicates that only procyanidin fraction C, at 100μg/mL, could induce NO production by monocytes/macrophages. Basal NO production by these cells was undetectable and no nitrite could be detected in any of the cocoa procyanidin fractions used at 100μg/mL. Figure 34 indicates that procyanidin fractions A and D enhanced LPS-induced NO production by Υ-interferon primed monocytes/macrophages. Procyanidin fraction C was marginally effective, since LPS-stimulated monocytes/macrophages cultured in the absence of procyanidin fractions produced only 4μmole/10^5 cells/48 hours. Υ-Interferon alone was ineffective in inducing NO.

Collectively, these results demonstrate that mixtures of the inventive compounds used at specific concentrations are capable of inducing monocyte/macrophage NO production both independent and dependent of stimulation by LPS or cytokines.

From the foregoing, it is clear that the extract and cocoa polyphenols, particularly the inventive compounds, as well as the compositions, methods, and kits, of the invention have significant and numerous utilities.

The inventive compounds can be used in food preservation or preparation, as well as in preventing or treating maladies of bacterial origin. Simply the inventive compounds can be used as an antimicrobial.

Further, the invention comprehends the use of the compounds or extracts as a vehicle for pharmaceutical preparations. Accordingly, there are many compositions and methods envisioned by the invention.

In this regard, it is mentioned that the invention is from an edible source and, that the activity

in vitro can demonstrate at least some activity *in vivo*; and from the *in vitro* and *in vivo* data herein, doses, routes of administration, and formulations can be obtained without undue experimentation

Example 21: Micellar Electrokinetic Capillary Chromatography of Cocoa Procyanidins

A rapid method was developed using micellar electrokinetic capillary chromatography (MECC) to separate procyanidin oligomers. The method is a modification of that reported by Delgado et al., 1994. The MECC method requires only 12 minutes to achieve the same separation as that obtained by a 70 minute normal phase HPLC analysis. Figure 28 represents a MECC separation of cocoa procyanidins obtained by Example 2.

MECC Conditions:

The cocoa procyanidin extract was prepared by the method described in Example 2 and dissolved at a concentration of 1 mg/mL in MECC buffer consisting of 200mM boric acid, 50mM sodium dodecyl sulfate (electrophoresis pure) and NaOH to adjust to pH = 8.5.

The sample was passed through a 0.45um filter and electrophoresed using a Hewlett Packard HP-3D CZE System operated at the following conditions:

Inlet buffer: Run buffer as described above

Outlet buffer: Run buffer as described above

Capillary: 50cm x 75um i.d. uncoated fused silica

Detection: 200nm, with Diode Array Detector

Injection: 50 mBar for 3 seconds (150 mBar sec)

Voltage: 6 watts

Amperage: System limit (<300uA)

Temperature: 25°C

Capillary Condition: 5 min flush with run buffer before and after each run.

This method can be modified by profiling temperature, pressure, and voltage parameters, as well as including organic modifiers and chiral selective agents in the run buffer.

Example 22: MALDI - TOF/MS Analysis of Procyanidin Oligomers with Metal Salt Solutions

A series of MALDI -TOF/MS analyses were performed on trimers combined with various metal salt solutions to determine whether cation adducts of the oligomer could be detected. The significance of the experiment was to provide evidence that the procyanidin oligomers play a physiological role *in vitro* and *in vivo* by sesquestering or delivering metal cations important to physiological processes and disease.

The method used was as described in Example 9. Briefly, 2uL of 10mM solutions of zinc sulfate dihydrate, calcium chloride, magnesium sulfate, ferric chloride hexahydrate, ferrous sulfate heptahydrate, and cupric sulfate were individually combined with 4uL of a trimer (10mg/mL) purified to apparent homogeneity as described in Example 8, and 44uL of DHB added.

The results (Figures 29A-F) showed [Metal-Trimer + H]$^+$ ions for copper and iron (ferrous and ferric) whose m/z values matched ± 1 amu standard deviation value for the theoretical calculated masses. The [Metal-Trimer + H]$^+$ masses for calcium and magnesium

could not be unequivocally resolved from the [Metal-Trimer + H]$^+$ masses for sodium and potassium, whose m/z values were within the ± 1 amu standard deviation values. No [Zn^{+2} - Trimer + H]$^+$ ion could be detected. Since some of these cations are multi-valent, the possibility for multimetal-oligomer(s) ligand species and /or metal-multioligomer species were possible. However, scanning for these adducts at their predicted masses proved unsuccessful.

The results shown above for copper, iron, calcium, magnesium and zinc may be used as general teachings for subsequent analysis of the reaction between other metal ions and the inventive compounds, taking into account such factors as oxidation state and the relative position in the periodic table of the ion in question.

Example 23. MALDI - TOF/MS Analysis of High Molecular Weight Procyanidin Oligomers

An analytical examination was made on GPC eluants associated with high molecular weight procyanidin oligomers as prepared in Example 3, Method A. The objective was to determine whether procyanidin oligomers with n > 12 were present. If present, these oligomers represent additional compounds of the invention. Adjustments to existing methods of isolation, separation and purification embodied in the invention can be made to obtain these oligomers for subsequent *in vitro* and *in vivo* evaluation.

Figure 30 represents a MALDI-TOF mass spectrum of the GPC eluant sample described above. The $[M + Na]^+$ and/or $[M + K]^+$ and/or $[M + 2Na]^+$ ions characterizing procyanidin oligomers representative of tetramers through octadecamers are clearly evident.

It was learned that an acid and heat treatment will cause the hydrolysis of procyanidin oligomers. Therefore, the invention comprehends the controlled hydrolysis of high molecular weight procyanidin oligomers (e.g. where n is 13 to 18) as a method to prepare lower molecular weight procyanidin oligomers (e.g. where n is 2 to 12).

Example 24. **Tablet Formulations**

A tablet formulation was prepared using cocoa solids obtained by methods described in U.S. Application Serial No. 08/709,406 filed 6 September 1996, hereby incorporated herein by reference. Briefly, this edible material is prepared by a process which enhances the natural occurrence of the compounds of the invention in contrast to their levels found in traditionally processed cocoa, such that the ratio of the initial amount of the compounds of the invention found in the unprocessed bean to that obtained after processing is less than or equal to 2. For simplicity, this cocoa solids material is designated herein as CP-cocoa solids. The inventive compound or compounds, e.g., in isolated and/or purified form may be used in tablets as described in this Example, instead of or in combination with CP-cocoa solids.

A tablet formula comprises the following (percentages expressed as weight percent):

CP-cocoa solids	24.0%
4-Fold Natural vanilla extract (Bush Boake Allen)	1.5%

Magnesium stearate (dry lubricant)(AerChem, Inc.)	0.5%
Dipac tabletting sugar (Amstar Sugar Corp.)	37.0%
Xylitol (American Xyrofin, Inc.)	37.0%

	100.0%

The CP-cocoa solids and vanilla extract are blended together in a food processor for 2 minutes. The sugars and magnesium stearate are gently mixed together, followed by blending in the CP-cocoa solids/vanilla mix. This material is run through a Manesty Tablet Press (B3B) at maximum pressure and compaction to produce round tablets (15mm x 5mm) weighing 1.5 - 1.8 gram. Another tablet of the above mentioned formula was prepared with a commercially available low fat natural cocoa powder (11% fat) instead of the CP-cocoa solids (11% fat). Both tablet formulas produced products having acceptable flavor characteristics and texture attributes.

An analysis of the two tablet formulas was performed using the procedures described in Example 4, Method 2. In this case, the analysis focused on the concentration of the pentamer and the total level of monomers and compounds of the invention where n is 2 to 12 which are reported below.

Tablet sample	pentamer (ug/g)	total (ug/g)	pentamer (ug/1.8g serving)	total (ug/1.8g serving)
tablet with CP-cocoa solids	239	8,277	430	14,989
tablet with commercial low fat cocoa powder	ND	868	ND	1563

ND = not detected

The data clearly showed a higher level of pentamer and total level of compounds of the invention in the CP-cocoa solids tablet than in the other tablet formula. Thus, tablet formulas prepared with CP-cocoa solids are an ideal delivery vehicle for the oral administration of compounds of the invention, for pharmaceutical, supplement and food applications.

The skilled artisan in this area can readily prepare other tablet formulas covering a wide range of flavors, colors, excipients, vitamins, minerals, OTC medicaments, sugar fillers, UV protectants (e.g., titanium dioxide, colorants, etc.), binders, hydrogels, and the like except for polyvinyl pyrrolidone which would irreversibly bind the compounds of the invention or combination of compounds. The amount of sugar fillers may be adjusted to manipulate the dosages of the compounds of the invention or combination of compounds.

Many apparent variations of the above are self-evident and possible without departing from the spirit and scope of the example.

Example 25. Capsule Formulations

A variation of Example 24 for the oral delivery of the compounds of the invention is made with push-fit capsules made of gelatin, as well as soft sealed capsules made of gelatin and a plasticizer such as glycerol. The push-fit capsules contain the compound of the invention or combination of compounds or CP-cocoa solids as described in Examples 24 and 26 in the form of a powder which can be optionally mixed with fillers such as lactose or sucrose to manipulate the dosages of the compounds of the invention. In soft capsules, the compound of the invention or combination of compounds or CP-cocoa solids are suspended in a suitable liquid such as fatty oils or cocoa butter or combinations therein. Since an inventive compound or compounds may be light-sensitive, e.g., sensitive to UV, a capsule can contain UV protectants such as titanium dioxide or suitable colors to protect against UV. The capsules can also contain fillers such as those mentioned in the previous Example.

Many apparent variations of the above are self-evident and possible to one skilled in the art without departing from the spirit and scope of the example.

Example 26. Standard of Identity (SOI) and Non-Standard of Identity (non-SOI) Dark and Milk Chocolate Formulations

Formulations of the compounds of the invention or combination of compounds derived by methods embodied in the invention can be prepared into SOI and non-SOI

dark and milk chocolates as a delivery vehicle for human and veterinary applications. Reference is made to copending U.S. Application Serial No. 08/709,406, filed September 6, 1996, hereby incorporated herein by reference. USSN 08/709,406 relates to a method of producing cocoa butter and/or cocoa solids having conserved levels of the compounds of the invention from cocoa beans using a unique combination of processing steps. Briefly, the edible cocoa solids obtained by this process conserves the natural occurrence of the compounds of the invention in contrast to their levels found in traditionally processed cocoa, such that the ratio of the initial amount of the compounds of the invention found in the unprocessed bean to that obtained after processing is less than or equal to 2. For simplicity, this cocoa solids material is designated herein as CP-cocoa solids. The CP-cocoa solids are used as a powder or liquor to prepare SOI and non-SOI chocolates, beverages, snacks, baked goods, and as an ingredient for culinary applications.

The term "SOI chocolate" as used herein shall mean any chocolate used in food in the United States that is subject to a Standard of Identity established by the U.S. Food and Drug Administration under the Federal Food, Drug and Cosmetic Act. The U.S. definitions and standards for various types of chocolate are well established. The term "non-SOI chocolate" as used herein shall mean any nonstandardized chocolates which have compositions which fall outside the specified ranges of the standardized chocolates.

Examples of nonstandardized chocolates result when the cocoa butter or milk fat are replaced partially or completely; or when the nutrative carbohydrate sweetener is replaced partially or completely; or flavors imitating milk, butter, cocoa powder, or chocolate are added or other additions or deletions in the formula are

made outside the U.S. FDA Standards of Identity for chocolate or combinations thereof.

As a confection, chocolate can take the form of solid pieces of chocolate, such as bars or novelty shapes, and can also be incorporated as a component of other, more complex confections where chocolate is optionally combined with any Flavor & Extract Manufacturers Association (FEMA) material, natural juices, spices, herbs and extracts categorized as natural-flavoring substances; nature-identical substances; and artificial flavoring substances as defined by FEMA GRAS lists, FEMA and FDA lists, Council of Europe (CoE) lists, International Organization of the Flavor Industry (IOFI) adopted by the FAO/WHO Food Standard Programme, Codex Alimentarius, and Food Chemicals Codex and generally coats other foods such as caramel, nougat, fruit pieces, nuts, wafers or the like. These foods are characterized as microbiologically shelf-stable at 65-85°F under normal atmospheric conditions. Other complex confections result from surrounding with chocolate soft inclusions such as cordial cherries or peanut butter. Other complex confections result from coating ice cream or other frozen or refrigerated desserts with chocolate. Generally, chocolate used to coat or surround foods must be more fluid than chocolates used for plain chocolate solid bars or novelty shapes.

Additionally, chocolate can also be a low fat chocolate comprising a fat and nonfat solids, having nutrative carbohydrate sweetener(s), and an edible emulsifier. As to low fat chocolate, reference is made to U.S. Patent Nos. 4,810,516, 4,701,337, 5,464,649, 5,474,795, and WO 96/19923.

Dark chocolates derive their dark color from the amount of chocolate liquor, or alkalized liquor or cocoa solids or alkalized cocoa solids used in any given

formulation. However, the use of alkalized cocoa solids or liquor would not be used in the dark chocolate formulations in the invention, since Reference Example 2 teaches the loss of the compounds of the invention due to the alkalization process.

Examples of formulations of SOI and non-SOI dark and milk chocolates are listed in Tables 16 and 17. In these formulations, the amount of the compounds of the invention present in CP-cocoa solids was compared to the compounds of the invention present in commercially available cocoa solids.

The following describes the processing steps used in preparing these chocolate formulations.

Process for non-SOI Dark Chocolate

1. Keep all mixers and refiners covered throughout process to avoid light.

2. Batch all the ingredients excluding 40% of the free fat (cocoa butter and anhy. milk fat) maintaining temperature between 30-35°C.

3. Refine to 20 microns.

4. Dry conche for 1 hour at 35°C.

5. Add full lechithin and 10% cocoa butter at the beginning of the wet conche cycle; wet conche for 1 hour.

6. Add all remaining fat, standardize if necessary and mix for 1 hour at 35°C.

7. Temper, mould and package chocolate.

Process for SOI Dark Chocolate

1. Batch all ingredients excluding milk fat at a temperature of 60°C.

2. Refine to 20 microns.

3. Dry conche for 3.5 hours at 60°C.

4. Add lecithin and milk fat and wet conche for 1 hour at 60°C.

5. Standardize if necessary and mix for 1 hour at 35°C.

Temper, mould and package chocolate.

Process for non-SOI Milk Chocolate

1. Keep all mixers and refiners covered throughout process to avoid light.

2. Batch sugar, whole milk powder, malted milk powder, and 66% of the cocoa butter, conche for 2 hours at 75°C.

3. Cool batch to 35°C and add cocoa powder, ethyl vanillin, chocolate liquor and 21% of cocoa butter, mix 20 minutes at 35°C.

4. Refine to 20 microns.

5. Add remainder of cocoa butter, dry conche for 1.5 hour at 35°C.

6. Add anhy. milk fat and lecithin, wet conche for 1 hour at 35°C.

7. Standardize, temper, mould and package the chocolate.

Process for SOI Milk Chocolate

1. Batch all ingredients excluding 65% of cocoa butter and milk fat at a temperature of 60°C.

2. Refine to 20 microns.

3. Dry conche for 3.5 hours at 60°C.

4. Add lecithin, 10% of cocoa butter and anhy. milk fat; wet conche for 1 hour at 60°C.

5. Add remaining cocoa butter, standardize if necessary and mix for 1 hour at 35°C.

6. Temper, mould and package the chocolate.

The CP-cocoa solids and commercial chocolate liquors used in the formulations were analyzed for the pentamer and total level of monomers and compounds of the invention where n is 2 to 12 as described in Method 2, Example 4 prior to incorporation in the formulations. These values were then used to calculate the expected levels in each chocolate formula as shown in Tables 16 and 17. In the cases for the non-SOI dark chocolate and non-SOI milk chocolate, their products were similarly analyzed for the pentamer, and the total level of monomers and the compounds of the invention where n is 2 to 12. The results appear in Tables 10 and 11.

The results from these formulation examples indicated that SOI and non-SOI dark and milk chocolates formulated with CP-cocoa solids contained approximately 6.5 times more expected pentamer, and 3.5 times more expected total levels in the SOI and non-SOI dark chocolates; and approximately 4.5; 7.0 times more expected pentamer and 2.5; 3.5 times more expected total levels in the SOI and non-SOI milk chocolates, respectively.

Analyses of some of the chocolate products were not performed since the difference between the expected levels of the compounds of the invention present in finished chocolates prepared with CP-cocoa solids were dramatically higher than those formulas prepared with commercially available cocoa solids. However, the effects of processing was evaluated in the non-SOI dark and milk chocolate products. As shown in the tables, a 25-50% loss of the pentamer occurred, while slight differences in total levels were observed. Without wishing to be bound by any theory, it is believed that these losses are due to heat and/or low chain fatty acids from the milk ingredient (e.g. acetic acid, propionic acid and butyric acid) which can hydrolyze the oligomers (e.g. a trimer can hydrolyze to a monomer and dimer). Alternatively, time consuming processing steps can allow for oxidation or irreversible binding of the compounds of the invention to protein sources within the formula. Thus, the invention comprehends altering methods of chocolate formulation and processing to address these effects to prevent or minimize these losses.

The skilled artisan will recognize many variations in these examples to cover a wide range of formulas, ingredients, processing, and mixtures to rationally adjust the naturally occurring levels of the compounds of the invention for a variety of chocolate applications.

Table 10: Dark Chocolate Formulas Prepared with non-Alkalized Cocoa Ingredients.

Non-SOI Dark Chocolate Using CP-cocoa solids	SOI Dark Chocolate Using CP-Cocoa Solids	SOI Dark Chocolate Using Commercial Cocoa Solids
Formulation:	Formulation:	Formulation:
41.49 % Sugar 3% whole milk powder 26% CP-cocoa solids 4.5% com. liquor 21.75% cocoa butter 2.75% anhy. milk fat 0.01% vanillin 0.5% lecithin	41.49% sugar 3% whole milk powder 52.65% CP-liquor 2.35% anhy. milk fat 0.01% vanillin 0.5% lecithin	41.49% sugar 3% whole milk powder 52.65% com. liquor 2.35% anhy. milk fat 0.01% vanillin 0.5% lecithin
Total fat: 31%	Total fat: 31%	Total fat: 31%
Particle size: 20 microns	Particle size: 20 microns	Particle size: 20 microns

Expected Levels of pentamer and total oligomeric procyanidins (monomers and n = 2-12; units of µg/g)

Pentamer: 1205	Pentamer: 1300	Pentamer: 185
Total: 13748	Total: 14646	Total: 3948

Actual Levels of pentamer and total oligomeric procyandins (monomers and n = 2-12; units of µg/g)

Pentamer: 561	Not performed	Not performed
Total: 14097		

Table 11: Milk Chocolate Formulas Prepared with non-Alkalized Cocoa Ingredients.

Non-SOI Milk Chocolate Using CP-cocoa solids	SOI Milk Chocolate Using CP-Cocoa Solids	SOI Milk Chocolate Using Commercial Cocoa Solids
Formulation:	Formulation:	Formulation:
46.9965 % Sugar 15.5% whole milk powder 4.5% CP-cocoa solids 5.5% com. liquor 21.4% cocoa butter 1.6% anhy. milk fat 0.035% vanillin 0.5% lecithin 4.0% malted milk powder	46.9965% sugar 15.5% whole milk powder 13.9% CP-liquor 1.6% anhy. milk fat 0.0035% vanillin 0.5% lecithin 17.5% cocoa butter 4.0% malted milk powder	46.9965% sugar 15.5% whole milk powder 13.9% com. liquor 1.60% anhy. milk fat 0.0035% vanillin 0.5% lecithin 17.5% cocoa butter 4.0% malted milk powder
Total fat: 31.75%	Total fat: 31.75%	Total fat: 31.75%
Particle size: 20 microns	Particle size: 20 microns	Particle size: 20 microns

Expected Levels of pentamer and total oligomeric procyanidins (monomers and n = 2-12; units of ug/g)

Pentamer: 225	Pentamer: 343	Pentamer: 49
Total: 2734	Total: 3867	Total: 1042

Actual Levels of pentamer and total oligomeric procyanidins (monomers and n = 2-12; units of ug/g)

Pentamer: 163	Not performed	Not performed
Total: 2399		

Example 27. Hydrolysis of Procyanidin Oligomers

Example 8, Method D describes the preparation normal phase HPLC procedure to purify the compounds of the invention. The oligomers are obtained as fractions dissolved in mobile phase. Solvent is then removed by standard vacuum distillation (20-29 in. Hg: 40°C) on a Rotovap apparatus. It was observed that losses of a particular oligomer occurred with increases in smaller oligomers when the vacuum distillation residence time was prolonged or temperatures >40°C were used.

The losses of a particular oligomer with accompanying increases in smaller oligomers was attributed to a time-temperature acid hydrolysis from residual acetic acid present in the mobile phase solvent mixture. This observation was confirmed by the following experiment where 100mg of hexamer was dissolved in 50mL of the mobile phase containing methylene chloride, acetic acid, water, and methanol (see Example 8, Method D for solvent proportions) and subjected to a time-temperature dependent distillation. At specific times, an aliquot was removed for analytical normal phase HPLC analysis as described in Example 4, Method 2. The results are illustrated in Figures 31 and 32, where hexamer levels decreased in a time-temperature dependent fashion. Figure 32 illustrates the appearance of one of the hydrolysis products (Trimer) in a time-temperature dependent fashion. Monomer and other oligomers (dimer to pentamer) also appeared in a time-temperature dependent fashion.

These results indicated that extreme care and caution must be taken during the handling of the inventive polymeric compounds.

The results provided above, together with that found in Examples 5, 9, 12, 13, 14 and 19, demonstrate that the method described above can be used to complement

other methods embodied in the invention to identify any given oligomer of the invention.

For instance, the complete hydrolysis of any given oligomer which yields exclusively (+)-catechin or (-)-epicatechin eliminates many "mixed" monomer-based oligomer structure possibilities and reduces the stereochemical linkage possibilities characteristic for each monomer comprising any given oligomer.

Further, the complete hydrolysis of any given oligomer which yields both (+)-catechin and (-)-epicatechin in specific proportions provides the skilled artisan with information on the monomer composition of any given oligomer, and hence, the stereochemical linkage possibilities characteristic for each monomer comprising the oligomer.

The skilled artisan would recognize the fact that acid catalyzed epimerization of individual monomers can occur and suitable control experiments and nonvigorous hydrolysis conditions should be taken into account (e.g., the use of an organic acids, such as acetic acid, in lieu of concentrated HCl, HNO_3, etc).

Having thus described in detail the preferred embodiments of the present invention, it is to be understood that the invention defined by the appended claims is not to be limited by particular details set forth in the above descriptions as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

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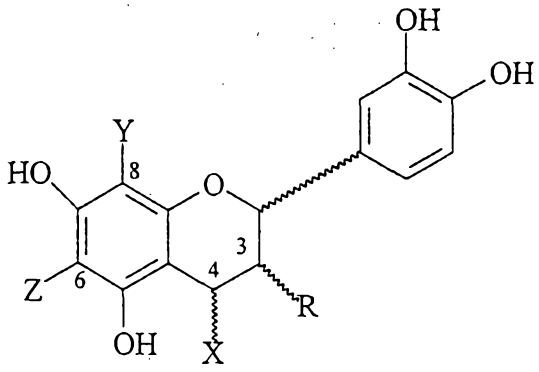
CLAIMS

1. A method for treating a subject in need of treatment with an antimicrobial agent comprising administering to the subject a composition comprising a polymeric compound of the formula A_n in an amount to effect said treatment, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof;

and a pharmaceutically, veterinarily or food science acceptable carrier.

2. The method according to claim 1, wherein n is 2 to 10.

3. The method according to claim 1, wherein the sugar is selected from the group consisting of glucose, galactose, xylose, rhamnose and arabinose.

4. The method according to claim 1, wherein the phenolic moiety is selected from the group consisting of caffeic, cinnamic, coumaric, ferulic, gallic, hydroxybenzoic and sinapic acids.

5. The method according to claim 1, wherein n is 2, 5, 6, 8 or 10.

6. The method according to claim 1, further comprising administering at least one additional antimicrobial agent.

7. The method according to claim 1, wherein, in the compound of the formula A_n, bonding occurs between C-4 and C-6 of adjacent monomers or between C-4 and C-8 of adjacent monomers; and

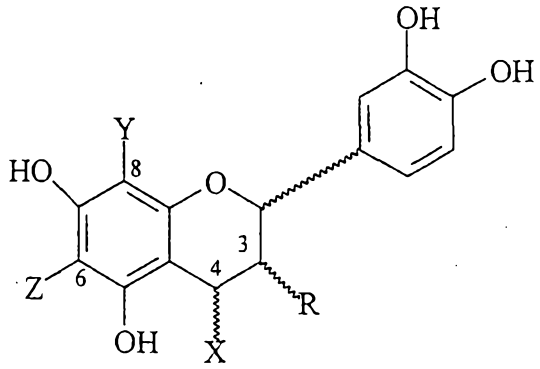
each of X, Z, and Y is H, a sugar or an adjacent monomer, with the provisos that if X and Y are adjacent monomers, Z is H or sugar and if X and Z are adjacent monomers, Y is H or sugar, and that as to at least one of the two terminal monomers, bonding of the adjacent monomer is at C-4 and optionally, Y = Z = hydrogen.

8. The method according to claim 1, wherein the compound is selected from the group consisting of a dimer of formula EC-(4β→8)-EC, a trimer selected from the group consisting of $[EC-(4\beta\rightarrow8)]_2$-EC, $[EC-(4\beta\rightarrow8)]_2$-C and $[EC-(4\beta\rightarrow6)]_2$-EC, a tetramer selected from the group consisting of $[EC-(4\beta\rightarrow8)]_3$-EC, $[EC-(4\beta\rightarrow8)]_3$-C and $[EC-(4\beta\rightarrow8)]_2$-EC-(4β→6)-C, a pentamer selected from the group consisting of $[EC-(4\beta\rightarrow8)]_4$-EC, $[EC-(4\beta\rightarrow8)]_3$-EC-(4β→6)-EC, $[EC-(4\beta\rightarrow8)]_3$-EC-(4β→8)-C and $[EC-(4\beta\rightarrow8)]_3$-EC-(4β→6)-C, a hexamer selected from the group consisting of $[EC-(4\beta\rightarrow8)]_5$-EC, $[EC-(4\beta\rightarrow8)]_4$-EC-(4β→6)-EC, $[EC-(4\beta\rightarrow8)]_4$-EC-(4β→8)-C, and $[EC-(4\beta\rightarrow8)]_4$-EC-(4β→6)-C, a heptamer selected from the group consisting of $[EC-(4\beta\rightarrow8)]_6$-EC, $[EC-(4\beta\rightarrow8)]_5$-EC-(4β→6)-EC, $[EC-(4\beta\rightarrow8)]_5$-EC-(4β→8)-C, and $[EC-(4\beta\rightarrow8)]_5$-EC-(4β→6)-C, an octamer selected from the group consisting of $[EC-(4\beta\rightarrow8)]_7$-EC, $[EC-(4\beta\rightarrow8)]_6$-EC-

(4β→6)-EC, [EC-(4β→8)]$_6$-EC-(4β→8)-C, and [EC-(4β→8)]$_6$-EC-(4β→6)-C, a nonamer selected from the group consisting of [EC-(4β→8)]$_8$-EC, [EC-(4β→8)]$_7$-EC-(4β→6)-EC, [EC-(4β→8)]$_7$-EC-(4β→8)-C, and [EC-(4β→8)]$_7$-EC-(4β→6)-C, and a decamer selected from the group consisting of [EC-(4β→8)]$_9$-EC, [EC-(4β→8)]$_8$-EC-(4β→6)-EC, [EC-(4β→8)]$_8$-EC-(4β→8)-C, and [EC-(4β→8)]$_8$-EC-(4β→6)-C.

9. The method according to claim 1, wherein the composition is selected from the group consisting of a tablet, capsule, Standard of Identity chocolate and non-Standard of Identity chocolate.

10. A method of inhibiting platelet aggregation comprising administering to a mammal in need of such treatment a composition comprising a polymeric compound of the formula A_n wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof; and a pharmaceutically, veterinarily or food science acceptable carrier.

11. A method according to claim 10, wherein the sugar is selected from the group consisting of glucose, galactose, xylose, rhamnose and arabinose.

12. A method according to claim 10, wherein the phenolic moiety is selected from the group consisting of caffeic, cinnamic, coumaric, ferulic, gallic, hydroxybenzoic and sinapic acids.

13. A method according to claim 10, wherein, in the compound of the formula A_n, bonding occurs between C-4 and C-6 of adjacent monomers or between C-4 and C-8 of adjacent monomers; and

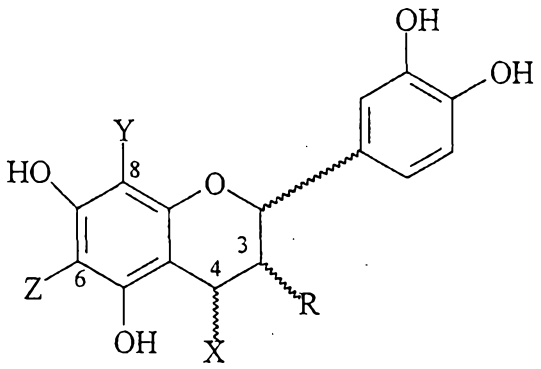
each of X, Z, and Y is H, a sugar or an adjacent monomer, with the provisos that if X and Y are adjacent monomers, Z is H or sugar and if X and Z are adjacent monomers, Y is H or sugar, and that as to at least one of the two terminal monomers, bonding of the adjacent monomer is at C-4 and optionally, Y=Z=hydrogen.

14. A method according to claim 10, wherein the compound is selected from the group consisting of a dimer of formula EC-(4β→8)-EC, a trimer selected from the group consisting of [EC-(4β→8)]$_2$-EC, [EC-(4β→8)]$_2$-C and [EC-(4β→6)]$_2$-EC, a tetramer selected from the group consisting of [EC-(4β→8)]$_3$-EC, [EC-(4β→8)]$_3$-C and [EC-(4β→8)]$_2$-EC-(4β→6)-C, a pentamer selected from the group consisting of [EC-(4β→8)]$_4$-EC, [EC-(4β→8)]$_3$-EC-(4β→6)-EC, [EC-(4β→8)]$_3$-EC-(4β→8)-C and [EC-(4β→8)]$_3$-EC-(4β→6)-C, a hexamer selected from the group consisting of [EC-(4β→8)]$_5$-EC, [EC-(4β→8)]$_4$-EC-(4β→6)-EC, [EC-(4β→8)]$_4$-EC-(4β→8)-C, and [EC-(4β→8)]$_4$-EC-(4β→6)-C, a heptamer selected from the group consisting of

[EC-(4β→8)]$_6$-EC, [EC-(4β→8)]$_5$-EC-(4β→6)-EC, [EC-(4β→8)]$_5$-EC-(4β→8)-C, and [EC-(4β→8)]$_5$-EC-(4β→6)-C, an octamer selected from the group consisting of [EC-(4β→8)]$_7$-EC, [EC-(4β→8)]$_6$-EC-(4β→6)-EC, [EC-(4β→8)]$_6$-EC-(4β→8)-C, and [EC-(4β→8)]$_6$-EC-(4β→6)-C, a nonamer selected from the group consisting of [EC-(4β→8)]$_8$-EC, [EC-(4β→8)]$_7$-EC-(4β→6)-EC, [EC-(4β→8)]$_7$-EC-(4β→8)-C, and [EC-(4β→8)]$_7$-EC-(4β→6)-C, a decamer selected from the group consisting of [EC-(4β→8)]$_9$-EC, [EC-(4β→8)]$_8$-EC-(4β→6)-EC, [EC-(4β→8)]$_8$-EC-(4β→8)-C, and [EC-(4β→8)]$_8$-EC-(4β→6)-C, an undecamer selected from the group consisting of [EC-(4β→8)]$_{10}$-EC, [EC-(4β→8)]$_9$-EC-(4β→6)-EC, [EC-(4β→8)]$_9$-EC-(4β→8)-C, and [EC-(4β→8)]$_9$-EC-(4β→6)-C, and a dodecamer selected from the group consisting of [EC-(4β→8)]$_{11}$-EC, [EC-(4β→8)]$_{10}$-EC-(4β→6)-EC, [EC-(4β→8)]$_{10}$-EC-(4β→8)-C, and [EC-(4β→8)]$_{10}$-EC-(4β→6)-C.

15. A method according to claim 10, wherein the composition is selected from the group consisting of a tablet, capsule, Standard of Identity chocolate and non-Standard of Identity chocolate.

16. A method of treating, preventing or reducing atherosclerosis or restenosis in a mammal comprising administering to said mammal a composition comprising a polymeric compound of the formula A_n, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y

and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

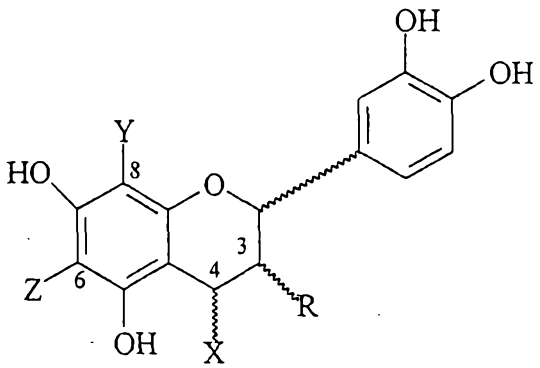
wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof,

and a pharmaceutically, veterinarily or food science acceptable carrier.

17. A method according to claim 16, wherein n is 2 to 10.

18. A method of modulating thrombosis in a mammal comprising administering to said mammal a composition comprising a polymeric compound of the formula A_n, wherein A is a monomer of the formula::



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and

Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

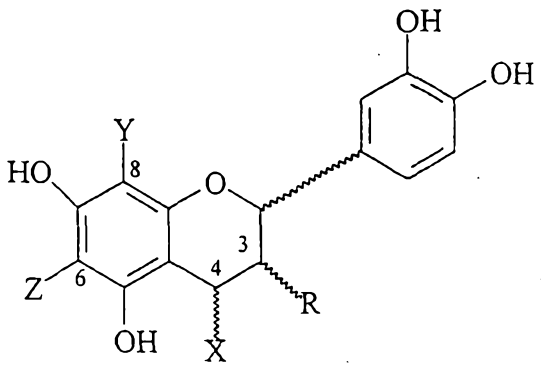
wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof,

and a pharmaceutically, veterinarily or food science acceptable carrier.

19. The method according to claim 18, wherein n is 2 to 12.

20. A method of inhibiting bacterial growth in a mammal comprising administering to said mammal a composition comprising a polymeric compound of the formula A_n, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

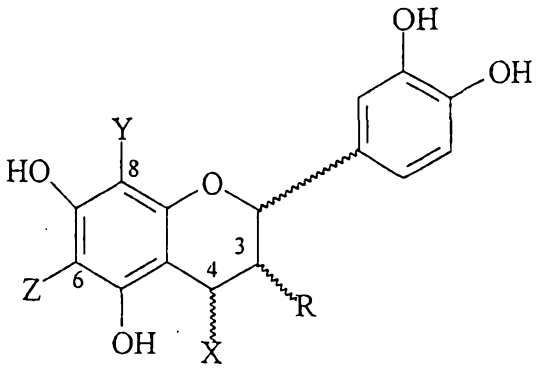
wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof,

and a pharmaceutically, veterinarily or food science acceptable carrier.

21. The method according to claim 20, wherein n is 2, 4, 5, 6, 8 or 10.

22. A method for the identification of at least one gene induced or repressed by a polymeric compound of the formula A_n, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

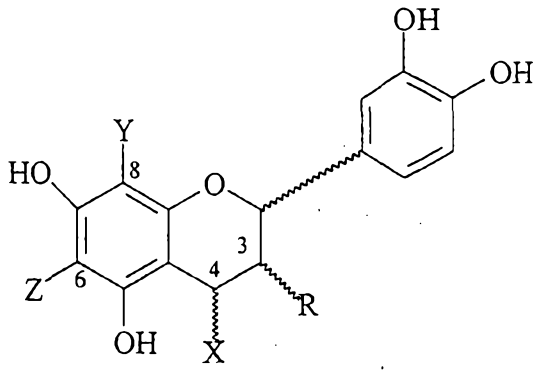
when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof, said method comprising contacting said at least one gene or a gene product thereof with the polymeric compound using a gene expression assay.

23. The method according to claim 22, wherein said gene expression assay is selected from the group consisting of Differential Display, sequencing of cDNA libraries, Serial Analysis of Gene Expression, and expression monitoring by hybridization to high density oligonucleotide arrays.

24. A chocolate composition comprising a Standard of Identity or a non-Standard of Identity chocolate comprising a polymeric compound of the formula A_n, in an antimicrobially effective amount, wherein A is a monomer of the formula::



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

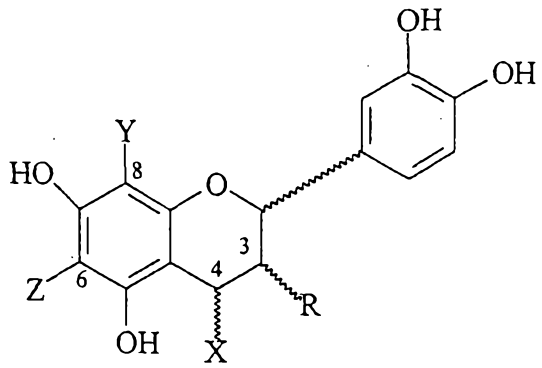
when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z

and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

25. A chocolate composition comprising a Standard of Identity or a non-Standard of Identity or a non-Standard of Identity chocolate comprising a polymeric compound of the formula A_n, in an amount effective to inhibit platelet aggregation, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

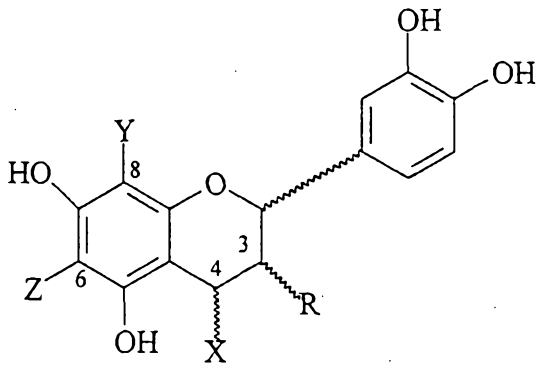
the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

26. A chocolate composition comprising a Standard of Identity or a non-Standard of Identity or a non-Standard of Identity chocolate comprising a polymeric compound of the formula A_n, in an amount effective to treat, prevent, or reduce atherosclerosis or restenosis in a mammal,wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

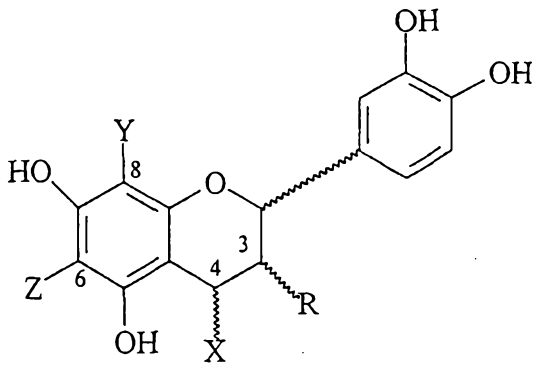
the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

27. A chocolate composition comprising a Standard of Identity or a non-Standard of Identity or a non-Standard of Identity chocolate comprising a polymeric compound of the formula A_n, in an amount effective to modulate thrombosis in a mammal, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

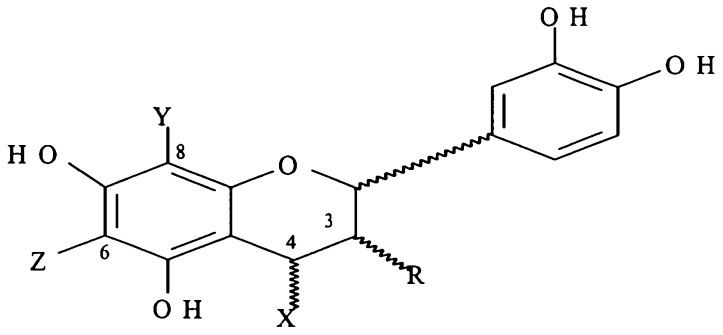
wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

28. A compound selected from the group consisting of a pentamer selected from the group consisting of [EC-(4β→8)]$_4$-EC, [EC-(4β→8)]$_3$-EC-(4β→6)-EC, [EC-(4β→8)]$_3$-EC-(4β→8)-C and [EC-(4β→8)]$_3$-EC-(4β→6)-C, a hexamer selected from the group consisting of [EC-(4β→8)]$_5$-EC, [EC-(4β→8)]$_4$-EC-(4β→6)-EC, [EC-4β→8)]$_4$-EC-(4β→8)-C, and [EC-(4β→8)]$_4$-EC-(4β→6)-C, a heptamer selected from the group consisting of [EC-(4β→8)]$_6$-EC, [EC-(4β→8)]$_5$-EC-(4 4β→6)-EC, [EC-(4β→8)]$_5$-EC-(4β→8)-C, and [EC-(4β→8)]$_5$-EC-(4β→6)-C, an octamer selected from the group consisting of [EC-(4β→8)]$_7$-EC, [EC-(4β→8)]$_6$-EC-(4β→6)-EC, [EC-(4β→8)]$_6$-EC-(4β→8)-C, and [EC-(4β→8)]$_6$-EC-(4β→6)-C, a nonamer selected from the group consisting of [EC-(4β→8)]$_8$-EC, [EC-(4β→8)]$_7$-EC-(4β→6)-EC, [EC-(4β→8)]$_7$-EC-(4β→8)-C, and {EC-(4β→8)]$_7$-EC-(4β→6)-C, a decamer

selected from the group consisting of [EC-(4β→8)]$_9$-EC, [EC-(4β→8)]$_8$-EC-(4β→6)-EC, [EC-(4β→8)]$_8$-EC-(4β→8)-C, and [EC-4β→8)]$_8$-EC-(4β→6)-C, an undecamer selected from the group consisting of [EC-(4β→8)]$_{10}$-EC, [EC-(4β→8)]$_9$-EC- (4β→6)-EC, [EC-(4β→8)]$_9$-EC-(4β→8)-C, and [EC-(4β→8)]$_9$- EC-(4β→6)-C, and a dodecamer selected from the group consisting of [EC-(4β→8)]$_{11}$-EC, [EC-(4β→8)]$_{10}$-EC-(4β→6)- EC, [EC-(4β→8)]$_{10}$-EC-(4β→8)-C, and [EC-(4β→8)]$_{10}$-EC- (4β→6)-C, wherein the 3-position of a terminal monomeric unit of said pentamer, hexamer, heptamer, octamer, nonamer, decamer, undecamer and dodecamer is derivatized with a gallate or a β-D-glucose.

29. Use, in the manufacture of a medicament or a food product for use as an antimicrobial agent, of a polymeric compound of formula A_n, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at

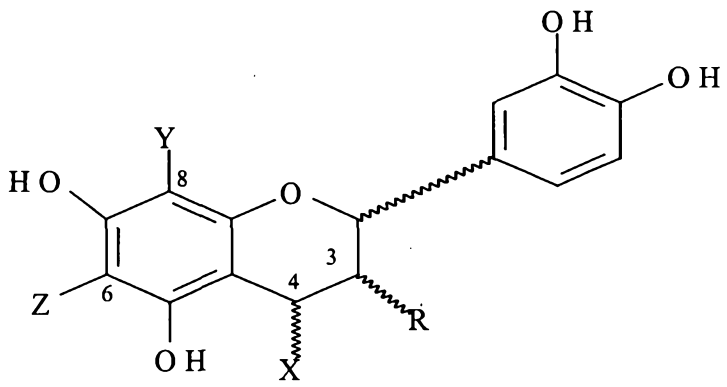
C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

30. Use, in the manufacture of a medicament or a food product for inhibiting platelet aggregation, of a polymeric compound of formula A_n, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at

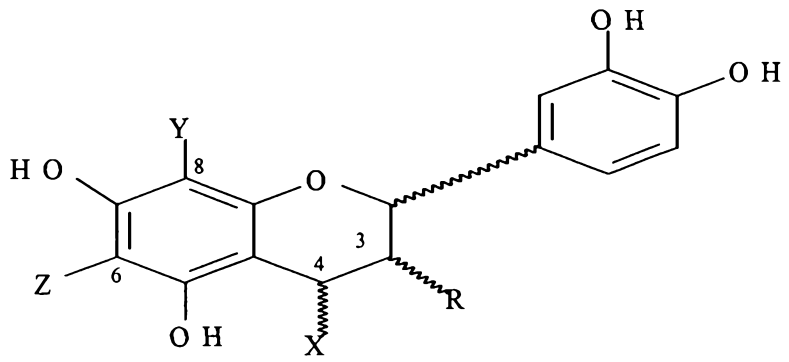
C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

31. Use, in the manufacture of a medicament or a food product for treating, preventing or reducing atherosclerosis or restenosis, of a polymeric compound of formula A_n, wherein A is monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and

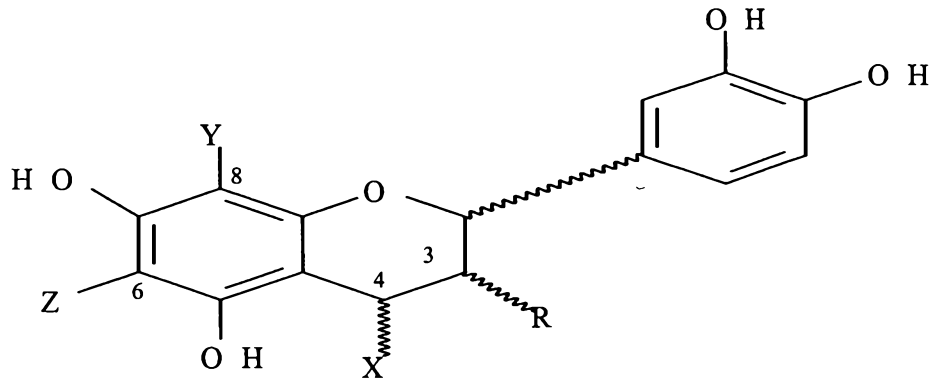
Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

32. Use, in the manufacturing of a medicament or a food product for modulating thrombosis, of a polymeric compound of formula A_n, wherein A is a monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and

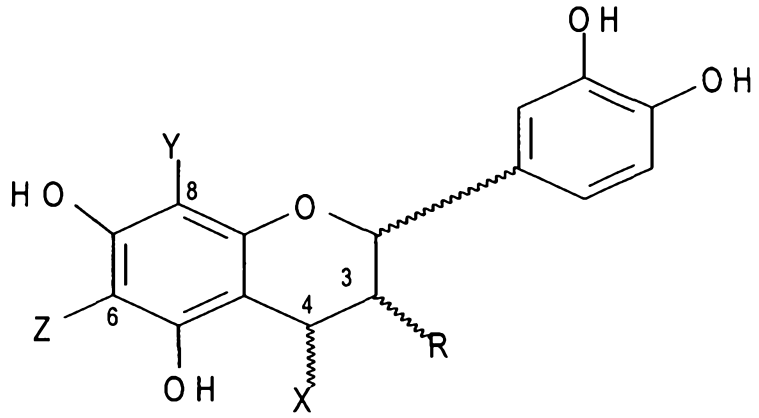
Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

33. Use, in the manufacture of a medicament or a food product for inhibiting bacterial growth in a mammal, of a polymeric compound of formula A_n, wherein A is monomer of the formula:



wherein

n is an integer from 2 to 18;

R and X each have either α or β stereochemistry;

R is OH or O-sugar;

the substitutents of C-4, C-6 and C-8 are X, Z, and Y, respectively, and bonding of monomeric units occurs at C-4, C-6 or C-8;

when any of C-4, C-6 or C-8 are not bonded to another monomeric unit, X, Y, and Z are hydrogen, and Z and Y are sugar and X is hydrogen, or X is sugar and Z and Y are H, or combinations thereof, provided that Y and Z of the first momomeric unit are hydrogen when the second monomeric unit is bonded to C-4 of said first unit; and

wherein the sugar is an unsubstituted sugar or a sugar substituted with a phenolic moiety via an ester bond;

or a derivative or oxidation product thereof.

FIG. 1

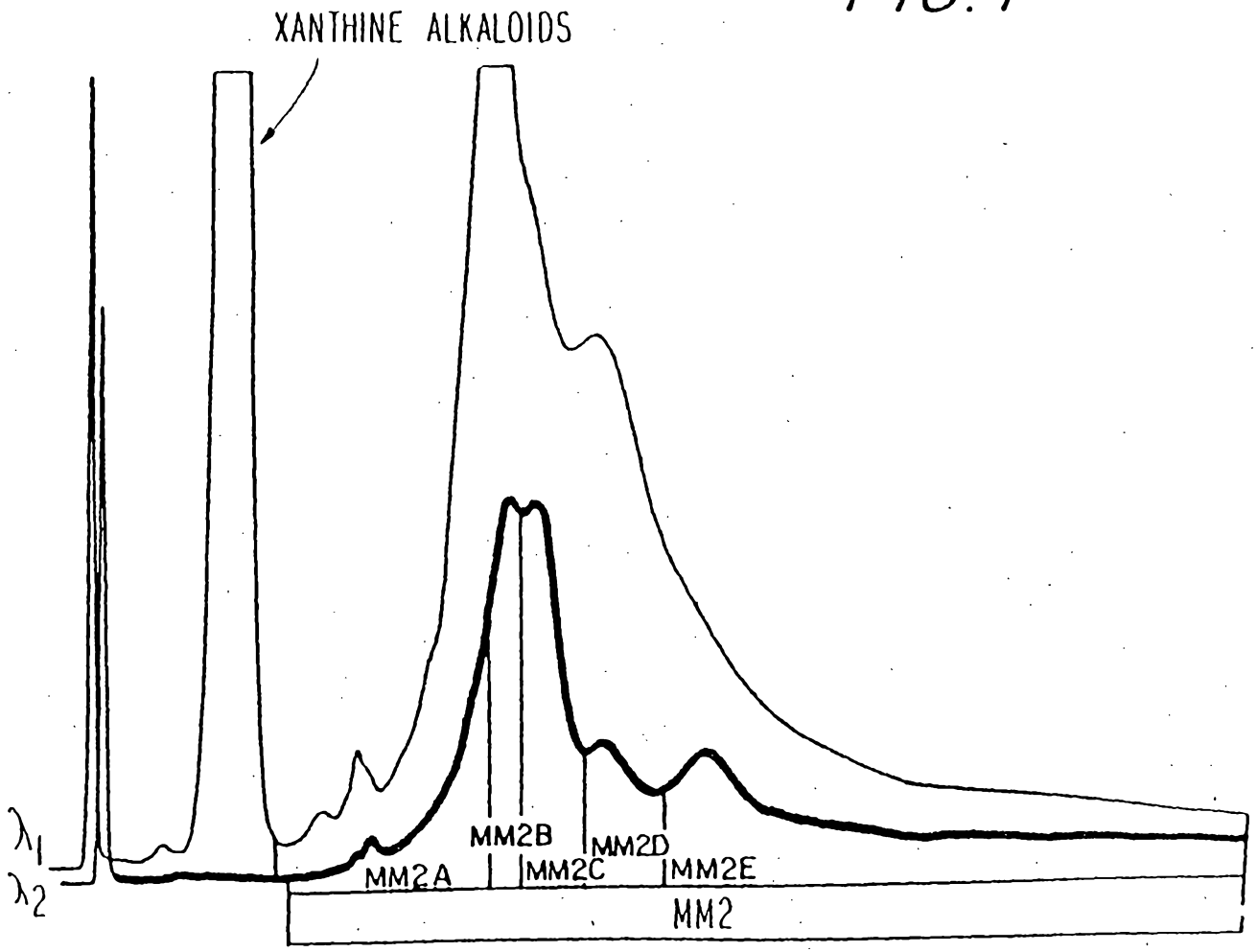
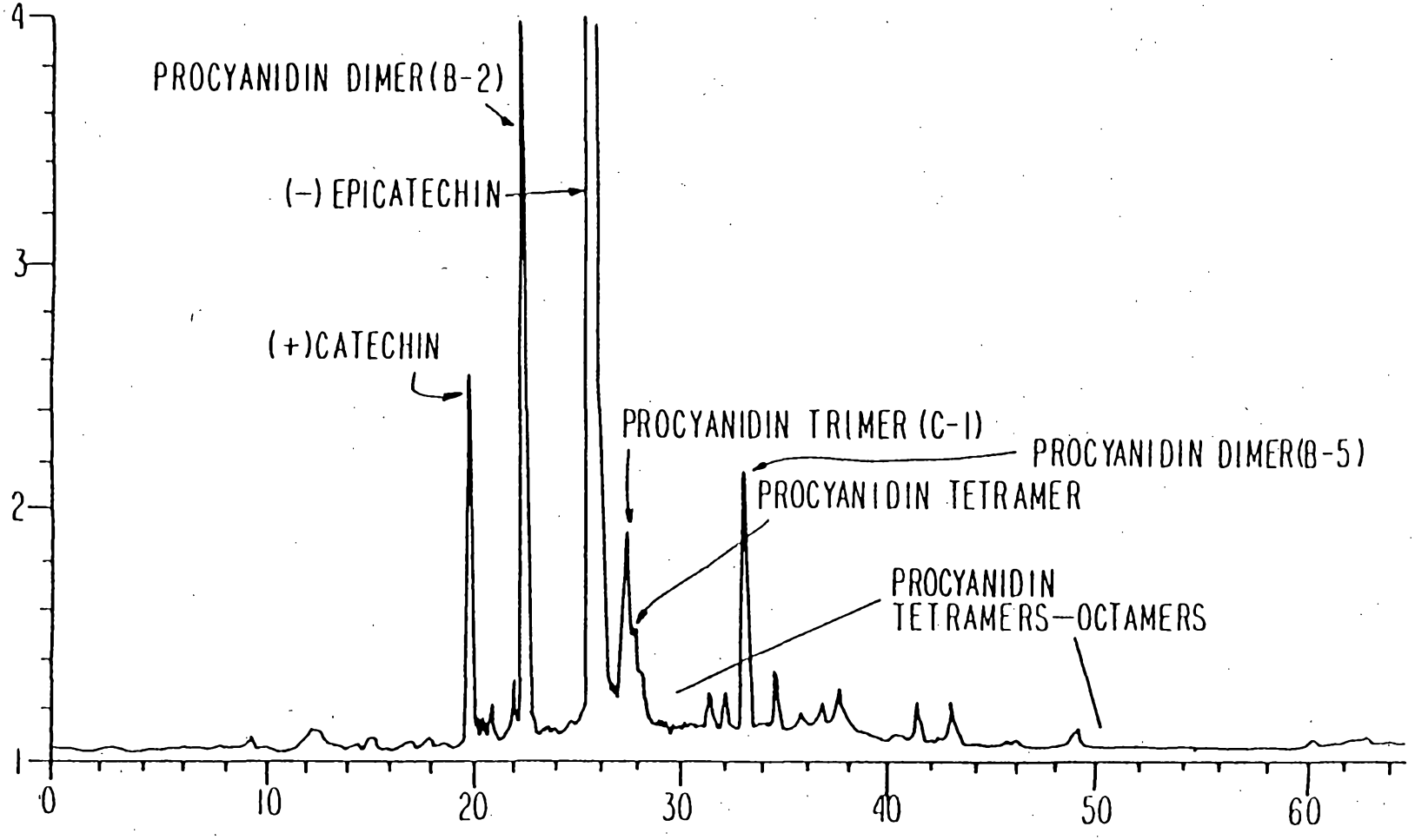


FIG. 2A


PROCYANIDIN DIMER(B-2)
(-) EPICATECHIN
(+)CATECHIN
PROCYANIDIN TRIMER (C-1)
PROCYANIDIN DIMER(B-5)
PROCYANIDIN TETRAMER
PROCYANIDIN TETRAMERS—OCTAMERS
4
3
2
1
0
10
20
30
40
50
60

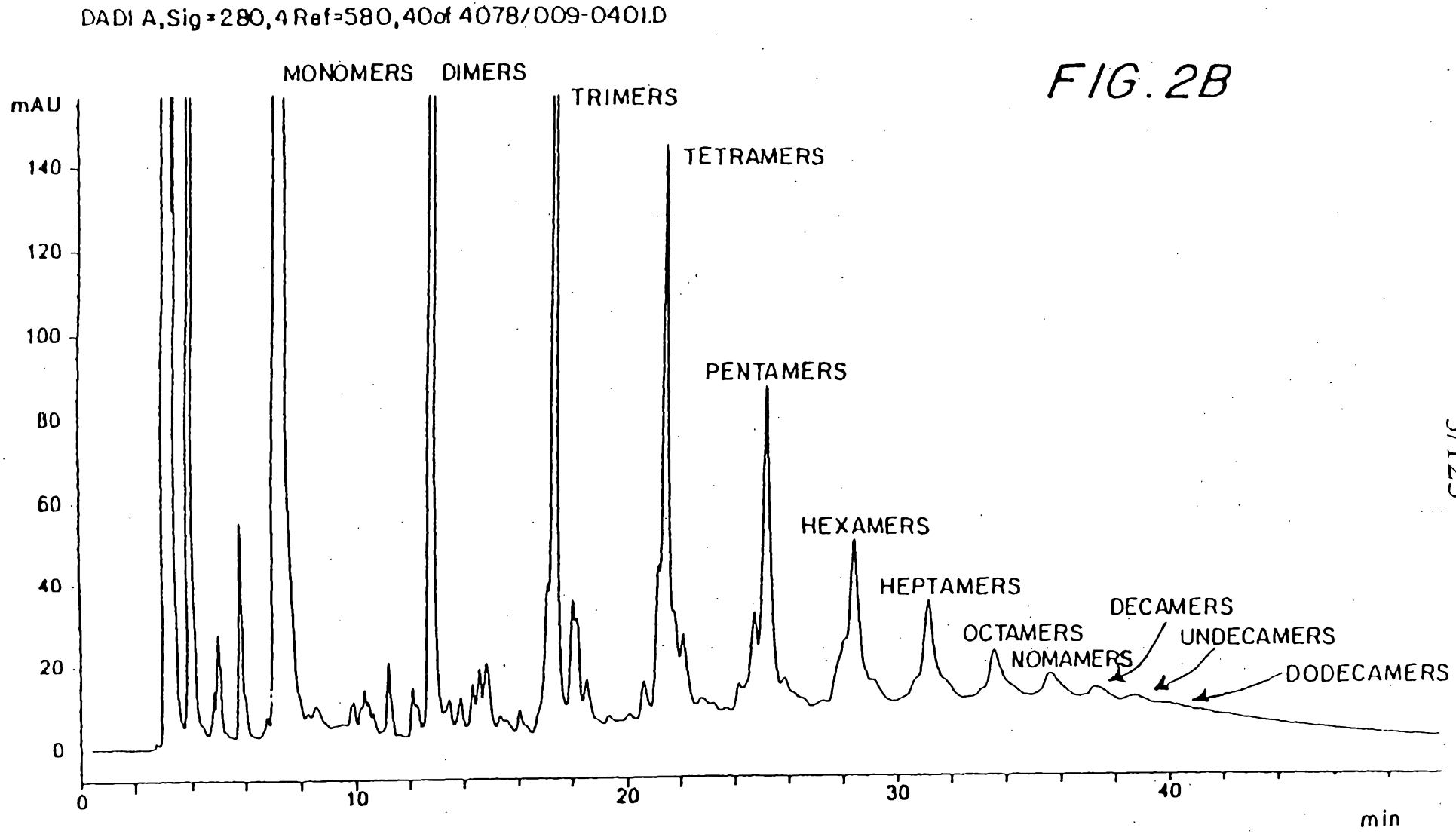
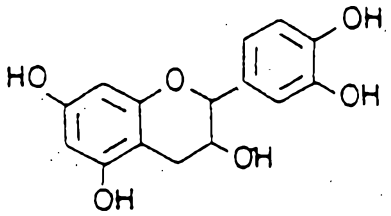
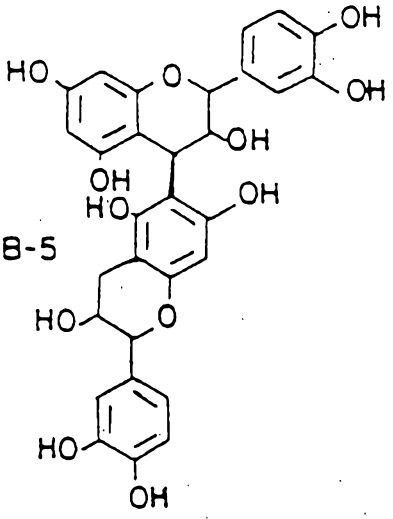

DADI A,Sig=280,4 Ref=580,40of 4078/009-0401.D
MONOMERS
DIMERS
TRIMERS
TETRAMERS
PENTAMERS
HEXAMERS
HEPTAMERS
OCTAMERS
NOMAMERS
DECAMERS
UNDECAMERS
DODECAMERS
mAU
140
120
100
80
60
40
20
0
0
10
20
30
40
min

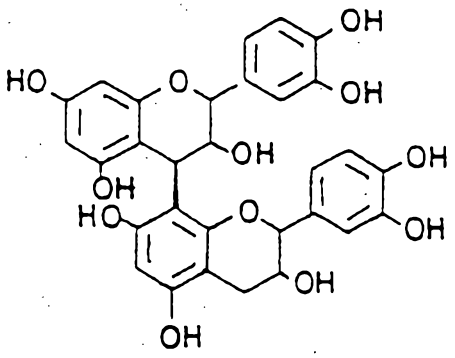
FIG. 2B



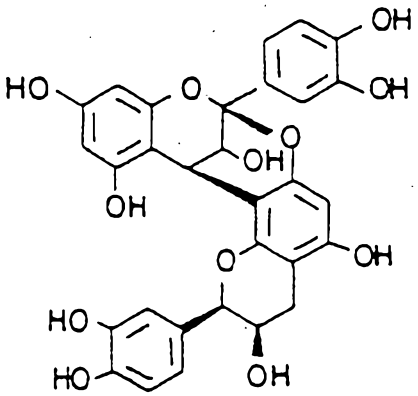
(−) EPICATECHIN



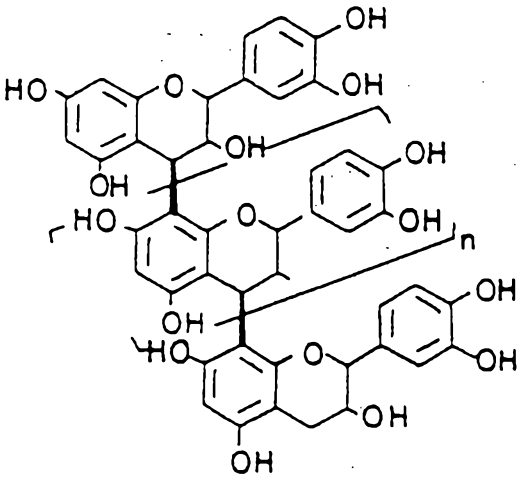
PROCYANIDIN B-5



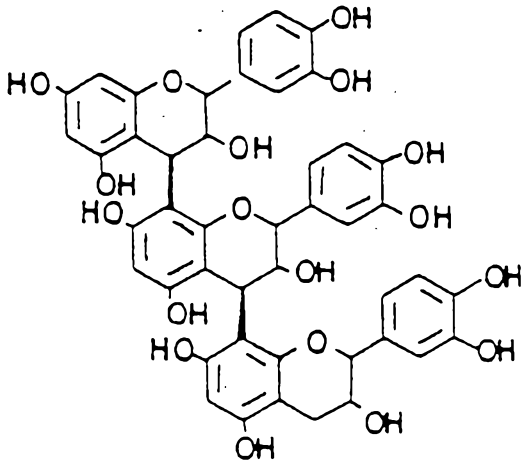
PROCYANIDIN B-2



PROCYANIDIN A-2



PROCYANIDIN OLIGONERS n = 2 THROUGH 5



PROCYANIDIN C-1

FIG. 3

FIG.4A

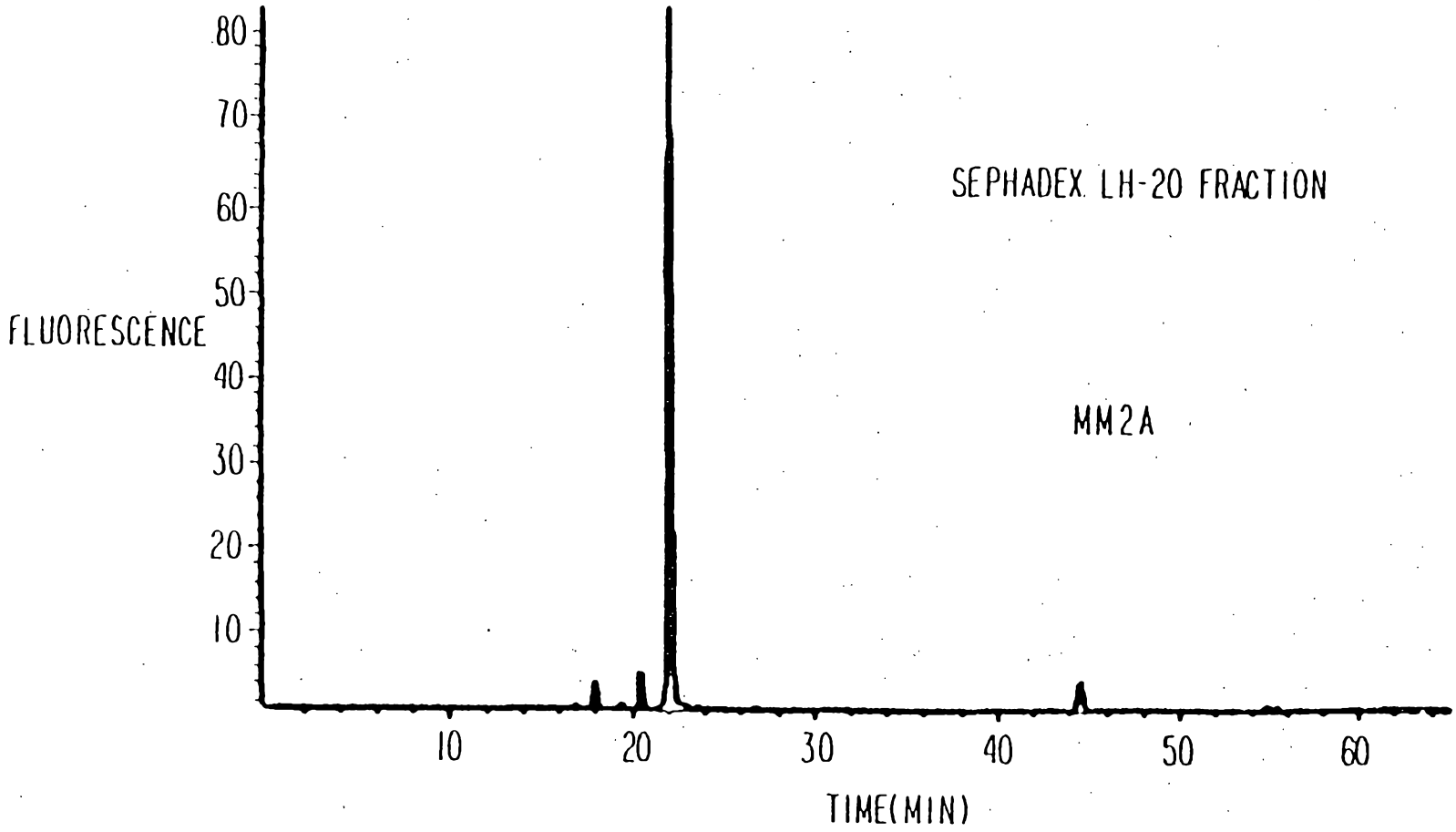

SEPHADEX LH-20 FRACTION
MM2A
FLUORESCENCE
80
70
60
50
40
30
20
10
10
20
30
40
50
60
TIME(MIN)

FIG. 4B

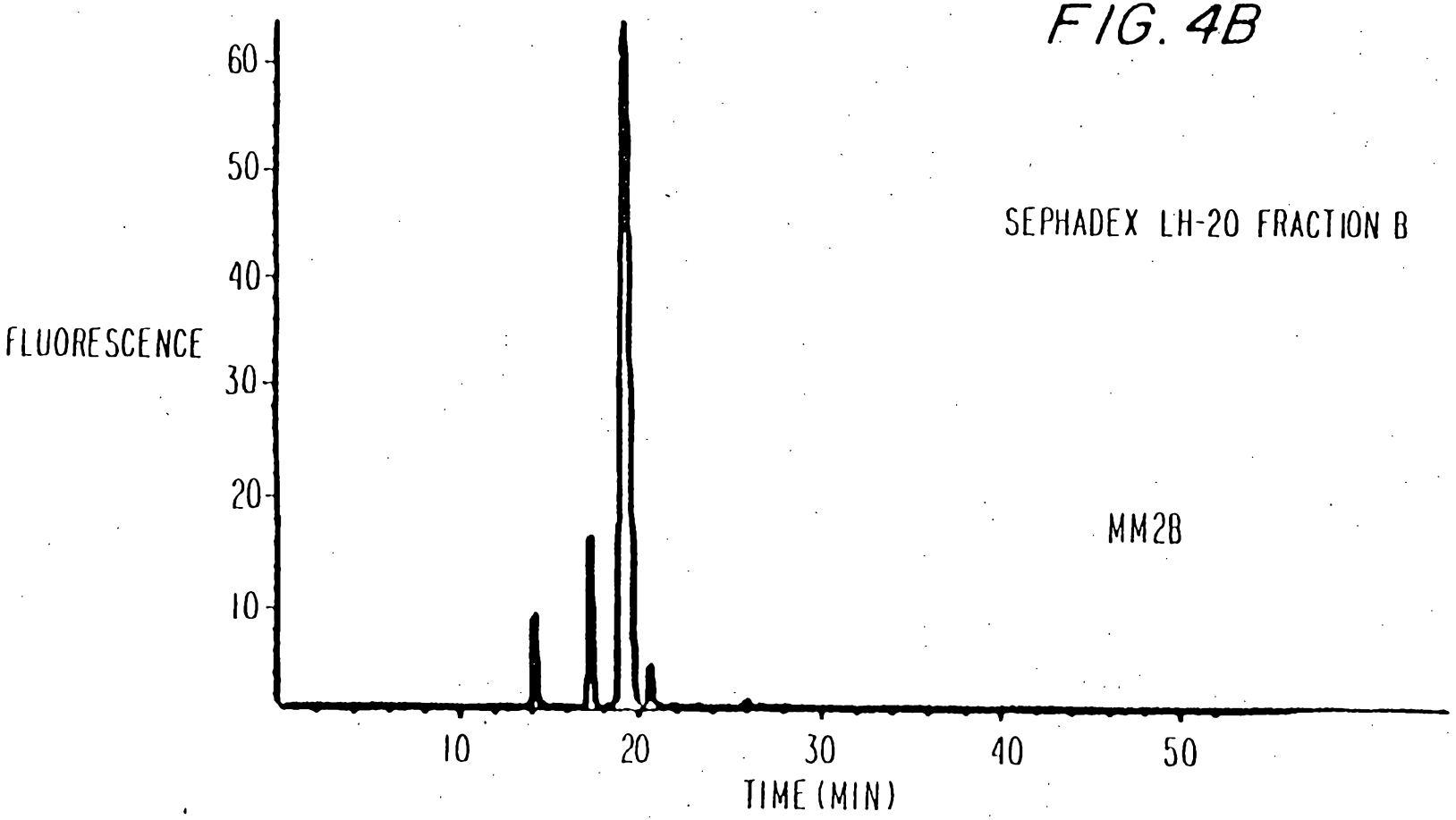

SEPHADEX LH-20 FRACTION B
MM2B
FLUORESCENCE
60
50
40
30
20
10
10
20
30
40
50
TIME (MIN)

FIG. 4C

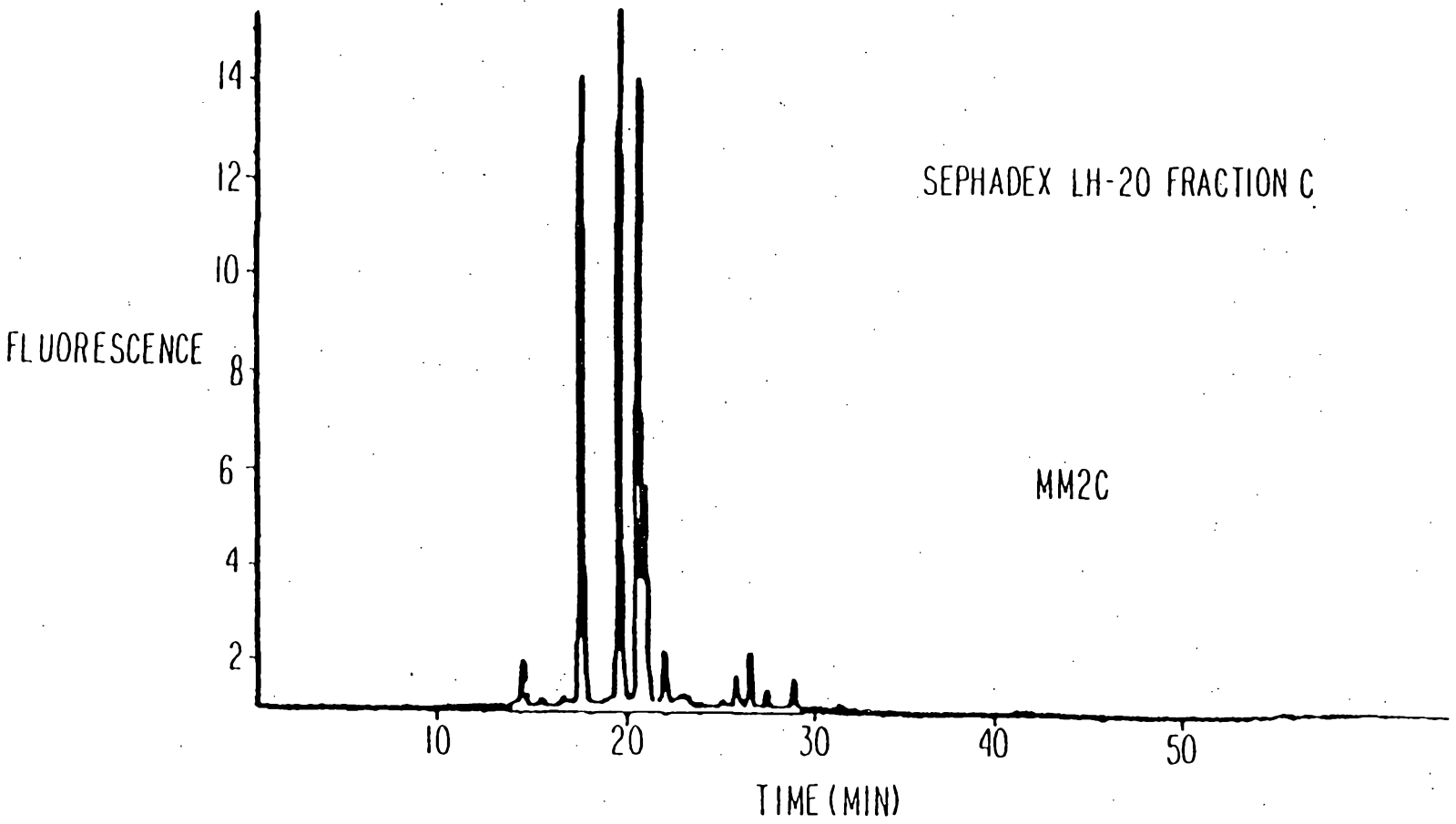

FLUORESCENCE
14
12
10
8
6
4
2
10
20
30
40
50
TIME (MIN)
SEPHADEX LH-20 FRACTION C
MM2C

FIG. 4D

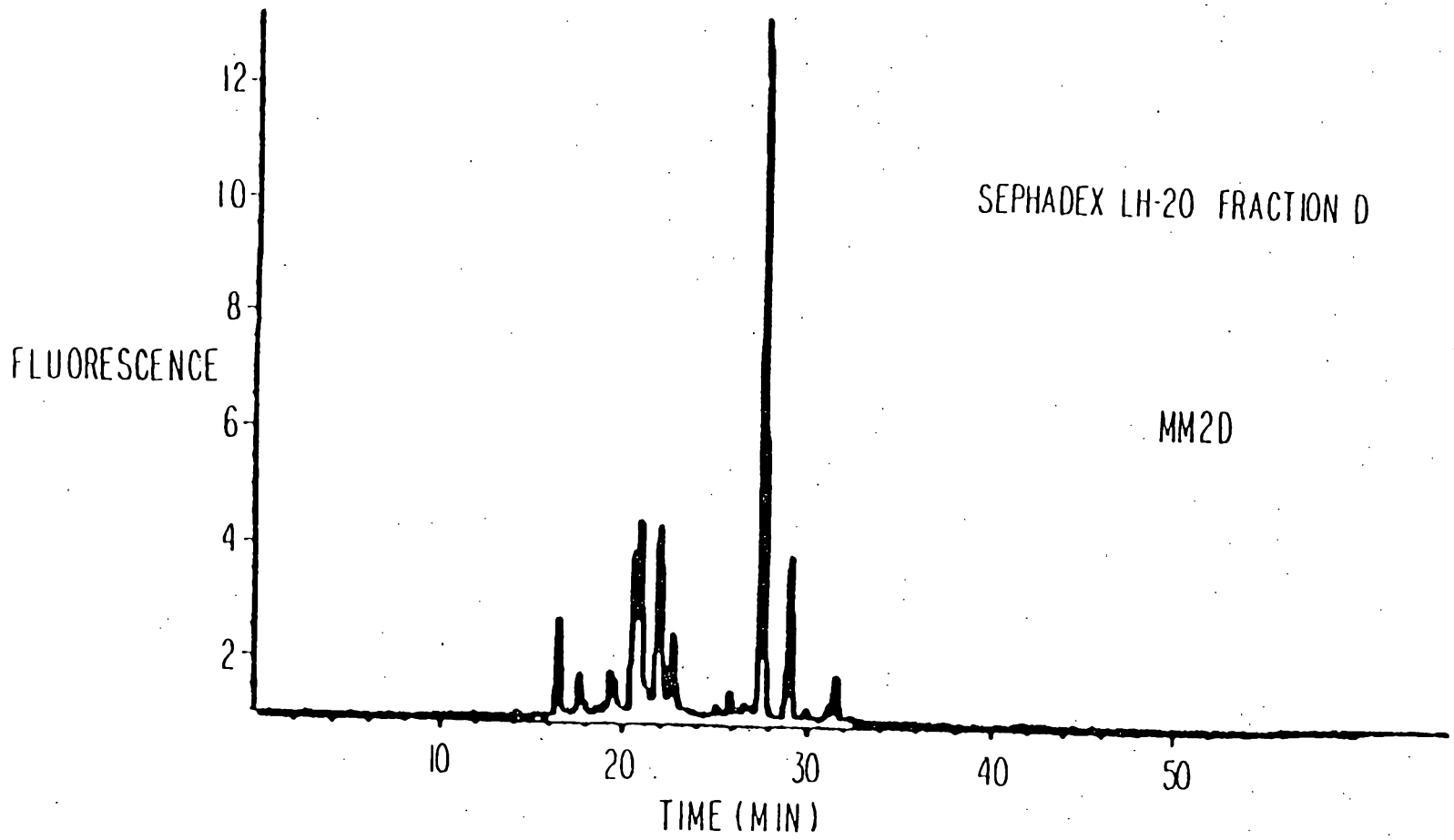

SEPHADEX LH-20 FRACTION D
MM2D
FLUORESCENCE
12
10
8
6
4
2
10
20
30
40
50
TIME (MIN)

FIG. 4E

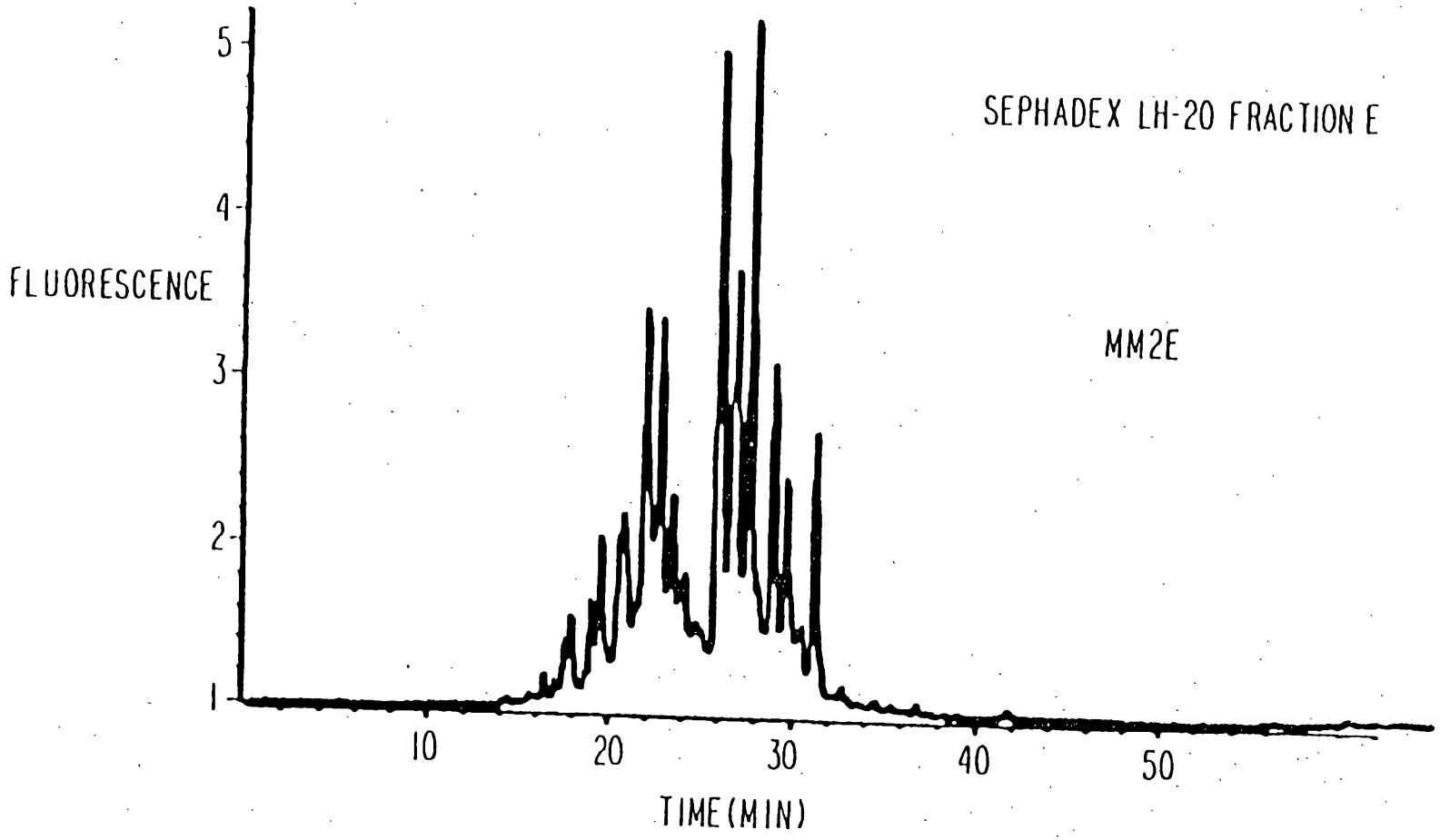

SEPHADEX LH-20 FRACTION E
MM2E
FLUORESCENCE
5
4
3
2
1
10
20
30
40
50
TIME (MIN)

FIG. 5A

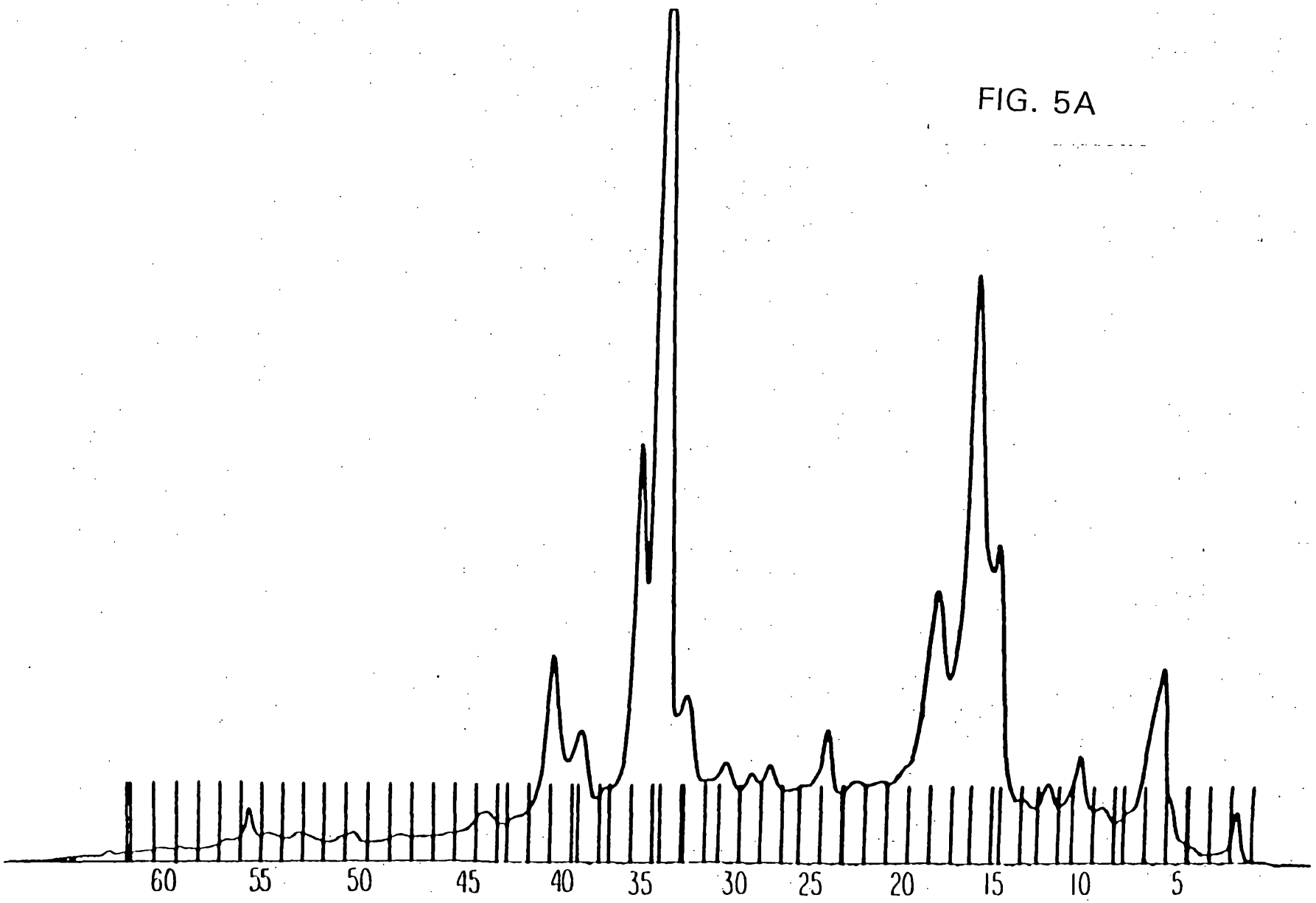


FIG. 5B

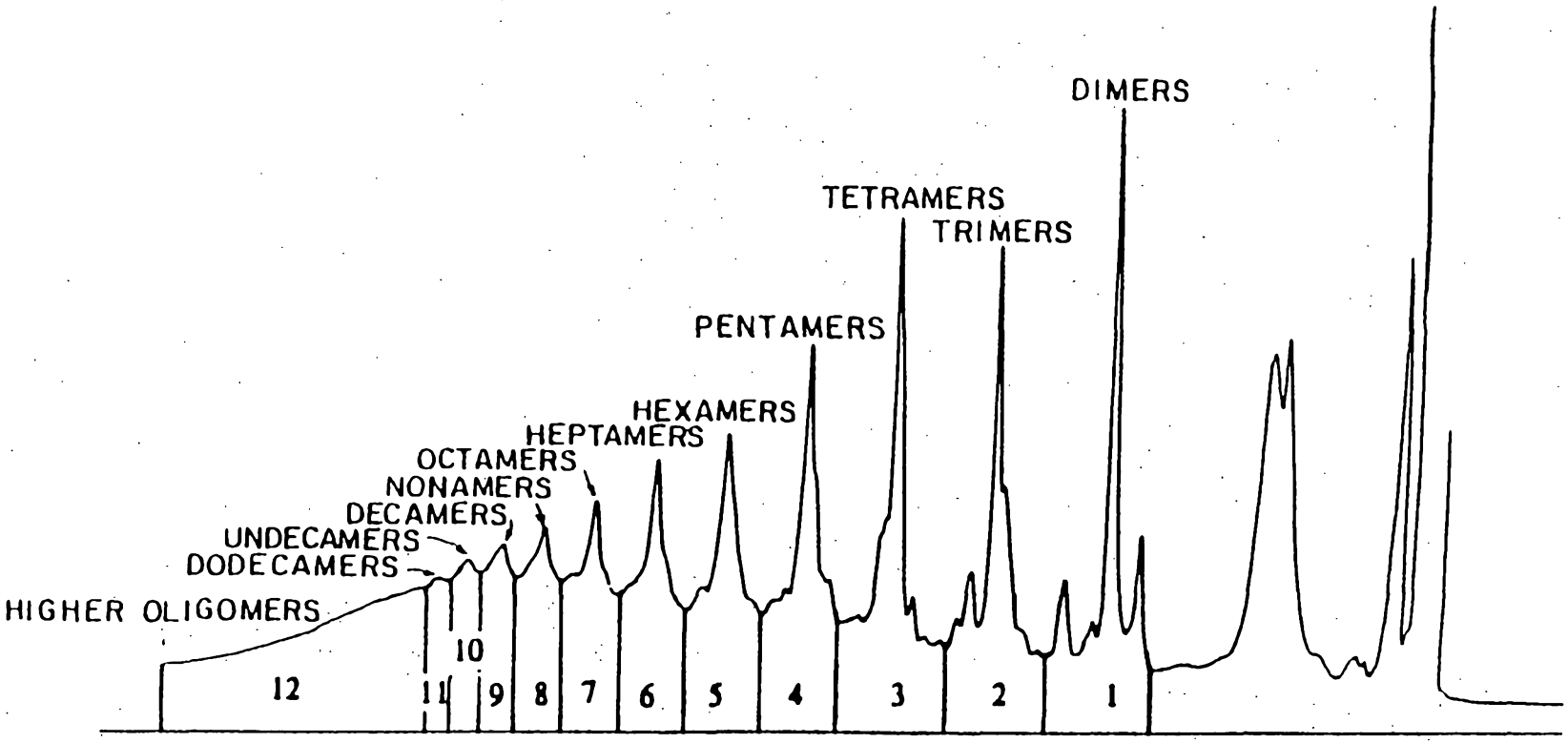

DIMERS
TETRAMERS
TRIMERS
PENTAMERS
HEXAMERS
HEPTAMERS
OCTAMERS
NONAMERS
DECAMERS
UNDECAMERS
DODECAMERS
HIGHER OLIGOMERS
12
11
10
9
8
7
6
5
4
3
2
1
FRACTION NUMBER

FIG. 5C

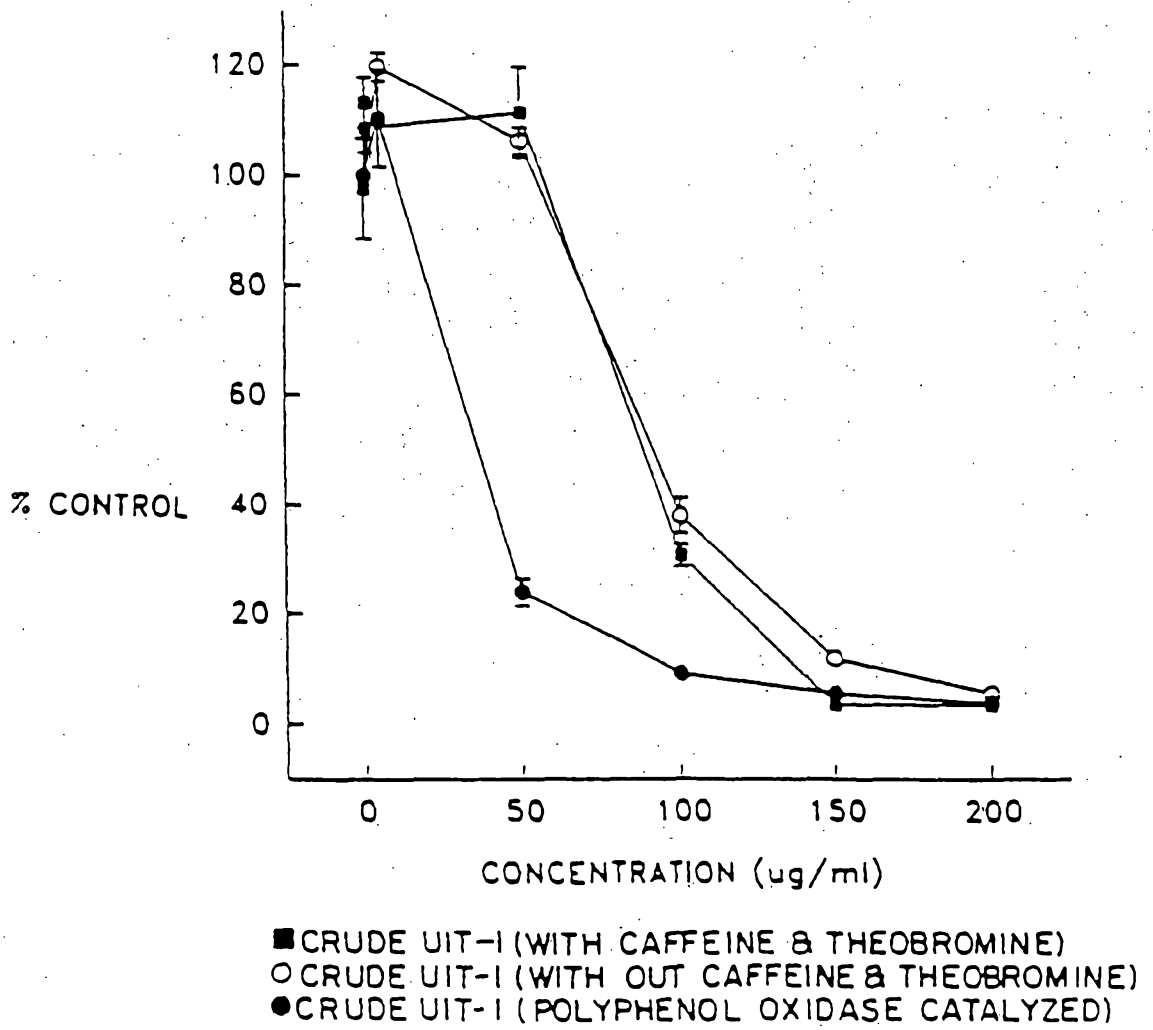

120
100
80
60
40
20
0
% CONTROL
0
50
100
150
200
CONCENTRATION (ug/ml)
■CRUDE UIT-I (WITH CAFFEINE & THEOBROMINE)
○CRUDE UIT-I (WITH OUT CAFFEINE & THEOBROMINE)
●CRUDE UIT-I (POLYPHENOL OXIDASE CATALYZED)

FIG. 6A

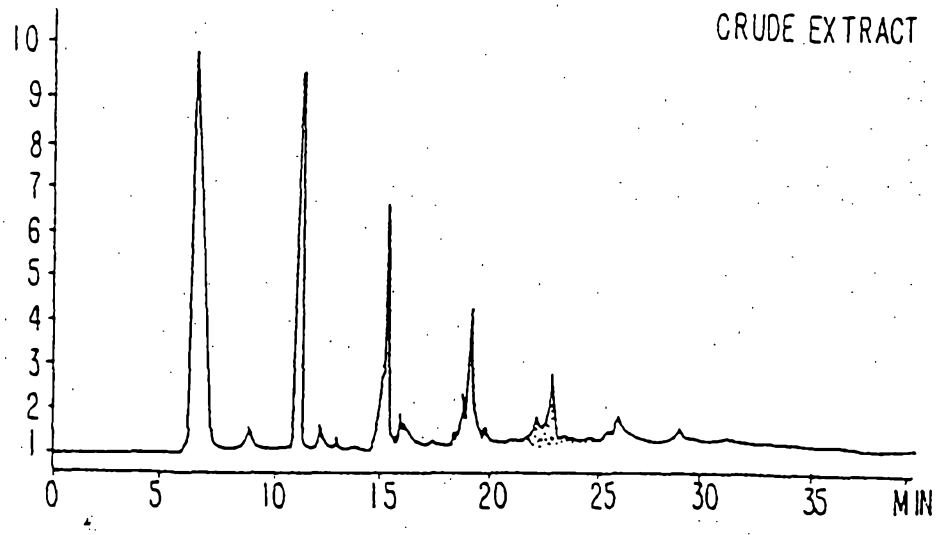


FIG. 6B

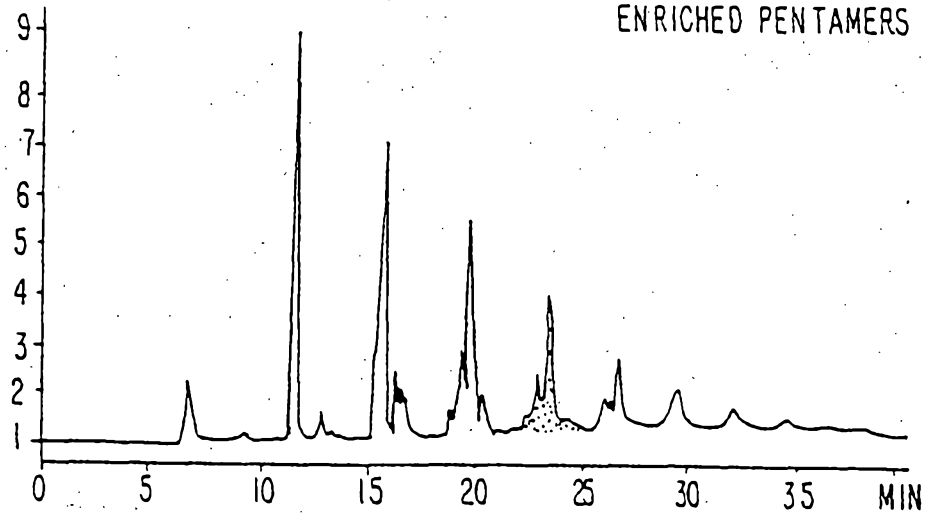


FIG. 6C

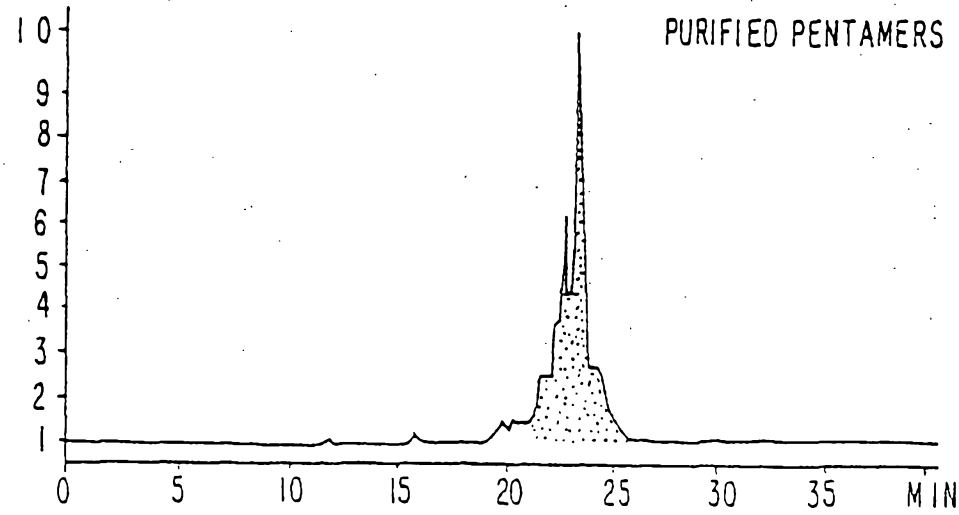


FIG. 7A

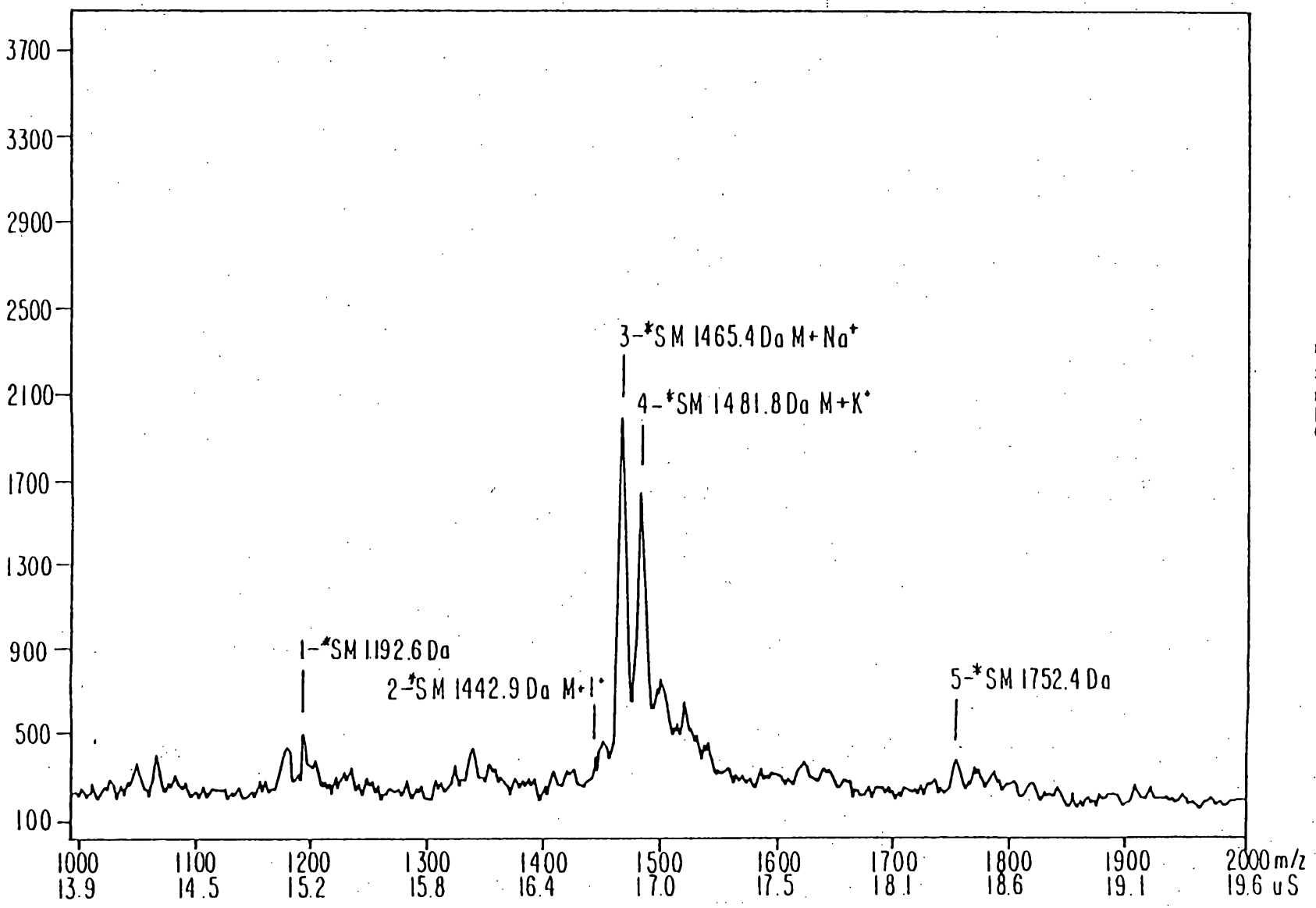
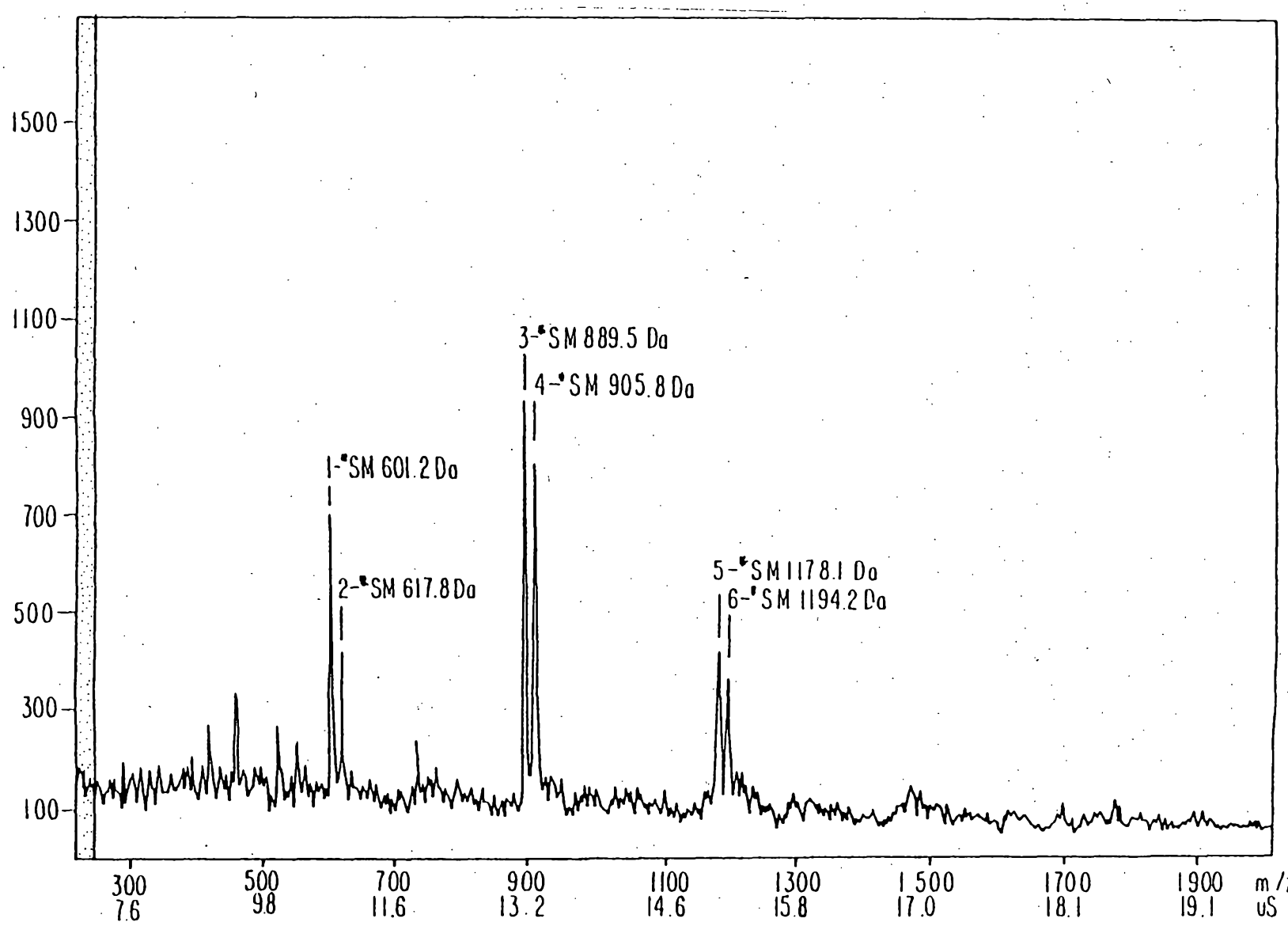

3700
3300
2900
2500
2100
1700
1300
900
500
100
3-*SM 1465.4 Da M+Na+
4-*SM 1481.8 Da M+K+
1-*SM 1192.6 Da
2-*SM 1442.9 Da M+1+
5-*SM 1752.4 Da
1000 13.9
1100 14.5
1200 15.2
1300 15.8
1400 16.4
1500 17.0
1600 17.5
1700 18.1
1800 18.6
1900 19.1
2000 m/z 19.6 uS

FIG. 7B


1500
1300
1100
900
700
500
300
100
1-*SM 601.2 Da
2-*SM 617.8 Da
3-*SM 889.5 Da
4-*SM 905.8 Da
5-*SM 1178.1 Da
6-*SM 1194.2 Da
300
7.6
500
9.8
700
11.6
900
13.2
1100
14.6
1300
15.8
1500
17.0
1700
18.1
1900
19.1
m/z
uS

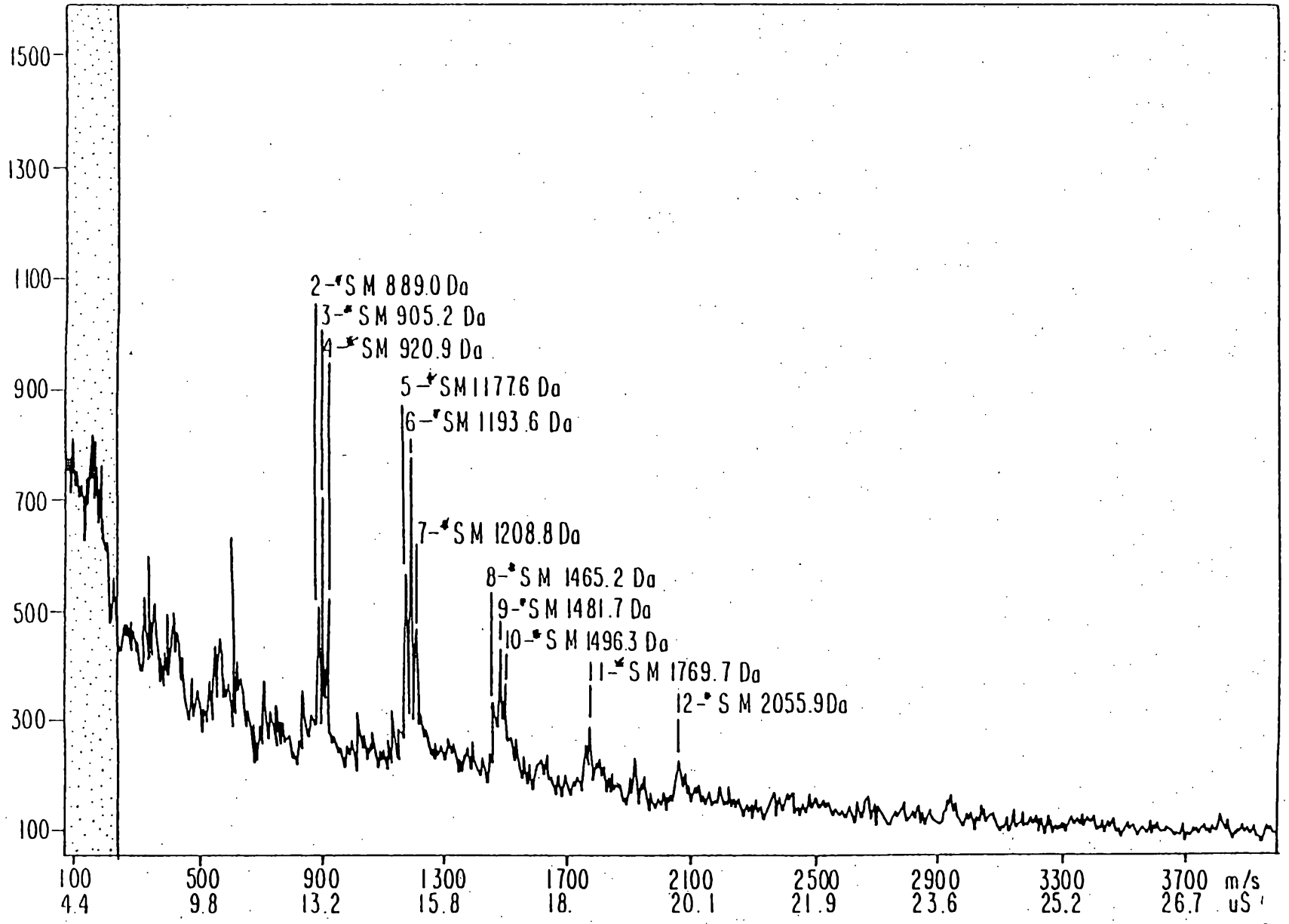

1500
1300
1100
900
700
500
300
100
2-*SM 889.0 Da
3-*SM 905.2 Da
4-*SM 920.9 Da
5-*SM 1177.6 Da
6-*SM 1193.6 Da
7-*SM 1208.8 Da
8-*SM 1465.2 Da
9-*SM 1481.7 Da
10-*SM 1496.3 Da
11-*SM 1769.7 Da
12-*SM 2055.9 Da
100 500 900 1300 1700 2100 2500 2900 3300 3700 m/s
4.4 9.8 13.2 15.8 18. 20.1 21.9 23.6 25.2 26.7 uS

FIG. 7C

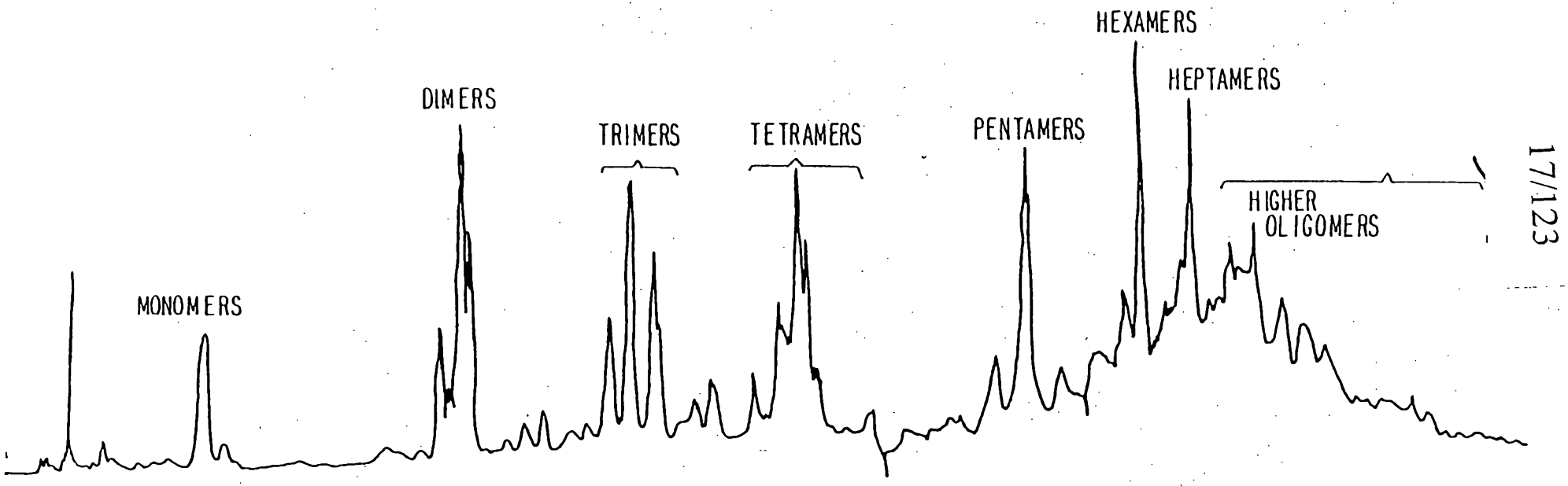

MONOMERS
DIMERS
TRIMERS
TETRAMERS
PENTAMERS
HEXAMERS
HEPTAMERS
HIGHER OLIGOMERS

FIG. 8A

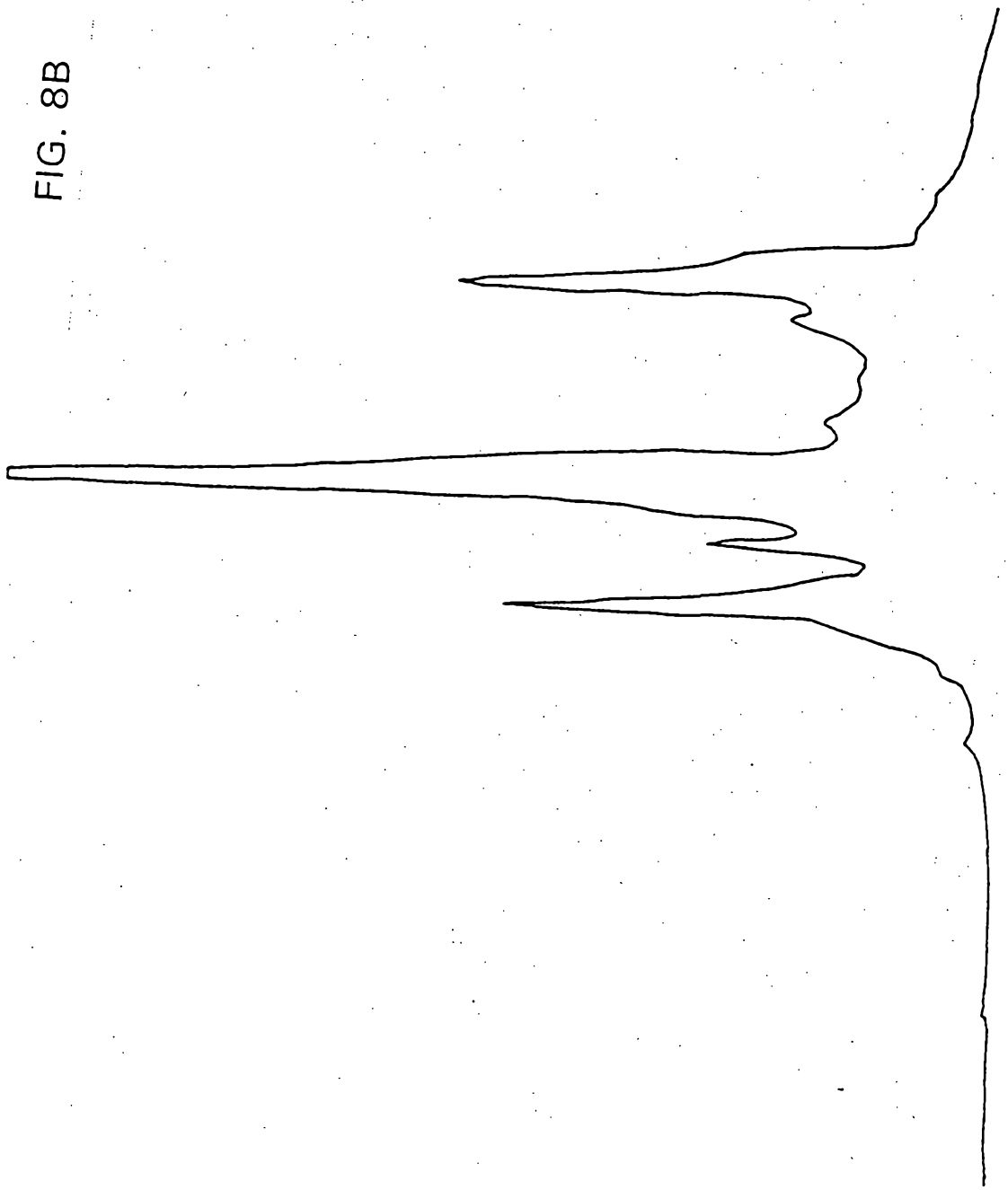


FIG. 8B

FIG. 9A

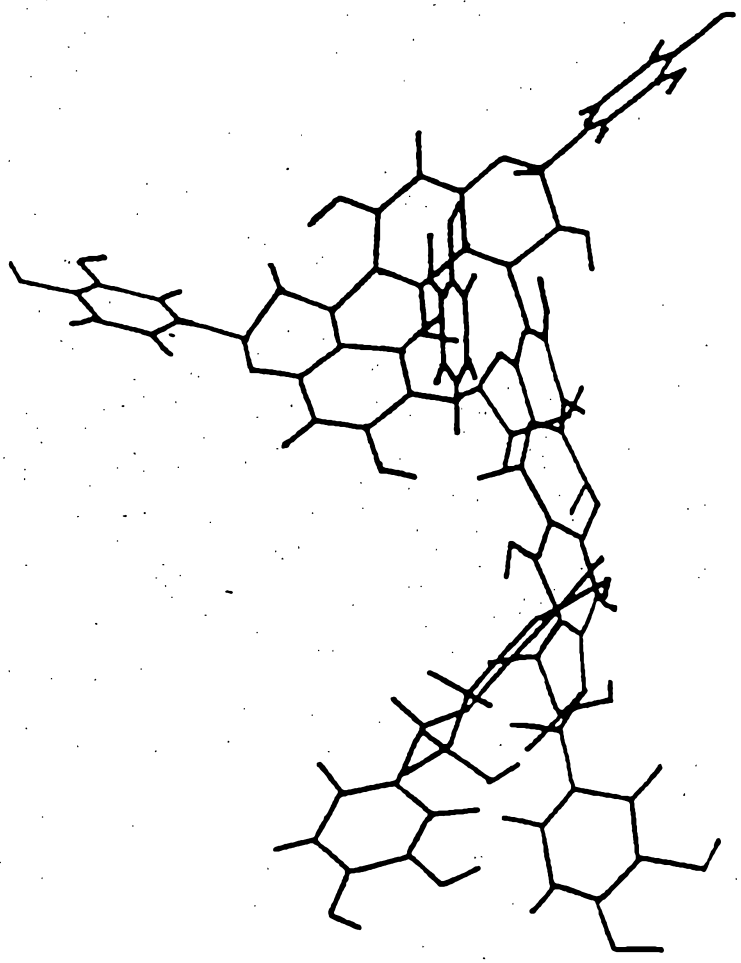


FIG. 9B

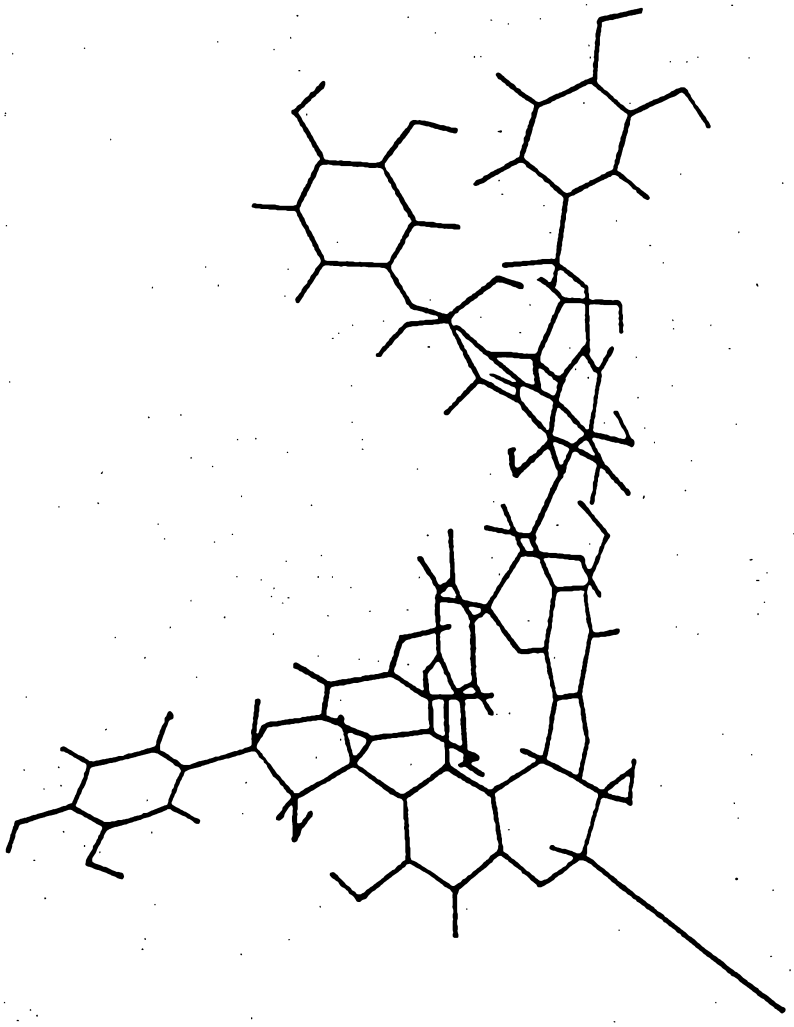


FIG. 9C

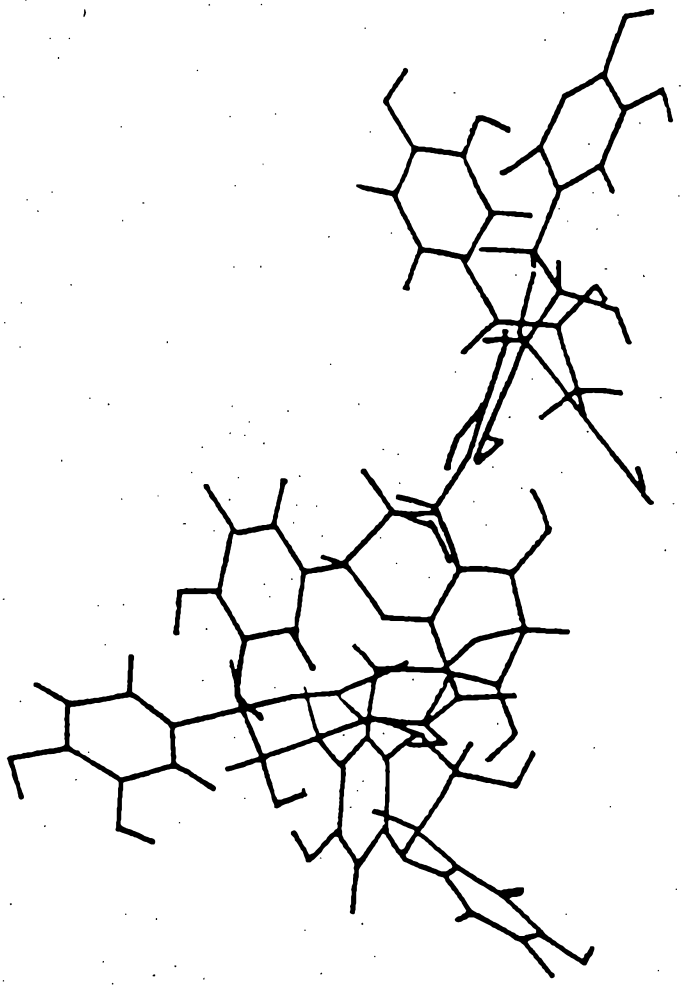
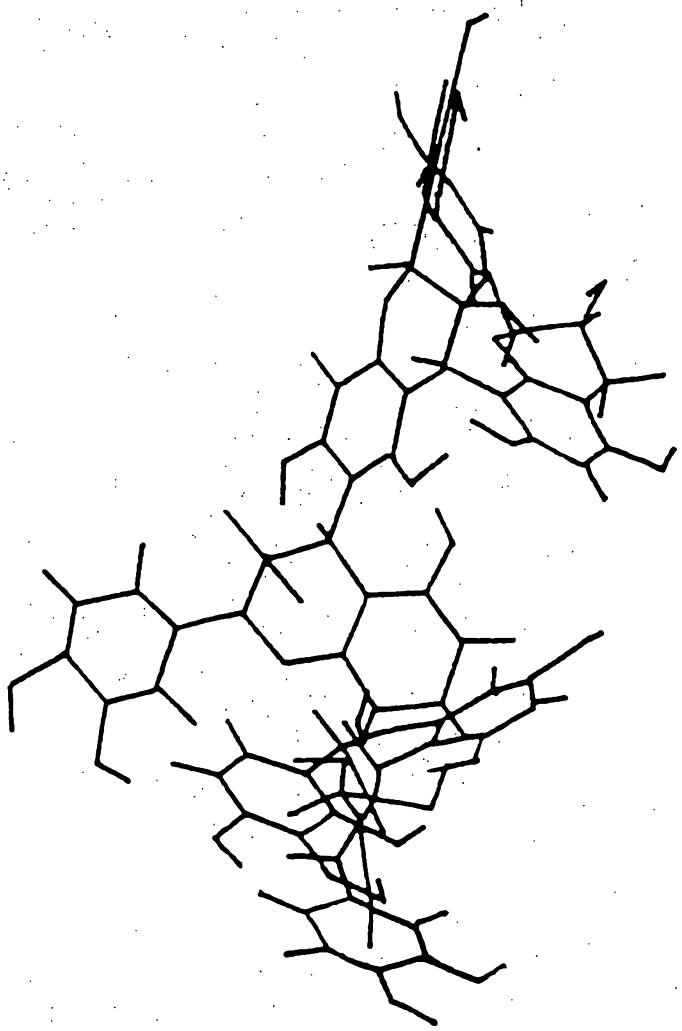


FIG. 9D



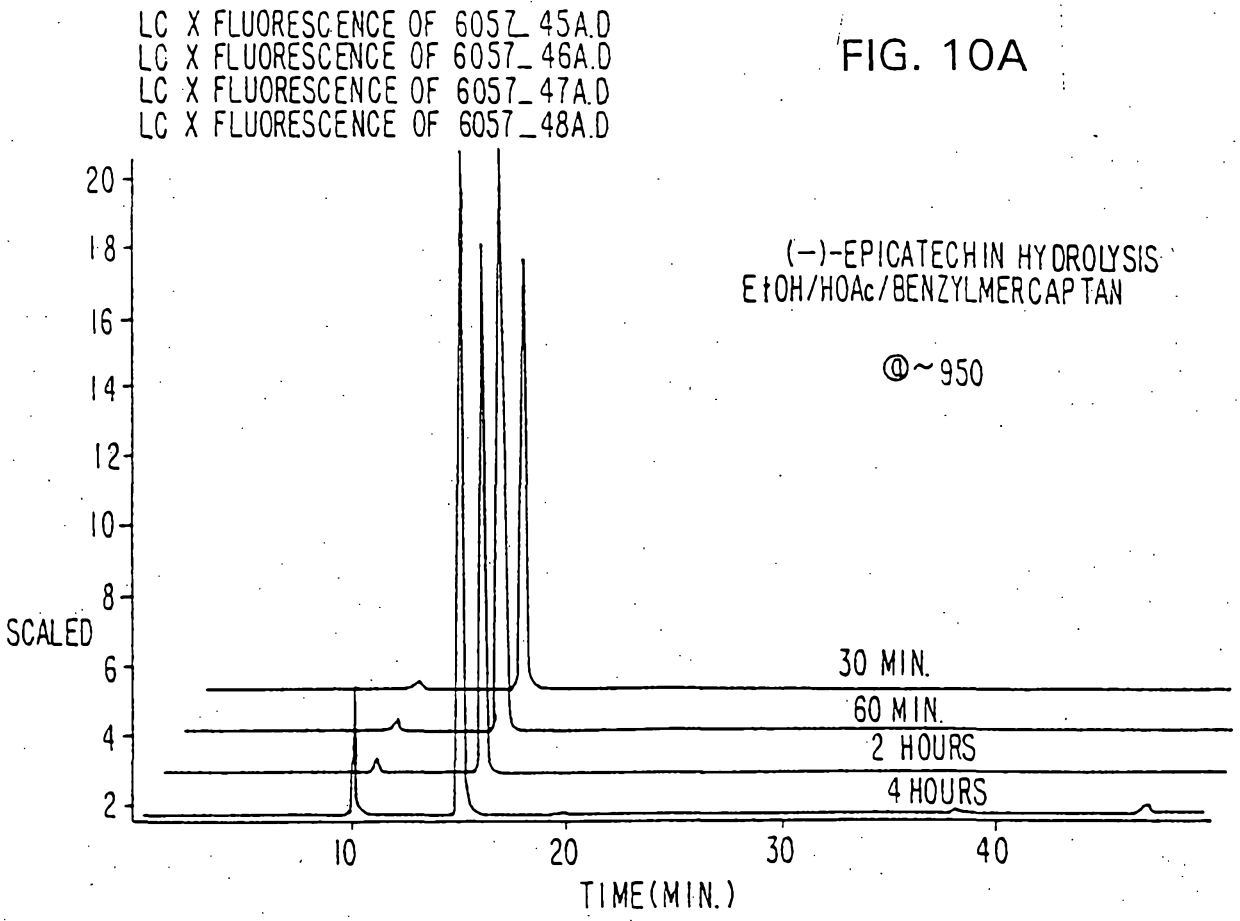


FIG. 10A

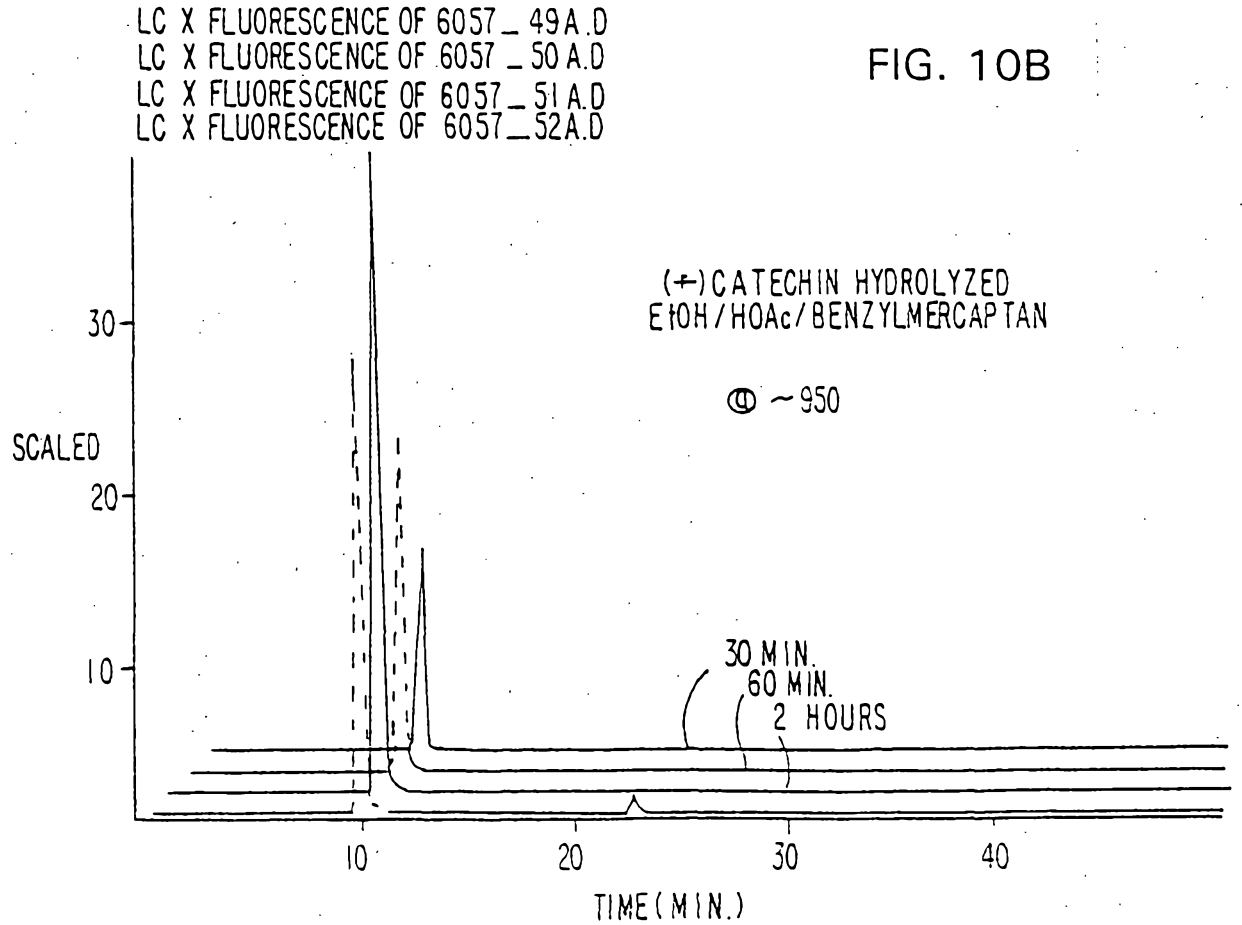


FIG. 10B

FIG. 10C

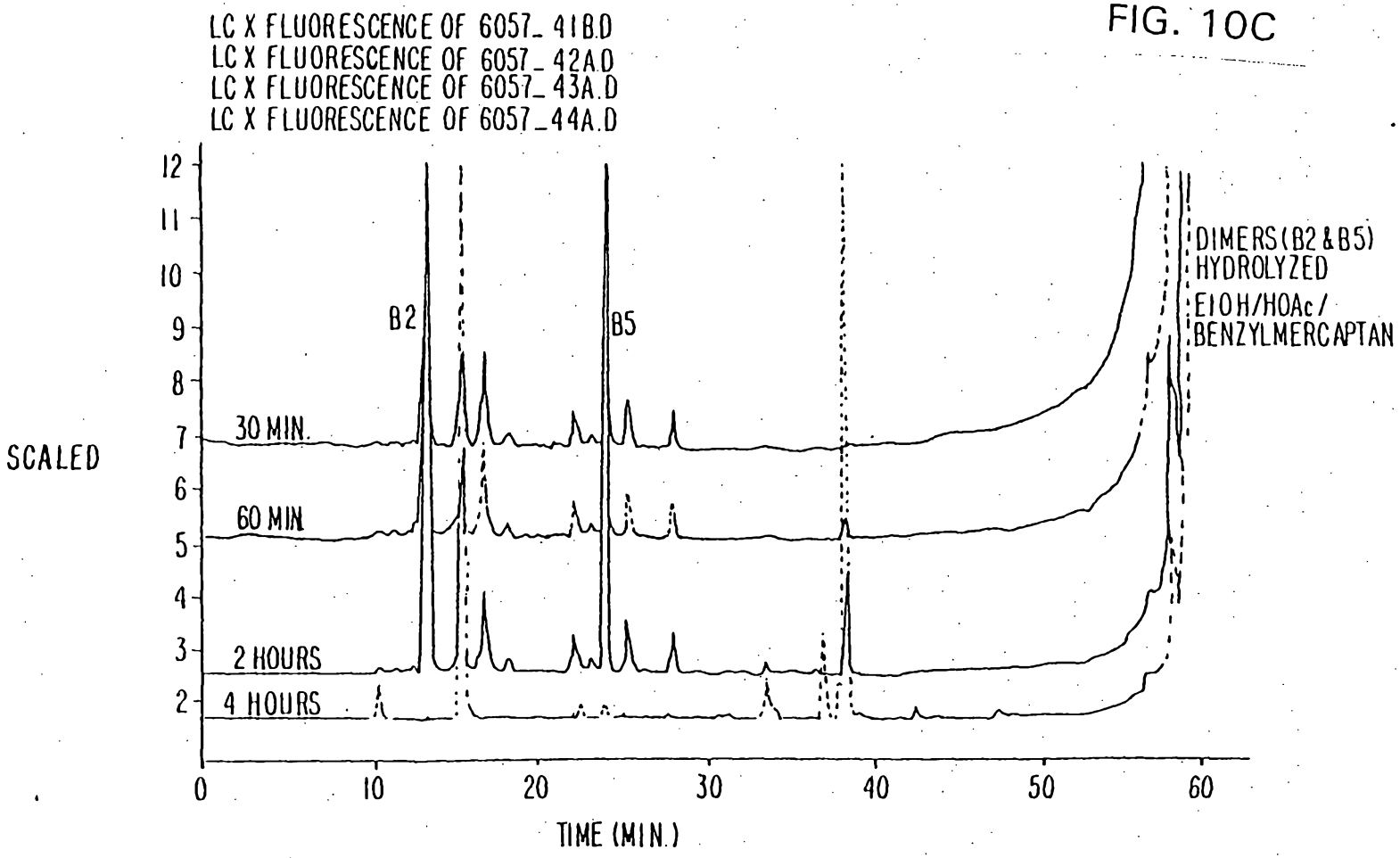

LC X FLUORESCENCE OF 6057_41B.D
LC X FLUORESCENCE OF 6057_42A.D
LC X FLUORESCENCE OF 6057_43A.D
LC X FLUORESCENCE OF 6057_44A.D
SCALED
12
11
10
9
8
7
6
5
4
3
2
B2
B5
30 MIN.
60 MIN
2 HOURS
4 HOURS
DIMERS (B2 & B5)
HYDROLYZED
EIOH/HOAc/
BENZYLMERCAPTAN
0
10
20
30
40
50
60
TIME (MIN.)

FIG. 11A

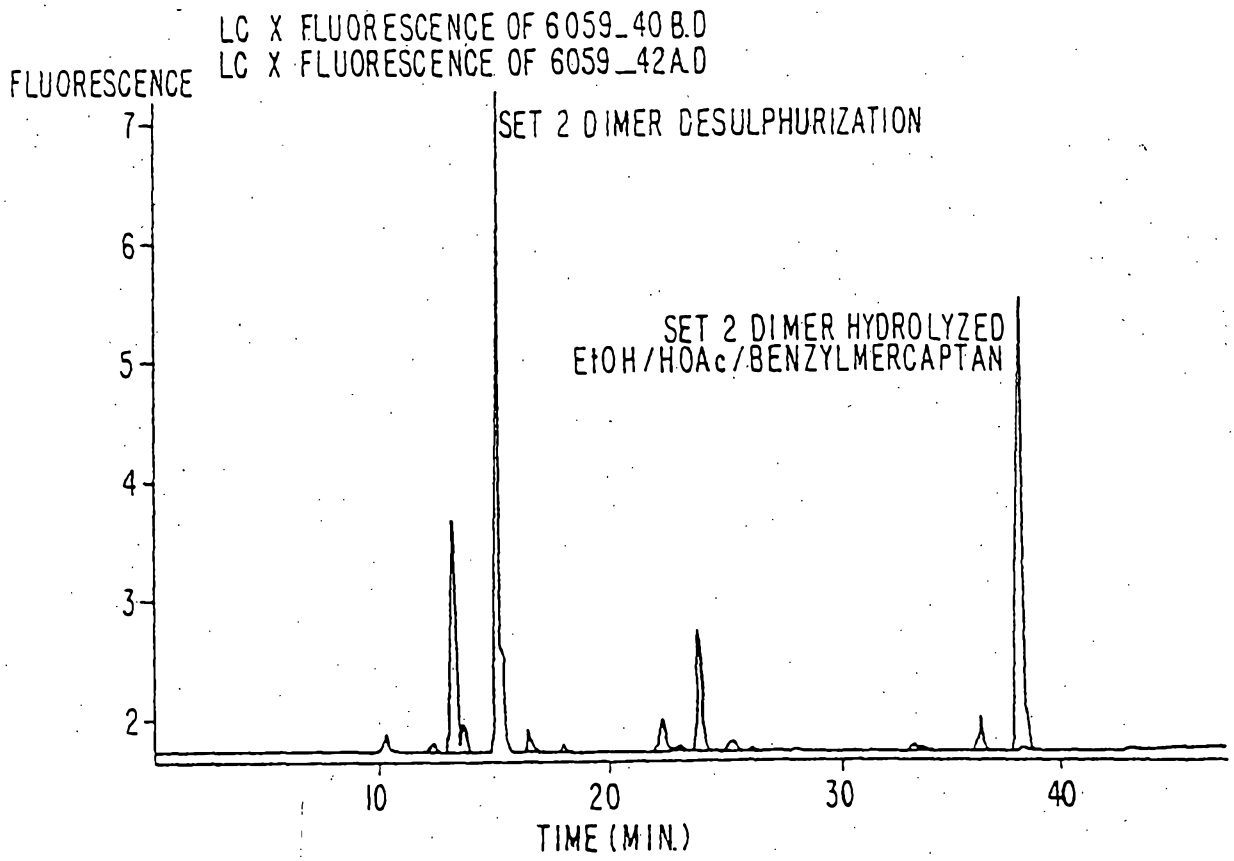
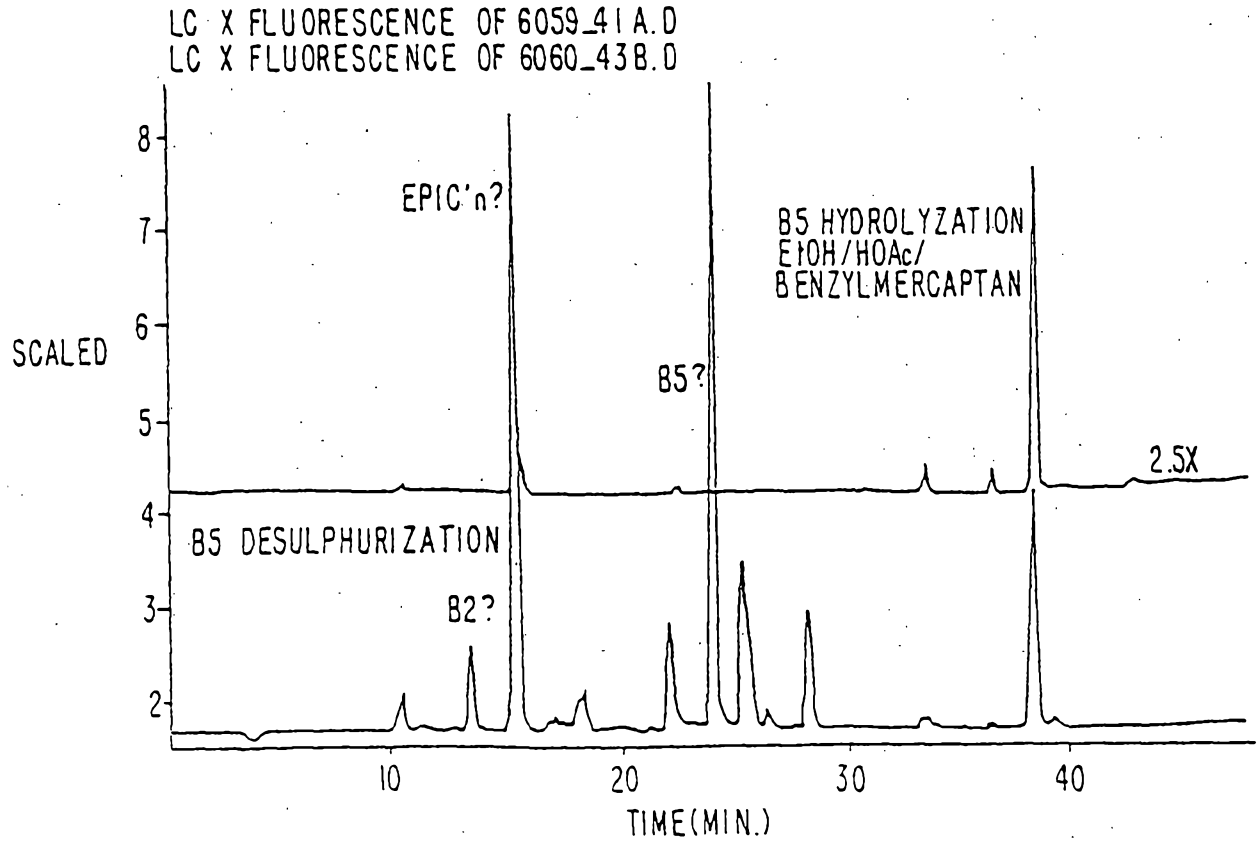


FIG. 11B



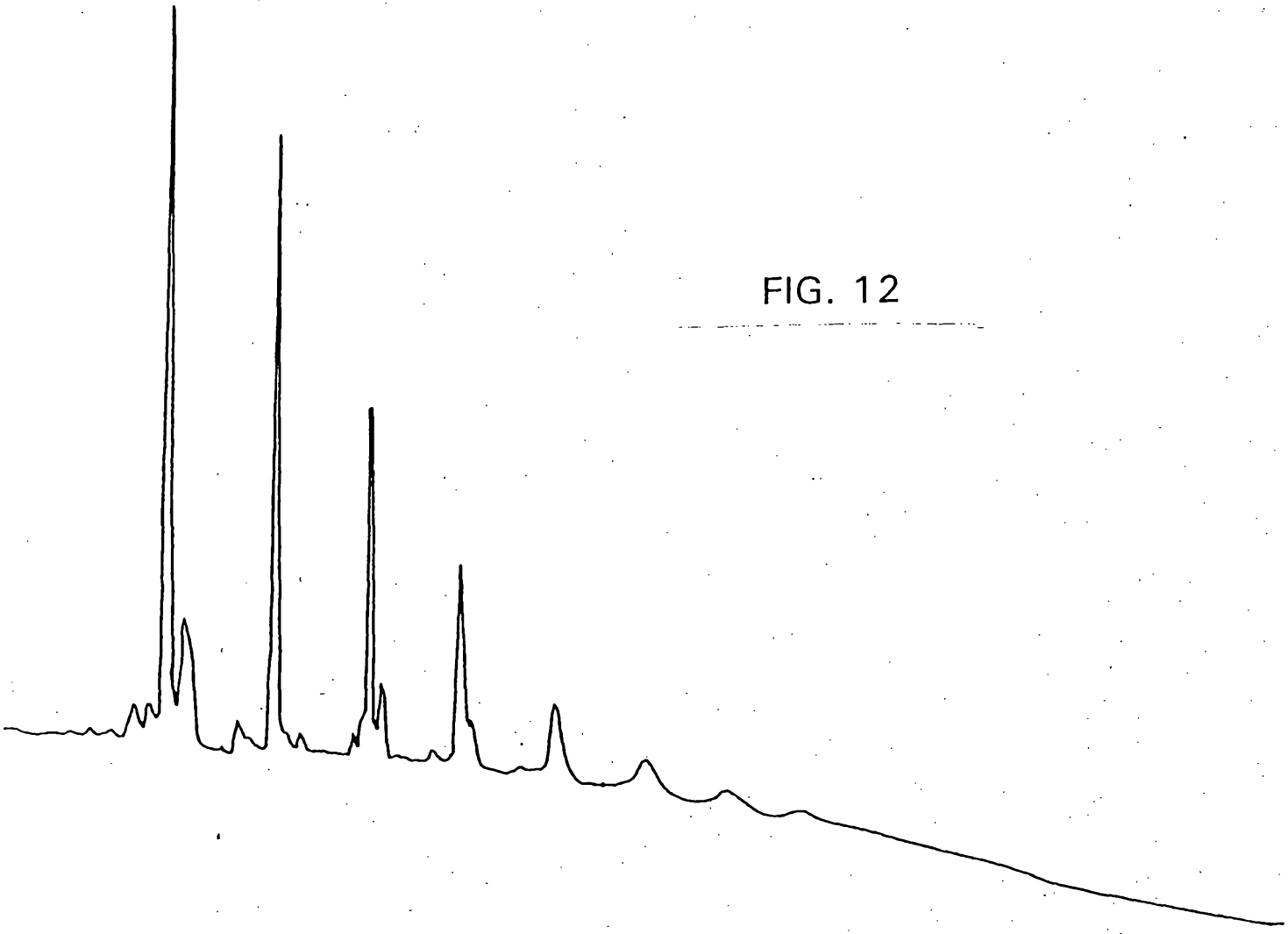


FIG. 12

FIG. 13A

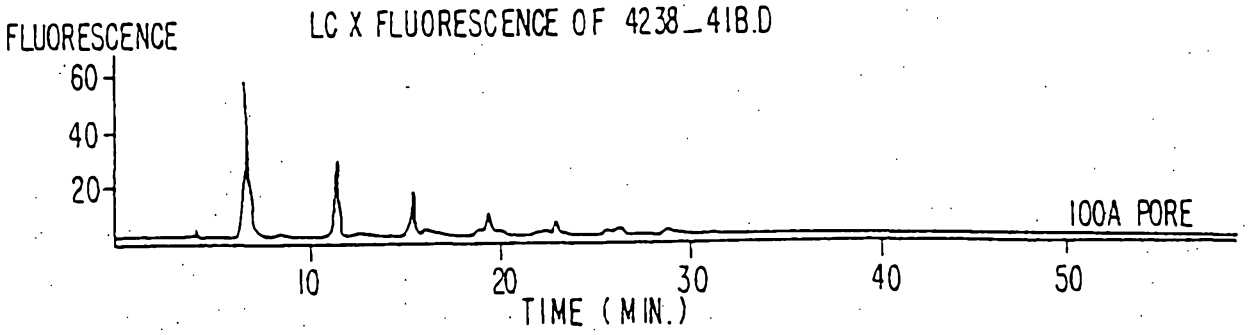


FIG. 13B

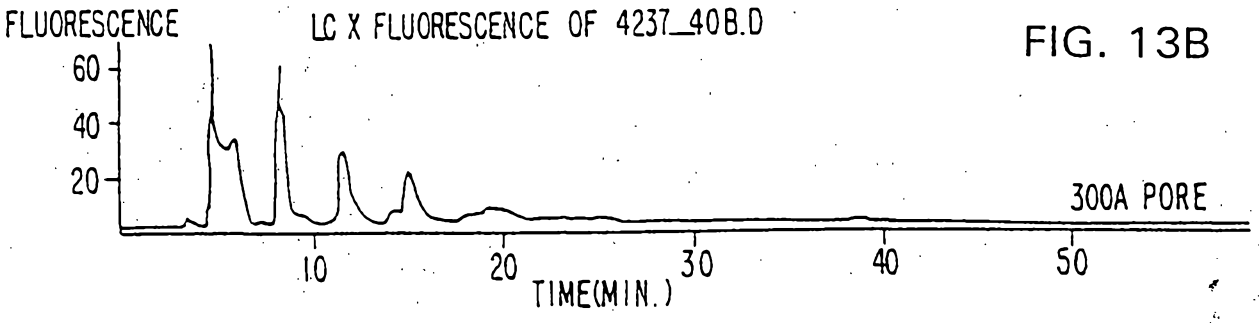


FIG. 13C

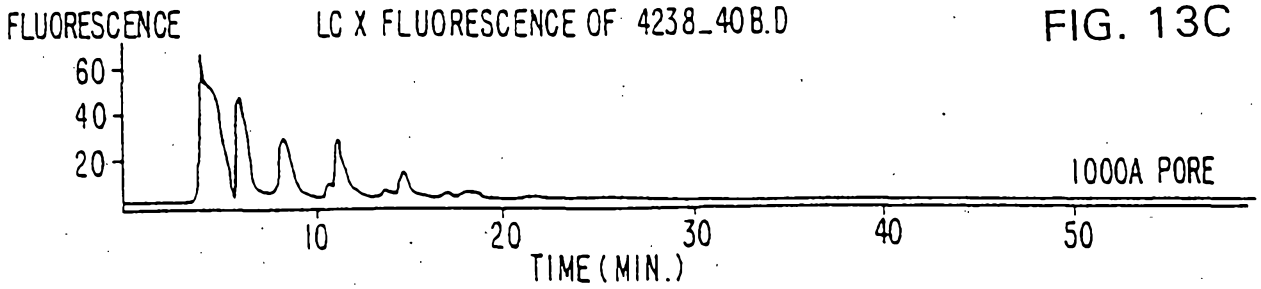


FIG. 14A

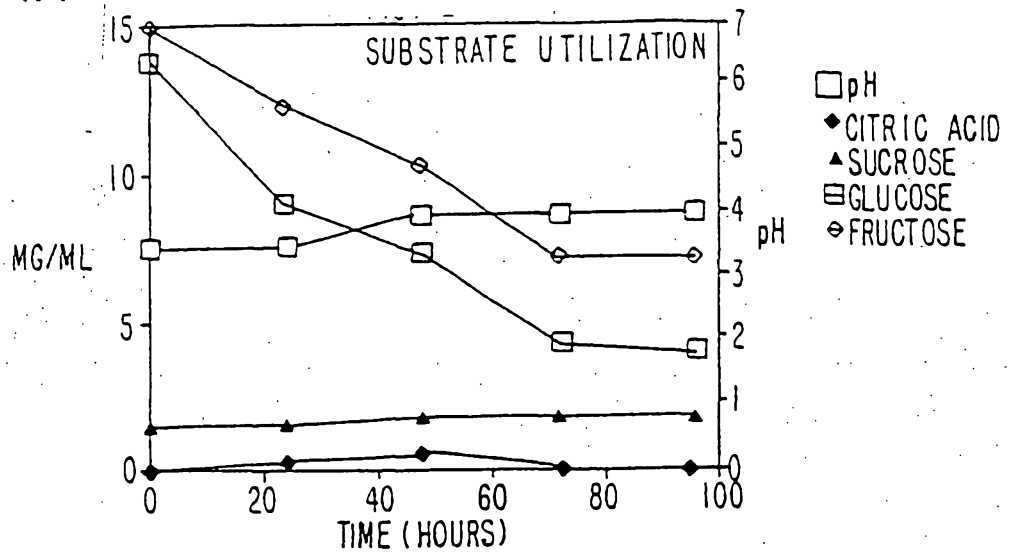


FIG. 14B

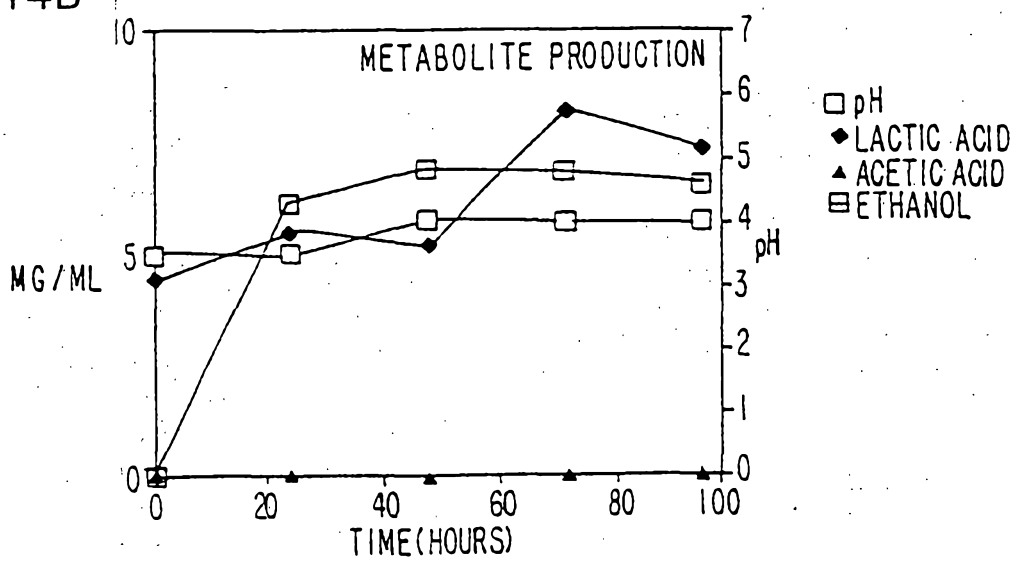


FIG. 14C

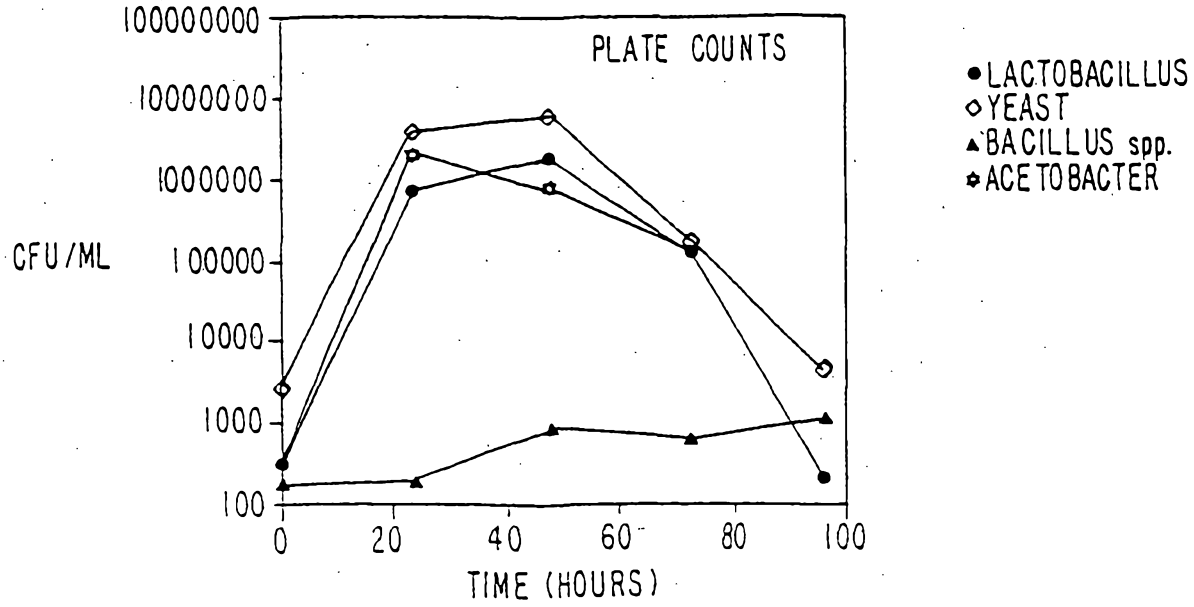


FIG. 14D

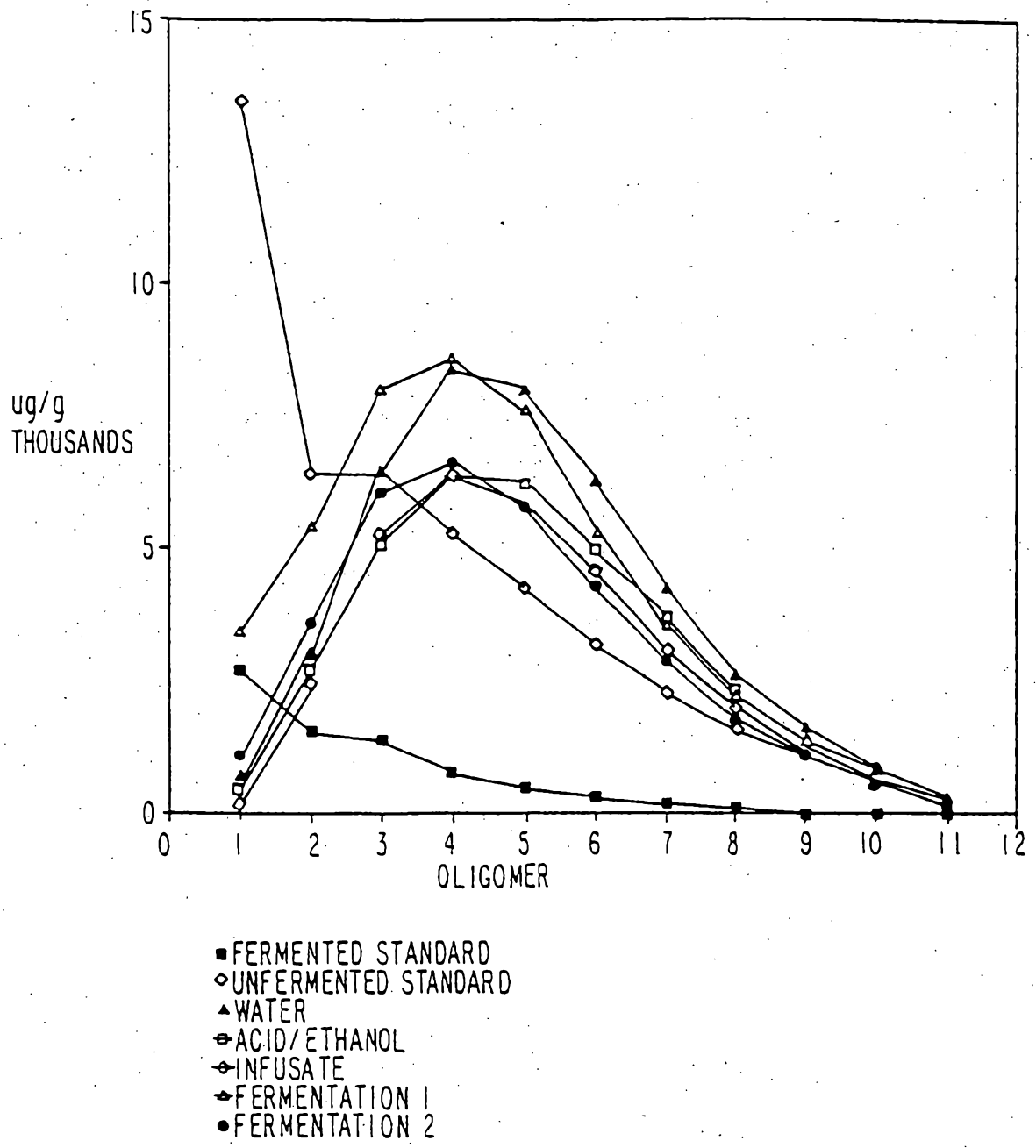
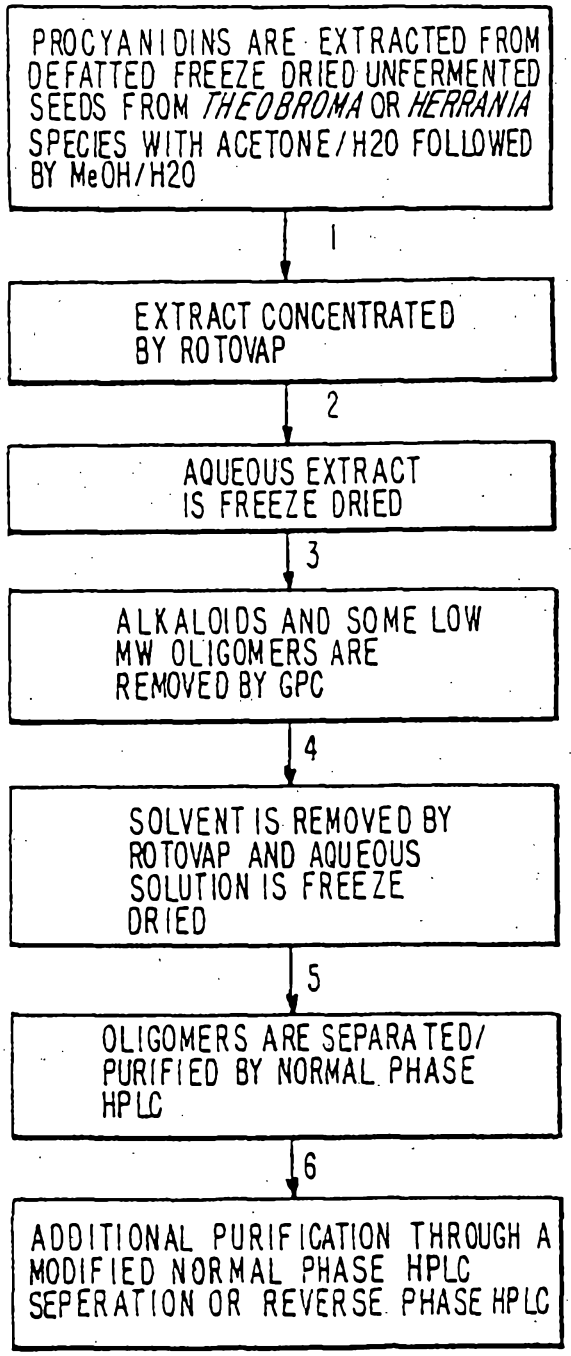

15
10
5
0
ug/g
THOUSANDS
0
1
2
3
4
5
6
7
8
9
10
11
12
OLIGOMER
FERMENTED STANDARD
UNFERMENTED STANDARD
WATER
ACID/ETHANOL
INFUSATE
FERMENTATION 1
FERMENTATION 2

FIG. 15


PROCYANIDINS ARE EXTRACTED FROM DEFATTED FREEZE DRIED UNFERMENTED SEEDS FROM *THEOBROMA* OR *HERRANIA* SPECIES WITH ACETONE/H20 FOLLOWED BY MeOH/H20
1
EXTRACT CONCENTRATED BY ROTOVAP
2
AQUEOUS EXTRACT IS FREEZE DRIED
3
ALKALOIDS AND SOME LOW MW OLIGOMERS ARE REMOVED BY GPC
4
SOLVENT IS REMOVED BY ROTOVAP AND AQUEOUS SOLUTION IS FREEZE DRIED
5
OLIGOMERS ARE SEPARATED/ PURIFIED BY NORMAL PHASE HPLC
6
ADDITIONAL PURIFICATION THROUGH A MODIFIED NORMAL PHASE HPLC SEPERATION OR REVERSE PHASE HPLC

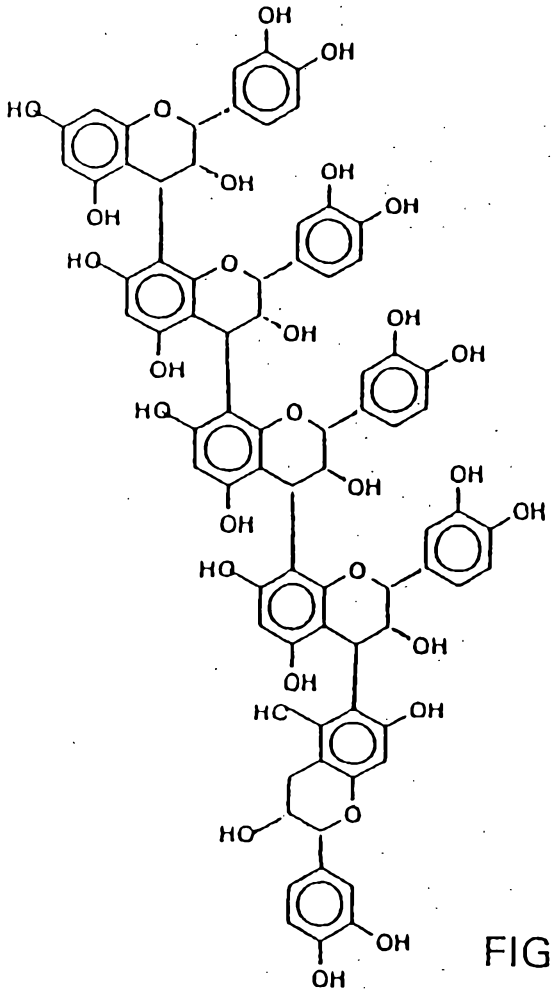


FIG. 16A

(4-8)(4-8)(4-8)(4-6)PENTAMER

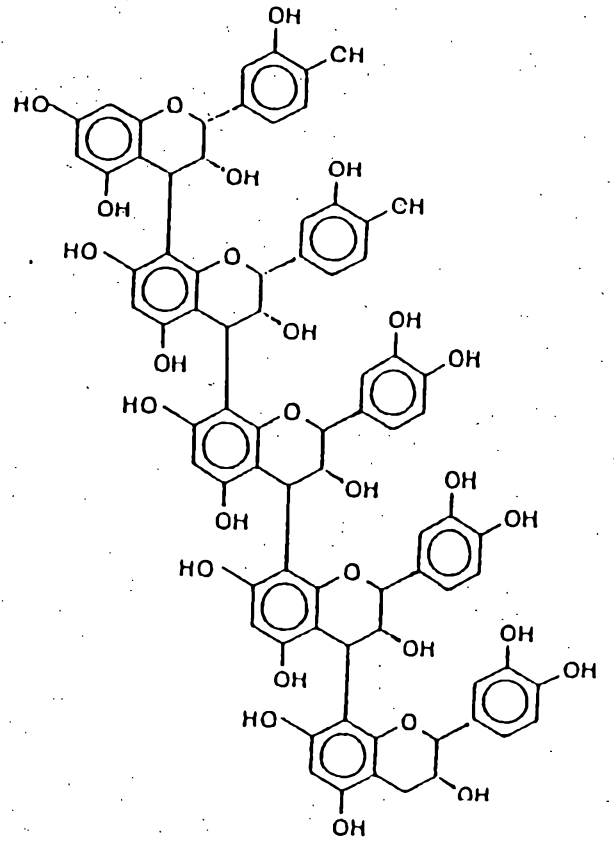
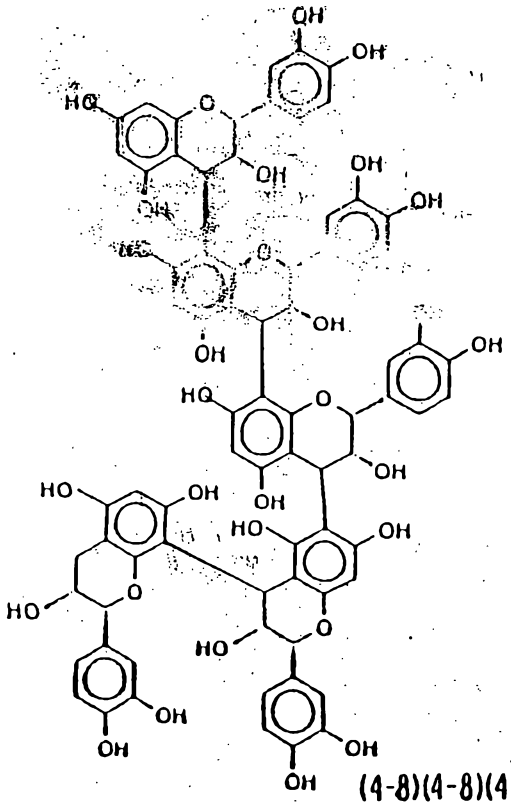


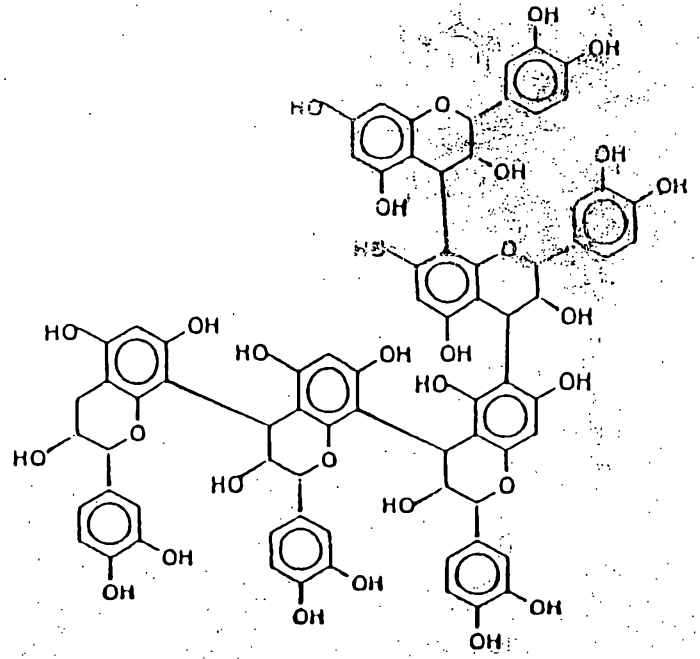
FIG. 16B

(4-8)(4-8)(4-8)(4-6)CENTAMER



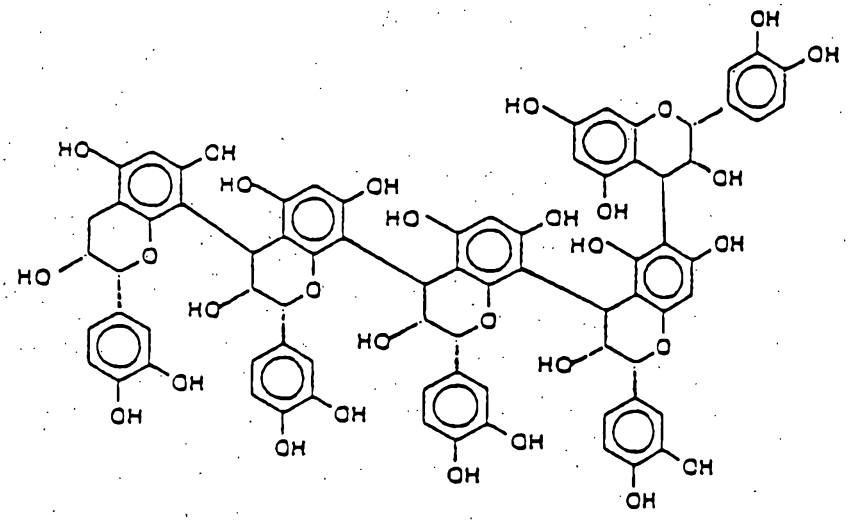
(4-8)(4-8)(4-6)(4-6)PENTAMER

FIG. 16C



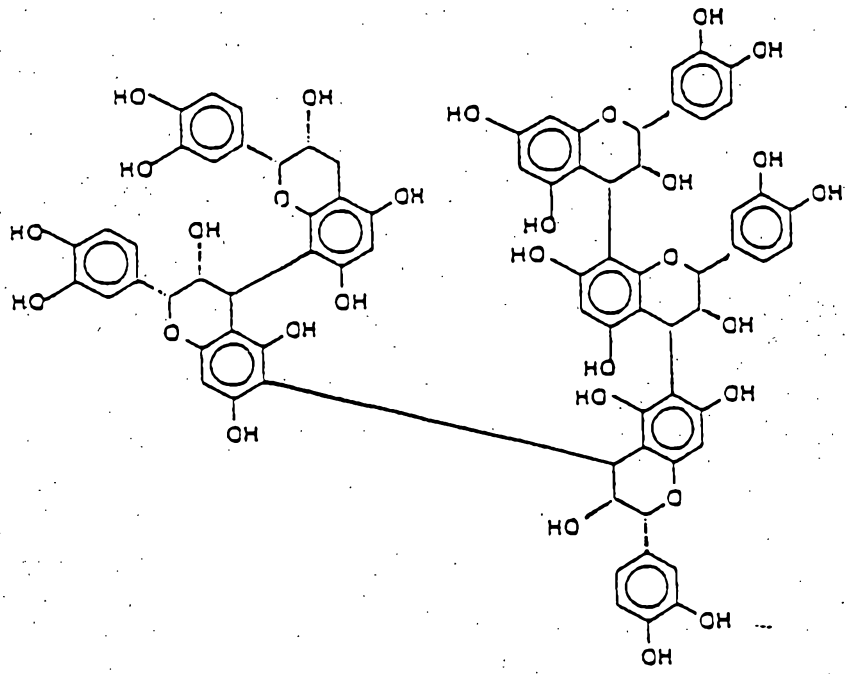
(4-8)(4-6)(4-8)(4-8) PENTAMER

FIG. 16D



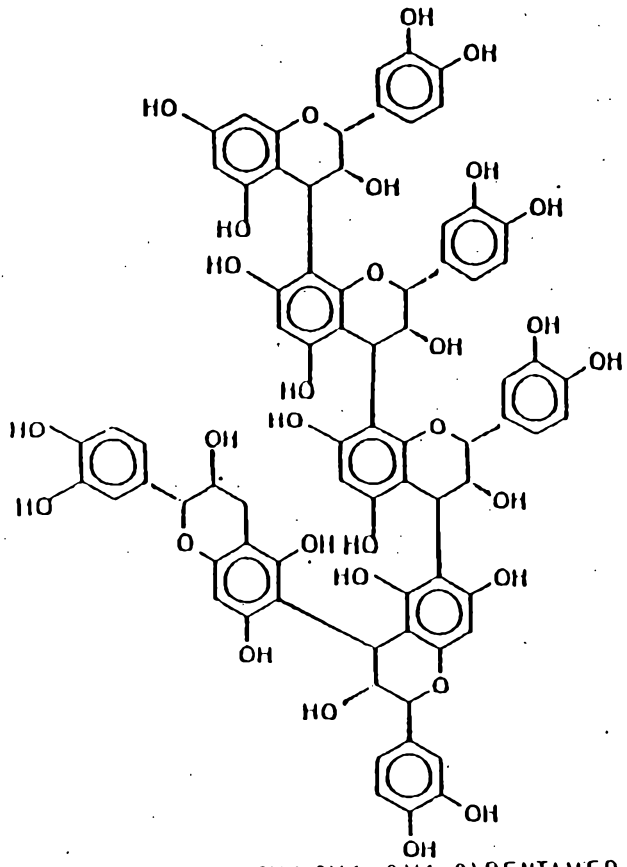
(4-6)(4-8)(4-8)(4-8) PENTAMER

FIG. 16E



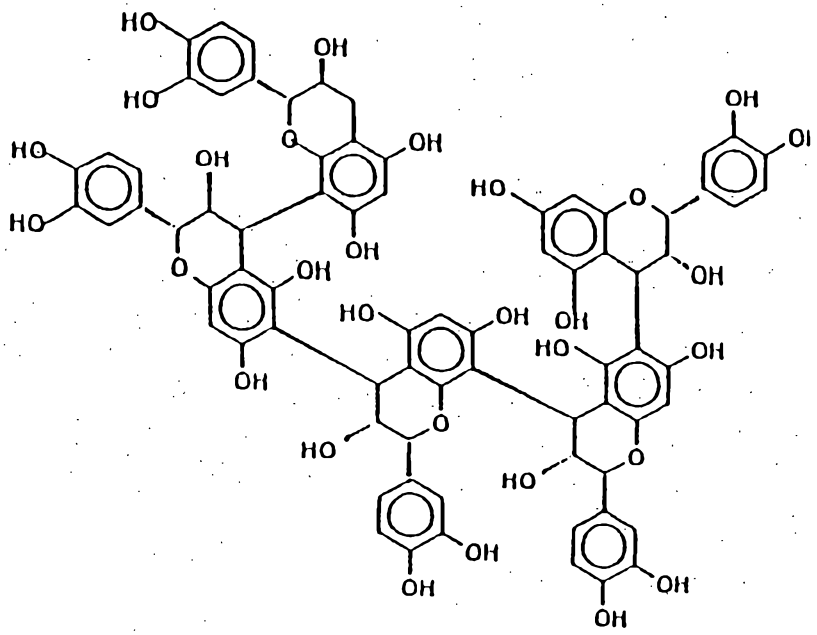
(4-8)(4-6)(4-6)(4-8) PENTAMER

FIG. 16F



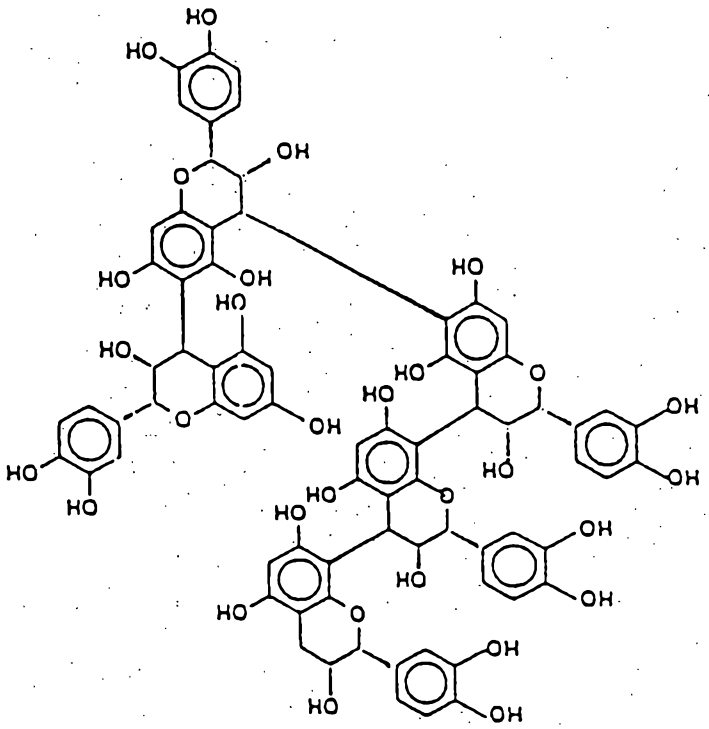
(4-8)(4-8)(4-6)(4-6) PENTAMER

FIG. 16G



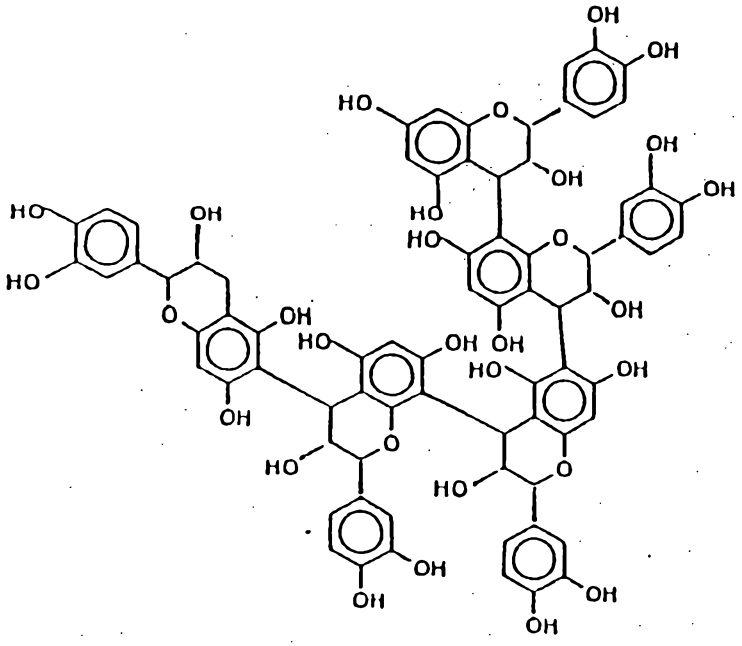
(4-6)(4-8)(4-6)(4-8) PENTAMER

FIG. 16H



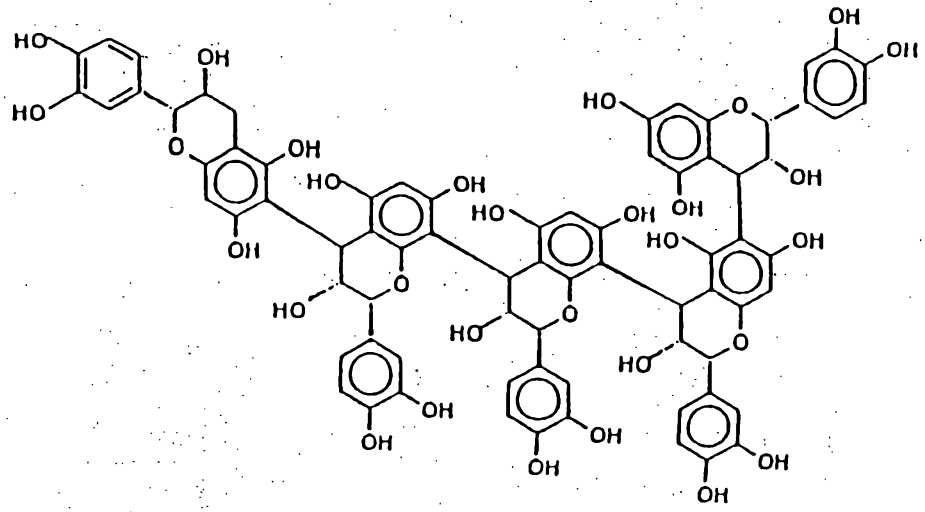
(4-6)(4-6)(4-8)(4-8)PENTAMER

FIG. 161



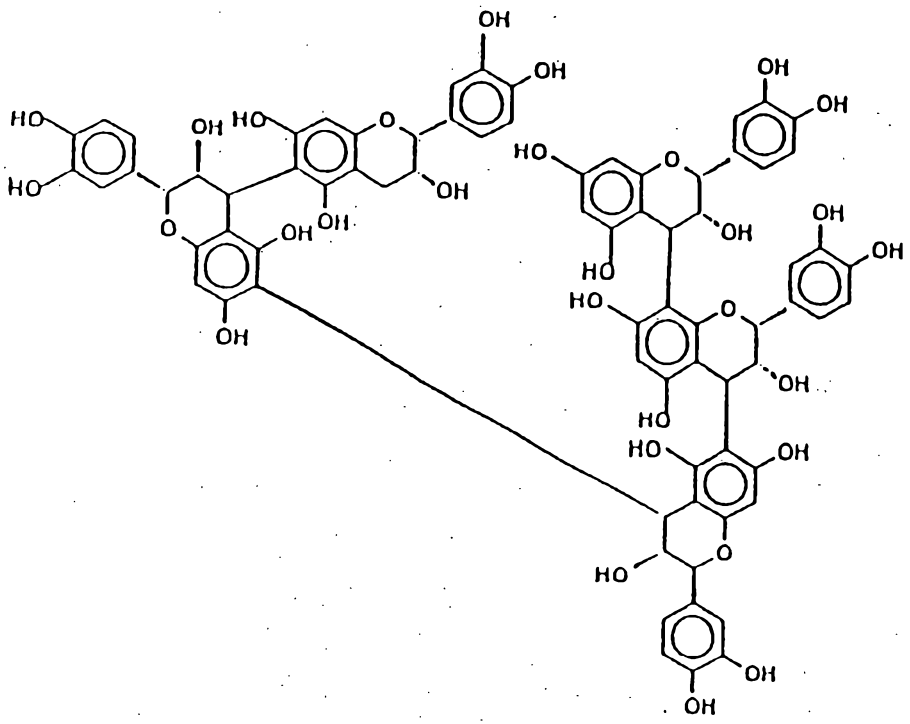
(4-8)(4-6)(4-8)(4-6) PENTAMER

FIG. 16J



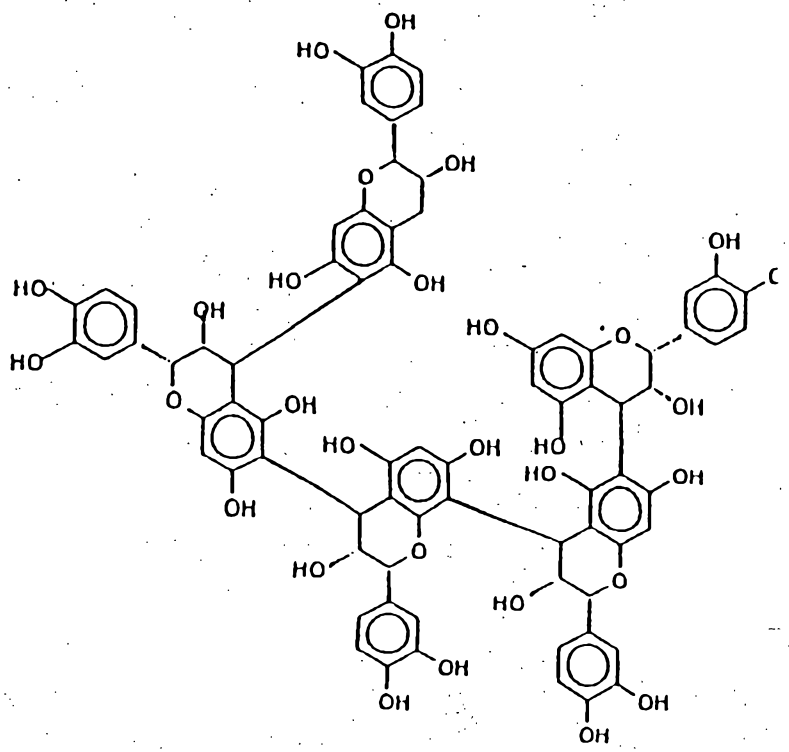
(4-6)(4-8)(4-8)(4-6) PENTAMER

FIG. 16K



(4-8)(4-6)(4-6)(4-6)PENTAMER

FIG. 16L



(4-6)(4-8)(4-6)(4-6)PENTAMER

FIG. 16M

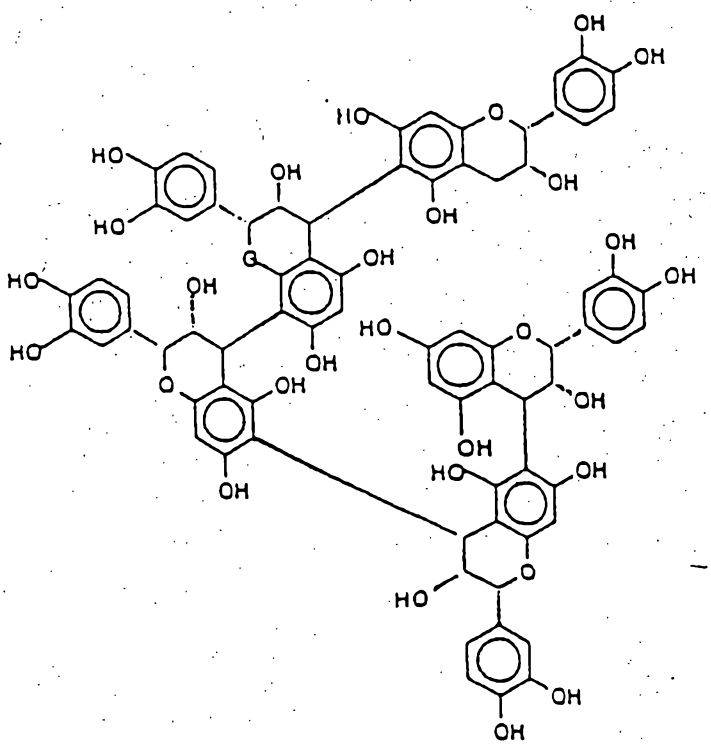
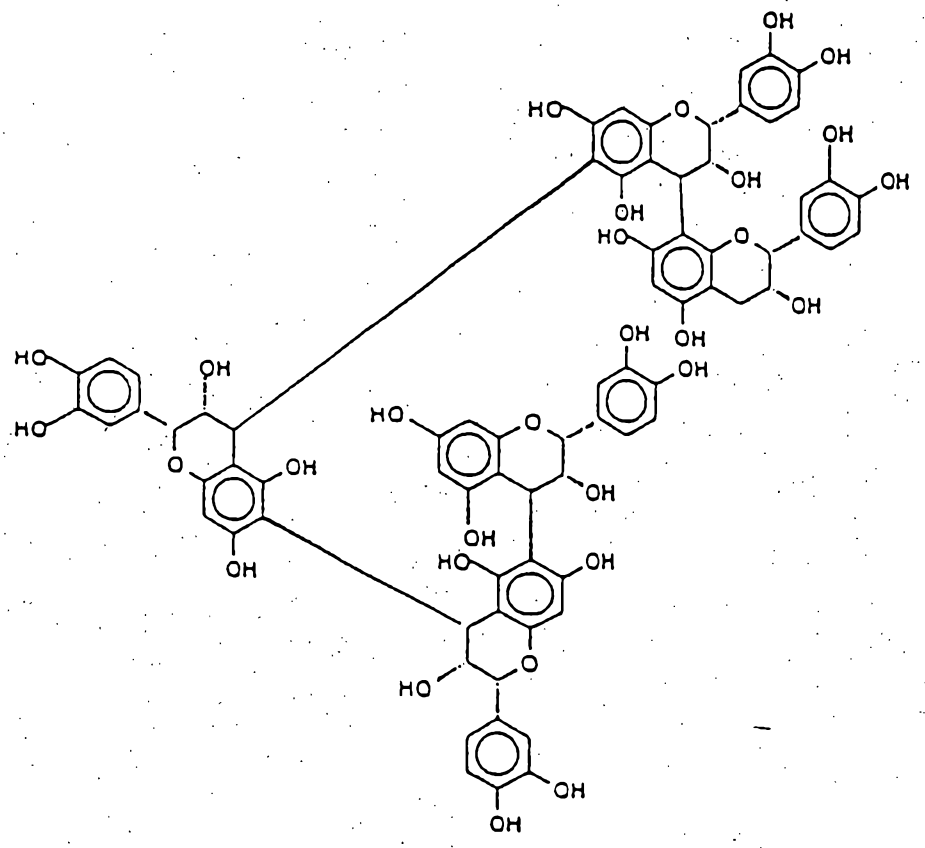
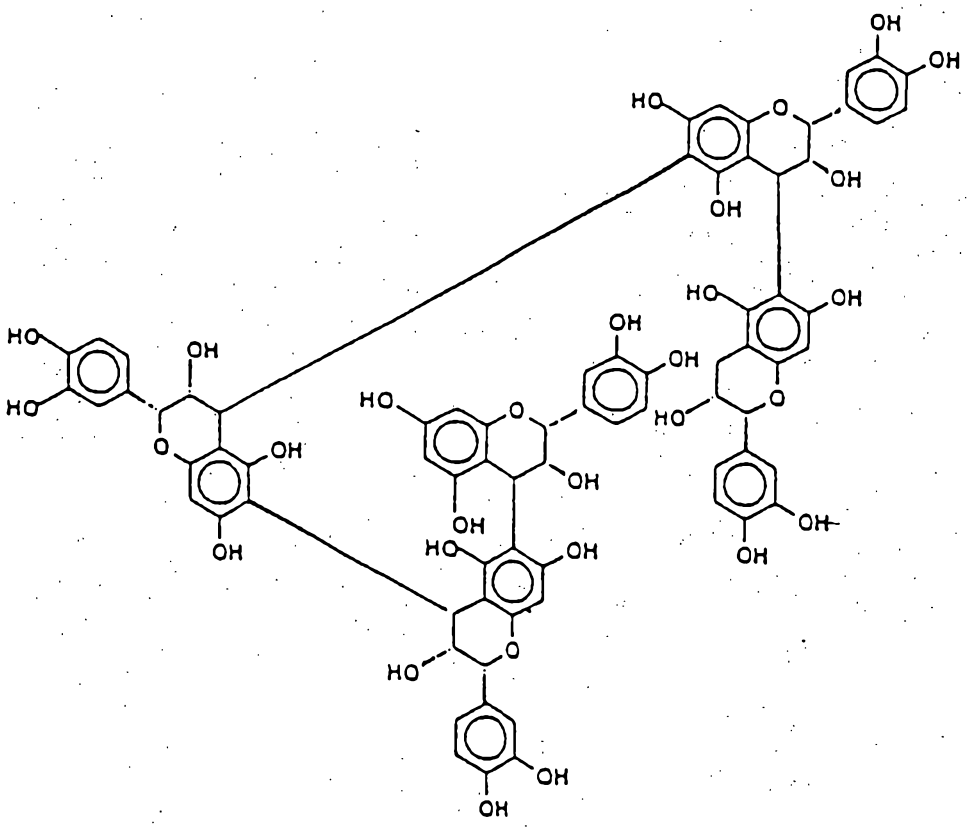


FIG. 16N



(4-6)(4-6)(4-6)(4-6)PENTAMER

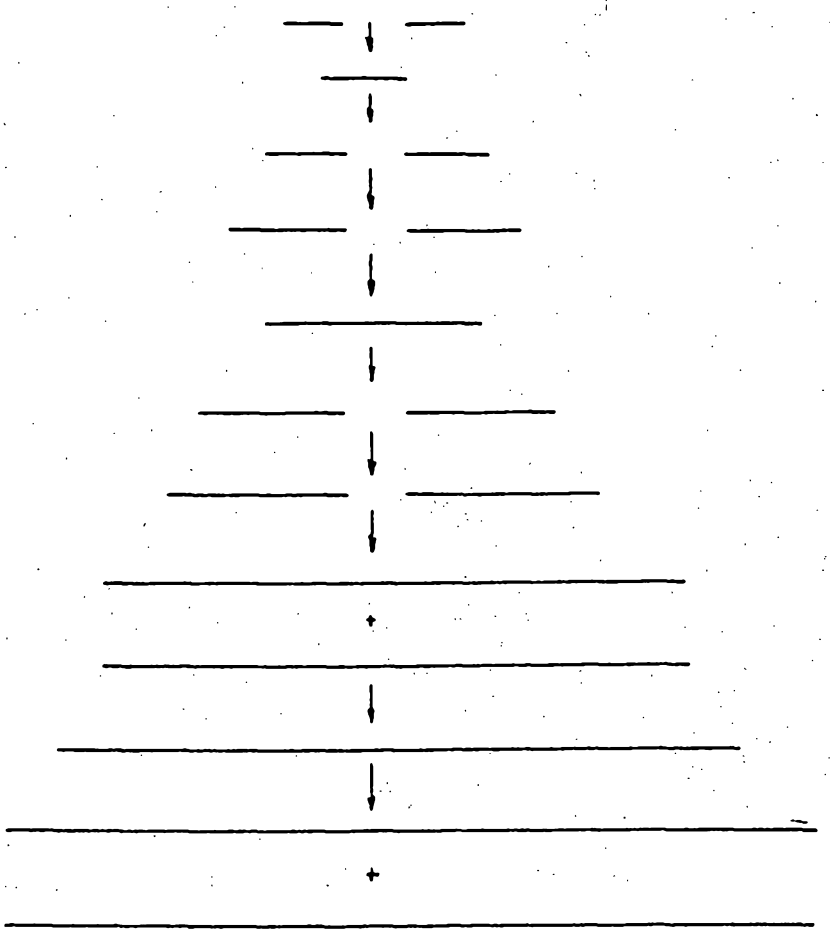
FIG. 160



(4-6)(4-6)(4-6)(4-8)PENTAMER

FIG. 16P

FIG 17A



X- CAT
0- EC

0-X-X-X-0	(4-8; 4-8; 4-8; 4-6)
0-X-X-X-0	(4-8; 4-8; 4-8; 4-8)
X-X-X-X-0	(4-6; 4-6; 4-6; 4-6)
X-X-X-X-0	(4-6; 4-6; 4-6; 4-8)
X-X-X-X-0	(4-6; 4-6; 4-8; 4-6)
X-X-X-X-0	(4-6; 4-6; 4-8; 4-8)
X-X-X-X-0	(4-6; 4-8; 4-6; 4-6)
X-X-X-X-0	(4-6; 4-8; 4-6; 4-8)
X-X-X-X-0	(4-6; 4-8; 4-8; 4-6)
X-X-X-X-0	(4-6; 4-8; 4-8; 4-8)
X-X-X-X-0	(4-8; 4-6; 4-6; 4-6)
X-X-X-X-0	(4-8; 4-6; 4-6; 4-8)
X-X-X-X-0	(4-8; 4-6; 4-8; 4-6)
X-X-X-X-0	(4-8; 4-6; 4-8; 4-8)
X-X-X-X-0	(4-8; 4-8; 4-6; 4-6)
X-X-X-X-0	(4-8; 4-8; 4-6; 4-8)
X-X-X-X-0	(4-8; 4-8; 4-8; 4-6)
X-X-X-X-0	(4-8; 4-8; 4-8; 4-8)

(CONTINUE LEVEL V)

FIG. 17AA

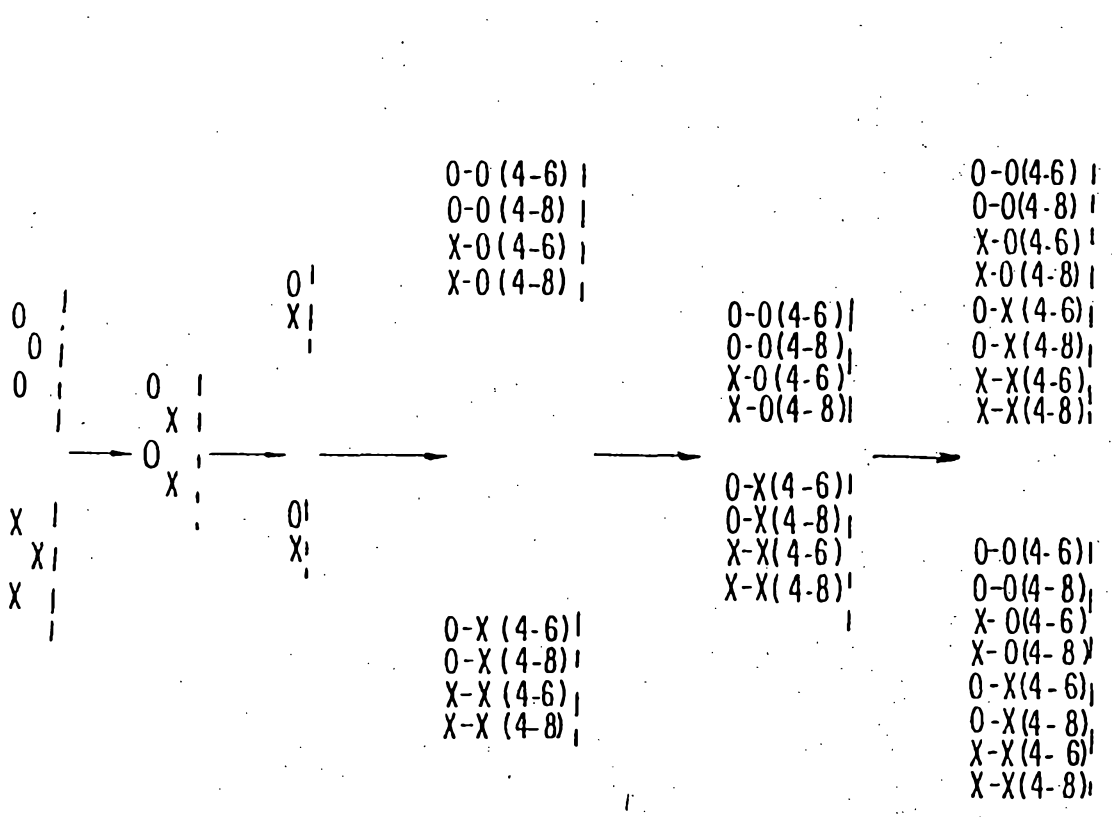


FIG. 17B

LEVEL I

3010013367

X=CAT
0=EC

(LEVEL I)

0-0-0 (4-6 ; 4-6)
0-0-0 (4-6 ; 4-8)
0-0-0 (4-8 ; 4-6)
0-0-0 (4-8 ; 4-8)
X-0-0 (4-6 ; 4-6)
X-0-0 (4-6 ; 4-8)
X-0-0 (4-8 ; 4-6)
X-0-0 (4-8 ; 4-8)
0-X-0 (4-6 ; 4-6)
0-X-0 (4-6 ; 4-8)
0-X-0 (4-8 ; 4-6)
0-X-0 (4-8 ; 4-8)
X-X-0 (4-6 ; 4-6)
X-X-0 (4-6 ; 4-8)
X-X-0 (4-8 ; 4-6)
X-X-0 (4-8 ; 4-8)

0-0-X (4-6 ; 4-6)
0-0-X (4-6 ; 4-8)
0-0-X (4-8 ; 4-6)
0-0-X (4-8 ; 4-8)
X-0-X (4-6 ; 4-6)
X-0-X (4-6 ; 4-8)
X-0-X (4-8 ; 4-6)
X-0-X (4-8 ; 4-8)
0-X-X (4-6 ; 4-6)
0-X-X (4-6 ; 4-8)
0-X-X (4-8 ; 4-6)
0-X-X (4-8 ; 4-8)
X-X-X (4-6 ; 4-6)
X-X-X (4-6 ; 4-8)

FIG. 17C

X-X-X (4-8; 4-6);
X-X-X (4-8; 4-8);

X=CAT
O=EC

FIG. 17D

O-O-O (4-6 ; 4-6)
O-O-O (4-6 ; 4-8)
O-O-O (4-8 ; 4-6)
O-O-O (4-8 ; 4-8)
X-O-O (4-6 ; 4-6)
X-O-O (4-6 ; 4-8)
X-O-O (4-8 ; 4-6)
X-O-O (4-8 ; 4-8)
O-X-O (4-6 ; 4-6)
O-X-O (4-6 ; 4-8)
O-X-O (4-8 ; 4-6)
O-X-O (4-8 ; 4-8)
X-X-O (4-6 ; 4-6)
X-X-O (4-6 ; 4-8)
X-X-O (4-8 ; 4-6)
X-X-O (4-8 ; 4-8)
O-O-X (4-6 ; 4-6)
O-O-X (4-6 ; 4-8)
O-O-X (4-8 ; 4-6)
O-O-X (4-8 ; 4-8)
X-O-X (4-6 ; 4-6)
X-O-X (4-6 ; 4-8)
X-O-X (4-8 ; 4-6)
X-O-X (4-8 ; 4-8)
O-X-X (4-6 ; 4-6)
O-X-X (4-6 ; 4-8)
O-X-X (4-8 ; 4-6)
O-X-X (4-8 ; 4-8)
X-X-X (4-6 ; 4-6)
X-X-X (4-6 ; 4-8)
X-X-X (4-8 ; 4-6)
X-X-X (4-8 ; 4-8)

X = CAT
O = EC

(LEVEL II)

FIG. 17E

O-O-O	(4-6	4-6)
O-O-O	(4-6	4-8)
O-O-O	(4-8	4-6)
O-O-O	(4-8	4-8)
X-O-O	(4-6	4-6)
X-O-O	(4-6	4-8)
X-O-O	(4-8	4-6)
X-O-O	(4-8	4-8)
O-X-O	(4-6	4-6)
O-X-O	(4-6	4-8)
O-X-O	(4-8	4-6)
O-X-O	(4-8	4-8)
X-X-O	(4-6	4-6)
X-X-O	(4-6	4-8)
X-X-O	(4-8	4-6)
X-X-O	(4-8	4-8)
O-O-X	(4-6	4-6)
O-O-X	(4-6	4-8)
O-O-X	(4-8	4-6)
O-O-X	(4-8	4-8)
X-O-X	(4-6	4-6)
X-O-X	(4-6	4-8)
X-O-X	(4-8	4-6)
X-O-X	(4-8	4-8)
O-X-X	(4-6	4-6)
O-X-X	(4-6	4-8)
O-X-X	(4-8	4-6)
O-X-X	(4-8	4-8)
X-X-X	(4-6	4-6)
X-X-X	(4-6	4-8)
X-X-X	(4-8	4-6)
X-X-X	(4-8	4-8)

X=CAT
O=EC

(CONTINUE-LEVEL II)

FIG. 17F

X = CAT
O = EC

(LEVEL III)

O-O-O-O	(4-6; 4-6; 4-6)
O-O-O-O	(4-6; 4-6; 4-8)
O-O-O-O	(4-6; 4-8; 4-6)
O-O-O-O	(4-6; 4-8; 4-8)
O-O-O-O	(4-8; 4-6; 4-6)
O-O-O-O	(4-8; 4-6; 4-8)
O-O-O-O	(4-8; 4-8; 4-6)
O-O-O-O	(4-8; 4-8; 4-8)
X-O-O-O	(4-6; 4-6; 4-6)
X-O-O-O	(4-6; 4-6; 4-8)
X-O-O-O	(4-6; 4-8; 4-6)
X-O-O-O	(4-6; 4-8; 4-8)
X-O-O-O	(4-8; 4-6; 4-6)
X-O-O-O	(4-8; 4-6; 4-8)
X-O-O-O	(4-8; 4-8; 4-6)
X-O-O-O	(4-8; 4-8; 4-8)
X-O-O-O	(4-6; 4-6; 4-6)
O-X-O-O	(4-6; 4-6; 4-6)
O-X-O-O	(4-6; 4-6; 4-8)
O-X-O-O	(4-6; 4-8; 4-6)
O-X-O-O	(4-6; 4-8; 4-8)
O-X-O-O	(4-8; 4-6; 4-6)
O-X-O-O	(4-8; 4-6; 4-8)
O-X-O-O	(4-8; 4-8; 4-6)
O-X-O-O	(4-8; 4-8; 4-8)
X-X-O-O	(4-6; 4-6; 4-6)
X-X-O-O	(4-6; 4-6; 4-8)
X-X-O-O	(4-6; 4-8; 4-6)
X-X-O-O	(4-6; 4-8; 4-8)
X-X-O-O	(4-8; 4-6; 4-6)
X-X-O-O	(4-8; 4-6; 4-8)
X-X-O-O	(4-8; 4-8; 4-6)
X-X-O-O	(4-8; 4-8; 4-8)
O-O-X-O	(4-6; 4-6; 4-6)

FIG. 17G

0-0-X-0 (4-6;4-6;4-8),
0-0-X-0 (4-6;4-8;4-6),
0-0-X-0 (4-6;4-8;4-8),
0-0-X-0 (4-8;4-6;4-6),
0-0-X-0 (4-8;4-6;4-8),
0-0-X-0 (4-8;4-8;4-6),
0-0-X-0 (4-8;4-8;4-8),
X-0-X-0 (4-6;4-6;4-6),
X-0-X-0 (4-6;4-6;4-8),
X-0-X-0 (4-6;4-8;4-6),
X-0-X-0 (4-6;4-8;4-8),
X-0-X-0 (4-8;4-6;4-6),
X-0-X-0 (4-8;4-6;4-8),
X-0-X-0 (4-8;4-8;4-6),
X-0-X-0 (4-8;4-8;4-8),
0-X-X-0 (4-6;4-6;4-6),
0-X-X-0 (4-6;4-6;4-8),
0-X-X-0 (4-6;4-8;4-6),
0-X-X-0 (4-6;4-8;4-8),
0-X-X-0 (4-8;4-6;4-6),
0-X-X-0 (4-8;4-6;4-8),
0-X-X-0 (4-8;4-8;4-6),
0-X-X-0 (4-8;4-8;4-8),
X-X-X-0 (4-6;4-6;4-6),
X-X-X-0 (4-6;4-6;4-8),
X-X-X-0 (4-6;4-8;4-6),
X-X-X-0 (4-6;4-8;4-8),
X-X-X-0 (4-8;4-6;4-6),
X-X-X-0 (4-8;4-6;4-8),
X-X-X-0 (4-8;4-8;4-6),
X-X-X-0 (4-8;4-8;4-8),

X= CAT
0=EC

(CONTINUE LEVEL III)

FIG. 17H

X-X-0-X (4-8; 4-8; 4-8);

X=CAT
0=EC

0-0-X-X (4-6; 4-6; 4-6)
0-0-X-X (4-6; 4-6; 4-8)
0-0-X-X (4-6; 4-8; 4-6)
0-0-X-X (4-6; 4-8; 4-8)
0-0-X-X (4-8; 4-6; 4-6)
0-0-X-X (4-8; 4-6; 4-8)
0-0-X-X (4-8; 4-8; 4-6)
0-0-X-X (4-8; 4-8; 4-8)
X-0-X-X (4-6; 4-6; 4-6)
X-0-X-X (4-6; 4-6; 4-8)
X-0-X-X (4-6; 4-8; 4-6)
X-0-X-X (4-6; 4-8; 4-8)
X-0-X-X (4-8; 4-6; 4-6)
X-0-X-X (4-8; 4-6; 4-8)
X-0-X-X (4-8; 4-8; 4-6)
X-0-X-X (4-8; 4-8; 4-8)
0-X-X-X (4-6; 4-6; 4-6)
0-X-X-X (4-6; 4-6; 4-8)
0-X-X-X (4-6; 4-8; 4-6)
0-X-X-X (4-6; 4-8; 4-8)
0-X-X-X (4-8; 4-6; 4-6)
0-X-X-X (4-8; 4-6; 4-8)
0-X-X-X (4-8; 4-8; 4-6)
0-X-X-X (4-8; 4-8; 4-8)
X-X-X-X (4-6; 4-6; 4-6)
X-X-X-X (4-6; 4-6; 4-8)
X-X-X-X (4-6; 4-8; 4-6)
X-X-X-X (4-6; 4-8; 4-8)

(CONTINUE OF LEVEL III)

FIG. 17I

O-O-O-X	(4-6; 4-6; 4-6)
O-O-O-X	(4-6; 4-6; 4-8)
O-O-O-X	(4-6; 4-8; 4-6)
O-O-O-X	(4-6; 4-8; 4-8)
O-O-O-X	(4-8; 4-6; 4-6)
O-O-O-X	(4-8; 4-6; 4-8)
O-O-O-X	(4-8; 4-8; 4-6)
O-O-O-X	(4-8; 4-8; 4-8)
X-O-O-X	(4-6; 4-6; 4-6)
X-O-O-X	(4-6; 4-6; 4-8)
X-O-O-X	(4-6; 4-8; 4-6)
X-O-O-X	(4-6; 4-8; 4-8)
X-O-O-X	(4-8; 4-6; 4-6)
X-O-O-X	(4-8; 4-6; 4-8)
X-O-O-X	(4-8; 4-8; 4-6)
X-O-O-X	(4-8; 4-8; 4-8)
O-X-O-X	(4-6; 4-6; 4-6)
O-X-O-X	(4-6; 4-6; 4-8)
O-X-O-X	(4-6; 4-8; 4-6)
O-X-O-X	(4-6; 4-8; 4-8)
O-X-O-X	(4-8; 4-6; 4-6)
O-X-O-X	(4-8; 4-6; 4-8)
O-X-O-X	(4-8; 4-8; 4-6)
O-X-O-X	(4-8; 4-8; 4-8)
X-X-O-X	(4-6; 4-6; 4-6)
X-X-O-X	(4-6; 4-6; 4-8)
X-X-O-X	(4-6; 4-8; 4-6)
X-X-O-X	(4-6; 4-8; 4-8)
X-X-O-X	(4-8; 4-6; 4-6)
X-X-O-X	(4-8; 4-6; 4-8)
X-X-O-X	(4-8; 4-8; 4-6)

X = CAT
O = EC

(CONTINUE LEVEL III)

FIG. 17J

X-CAT
O-EC

X-X-X-X (4-8; 4-6; 4-6)
X-X-X-X (4-8; 4-6; 4-8)
X-X-X-X (4-8; 4-8; 4-6)
X-X-X-X (4-8; 4-8; 4-8)

FIG. 17K

0-0-0-0-0	(4-6;4-6;4-6;4-6)
0-0-0-0-0	(4-6;4-6;4-6;4-8)
0-0-0-0-0	(4-6;4-6;4-8;4-6)
0-0-0-0-0	(4-6;4-6;4-8;4-8)
0-0-0-0-0	(4-6;4-8;4-6;4-6)
0-0-0-0-0	(4-6;4-8;4-6;4-8)
0-0-0-0-0	(4-6;4-8;4-8;4-6)
0-0-0-0-0	(4-6;4-8;4-8;4-8)
0-0-0-0-0	(4-8;4-6;4-6;4-6)
0-0-0-0-0	(4-8;4-6;4-6;4-8)
0-0-0-0-0	(4-8;4-6;4-8;4-6)
0-0-0-0-0	(4-8;4-6;4-8;4-8)
0-0-0-0-0	(4-8;4-8;4-6;4-6)
0-0-0-0-0	(4-8;4-8;4-6;4-8)
0-0-0-0-0	(4-8;4-8;4-8;4-6)
0-0-0-0-0	(4-8;4-8;4-8;4-8)
X-0-0-0-0	(4-6;4-6;4-6;4-6)
X-0-0-0-0	(4-6;4-6;4-6;4-8)
X-0-0-0-0	(4-6;4-6;4-8;4-6)
X-0-0-0-0	(4-6;4-6;4-8;4-8)
X-0-0-0-0	(4-6;4-8;4-6;4-6)
X-0-0-0-0	(4-6;4-8;4-6;4-8)
X-0-0-0-0	(4-6;4-8;4-8;4-6)
X-0-0-0-0	(4-6;4-8;4-8;4-8)
X-0-0-0-0	(4-8;4-6;4-6;4-6)
X-0-0-0-0	(4-8;4-6;4-6;4-8)
X-0-0-0-0	(4-8;4-6;4-8;4-6)
X-0-0-0-0	(4-8;4-6;4-8;4-8)
X-0-0-0-0	(4-8;4-8;4-6;4-6)
X-0-0-0-0	(4-8;4-8;4-6;4-8)
X-0-0-0-0	(4-8;4-8;4-8;4-6)
X-0-0-0-0	(4-8;4-8;4-8;4-8)
0-X-0-0-0	(4-6;4-6;4-6;4-6)
0-X-0-0-0	(4-6;4-6;4-6;4-8)

X=CAT
0=EC

(LEVEL IV)

FIG. 17L

0-X-0-0-0 (4-6; 4-6; 4-8; 4-6)
0-X-0-0-0 (4-6; 4-6; 4-8; 4-8)
0-X-0-0-0 (4-6; 4-8; 4-6; 4-6)
0-X-0-0-0 (4-6; 4-8; 4-6; 4-8)
0-X-0-0-0 (4-6; 4-8; 4-8; 4-6)
0-X-0-0-0 (4-6; 4-8; 4-8; 4-8)
0-X-0-0-0 (4-8; 4-6; 4-6; 4-6)
0-X-0-0-0 (4-8; 4-6; 4-6; 4-8)
0-X-0-0-0 (4-8; 4-6; 4-8; 4-6)
0-X-0-0-0 (4-8; 4-6; 4-8; 4-8)
0-X-0-0-0 (4-8; 4-8; 4-6; 4-6)
0-X-0-0-0 (4-8; 4-8; 4-6; 4-8)
0-X-0-0-0 (4-8; 4-8; 4-8; 4-6)
0-X-0-0-0 (4-8; 4-8; 4-8; 4-8)
X-X-0-0-0 (4-6; 4-6; 4-6; 4-6)
X-X-0-0-0 (4-6; 4-6; 4-6; 4-8)
X-X-0-0-0 (4-6; 4-6; 4-8; 4-6)
X-X-0-0-0 (4-6; 4-6; 4-8; 4-8)
X-X-0-0-0 (4-6; 4-8; 4-6; 4-6)
X-X-0-0-0 (4-6; 4-8; 4-6; 4-8)
X-X-0-0-0 (4-6; 4-8; 4-8; 4-6)
X-X-0-0-0 (4-6; 4-8; 4-8; 4-8)
X-X-0-0-0 (4-8; 4-6; 4-6; 4-6)
X-X-0-0-0 (4-8; 4-6; 4-6; 4-8)
X-X-0-0-0 (4-8; 4-6; 4-8; 4-6)
X-X-0-0-0 (4-8; 4-6; 4-8; 4-8)
X-X-0-0-0 (4-8; 4-8; 4-6; 4-6)
X-X-0-0-0 (4-8; 4-8; 4-6; 4-8)
X-X-0-0-0 (4-8; 4-8; 4-8; 4-6)
X-X-0-0-0 (4-8; 4-8; 4-8; 4-8)
0-0-X-0-0 (4-6; 4-6; 4-6; 4-6)
0-0-X-0-0 (4-6; 4-6; 4-6; 4-8)
0-0-X-0-0 (4-6; 4-6; 4-8; 4-6)
0-0-X-0-0 (4-6; 4-6; 4-8; 4-8)

X=CAT
0=EC

(CONTINUE-LEVEL IV)

FIG. 17M

X=CAT
O=EC

O-O-X-O-O (4-6;4-8;4-6;4-6)
O-O-X-O-O (4-6;4-8;4-6;4-8)
O-O-X-O-O (4-6;4-8;4-8;4-6)
O-O-X-O-O (4-6;4-8;4-8;4-8)
O-O-X-O-O (4-8;4-6;4-6;4-6)
O-O-X-O-O (4-8;4-6;4-6;4-8)
O-O-X-O-O (4-8;4-6;4-8;4-6)
O-O-X-O-O (4-8;4-6;4-8;4-8)
O-O-X-O-O (4-8;4-8;4-6;4-6)
O-O-X-O-O (4-8;4-8;4-6;4-8)
O-O-X-O-O (4-8;4-8;4-8;4-6)
O-O-X-O-O (4-8;4-8;4-8;4-8)
X-O-X-O-O (4-6;4-6;4-6;4-6)
X-O-X-O-O (4-6;4-6;4-6;4-8)
X-O-X-O-O (4-6;4-6;4-8;4-6)
X-O-X-O-O (4-6;4-6;4-8;4-8)
X-O-X-O-O (4-6;4-8;4-6;4-6)
X-O-X-O-O (4-6;4-8;4-6;4-8)
X-O-X-O-O (4-6;4-8;4-8;4-6)
X-O-X-O-O (4-6;4-8;4-8;4-8)
X-O-X-O-O (4-8;4-6;4-6;4-6)
X-O-X-O-O (4-8;4-6;4-6;4-8)
X-O-X-O-O (4-8;4-6;4-8;4-6)
X-O-X-O-O (4-8;4-6;4-8;4-8)
X-O-X-O-O (4-8;4-8;4-6;4-6)
X-O-X-O-O (4-8;4-8;4-6;4-8)
X-O-X-O-O (4-8;4-8;4-8;4-6)
X-O-X-O-O (4-8;4-8;4-8;4-8)
O-X-X-O-O (4-6;4-6;4-6;4-6)
O-X-X-O-O (4-6;4-6;4-6;4-8)
O-X-X-O-O (4-6;4-6;4-8;4-6)
O-X-X-O-O (4-6;4-6;4-8;4-8)
O-X-X-O-O (4-6;4-8;4-6;4-6)
O-X-X-O-O (4-6;4-8;4-6;4-8)

(CONTINUE LEVEL IV)

FIG. 17N

O-X-X-O-O (4-6; 4-8; 4-8; 4-6)
O-X-X-O-O (4-6; 4-8; 4-8; 4-8)
O-X-X-O-O (4-8; 4-6; 4-6; 4-6)
O-X-X-O-O (4-8; 4-6; 4-6; 4-8)
O-X-X-O-O (4-8; 4-6; 4-8; 4-6)
O-X-X-O-O (4-8; 4-6; 4-8; 4-8)
O-X-X-O-O (4-8; 4-8; 4-6; 4-6)
O-X-X-O-O (4-8; 4-8; 4-6; 4-8)
O-X-X-O-O (4-8; 4-8; 4-8; 4-6)
O-X-X-O-O (4-8; 4-8; 4-8; 4-8)
O-X-X-O-O (4-6; 4-6; 4-6; 4-6)
O-X-X-O-O (4-6; 4-6; 4-6; 4-8)
X-X-X-O-O (4-6; 4-6; 4-8; 4-6)
X-X-X-O-O (4-6; 4-6; 4-8; 4-8)
X-X-X-O-O (4-6; 4-6; 4-6; 4-6)
X-X-X-O-O (4-6; 4-6; 4-6; 4-8)
X-X-X-O-O (4-6; 4-8; 4-8; 4-6)
X-X-X-O-O (4-6; 4-8; 4-8; 4-8)
X-X-X-O-O (4-6; 4-8; 4-6; 4-6)
X-X-X-O-O (4-6; 4-8; 4-6; 4-8)
X-X-X-O-O (4-8; 4-6; 4-8; 4-6)
X-X-X-O-O (4-8; 4-6; 4-8; 4-8)
X-X-X-O-O (4-8; 4-6; 4-6; 4-6)
X-X-X-O-O (4-8; 4-6; 4-6; 4-8)
X-X-X-O-O (4-8; 4-8; 4-8; 4-6)
X-X-X-O-O (4-8; 4-8; 4-8; 4-8)
X-X-X-O-O (4-8; 4-8; 4-6; 4-6)
X-X-X-O-O (4-8; 4-8; 4-6; 4-8)
O-O-O-X-X (4-6; 4-6; 4-8; 4-6)
O-O-O-X-X (4-6; 4-6; 4-8; 4-8)
O-O-O-X-X (4-6; 4-6; 4-6; 4-6)
O-O-O-X-X (4-6; 4-6; 4-6; 4-8)
O-O-O-X-X (4-6; 4-8; 4-8; 4-6)
O-O-O-X-X (4-6; 4-8; 4-8; 4-8)

X = CAT
O = EC

(CONTINUE LEVEL IV)

FIG. 17O

O-O-O-X-X (4-8; 4-6; 4-6; 4-6)
O-O-O-X-X (4-8; 4-6; 4-6; 4-8)
O-O-O-X-X (4-8; 4-6; 4-8; 4-6)
O-O-O-X-X (4-8; 4-6; 4-8; 4-8)
O-O-O-X-X (4-8; 4-8; 4-6; 4-6)
O-O-O-X-X (4-8; 4-8; 4-6; 4-8)
O-O-O-X-X (4-8; 4-8; 4-8; 4-6)
O-O-O-X-X (4-8; 4-8; 4-8; 4-8)
X-O-O-X-X (4-6; 4-6; 4-6; 4-6)
X-O-O-X-X (4-6; 4-6; 4-6; 4-8)
X-O-O-X-X (4-6; 4-6; 4-8; 4-6)
X-O-O-X-X (4-6; 4-6; 4-8; 4-8)
X-O-O-X-X (4-6; 4-8; 4-6; 4-6)
X-O-O-X-X (4-6; 4-8; 4-6; 4-8)
X-O-O-X-X (4-6; 4-8; 4-8; 4-6)
X-O-O-X-X (4-6; 4-8; 4-8; 4-8)
X-O-O-X-X (4-8; 4-6; 4-6; 4-6)
X-O-O-X-X (4-8; 4-6; 4-6; 4-8)
X-O-O-X-X (4-8; 4-6; 4-8; 4-6)
X-O-O-X-X (4-8; 4-6; 4-8; 4-8)
X-O-O-X-X (4-8; 4-8; 4-6; 4-6)
X-O-O-X-X (4-8; 4-8; 4-6; 4-8)
X-O-O-X-X (4-8; 4-8; 4-8; 4-6)
X-O-O-X-X (4-8; 4-8; 4-8; 4-8)
O-X-O-X-X (4-6; 4-6; 4-6; 4-6)
O-X-O-X-X (4-6; 4-6; 4-6; 4-8)
O-X-O-X-X (4-6; 4-6; 4-8; 4-6)
O-X-O-X-X (4-6; 4-6; 4-8; 4-8)
O-X-O-X-X (4-6; 4-8; 4-6; 4-6)
O-X-O-X-X (4-6; 4-8; 4-6; 4-8)
O-X-O-X-X (4-6; 4-8; 4-8; 4-6)
O-X-O-X-X (4-6; 4-8; 4-8; 4-8)
O-X-O-X-X (4-8; 4-6; 4-6; 4-6)
O-X-O-X-X (4-8; 4-6; 4-6; 4-8)

X=CAT
O=EC

(CONTINUE LEVEL IV)

FIG. 17P

0-X-0-X-X	(4-8; 4-6; 4-8; 4-6)
0-X-0-X-X	(4-8; 4-6; 4-8; 4-8)
0-X-0-X-X	(4-8; 4-8; 4-6; 4-6)
0-X-0-X-X	(4-8; 4-8; 4-6; 4-8)
0-X-0-X-X	(4-8; 4-8; 4-8; 4-6)
0-X-0-X-X	(4-8; 4-8; 4-8; 4-8)
X-X-0-X-X	(4-6; 4-6; 4-6; 4-6)
X-X-0-X-X	(4-6; 4-6; 4-6; 4-8)
X-X-0-X-X	(4-6; 4-6; 4-8; 4-6)
X-X-0-X-X	(4-6; 4-6; 4-8; 4-8)
X-X-0-X-X	(4-6; 4-8; 4-6; 4-6)
X-X-0-X-X	(4-6; 4-8; 4-6; 4-8)
X-X-0-X-X	(4-6; 4-8; 4-8; 4-6)
X-X-0-X-X	(4-6; 4-8; 4-8; 4-8)
X-X-0-X-X	(4-8; 4-6; 4-6; 4-6)
X-X-0-X-X	(4-8; 4-6; 4-6; 4-8)
X-X-0-X-X	(4-8; 4-6; 4-8; 4-6)
X-X-0-X-X	(4-8; 4-6; 4-8; 4-8)
X-X-0-X-X	(4-8; 4-8; 4-6; 4-6)
X-X-0-X-X	(4-8; 4-8; 4-6; 4-8)
X-X-0-X-X	(4-8; 4-8; 4-8; 4-6)
X-X-0-X-X	(4-8; 4-8; 4-8; 4-8)
0-0-X-X-X	(4-6; 4-6; 4-6; 4-6)
0-0-X-X-X	(4-6; 4-6; 4-6; 4-8)
0-0-X-X-X	(4-6; 4-6; 4-8; 4-6)
0-0-X-X-X	(4-6; 4-6; 4-8; 4-8)
0-0-X-X-X	(4-6; 4-8; 4-6; 4-6)
0-0-X-X-X	(4-6; 4-8; 4-6; 4-8)
0-0-X-X-X	(4-6; 4-8; 4-8; 4-6)
0-0-X-X-X	(4-6; 4-8; 4-8; 4-8)
0-0-X-X-X	(4-8; 4-6; 4-6; 4-6)
0-0-X-X-X	(4-8; 4-6; 4-6; 4-8)
0-0-X-X-X	(4-8; 4-6; 4-8; 4-6)
0-0-X-X-X	(4-8; 4-6; 4-8; 4-8)

X = CAT
0 = EC

(CONTINUE LEVEL IV)

FIG. 17Q

0-0-X-X-X (4-8,4-8,4-6,4-6)
0-0-X-X-X (4-8,4-8,4-6,4-8)
0-0-X-X-X (4-8,4-8,4-8,4-6)
0-0-X-X-X (4-8,4-8,4-8,4-8)
X-0-X-X-X (4-6,4-6,4-6,4-6)
X-0-X-X-X (4-6,4-6,4-6,4-8)
X-0-X-X-X (4-6,4-6,4-8,4-6)
X-0-X-X-X (4-6,4-6,4-8,4-8)
X-0-X-X-X (4-6,4-8,4-6,4-6)
X-0-X-X-X (4-6,4-8,4-6,4-8)
X-0-X-X-X (4-6,4-8,4-8,4-6)
X-0-X-X-X (4-6,4-8,4-8,4-8)
X-0-X-X-X (4-8,4-6,4-6,4-6)
X-0-X-X-X (4-8,4-6,4-6,4-8)
X-0-X-X-X (4-8,4-6,4-8,4-6)
X-0-X-X-X (4-8,4-6,4-8,4-8)
X-0-X-X-X (4-8,4-8,4-6,4-6)
X-0-X-X-X (4-8,4-8,4-6,4-8)
X-0-X-X-X (4-8,4-8,4-8,4-6)
X-0-X-X-X (4-8,4-8,4-8,4-8)
0-X-X-X-X (4-6,4-6,4-6,4-6)
0-X-X-X-X (4-6,4-6,4-6,4-8)
0-X-X-X-X (4-6,4-6,4-8,4-6)
0-X-X-X-X (4-6,4-6,4-8,4-8)
0-X-X-X-X (4-6,4-8,4-6,4-6)
0-X-X-X-X (4-6,4-8,4-6,4-8)
0-X-X-X-X (4-6,4-8,4-8,4-6)
0-X-X-X-X (4-6,4-8,4-8,4-8)
0-X-X-X-X (4-8,4-6,4-6,4-6)
0-X-X-X-X (4-8,4-6,4-6,4-8)
0-X-X-X-X (4-8,4-6,4-8,4-6)
0-X-X-X-X (4-8,4-6,4-8,4-8)
0-X-X-X-X (4-8,4-8,4-6,4-6)
0-X-X-X-X (4-8,4-8,4-6,4-8)

X = CAT
0 = EC

(CONTINUE LEVEL IV)

FIG. 17R

X-CAT
O-EC

O-X-X-X-X (4-8;4-8;4-8;4-6)
O-X-X-X-X (4-8;4-8;4-8;4-8)
X-X-X-X-X (4-6;4-6;4-6;4-6)
X-X-X-X-X (4-6;4-6;4-6;4-8)
X-X-X-X-X (4-6;4-6;4-8;4-6)
X-X-X-X-X (4-6;4-6;4-8;4-8)
X-X-X-X-X (4-6;4-8;4-6;4-6)
X-X-X-X-X (4-6;4-8;4-6;4-8)
X-X-X-X-X (4-6;4-8;4-8;4-6)
X-X-X-X-X (4-6;4-8;4-8;4-8)
X-X-X-X-X (4-8;4-6;4-6;4-6)
X-X-X-X-X (4-8;4-6;4-6;4-8)
X-X-X-X-X (4-8;4-6;4-8;4-6)
X-X-X-X-X (4-8;4-6;4-8;4-8)
X-X-X-X-X (4-8;4-8;4-6;4-6)
X-X-X-X-X (4-8;4-8;4-6;4-8)
X-X-X-X-X (4-8;4-8;4-8;4-6)
X-X-X-X-X (4-8;4-8;4-8;4-8)

(CONTINUE LEVEL IV)

FIG. 17S

0-0-0-0-X	(4-6;4-6;4-6;4-6)
0-0-0-0-X	(4-6;4-6;4-6;4-8)
0-0-0-0-X	(4-6;4-6;4-8;4-6)
0-0-0-0-X	(4-6;4-6;4-8;4-8)
0-0-0-0-X	(4-6;4-8;4-6;4-6)
0-0-0-0-X	(4-6;4-8;4-6;4-8)
0-0-0-0-X	(4-6;4-8;4-8;4-6)
0-0-0-0-X	(4-6;4-8;4-8;4-8)
0-0-0-0-X	(4-8;4-6;4-6;4-6)
0-0-0-0-X	(4-8;4-6;4-6;4-8)
0-0-0-0-X	(4-8;4-6;4-8;4-6)
0-0-0-0-X	(4-8;4-6;4-8;4-8)
0-0-0-0-X	(4-8;4-8;4-6;4-6)
0-0-0-0-X	(4-8;4-8;4-6;4-8)
0-0-0-0-X	(4-8;4-8;4-8;4-6)
0-0-0-0-X	(4-8;4-8;4-8;4-8)
X-0-0-0-X	(4-6;4-6;4-6;4-6)
X-0-0-0-X	(4-6;4-6;4-6;4-8)
X-0-0-0-X	(4-6;4-6;4-8;4-6)
X-0-0-0-X	(4-6;4-6;4-8;4-8)
X-0-0-0-X	(4-6;4-8;4-6;4-6)
X-0-0-0-X	(4-6;4-8;4-6;4-8)
X-0-0-0-X	(4-6;4-8;4-8;4-6)
X-0-0-0-X	(4-6;4-8;4-8;4-8)
X-0-0-0-X	(4-8;4-6;4-6;4-6)
X-0-0-0-X	(4-8;4-6;4-6;4-8)
X-0-0-0-X	(4-8;4-6;4-8;4-6)
X-0-0-0-X	(4-8;4-6;4-8;4-8)
X-0-0-0-X	(4-8;4-8;4-6;4-6)
X-0-0-0-X	(4-8;4-8;4-6;4-8)
X-0-0-0-X	(4-8;4-8;4-8;4-6)
X-0-0-0-X	(4-8;4-8;4-8;4-8)
0-X-0-0-X	(4-6;4-6;4-6;4-6)
0-X-0-0-X	(4-6;4-6;4-6;4-8)

(LEVEL V)

X=CAT
0=EC

FIG. 17T

O-X-O-O-X	(4-6; 4-6; 4-8; 4-6)
O-X-O-O-X	(4-6; 4-6; 4-8; 4-8)
O-X-O-O-X	(4-6; 4-8; 4-6; 4-6)
O-X-O-O-X	(4-6; 4-8; 4-6; 4-8)
O-X-O-O-X	(4-6; 4-8; 4-8; 4-6)
O-X-O-O-X	(4-6; 4-8; 4-8; 4-8)
O-X-O-O-X	(4-8; 4-6; 4-6; 4-6)
O-X-O-O-X	(4-8; 4-6; 4-6; 4-8)
O-X-O-O-X	(4-8; 4-6; 4-8; 4-6)
O-X-O-O-X	(4-8; 4-6; 4-8; 4-8)
O-X-O-O-X	(4-8; 4-8; 4-6; 4-6)
O-X-O-O-X	(4-8; 4-8; 4-6; 4-8)
O-X-O-O-X	(4-8; 4-8; 4-8; 4-6)
O-X-O-O-X	(4-8; 4-8; 4-8; 4-8)
X-X-O-O-X	(4-6; 4-6; 4-6; 4-6)
X-X-O-O-X	(4-6; 4-6; 4-6; 4-8)
X-X-O-O-X	(4-6; 4-6; 4-8; 4-6)
X-X-O-O-X	(4-6; 4-6; 4-8; 4-8)
X-X-O-O-X	(4-6; 4-8; 4-6; 4-6)
X-X-O-O-X	(4-6; 4-8; 4-6; 4-8)
X-X-O-O-X	(4-6; 4-8; 4-8; 4-6)
X-X-O-O-X	(4-6; 4-8; 4-8; 4-8)
X-X-O-O-X	(4-8; 4-6; 4-6; 4-6)
X-X-O-O-X	(4-8; 4-6; 4-6; 4-8)
X-X-O-O-X	(4-8; 4-6; 4-8; 4-6)
X-X-O-O-X	(4-8; 4-6; 4-8; 4-8)
X-X-O-O-X	(4-8; 4-8; 4-6; 4-6)
X-X-O-O-X	(4-8; 4-8; 4-6; 4-8)
X-X-O-O-X	(4-8; 4-8; 4-8; 4-6)
X-X-O-O-X	(4-8; 4-8; 4-8; 4-8)
O-O-X-O-X	(4-6; 4-6; 4-6; 4-6)
O-O-X-O-X	(4-6; 4-6; 4-6; 4-8)
O-O-X-O-X	(4-6; 4-6; 4-8; 4-6)
O-O-X-O-X	(4-6; 4-6; 4-8; 4-8)

X = CAT
O = EC

(CONTINUE LEVEL V)

FIG. 17U

0-0-X-0-X (4-6;4-8;4-6;4-6)
0-0-X-0-X (4-6;4-8;4-6;4-8)
0-0-X-0-X (4-6;4-8;4-8;4-6)
0-0-X-0-X (4-6;4-8;4-8;4-8)
0-0-X-0-X (4-8;4-6;4-6;4-6)
0-0-X-0-X (4-8;4-6;4-6;4-8)
0-0-X-0-X (4-8;4-6;4-8;4-6)
0-0-X-0-X (4-8;4-6;4-8;4-8)
0-0-X-0-X (4-8;4-8;4-6;4-6)
0-0-X-0-X (4-8;4-8;4-6;4-8)
0-0-X-0-X (4-8;4-8;4-8;4-6)
0-0-X-0-X (4-8;4-8;4-8;4-8)
X-0-X-0-X (4-6;4-6;4-6;4-6)
X-0-X-0-X (4-6;4-6;4-6;4-8)
X-0-X-0-X (4-6;4-6;4-8;4-6)
X-0-X-0-X (4-6;4-6;4-8;4-8)
X-0-X-0-X (4-6;4-8;4-6;4-6)
X-0-X-0-X (4-6;4-8;4-6;4-8)
X-0-X-0-X (4-6;4-8;4-8;4-6)
X-0-X-0-X (4-6;4-8;4-8;4-8)
X-0-X-0-X (4-8;4-6;4-6;4-6)
X-0-X-0-X (4-8;4-6;4-6;4-8)
X-0-X-0-X (4-8;4-6;4-8;4-6)
X-0-X-0-X (4-8;4-6;4-8;4-8)
X-0-X-0-X (4-8;4-8;4-6;4-6)
X-0-X-0-X (4-8;4-8;4-6;4-8)
X-0-X-0-X (4-8;4-8;4-8;4-6)
X-0-X-0-X (4-8;4-8;4-8;4-8)
0-X-X-0-X (4-6;4-6;4-6;4-6)
0-X-X-0-X (4-6;4-6;4-6;4-8)
0-X-X-0-X (4-6;4-6;4-8;4-6)
0-X-X-0-X (4-6;4-6;4-8;4-8)
0-X-X-0-X (4-6;4-8;4-6;4-6)
0-X-X-0-X (4-6;4-8;4-6;4-8)

X-CAT
0-EC

(CONTINUE LEVEL V)

FIG. 17V

0-X-X-0-X	(4-6; 4-8; 4-8; 4-6)
0-X-X-0-X	(4-6; 4-8; 4-8; 4-8)
0-X-X-0-X	(4-8; 4-6; 4-6; 4-6)
0-X-X-0-X	(4-8; 4-6; 4-6; 4-8)
0-X-X-0-X	(4-8; 4-6; 4-8; 4-6)
0-X-X-0-X	(4-8; 4-6; 4-8; 4-8)
0-X-X-0-X	(4-8; 4-8; 4-6; 4-6)
0-X-X-0-X	(4-8; 4-8; 4-6; 4-8)
0-X-X-0-X	(4-8; 4-8; 4-8; 4-6)
0-X-X-0-X	(4-8; 4-8; 4-8; 4-8)
X-X-X-0-X	(4-6; 4-6; 4-6; 4-6)
X-X-X-0-X	(4-6; 4-6; 4-6; 4-8)
X-X-X-0-X	(4-6; 4-6; 4-8; 4-6)
X-X-X-0-X	(4-6; 4-6; 4-8; 4-8)
X-X-X-0-X	(4-6; 4-8; 4-6; 4-6)
X-X-X-0-X	(4-6; 4-8; 4-6; 4-8)
X-X-X-0-X	(4-6; 4-8; 4-8; 4-6)
X-X-X-0-X	(4-6; 4-8; 4-8; 4-8)
X-X-X-0-X	(4-8; 4-6; 4-6; 4-6)
X-X-X-0-X	(4-8; 4-6; 4-6; 4-8)
X-X-X-0-X	(4-8; 4-6; 4-8; 4-6)
X-X-X-0-X	(4-8; 4-6; 4-8; 4-8)
X-X-X-0-X	(4-8; 4-8; 4-6; 4-6)
X-X-X-0-X	(4-8; 4-8; 4-6; 4-8)
X-X-X-0-X	(4-8; 4-8; 4-8; 4-6)
X-X-X-0-X	(4-8; 4-8; 4-8; 4-8)
0-0-0-X-0	(4-6; 4-6; 4-6; 4-6)
0-0-0-X-0	(4-6; 4-6; 4-6; 4-8)
0-0-0-X-0	(4-6; 4-6; 4-8; 4-6)
0-0-0-X-0	(4-6; 4-6; 4-8; 4-8)
0-0-0-X-0	(4-6; 4-8; 4-6; 4-6)
0-0-0-X-0	(4-6; 4-8; 4-6; 4-8)
0-0-0-X-0	(4-6; 4-8; 4-8; 4-6)
0-0-0-X-0	(4-6; 4-8; 4-8; 4-8)

X = CAT
0 = EC

(CONTINUE LEVEL V)

FIG. 17W

O-O-O-X-O (4-8; 4-6; 4-6; 4-6)
O-O-O-X-O (4-8; 4-6; 4-6; 4-8)
O-O-O-X-O (4-8; 4-6; 4-8; 4-6)
O-O-O-X-O (4-8; 4-6; 4-8; 4-8)
O-O-O-X-O (4-8; 4-8; 4-6; 4-6)
O-O-O-X-O (4-8; 4-8; 4-6; 4-8)
O-O-O-X-O (4-8; 4-8; 4-8; 4-6)
O-O-O-X-O (4-8; 4-8; 4-8; 4-8)
X-O-O-X-O (4-6; 4-6; 4-6; 4-6)
X-O-O-X-O (4-6; 4-6; 4-6; 4-8)
X-O-O-X-O (4-6; 4-6; 4-8; 4-6)
X-O-O-X-O (4-6; 4-6; 4-8; 4-8)
X-O-O-X-O (4-6; 4-8; 4-6; 4-6)
X-O-O-X-O (4-6; 4-8; 4-6; 4-8)
X-O-O-X-O (4-6; 4-8; 4-8; 4-6)
X-O-O-X-O (4-6; 4-8; 4-8; 4-8)
X-O-O-X-O (4-8; 4-6; 4-6; 4-6)
X-O-O-X-O (4-8; 4-6; 4-6; 4-8)
X-O-O-X-O (4-8; 4-6; 4-8; 4-6)
X-O-O-X-O (4-8; 4-6; 4-8; 4-8)
X-O-O-X-O (4-8; 4-8; 4-6; 4-6)
X-O-O-X-O (4-8; 4-8; 4-6; 4-8)
X-O-O-X-O (4-8; 4-8; 4-8; 4-6)
X-O-O-X-O (4-8; 4-8; 4-8; 4-8)
O-X-O-X-O (4-6; 4-6; 4-6; 4-6)
O-X-O-X-O (4-6; 4-6; 4-6; 4-8)
O-X-O-X-O (4-6; 4-6; 4-8; 4-6)
O-X-O-X-O (4-6; 4-6; 4-8; 4-8)
O-X-O-X-O (4-6; 4-8; 4-6; 4-6)
O-X-O-X-O (4-6; 4-8; 4-6; 4-8)
O-X-O-X-O (4-6; 4-8; 4-8; 4-6)
O-X-O-X-O (4-6; 4-8; 4-8; 4-8)
O-X-O-X-O (4-8; 4-6; 4-6; 4-6)
O-X-O-X-O (4-8; 4-6; 4-6; 4-8)

X=CAT
O=EC

(CONTINUE LEVEL V)

FIG. 17X

0-X-0-X-0	(4-6; 4-6; 4-8; 4-6)
0-X-0-X-0	(4-6; 4-6; 4-8; 4-8)
0-X-0-X-0	(4-6; 4-6; 4-6; 4-6)
0-X-0-X-0	(4-6; 4-6; 4-6; 4-8)
0-X-0-X-0	(4-6; 4-8; 4-8; 4-6)
0-X-0-X-0	(4-6; 4-8; 4-8; 4-8)
X-X-0-X-0	(4-6; 4-6; 4-6; 4-6)
X-X-0-X-0	(4-6; 4-6; 4-6; 4-8)
X-X-0-X-0	(4-6; 4-6; 4-8; 4-6)
X-X-0-X-0	(4-6; 4-6; 4-8; 4-8)
X-X-0-X-0	(4-6; 4-8; 4-6; 4-6)
X-X-0-X-0	(4-6; 4-8; 4-6; 4-8)
X-X-0-X-0	(4-6; 4-8; 4-8; 4-6)
X-X-0-X-0	(4-6; 4-8; 4-8; 4-8)
X-X-0-X-0	(4-8; 4-6; 4-6; 4-6)
X-X-0-X-0	(4-8; 4-6; 4-6; 4-8)
X-X-0-X-0	(4-8; 4-6; 4-8; 4-6)
X-X-0-X-0	(4-8; 4-6; 4-8; 4-8)
X-X-0-X-0	(4-8; 4-8; 4-6; 4-6)
X-X-0-X-0	(4-8; 4-8; 4-6; 4-8)
X-X-0-X-0	(4-8; 4-8; 4-8; 4-6)
X-X-0-X-0	(4-8; 4-8; 4-8; 4-8)
0-0-X-X-0	(4-6; 4-6; 4-6; 4-6)
0-0-X-X-0	(4-6; 4-6; 4-6; 4-8)
0-0-X-X-0	(4-6; 4-6; 4-8; 4-6)
0-0-X-X-0	(4-6; 4-6; 4-8; 4-8)
0-0-X-X-0	(4-6; 4-8; 4-6; 4-6)
0-0-X-X-0	(4-6; 4-8; 4-6; 4-8)
0-0-X-X-0	(4-6; 4-8; 4-8; 4-6)
0-0-X-X-0	(4-6; 4-8; 4-8; 4-8)
0-0-X-X-0	(4-8; 4-6; 4-6; 4-6)
0-0-X-X-0	(4-8; 4-6; 4-6; 4-8)
0-0-X-X-0	(4-8; 4-6; 4-8; 4-6)
0-0-X-X-0	(4-8; 4-6; 4-8; 4-8)

X-CAT
0-EC

(CONTINUE LEVEL V)

FIG. 17Y

O-O-X-X-O (4-8; 4-8; 4-6; 4-6)
O-O-X-X-O (4-8; 4-8; 4-6; 4-8)
O-O-X-X-O (4-8; 4-8; 4-8; 4-6)
O-O-X-X-O (4-8; 4-8; 4-8; 4-8)
X-O-X-X-O (4-6; 4-6; 4-6; 4-6)
X-O-X-X-O (4-6; 4-6; 4-6; 4-8)
X-O-X-X-O (4-6; 4-6; 4-8; 4-6)
X-O-X-X-O (4-6; 4-6; 4-8; 4-8)
X-O-X-X-O (4-6; 4-8; 4-6; 4-6)
X-O-X-X-O (4-6; 4-8; 4-6; 4-8)
X-O-X-X-O (4-6; 4-8; 4-8; 4-6)
X-O-X-X-O (4-6; 4-8; 4-8; 4-8)
X-O-X-X-O (4-8; 4-6; 4-6; 4-6)
X-O-X-X-O (4-8; 4-6; 4-6; 4-8)
X-O-X-X-O (4-8; 4-6; 4-8; 4-6)
X-O-X-X-O (4-8; 4-6; 4-8; 4-8)
X-O-X-X-O (4-8; 4-8; 4-6; 4-6)
X-O-X-X-O (4-8; 4-8; 4-6; 4-8)
X-O-X-X-O (4-8; 4-8; 4-8; 4-6)
X-O-X-X-O (4-8; 4-8; 4-8; 4-8)
O-X-X-X-O (4-6; 4-6; 4-6; 4-6)
O-X-X-X-O (4-6; 4-6; 4-6; 4-8)
O-X-X-X-O (4-6; 4-6; 4-8; 4-6)
O-X-X-X-O (4-6; 4-6; 4-8; 4-8)
O-X-X-X-O (4-6; 4-8; 4-6; 4-6)
O-X-X-X-O (4-6; 4-8; 4-6; 4-8)
O-X-X-X-O (4-6; 4-8; 4-8; 4-6)
O-X-X-X-O (4-6; 4-8; 4-8; 4-8)
O-X-X-X-O (4-8; 4-6; 4-6; 4-6)
O-X-X-X-O (4-8; 4-6; 4-6; 4-8)
O-X-X-X-O (4-8; 4-6; 4-8; 4-6)
O-X-X-X-O (4-8; 4-6; 4-8; 4-8)
O-X-X-X-O (4-8; 4-8; 4-6; 4-6)
O-X-X-X-O (4-8; 4-8; 4-6; 4-8)

X=CAT
O=EC

(CONTINUE LEVEL V)

FIG. 17Z

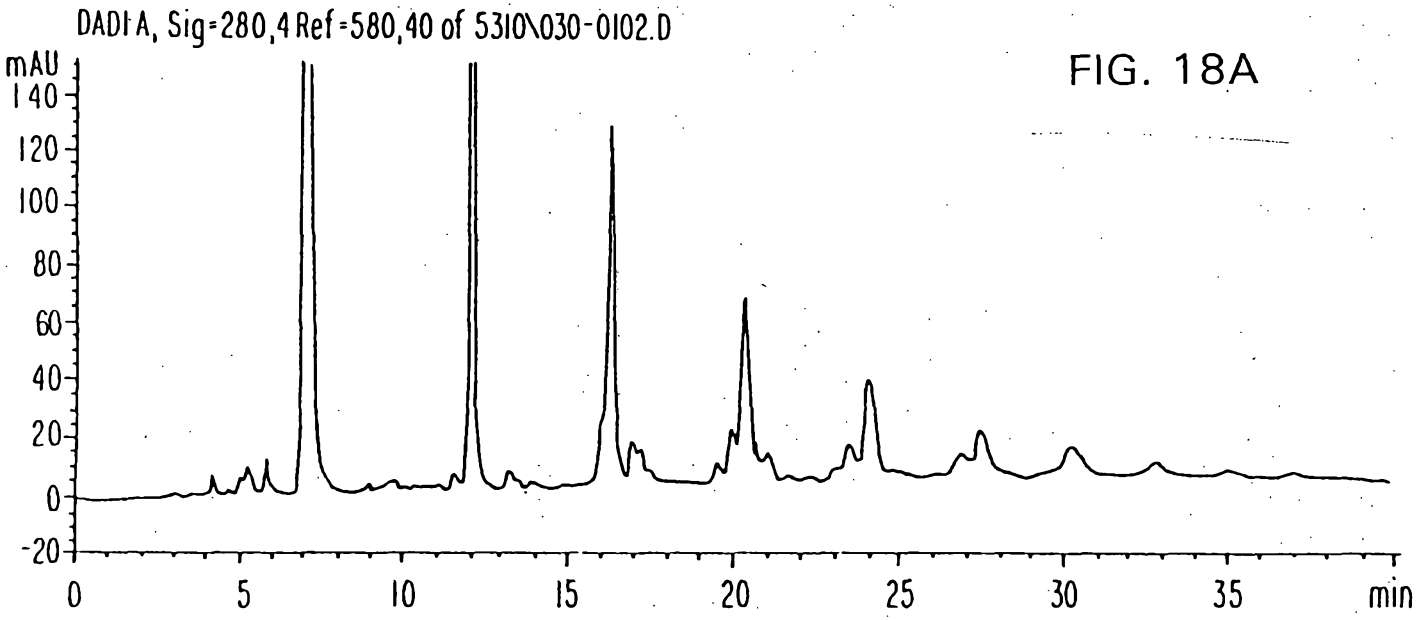


FIG. 18A

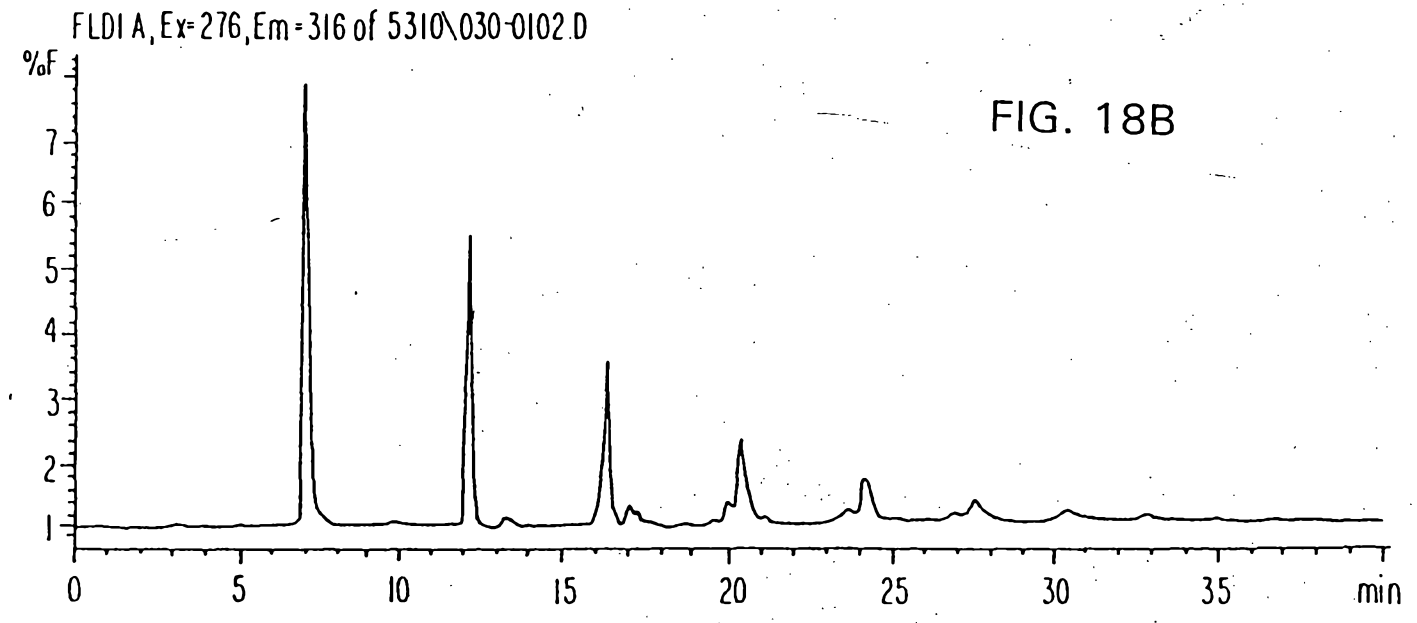


FIG. 18B

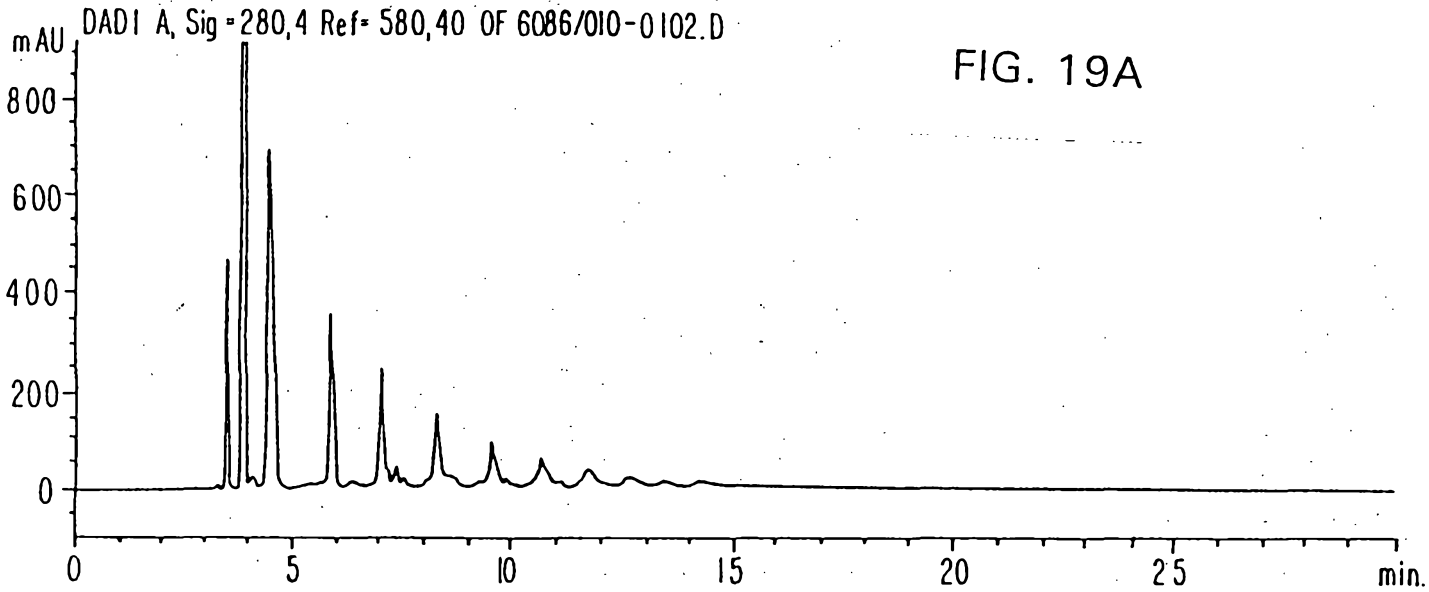


FIG. 19A

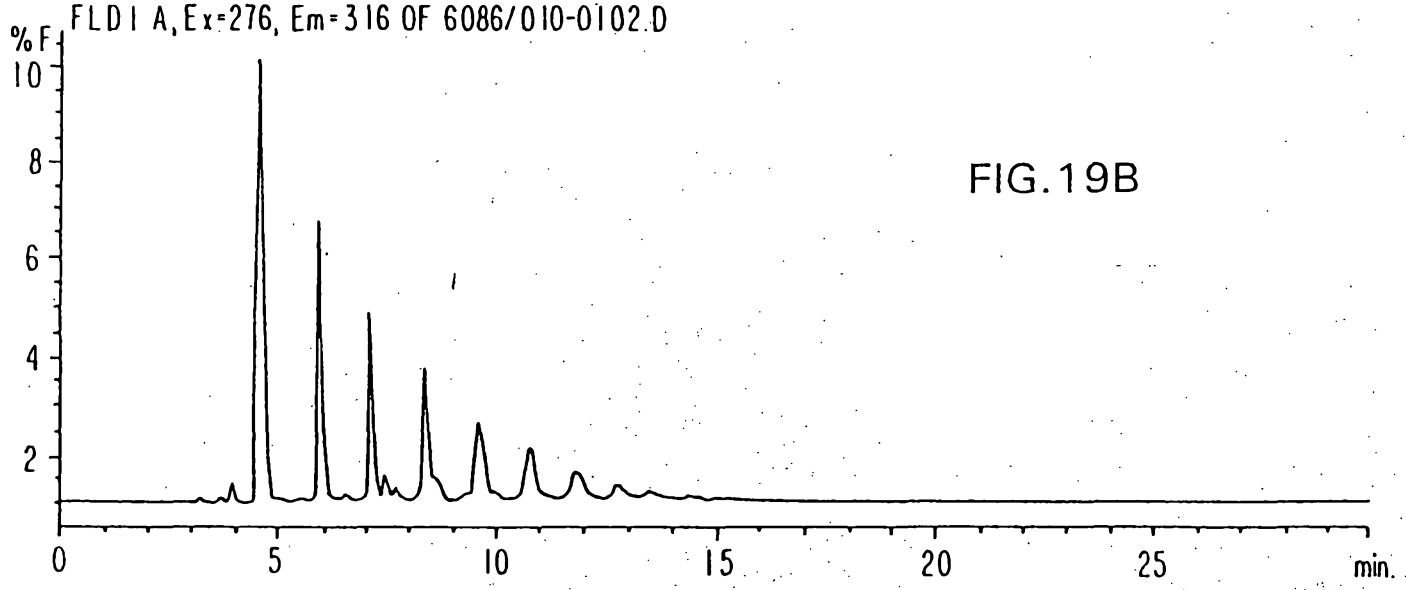


FIG. 19B

FIG. 20

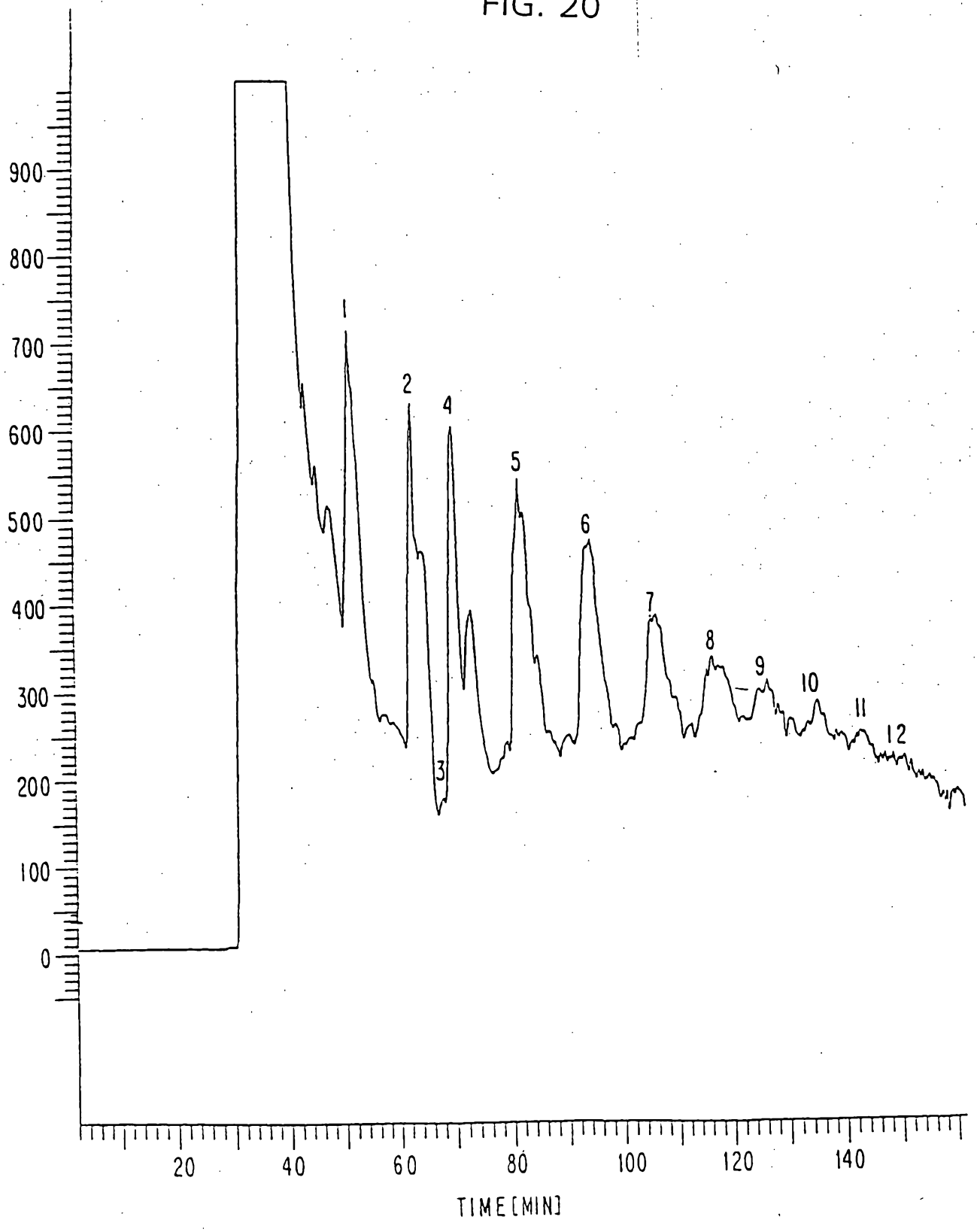

900
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
20
40
60
80
100
120
140
TIME [MIN]

FIG. 21A

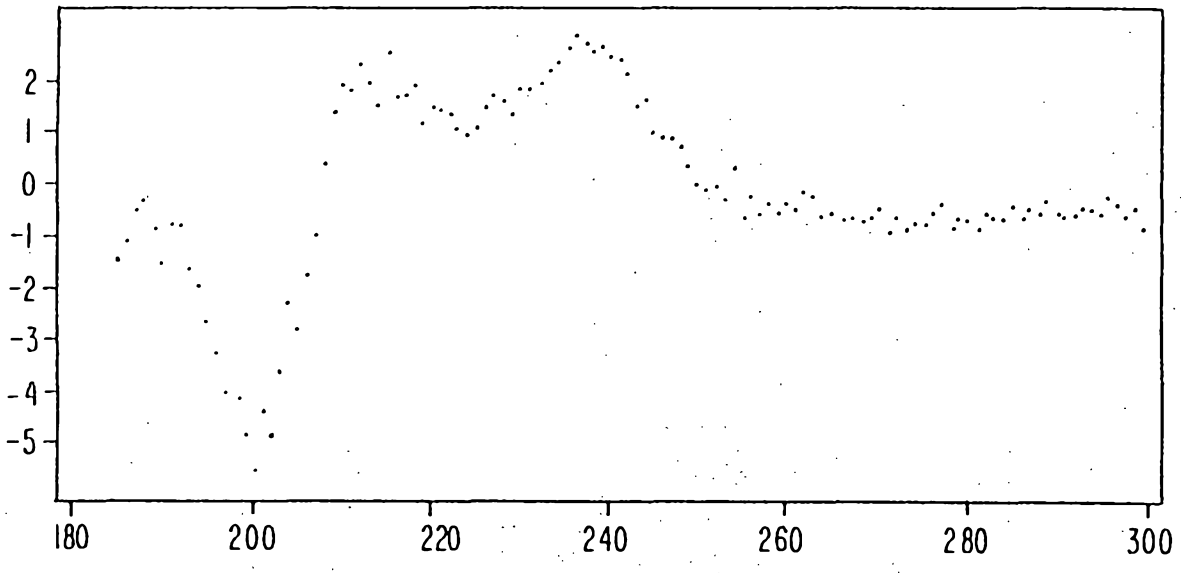


FIG. 21B

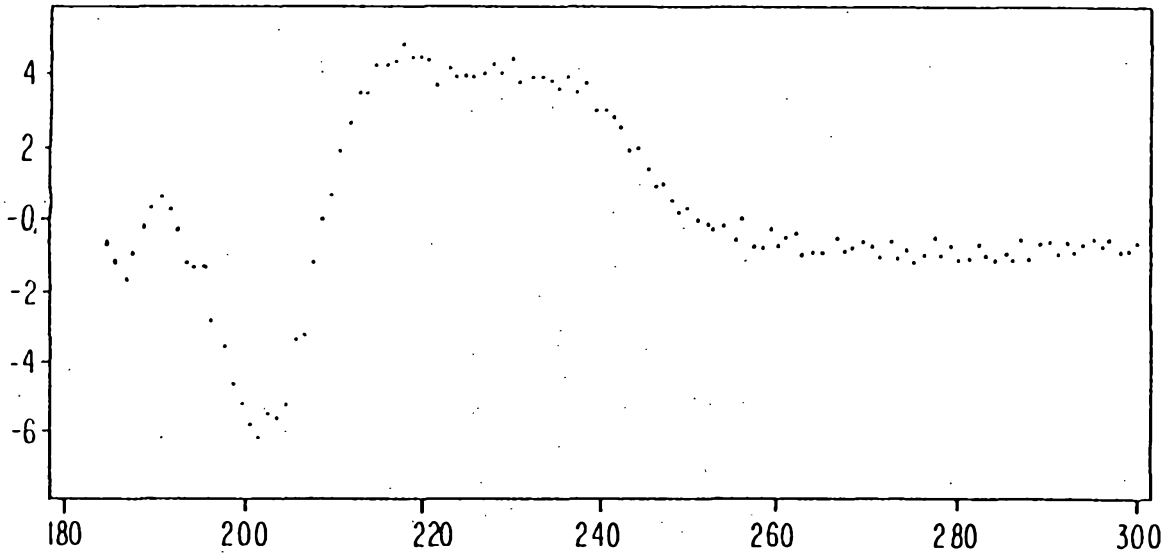


FIG. 21C

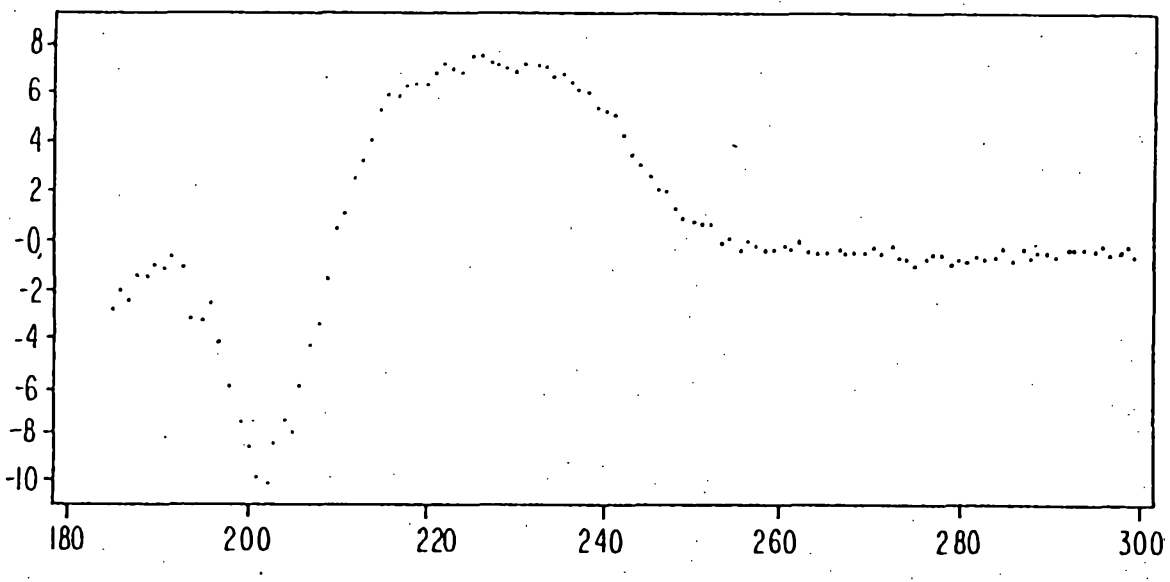


FIG. 21D

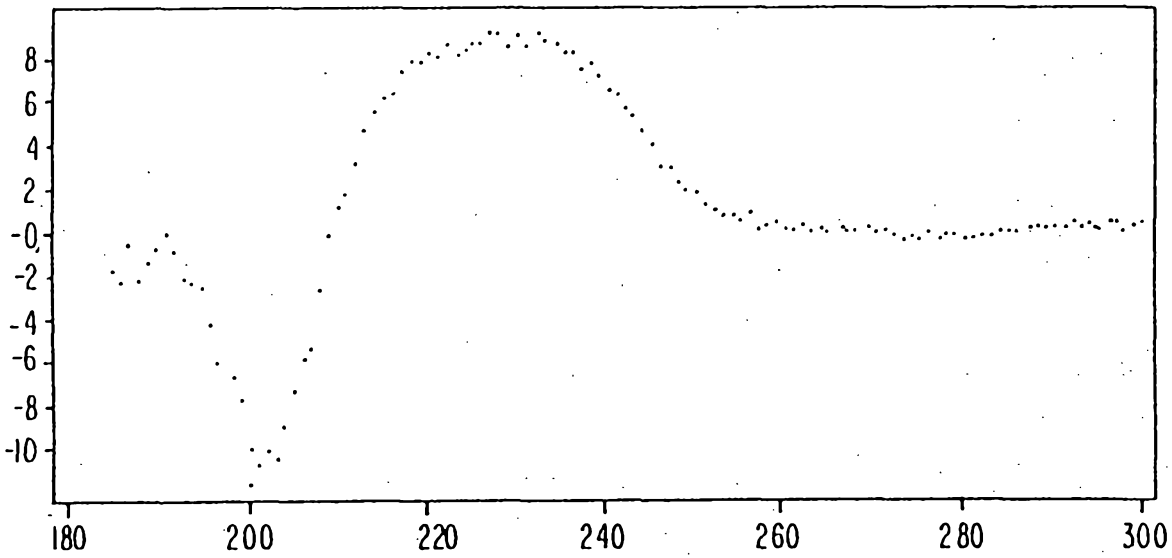


FIG. 21E

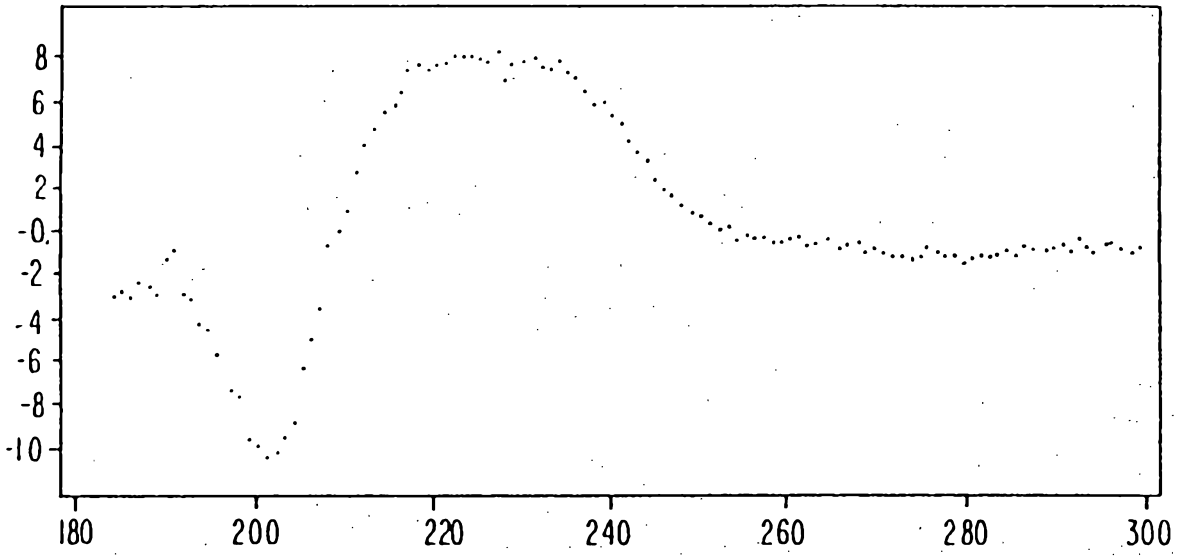


FIG. 21F

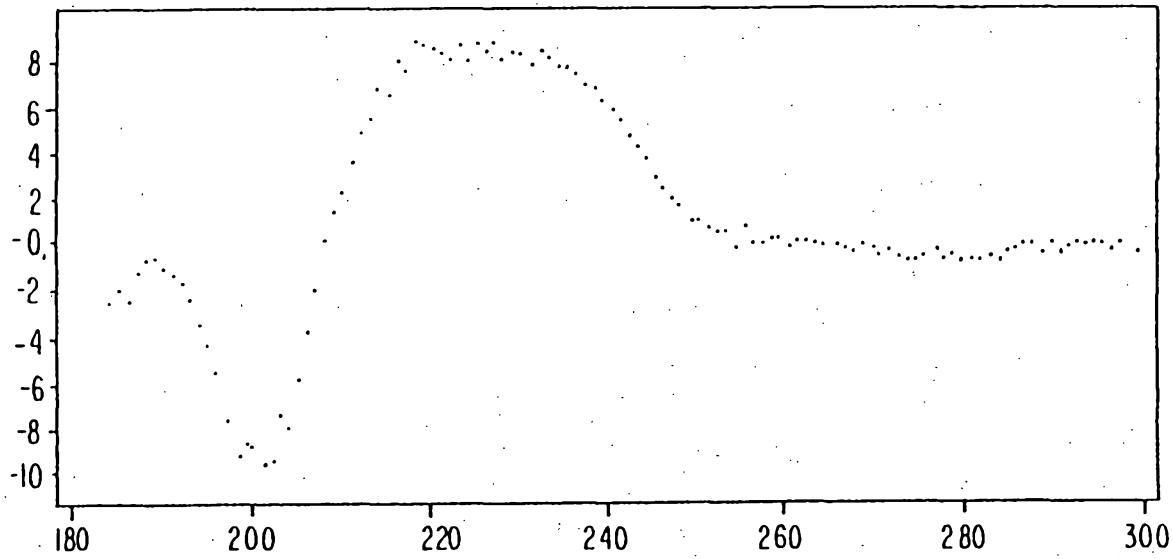
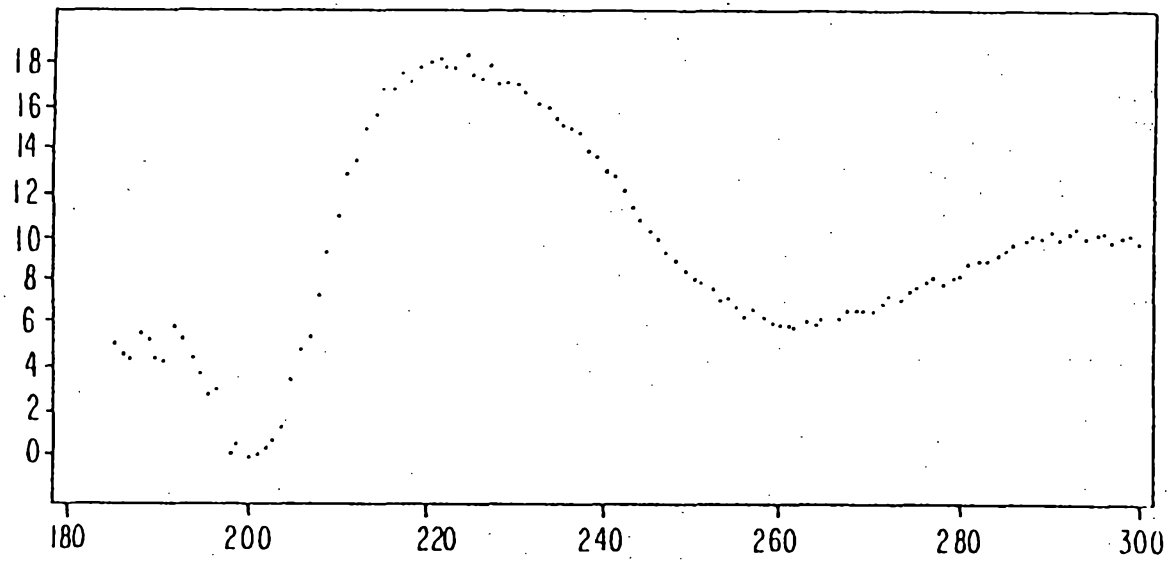
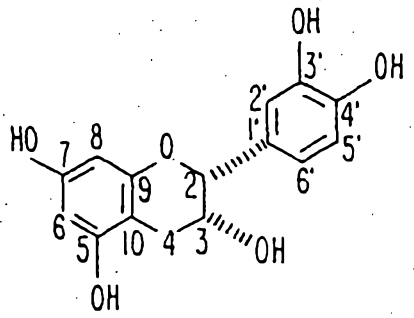


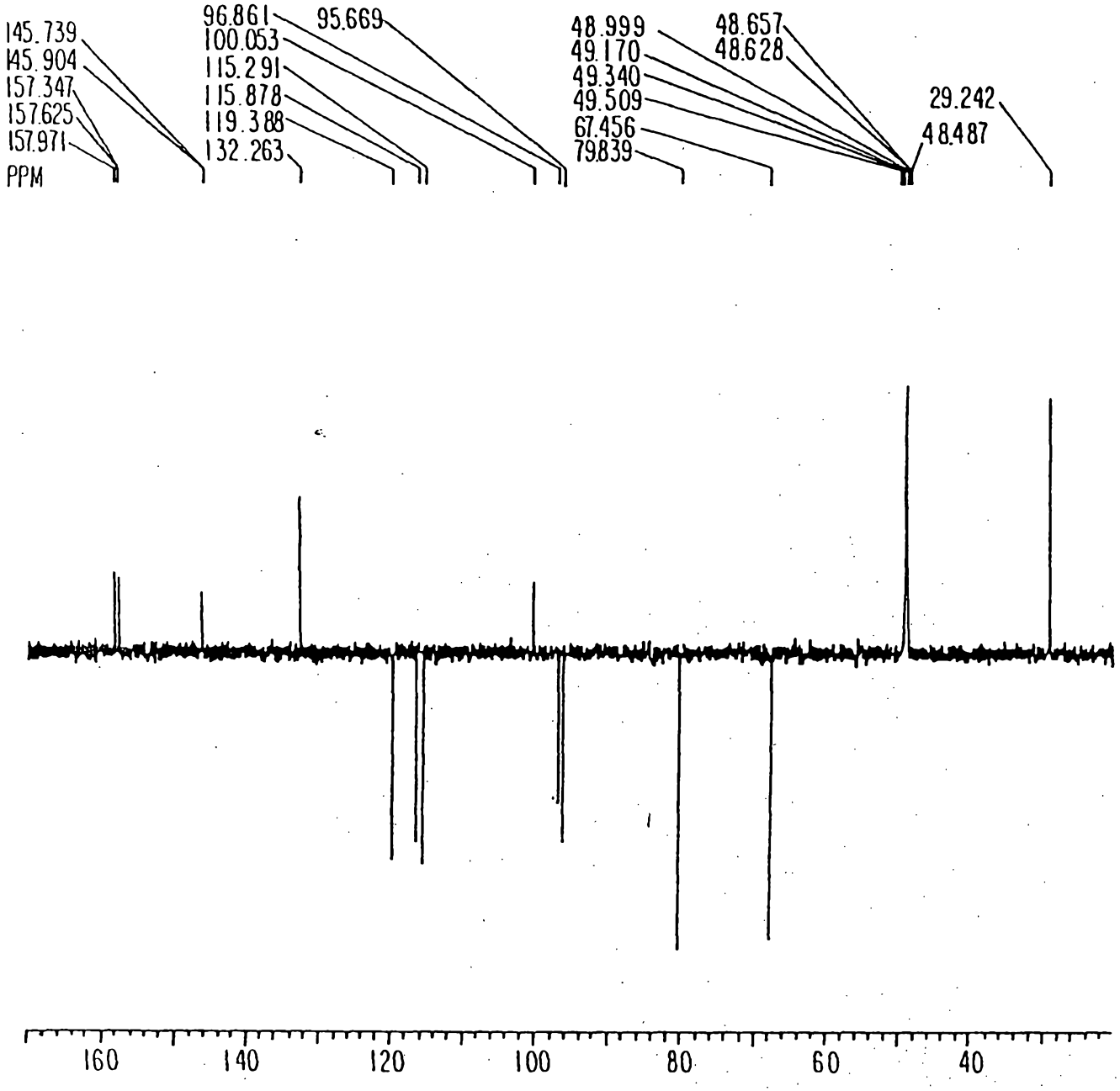
FIG. 21G





EPICATECHIN			
^{1}H	CHEMICAL SHIFT (ppm)	^{13}C	CHEMICAL SHIFT (ppm)
2	4.81	2	79.84
3	4.16	3	67.46
4α, 4β	2.73, 2.85	4	29.24
		6	95.87
6	5.94	8	96.36
8	5.91	2'	115.29
5', 6'	6.75, 6.79	5', 6'	115.88, 119.39
2'	6.97		

FIG. 22A

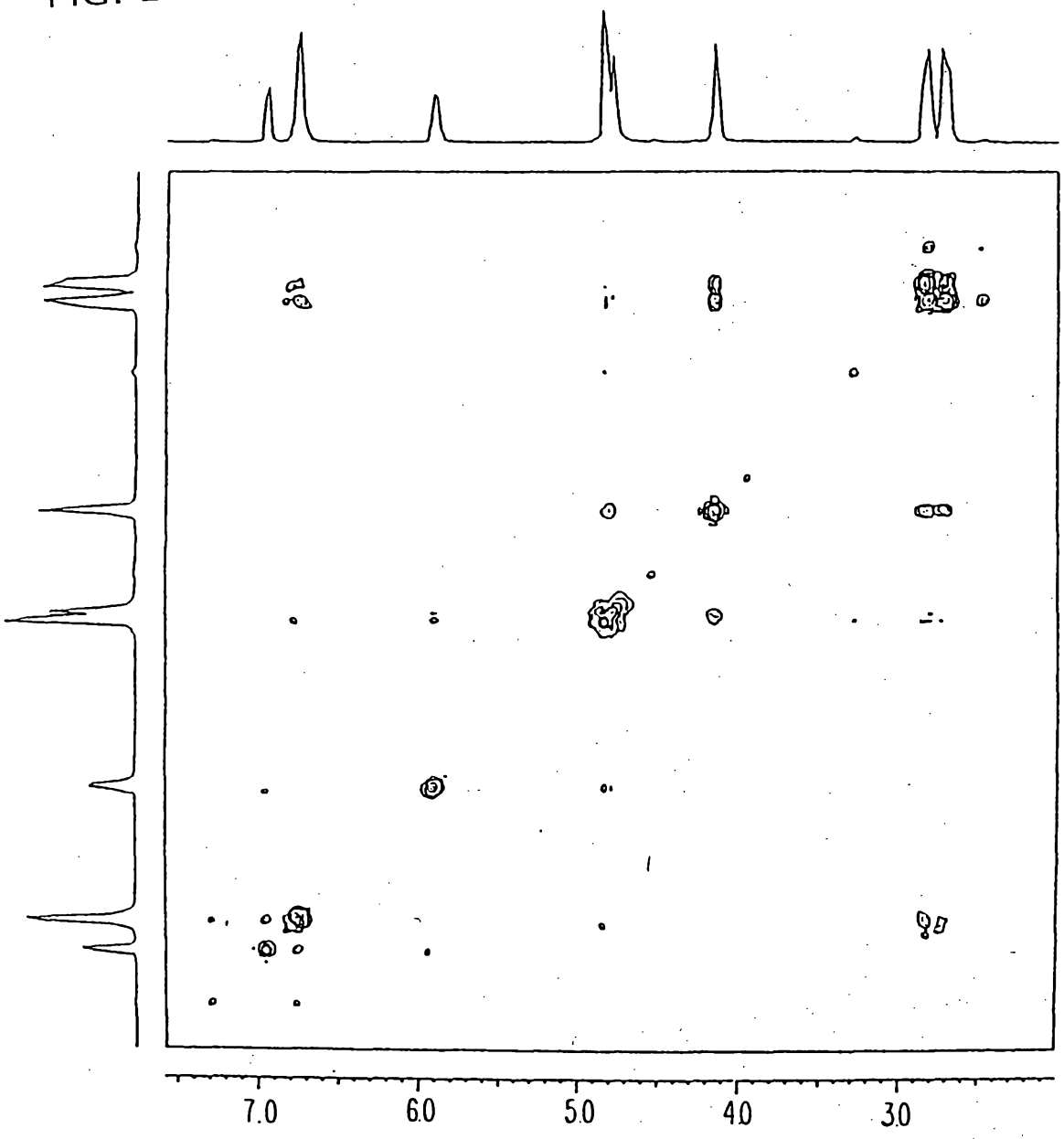


SOLVENT	CDCl3
SF	125.759
SFO	125.770
SFO2	500.130
SF2	125.759
SY	93.0
O1	3164.000
SI	65536
TD	65536
SW	29411.765
SW2	29411.765
HZ/PT	.898
VD	.0080
PW	0.0
RD	0.0
AQ	1.114
RG	100
NS	128
TE	300
DE	23.8
DR	12
DW	17
FW	36800
O2	9912.000
DP	30H PO
LB	1.000
GB	0.0
NC	2
CX	20.00
CY	5.00
FI	170.005P
F2	20.009P
MI	0.0
DC	1.000
HZ/CM	943.173
PPM/CM	7.500
IS	1
SR	-10527.08
DI	1.0000000

S1	30H
P9	110.00
D2	.0050000
S2	20H
P1	5.20
VD	.0080000
P2	10.40
RD	0.0
PW	0.0
DE	23.80

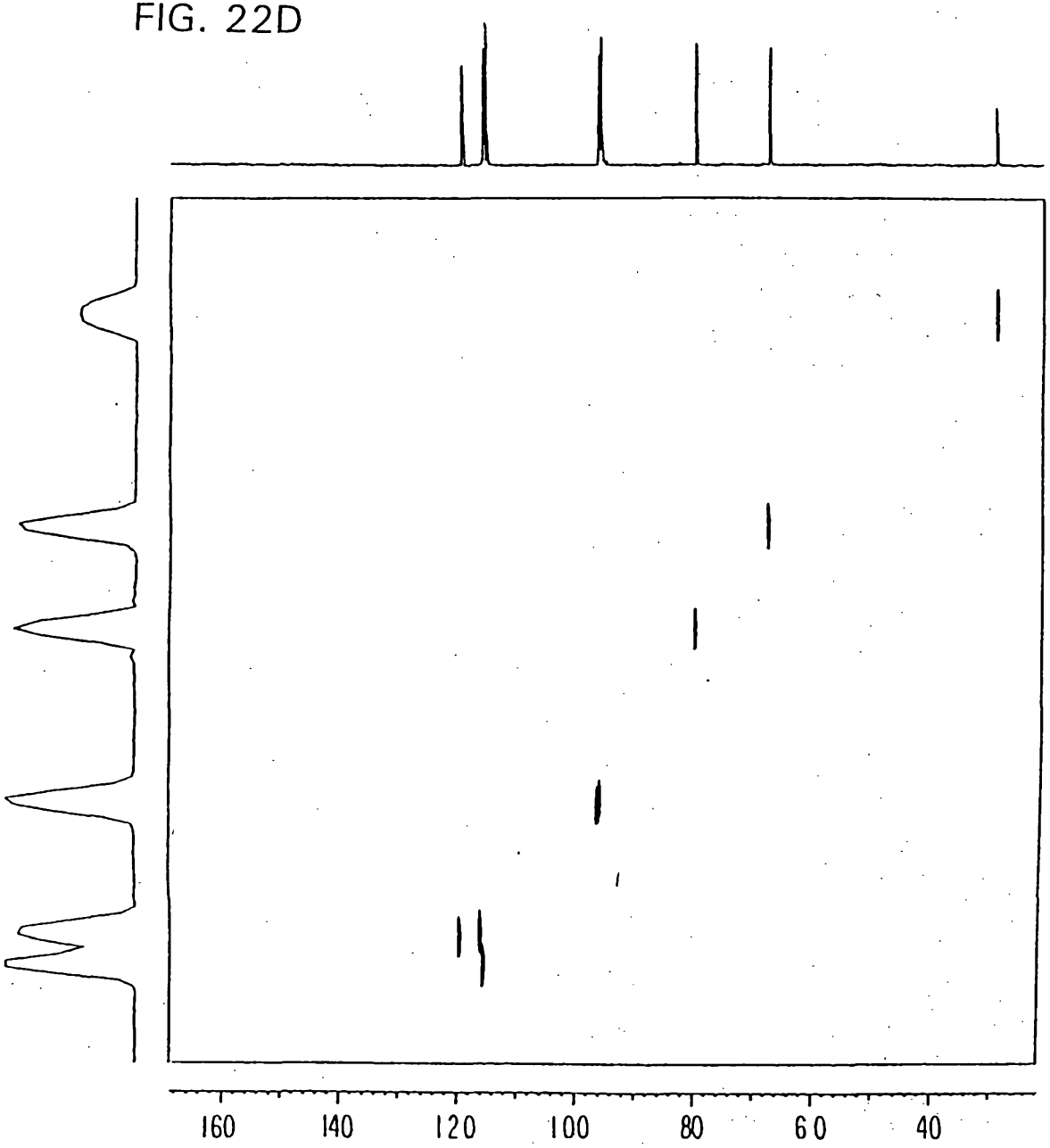
FIG. 22B

FIG. 22C



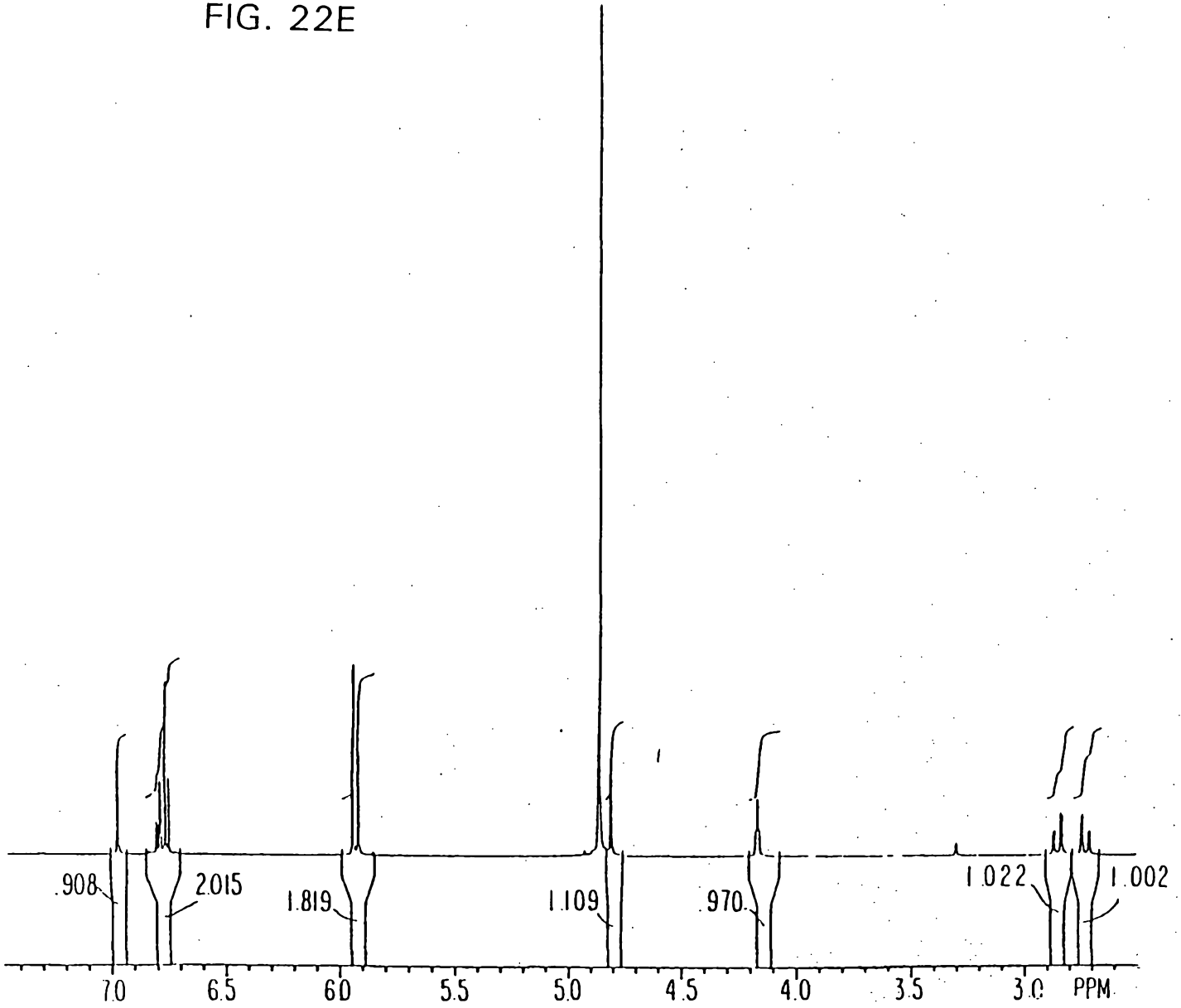
SI2	512
SI1	256
SW2	2793.296
SW1	1396.648
NDO	1
WDW2	S
WDW1	S
SSB2	0
SSB1	0
MC2	M
PLIM ROW:	
F1	7.573P
F2	2.010P
AND COLUMN:	
F1	7.573P
F2	2.010P
D1	1.0000000
P1	10.50
D0	.0000030
P2	5.20
RD	0.0
PW	0.0
DE	256.70
NS	4
DS	0
NE	128
IN	.0003580

FIG. 22D

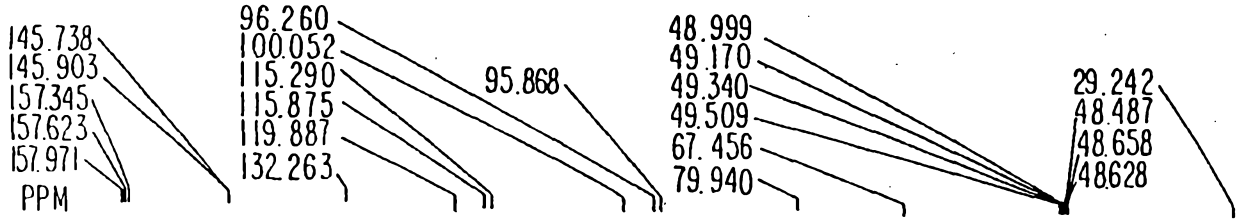


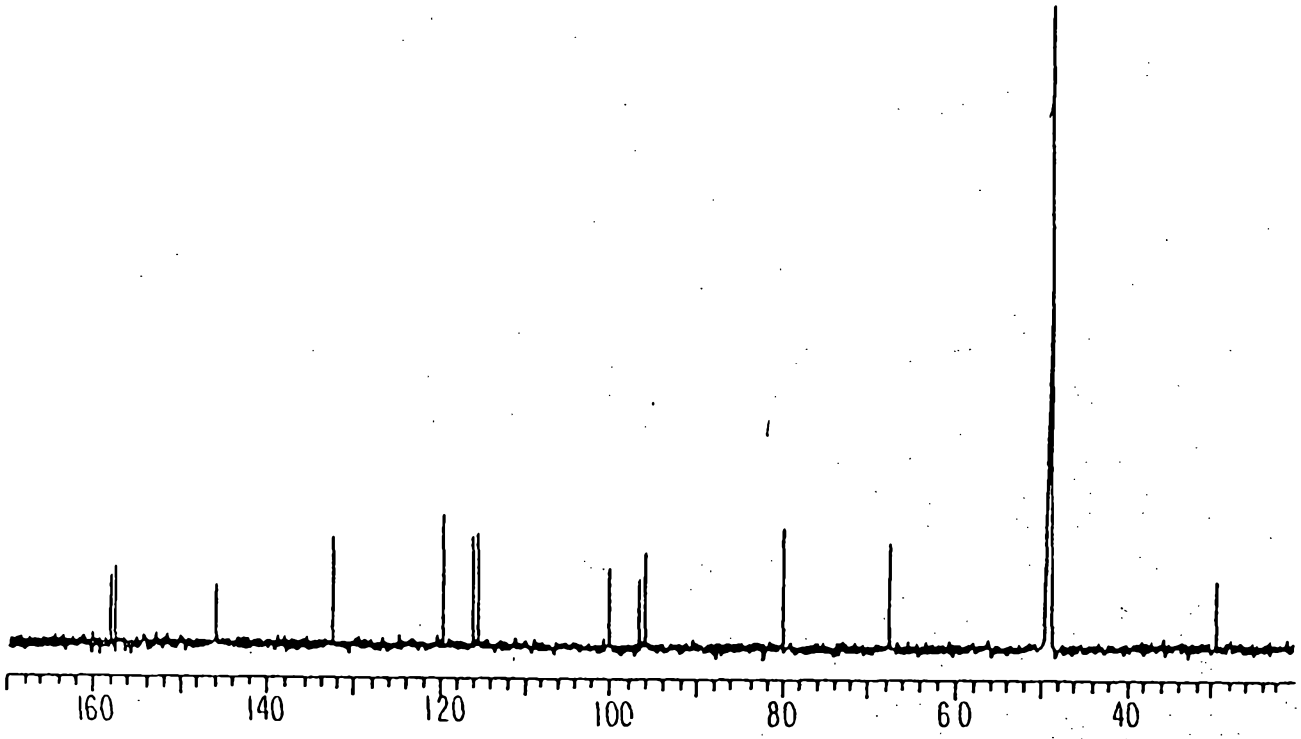
SI2	2048
SI1	128
SW2	18518.519
SW1	1399.776
NDO	2
WDW2	S
WDW1	S
SSB2	0
SSB1	0
MC2	M
PLIM ROW:	
F1	168.584P
F2	21.475P
AND COLUMN:	
F1	21.901P
F2	-.187P
D1	1.0000000
SI	0H
P1	10.50
D0	.0000030
P4	10.40
D3	.0040000
P3	5.20
D4	.0020000
S2	20H
RD	0.0
PW	0.0
DE	40.80
NS	64
DS	0
P9	110.00
NE	64
IN	.0001786

FIG. 22E



SOLVENT	CDCl3
SF	500.137
SFO	500.130
SFO2	500.130
SF2	500.137
SY	166.0
O1	9894.590
SI	32768
TD	32768
SW	6024.096
SW2	6024.096
HZ/PT	.368
VD	0.0
PW	3.0
RD	0.0
AQ	2.720
RG	8
NS	16
TE	300
DE	106.3
DR	12
DW	83
FW	7600
O2	5000.000
DP	63LPO
LB	0.0
GB	0.0
NC	0
CX	20.00
CY	15.00
FI	7.500P
F2	2.500P
MI	0.0
DC	1.000
HZ/CM	125.030
PPM/CM	.250
IS	3
SR	7394.06

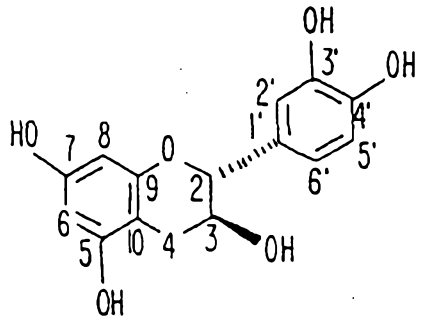




SOLVENT	CDCl3
SF	125.759
SFO	125.770
SFO2	500.130
SF2	125.759
SY	93.0
O1	3164.000
SI	65536
TD	65536
SW	29411.765
SW2	29411.765
HZ/PT	.898
VD	.0080
PW	2.6
RD	0.0
AQ	1.114
RG	100
NS	128
TE	300
DE	23.8
DR	12
DW	17
FW	36800
O2	9912.000
DP	20H D0
LB	1.000
GB	0.0
NC	3
CX	20.00
CY	10.00
FI	170.005P
F2	20.009P
MI	0.0
DC	1.000
HZ/CM	943.173
PPM/CM	7.500
IS	1
SR	-10527.08
D1	1.0000000

S2	20H
RD	0.0
PW	2.60
DE	23.80
NS	128
DS	1
P9	110.00
D2	.0050000

FIG. 22F



CATECHIN			
^{1}H	CHEMICAL SHIFT (ppm)	^{13}C	CHEMICAL SHIFT (ppm)
2	4.56	2	79.84
3	3.97	3	67.46
4α	2.50	4	29.24
4β	2.84	6	95.87
6	5.85	8	96.36
8	5.92	2'	115.29
2'	6.83	6'	116.08
5'	6.76	5'	120.08
6'	6.71		

FIG. 23A

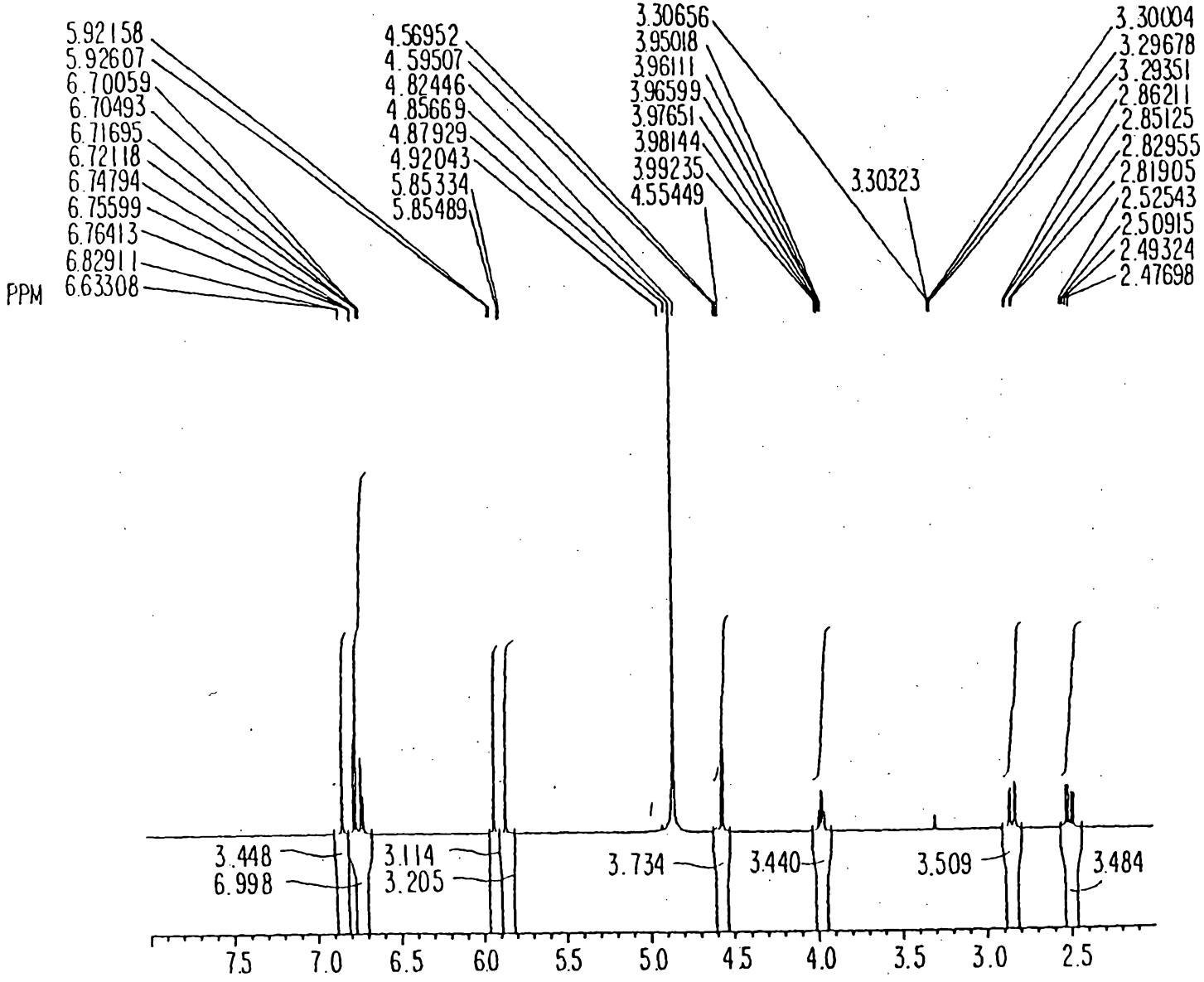
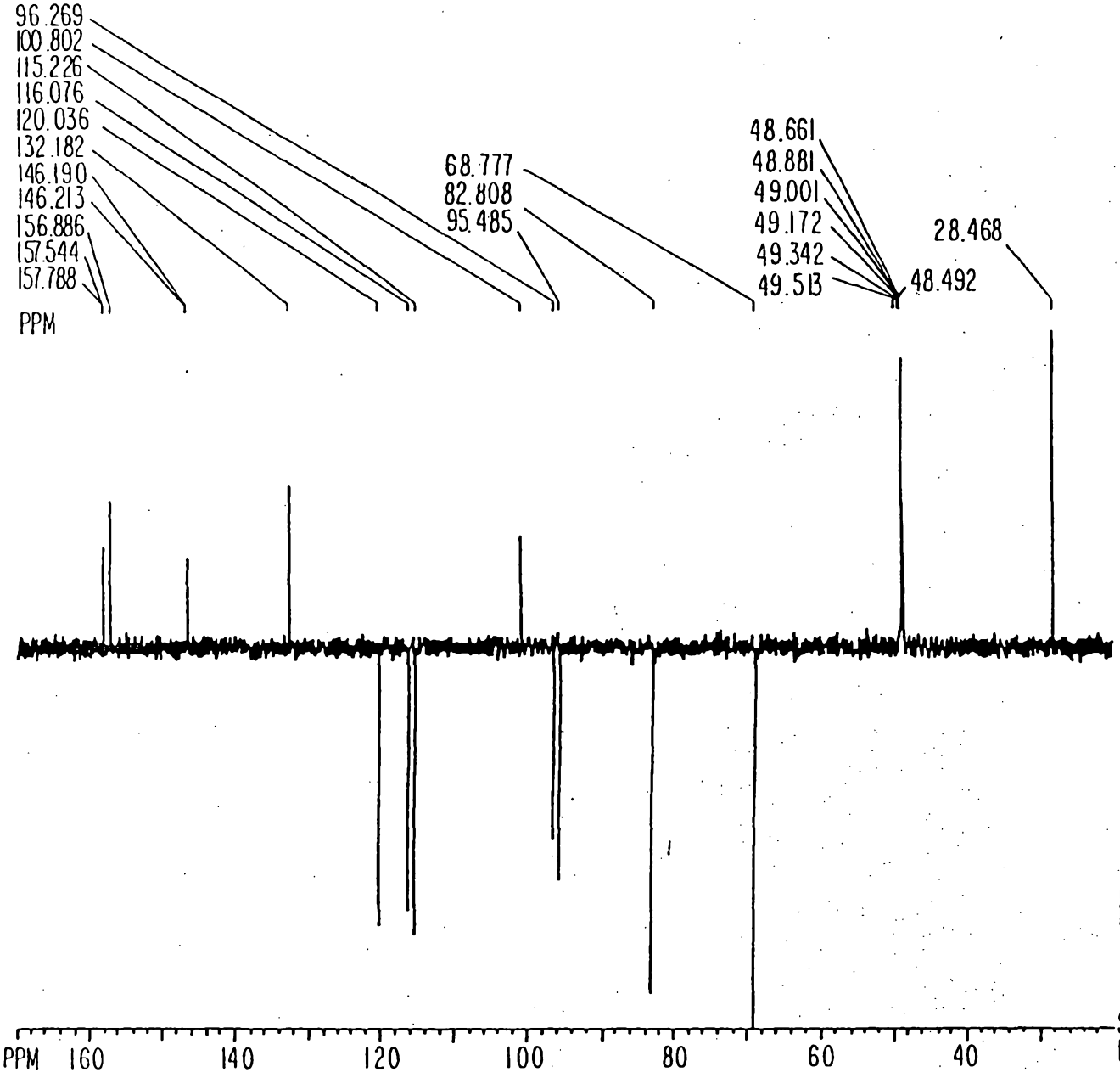


FIG. 23B

SOLVENT	CDCl3
SF	500.137
SFO	500.130
SFO2	500.130
SF2	500.137
SY	166.0
O1	9894.000
SI	32768
TD	32768
SW	6024.096
SW2	6024.096
HZ/PT	.368
VD	0.0
PW	3.0
RD	0.0
AQ	2.720
RG	8
NS	16
TE	300
DE	120.4
DR	12
DW	83
FW	7600
O2	50000.000
DP	63L PO
LB	0.0
GB	0.0
NC	-1
CX	20.00
CY	15.00
F1	8.001P
F2	2.001P
MI	0.0
DC	1.000
HZ/CM	150.033
PPM/CM	.300
IS	1
SR	7394.20

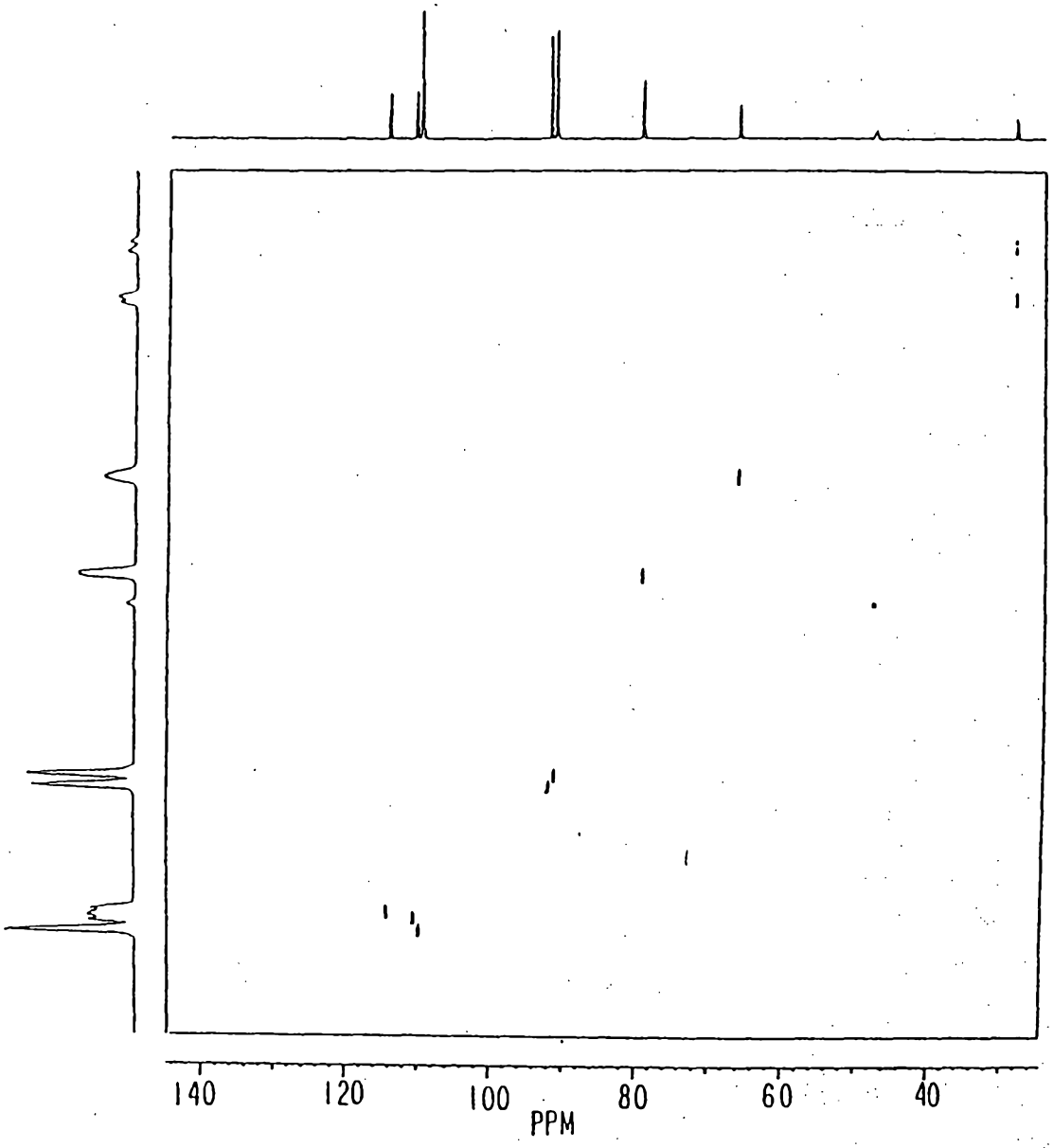


SOLVENT	CDCl3
SF	125.759
SFO	125.770
SFO2	500.130
SF2	125.759
SY	93.0
O1	3164.000
SI	65536
TD	65536
SW	29411.765
SW2	29411.765
HZ/PT	.898
VD	.0080
PW	0.0
RD	0.0
AQ	1.114
RG	100
NS	128
TE	300
DE	26.6
DR	12
DW	17
FW	36800
O2	9794.600
DP	30H PO
LB	1.000
GB	0.0
NC	2
CX	20.00
CY	7.00
F1	170.005P
F2	20.009P
MI	0.0
DC	1.000
HZ/CM	943.173
PPM/CM	7.500
IS	1
SR	-10527.08
D1	1.0000000

S1	30H
P9	110.00
D2	.0050000
S2	20H
P1	5.20
VD	.0080000
P2	10.40
RD	0.0
PW	0.0
DE	26.64
NS	128

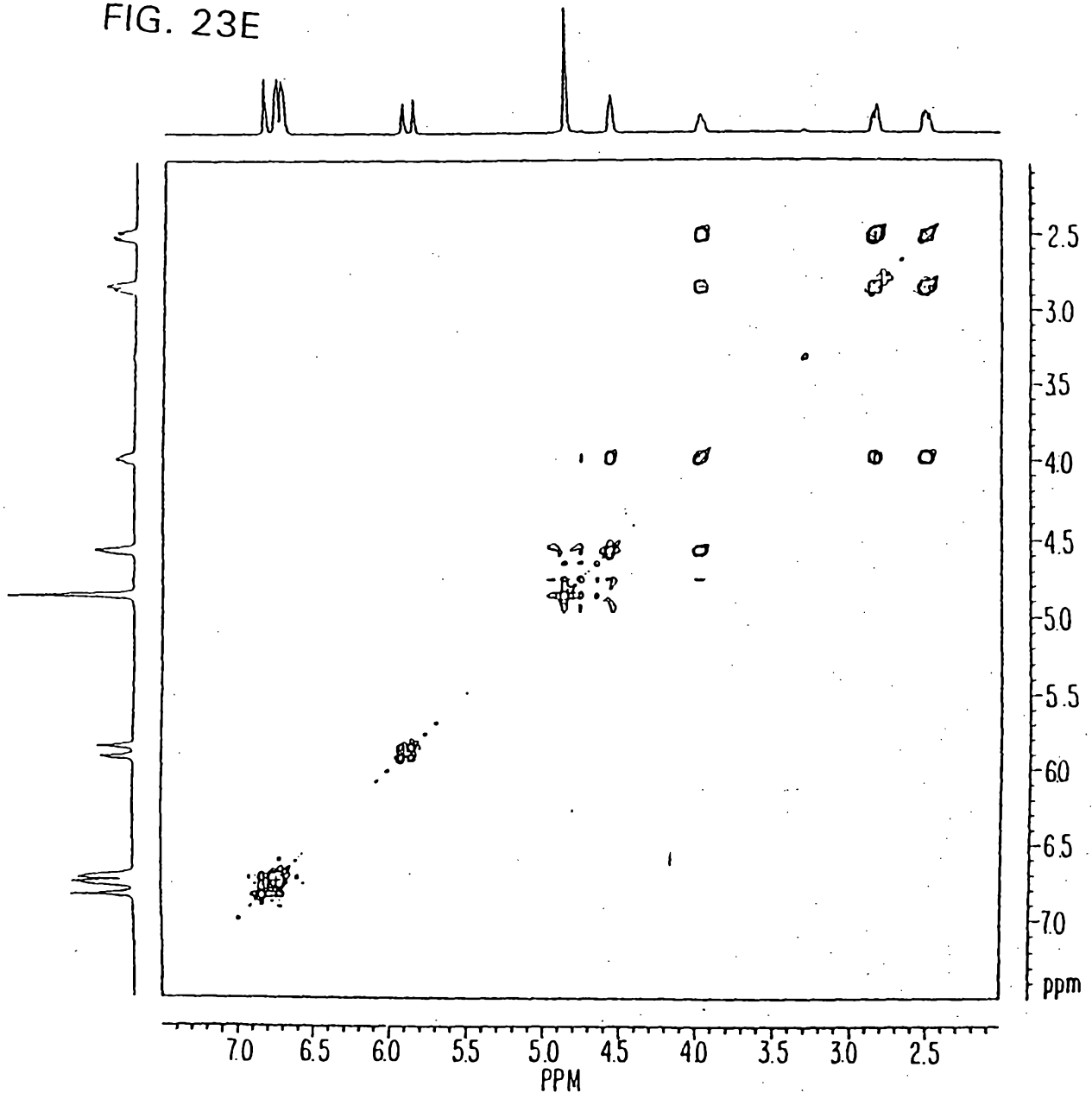
FIG. 23C

FIG. 23D



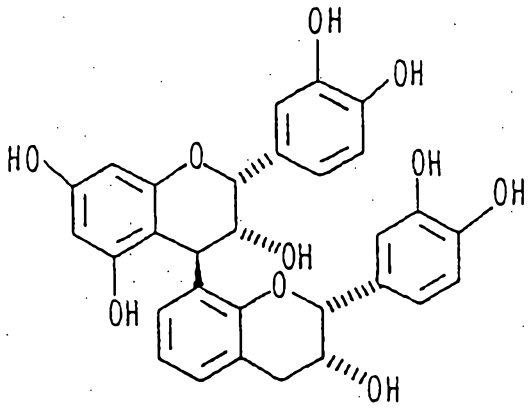
SI2	2048
SI1	512
SW2	16129.032
SW1	1375.138
NDO	2
WDW2	S
WDW1	S
SSB2	0
SSB1	0
MC2	M
PLIM ROW:	
F1	144.689P
F2	24.327P
AND COLUMN:	
F1	21.378P
F2	-.449P
D1	1.0000000
SI	0H
P1	10.50
D0	.0000030
P4	10.40
D3	.0040000
P3	5.20
D4	.0020000
S2	20H
RD	0.0
PW	0.0
DE	46.50
NS	32
DS	0
P9	110.00
NE	256
IN	.0001818

FIG. 23E



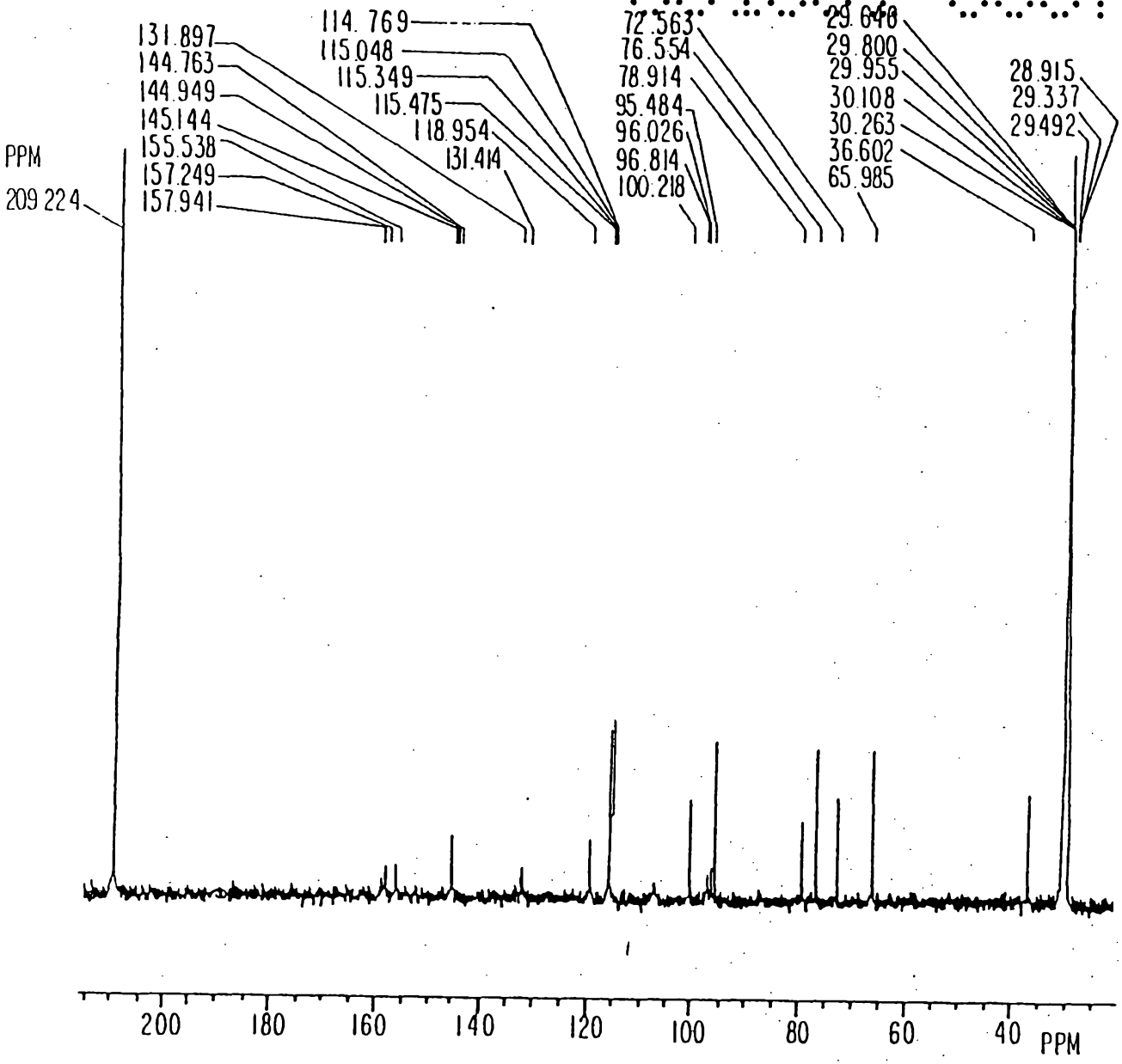

```
SI2     1024
SI1      512
SW2   2747.253
SW1   1373.626
NDO        1

WDW2       S
WDW1       S
SSB2       0
SSB1       0
MC2        M
PLIM ROW:
F1      7.496P
F2      2.014P
AND COLUMN:
F1      7.496P
F2      2.014P
DI      1.0000000
PI      10.50
DO       .0000030
P2      5.20
RD      0.0
PW      0.0
DE      260.90
NS      4
DS      0
NE      256
IN       .0003640
```



B2 DIMER			
^{1}H	CHEMICAL SHIFT (ppm)	^{13}C	CHEMICAL SHIFT (ppm)
B4	2.69 2.83	B4 T4	28.92 36.60
T4	4.63	B3	65.99
B3	4.29	T3	72.56
T3	3.85	T2	76.55
T2	4.99	B2	78.91
B2	4.92	T6 OR 8	95.48
B6	5.92	B6	96.03
T6 OR 8	5.91	T6 OR 8	96.81
T6 OR 8	5.98		100.22
B2'	7.12	B2'	114.77
T2'	6.92	T2'	115.05
T5 B5	6.68 6.70	T5' B5'	115.35 115.48
T6' B6'	6.87 6.58	T6' B6'	118.95
		T1'	131.41 131.90

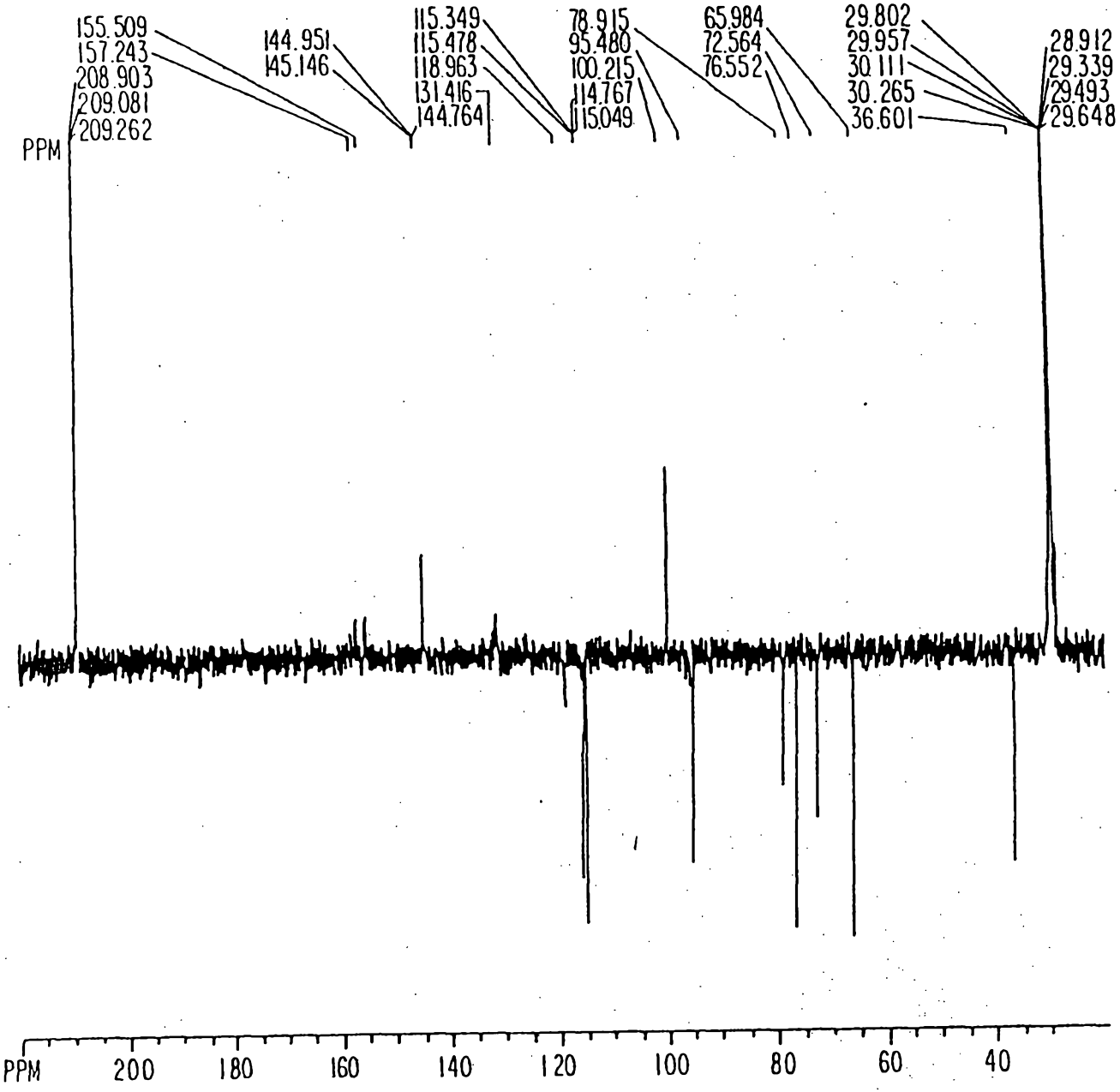
FIG. 24A



SA.NA GJ	
SA.NO TEMP 101	
SOLVENT D	
SF	125.760
SFO	125.770
SFO2	500.130
SF2	125.760
SY	93.0
O1	3316.000
SI	65536
TD	65536
SW	29411.765
SW2	29411.765
HZ/PT	.898
VD	.0080
PW	3.0
RD	0.0
AQ	1.114
RG	100
NS	4181
TE	300
DE	23.8
DR	12
DW	17
FW	36800
O2	105117.000
DP	20H CPD
LB	2.000
GB	0.0
NC	8
CX	20.00
CY	3.00
F1	215.00 P
F2	20.00 P
MI	.00
DC	1.000
HZ/CM	1.226E3
PPM/CM	9.750
IS	2

SR	-10278.05
D1	.10000000
P9	110.00
SI	30H
D2	.0050000
S2	20H
RD	0.0
PW	3.00
DE	23.80
NS	4181
DS	1

FIG. 24B

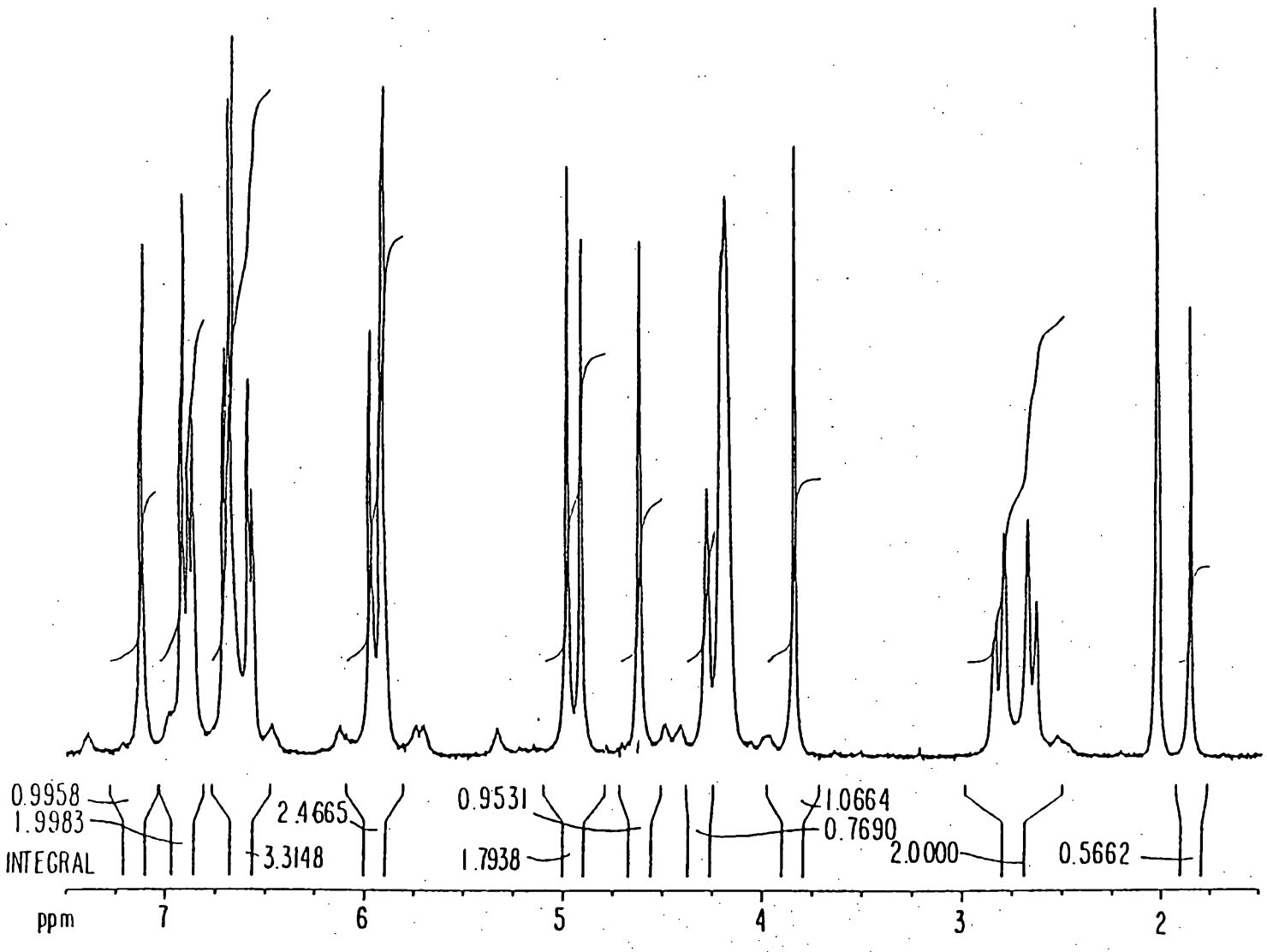


SOLVENT	CDCl3
SF	125.760
SFO	125.770
SFO2	500.130
SF2	125.760
SY	93.0
O1	3316.000
SI	65536
TD	65536
SW	29411.765
SW2	29411.765
HZ/PT	.898
VD	.0080
PW	2.6
RD	0.0
AQ	1.114
RG	100
NS	4096
TE	300
DE	23.8
DR	12
DW	17
FW	36800
O2	10517.000
DP	30H D0
LB	2.000
GB	0.0
NC	7
CX	20.00
CY	5.00
F1	220.000P
F2	20.001P
MI	.00
DC	1.000
HZ/CM	1.257E3
PPM/CM	10.000
IS	1
SR	-10278.05
D1	1.0000000

S1	30H
P9	110.00
D2	.0050000
S2	20H
P1	5.20
VD	.0080000
P2	10.40
RD	0.0
PW	0.0
DE	23.80
NS	4096

FIG. 24C

FIG. 24D



F2-ACQUISITION PARAMET

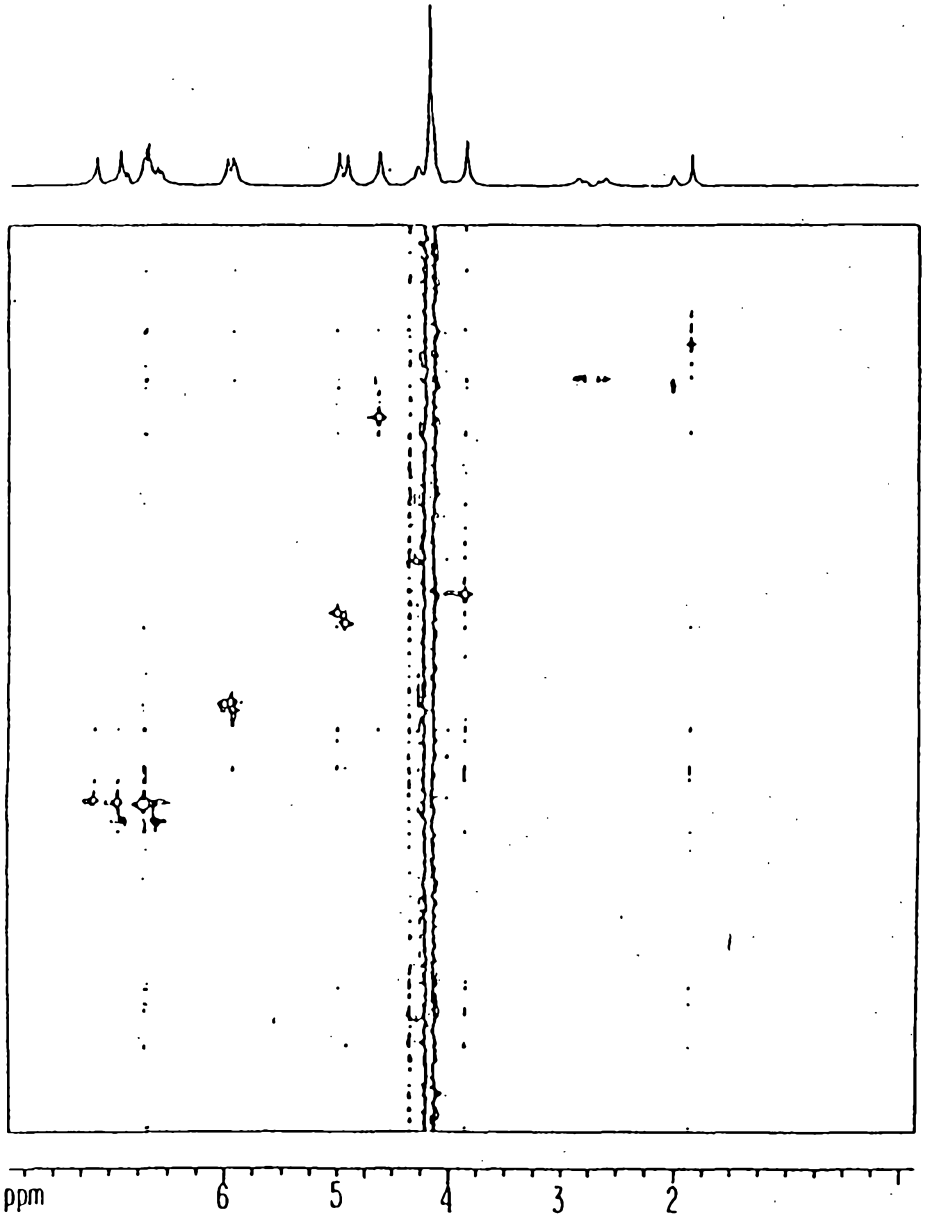
PULPROG	zgpr
SOLVENT	
AQ	3.7683384
FIDRES	0.132685
DW	115.0
RG	128
NUCLEUS	1H
HL1	1
D12	0.0000200
HL2	76
P18	1000000.0
D13	0.0000040
P1	5.0
DE	164.3
SFO1	360.1373784
SWH	4347.83
TD	32768
NS	16
DS	0

F2-PROCESSING PARAMETERS

SI	16384
SF	360.1358580
WDW	no
SSB	0
LB	0.00
GB	0
PC	1.00

1D NMR PLOT PARAMETERS

CX	22.00
FIP	7.500
FI	2701.02
F2P	1.500
F2	540.20
PPMCM	0.27273
HZCM	98.21887

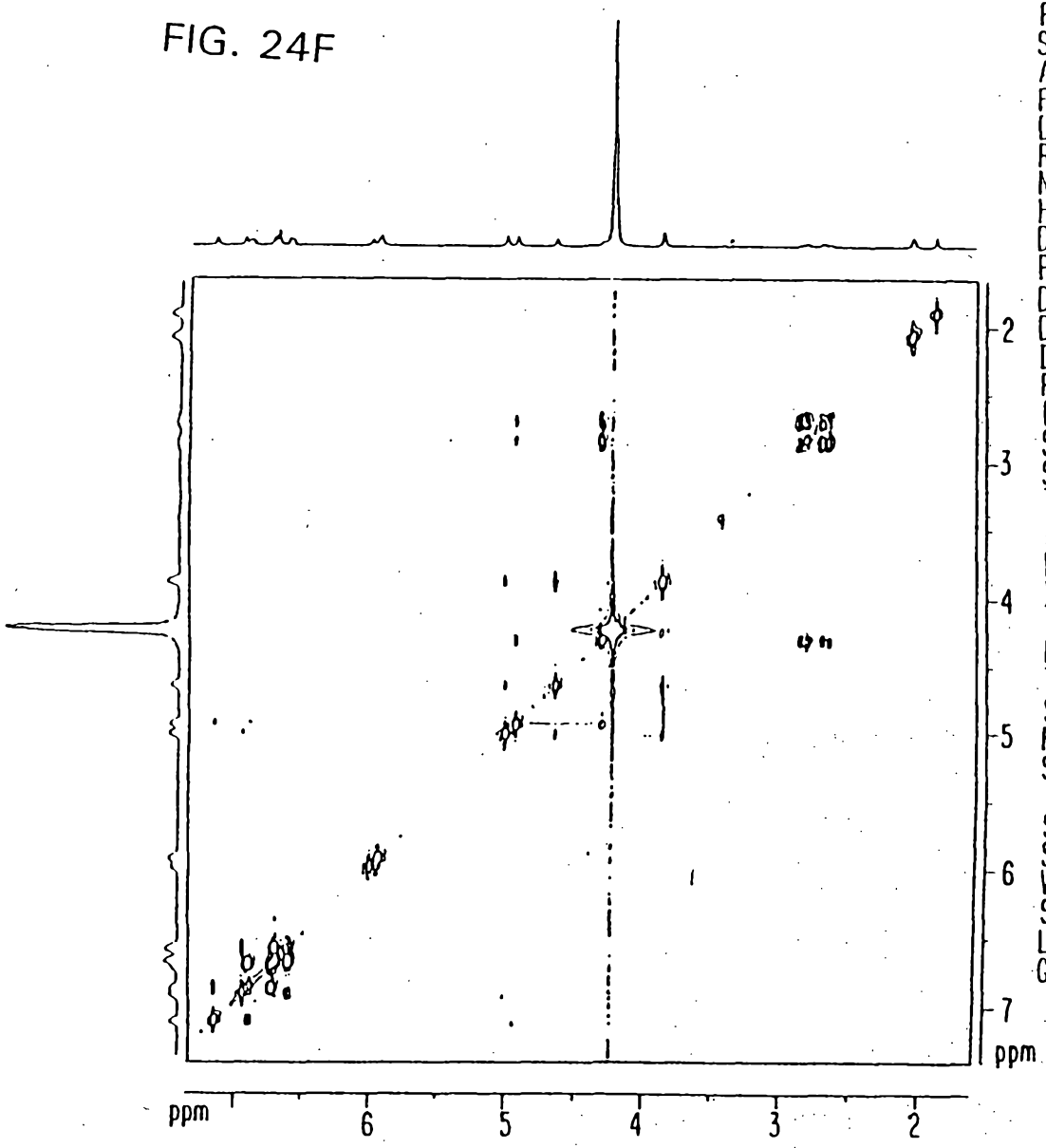


PULPROG	inv4	
SOLVENT	dioxane	
AQ	0.3543240	sec
FIDRES	1.411217	Hz
DW	173.0	usec
RG	256	
NUCLEUS	1H	
P1	7.5	usec
CNST2	145.0000000	
HL1	1	dB
D1	1.0000000	sec
D2	0.0034483	sec
P3	13.5	usec
SFO2	90.5643220	MHz
D0	0.0000030	sec
P2	15.0	usec
D13	0.0000040	sec
DL0	17.00	dB
DE	247.1	usec
SFO1	360.1372552	MHz
SWH	2890.17	Hz
TD	2048	
P31	65.0	usec
NS	16	
DS	4	
IN0	0.0000300	sec
F1-ACQUISITION PARAMETERS		
NDO	2	
TD	256	
SFO1	90.56254	MHz
FIDRES	65.091827	Hz
SW	184.000	ppm
F2-PROCESSING PARAMETERS		
SI	2048	
SF	360.1358599	MHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	
PC	1.40	

F1-PROCESSING PARAMETERS		
SI	2048	
MC2	QF	
SF	90.5543832	MHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	
2D NMR PLOT PARAMETERS		
CX2	12.29	cm
CX1	12.29	cm
F2PLO	7.887	ppm
F2LO	2840.39	Hz
F2PHI	-0.138	ppm
F2HI	-49.78	Hz
F1PLO	182.085	ppm
F1LO	16488.57	Hz
F1PHI	-1.932	ppm
F1HI	-174.94	Hz
F2PPMCM	0.65299	ppm/cm
F2HZCM	235.16463	Hz/cm
F1PPMCM	14.97287	ppm/cm
F1HZCM	1355.8593	Hz/cm

FIG. 24E

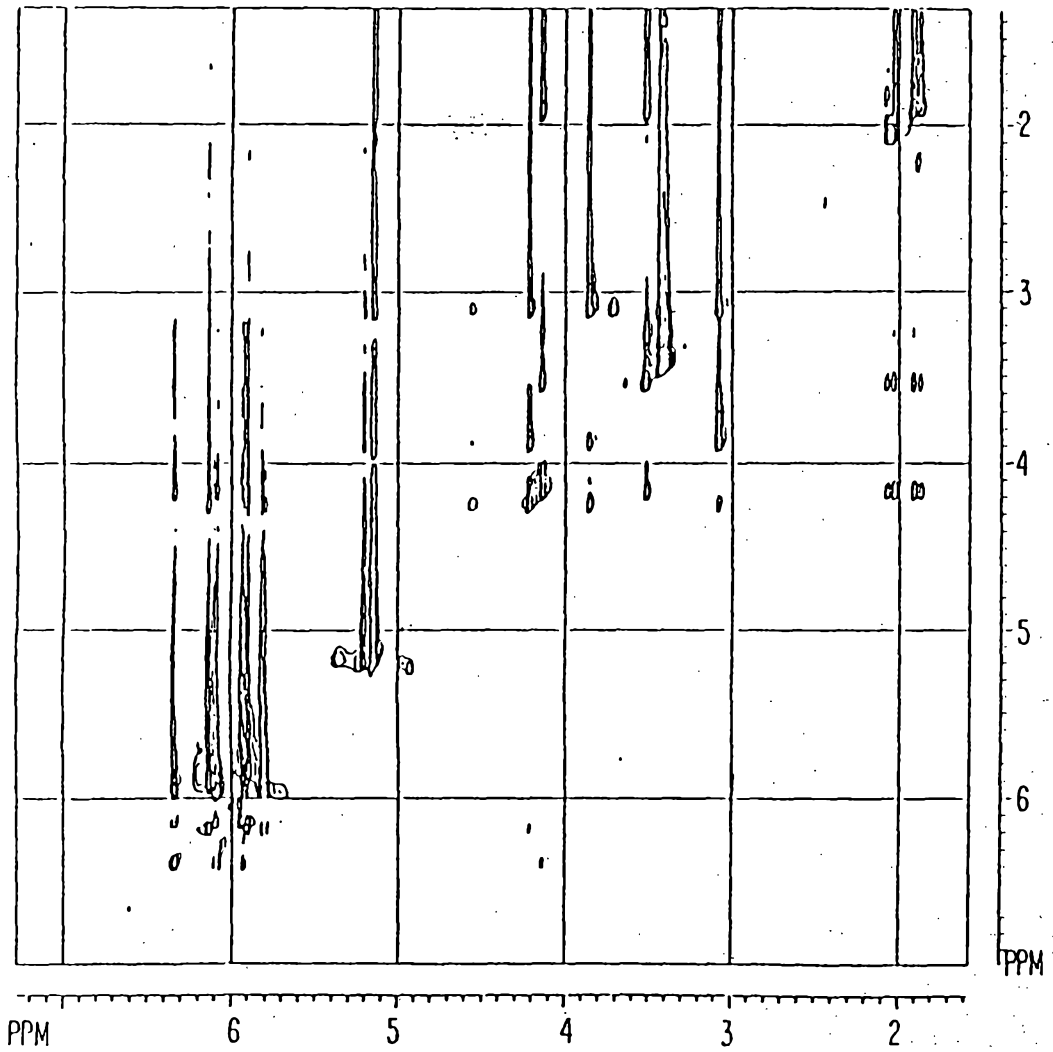
FIG. 24F



PULPROG	cosygr.ssp	
SOLVENT	DMSO	
AQ	0.3543240	sec
FIDRES	1.411217	Hz
DW	173.0	usec
RG	128	
NUCLEUS	1H	
HLI	1	dB
D1	1.0000000	sec
P1	7.5	usec
D0	0.0000030	sec
D31	0.0010000	sec
D20	0.0005000	sec
P0	3.8	usec
DE	247.1	usec
SFO1	360.1372311	Hz
SWH	2890.17	Hz
TD	2048	
NS	4	
DS	4	
INO	0.0003460	sec
F1-ACQUISITION PARAMETERS		
NDO	1	
TD	256	
SFO1	360.1372	
FIDRES	11.289726	
SW	8.025	ppm
F2-PROCESSING PARAMETERS		
SI	2048	
SF	360.1358488	MHz
WDW	SINE	
SSB	0	
LB	0.00	Hz
GB	0	

F1-PROCESSING PARAMETERS		
SI	1024	
MC2	QF	
SF	360.1357777	MHz
WDW	SINE	
SSB	0	
LB	0.00	Hz
GB	0	
2D NMR PLOT PARAMETERS		
CX2	12.29	cm
CX1	12.29	cm
F2PLO	7.334	ppm
F2LO	2641.09	Hz
F2PHI	1.597	ppm
F2HI	575.06	Hz
F1PLO	7.412	ppm
F1LO	2669.31	Hz
F1PHI	1.659	ppm
F1HI	597.64	Hz
F2PPMCM	0.46669	ppm/cm
F2HZCM	168.07179	Hz/cm
F1PPMCM	0.46796	ppm/cm
F1HZCM	168.53079	Hz/cm

FIG. 24G



PULPROG		
SOLVENT		
AQ	0.3543240	sec
FIDRES	1.411217	Hz
DW	173.0	usec
RG	128	
NUCLEUS	1H	
P6	52.0	usec
HL1	1	dB
DI1	0.0300000	sec
DI2	0.0000200	sec
HL2	76	dB
P18	1000000.0	usec
DI3	0.0000040	sec
P1	7.5	usec
DO	0.0000030	sec
HL3	17	dB
P17	2500.0	usec
P7	104.0	usec
LI	56	
P5	34.7	usec
DE	247.1	usec
SFO1	360.13744	MHz
SWH	2890.17	Hz
TD	2048	
NS	8	
DS	4	
INO	0.0001730	sec
F1-ACQUISITION PARAMETERS		
NDO	2	
TD	128	
SFO1	360.1373	MHz
FIDRES	22.579477	Hz

SW	8.025	ppm
F2-PROCESSING PARAMETERS		
SI	1024	
SF	360.13677	MHz
WDW	SINE	
SSB	4	
LB	0.00	Hz
GB	0	
PC	1.40	
F1-.PROCESSING PARAMETERS		
SI	1024	
MC2	TPPI	
SF	360.1360418	MHz
WDW	SINE	
SSB	4	
LB	0.00	Hz
GB	0	
2D NMR PLOT PARAMETERS		
CX2	15.00	cm
CX1	15.00	cm
F2PLO	7.283	ppm
F2LO	2622.72	Hz
F2PH1	1.577	ppm
F2H1	567.99	Hz
F1PLO	6.990	ppm
F1LO	2517.31	Hz
F1PH1	1.331	ppm
F1H1	479.51	Hz
F2PPMCM	0.38036	ppm/cm
F2HZCM	136.98227	Hz/cm
F1PPMCM	0.37723	ppm/cm
F1HZCM	135.85324	Hz/cm

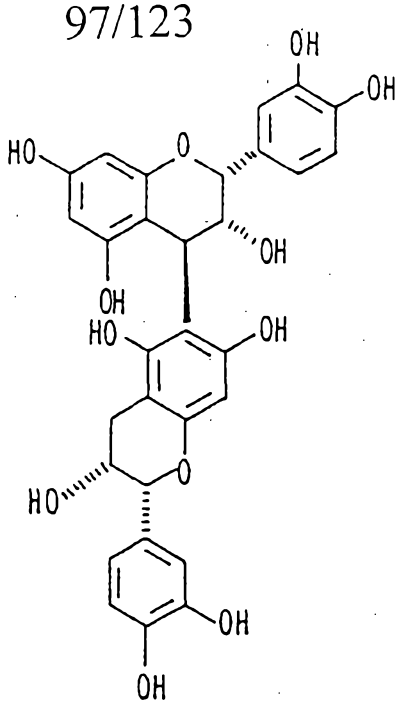
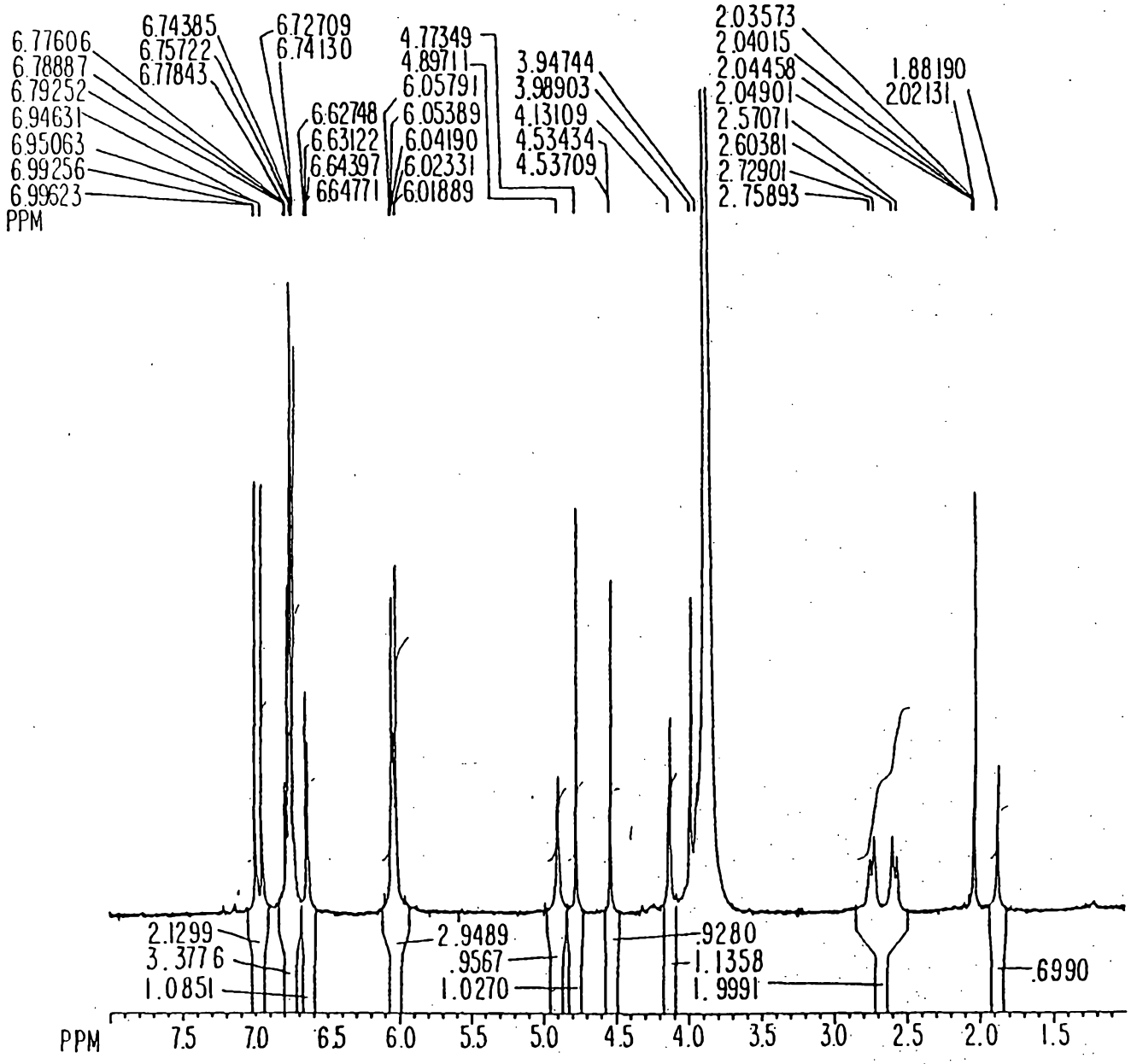


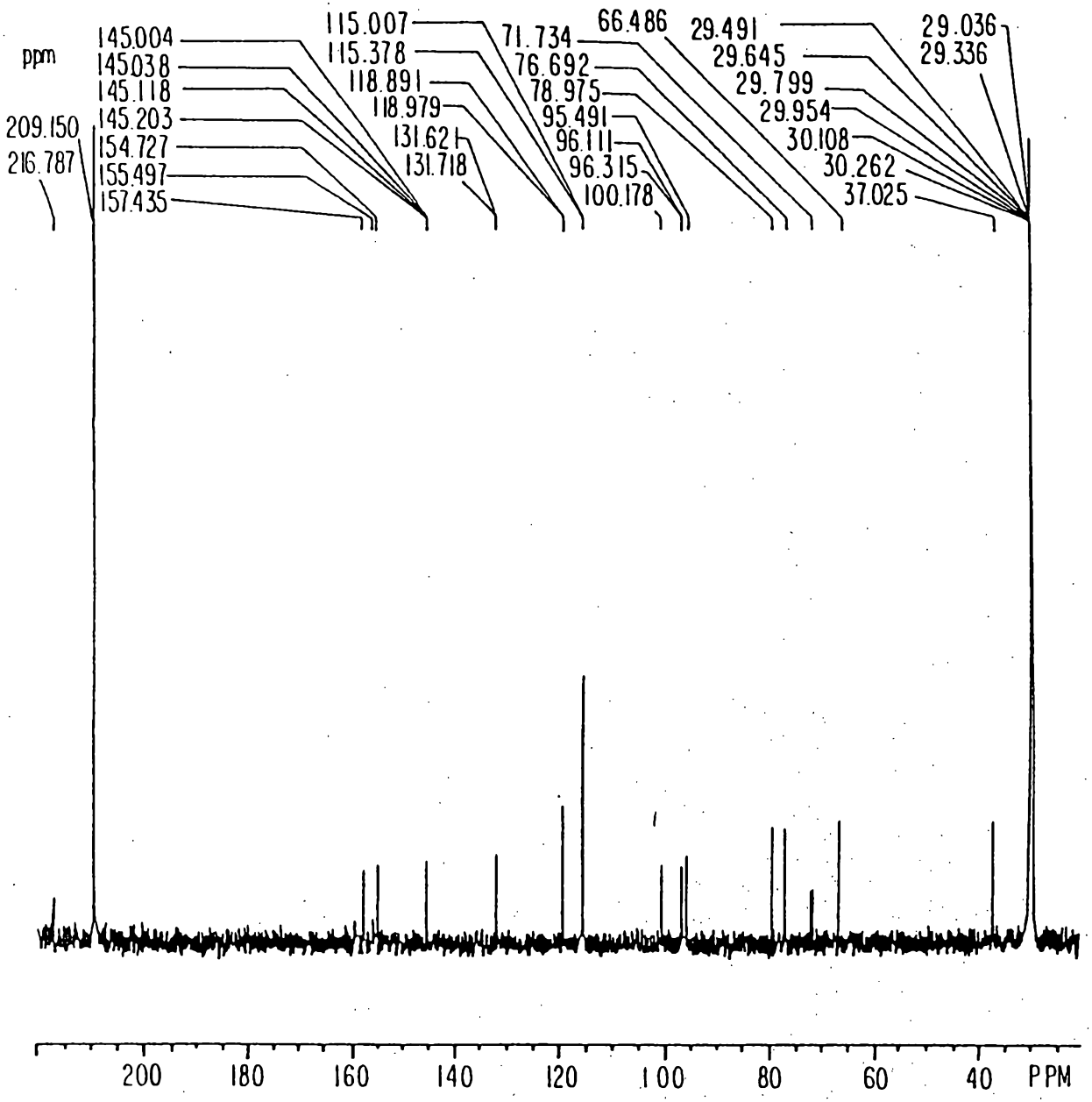
FIG. 25A

B5 DIMER			
^{1}H	CHEMICAL SHIFT (ppm)	^{13}C	CHEMICAL SHIFT (ppm)
B4	2.585 7.745	B4 T4	29.04 37.03
T3	3.99	B3	66.49
B3	4.13	T3	71.73
T4	4.54	T2	76.69
B2	4.77	B2	78.98
T2	4.90	B6+8	95.49
T6+	6.02		96.11
T8	6.055	T6+8	96.32
B8	6.04		100.18
T6'	6.64	T2'+5'	115.01
T5'	6.74	B2'+5'	115.38
B5'	6.75	B6	118.90
B6'	6.78	T6	118.98
T2'	6.95	B1'	131.62
B2'	6.99	T1'	131.72
		B & T	145.00
		3' + 4'	145.04 145.04 145.12 145.20
		B + T 5, 7 + 8a	154.73 155.50 157.44



SOLVENT	CDCl3
SF	500.138
SFO	500.130
SFO2	500.130
SF2	500.138
SY	166.0
O1	10517.000
SI	32768
TD	32768
SW	6024.096
SW2	6024.096
HZ/PT	.368
VD	0.0
PW	3.0
RD	0.0
AQ	2.720
RG	8
NS	64
TE	300
DE	106.3
DR	12
DW	83
FW	7600
O2	50000.000
DP	63L PO
LB	0.0
GB	0.0
NC	1
CX	20.00
CY	12.00
FI	8.000P
F2	1.00
MI	.00
DC	1.000
HZ/CM	175.035
PPM/CM	.350
IS	1
SR	8023.42

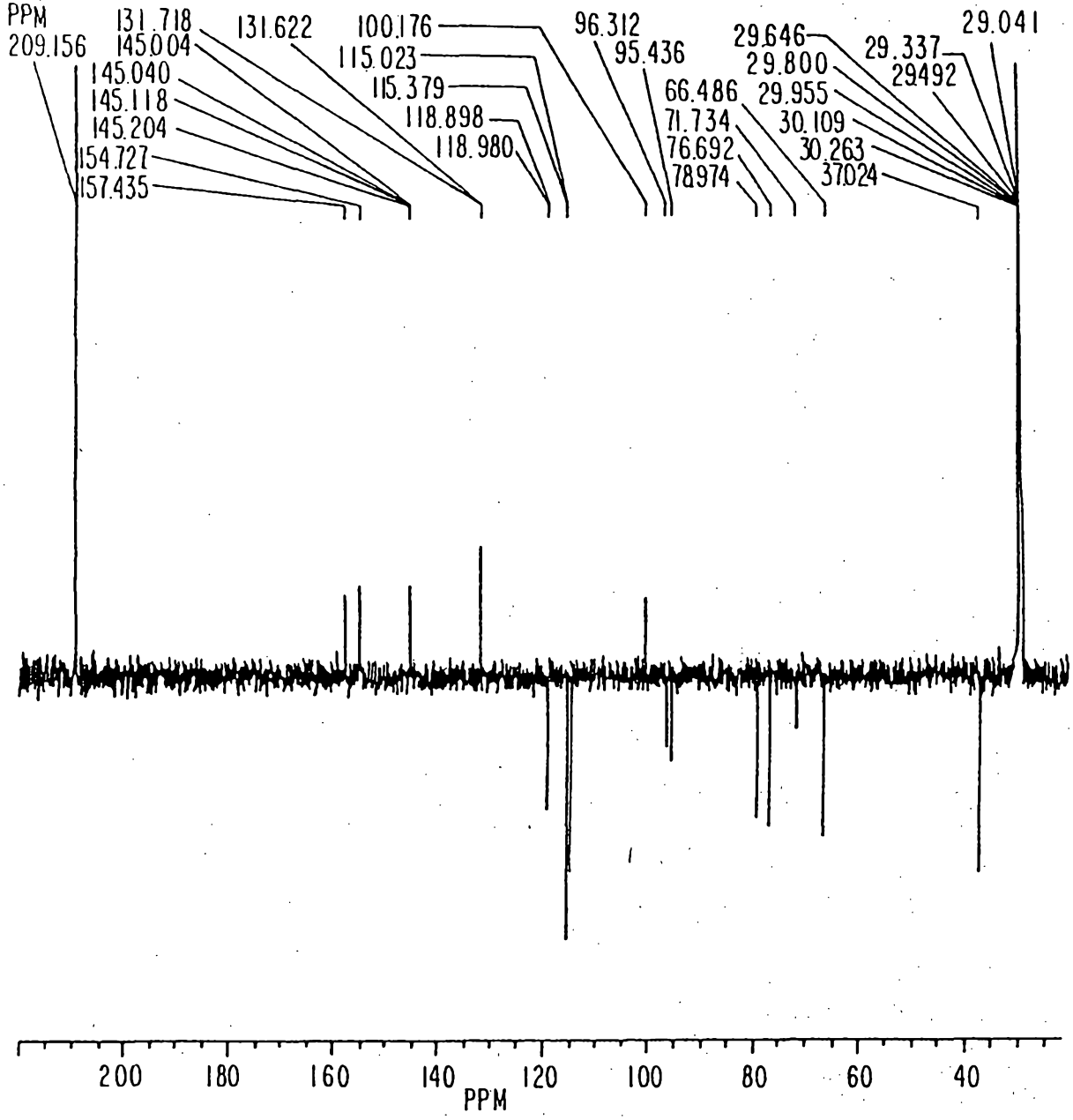
FIG. 25B



SOLVENT	CDCl3
SF	125.760
SFO	125.770
SFO2	500.130
SF2	125.760
SY	93.0
O1	3316.000
SI	65536
TD	65536
SW	29411.765
SW2	29411.765
HZ/PT	.898
VD	.0080
PW	3.0
RD	0.0
AQ	1.114
RG	100
NS	4096
TE	300
DE	23.8
DR	12
DW	17
FW	36800
O2	10517.000
DP	30H PO
LB	2.000
GB	0.0
NC	7
CX	20.00
CY	5.00
FI	220.000P
F2	20.001P
MI	.00
DC	1.000
HZ/CM	1.257E3
PPM/CM	10.000
IS	1
SR	-10277.15
DI	.10000000

P9	110.00
SI	30H
D2	.0050000
S2	20H
RD	0.0
PW	3.00
DE	23.80
NS	4096
DS	1

FIG. 25C

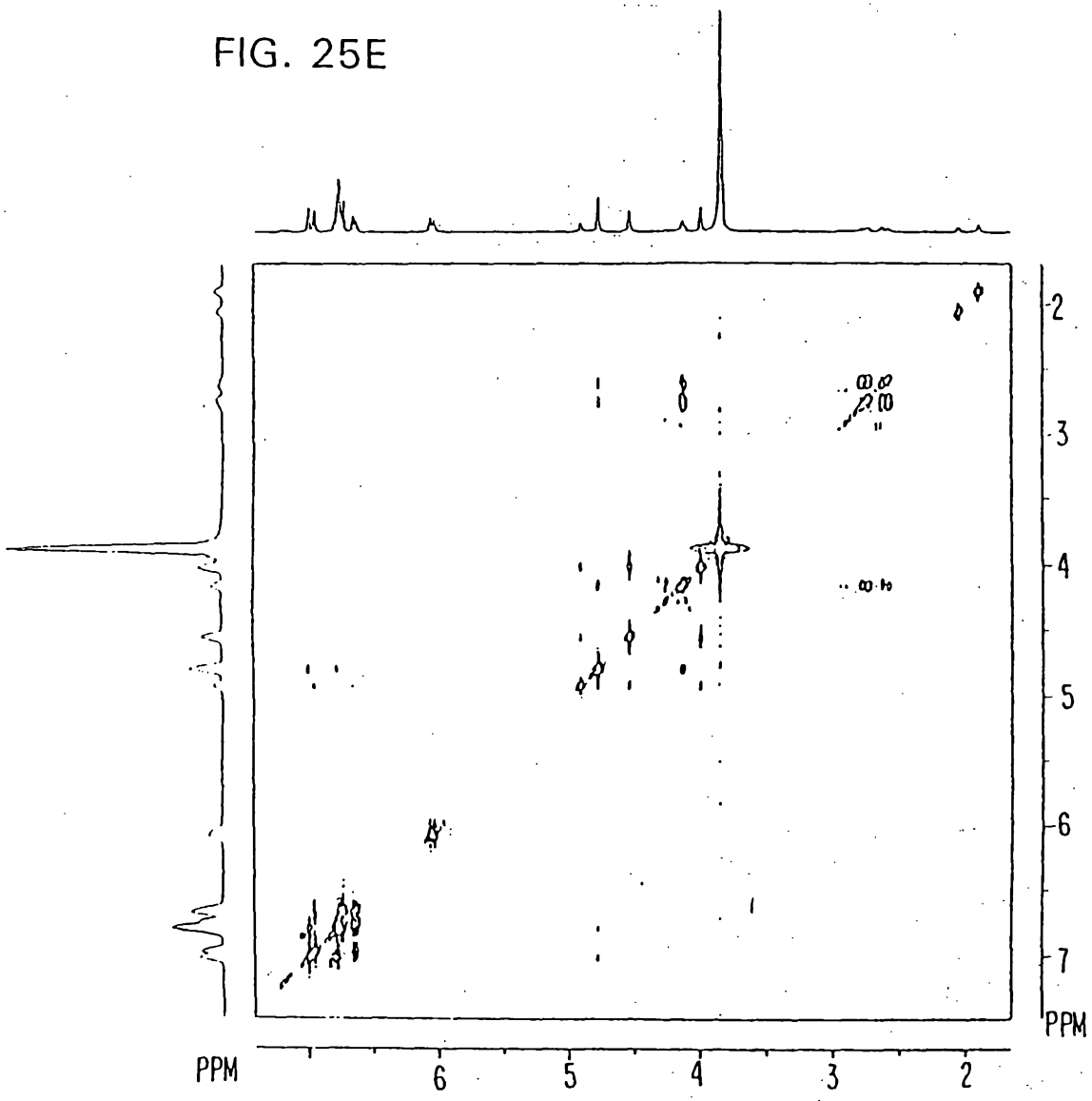


SOLVENT	CDCl3
SF	125.760
SFO	125.770
SFO2	500.130
SF2	125.760
SY	93.0
O1	3316.000
SI	65536
TD	65536
SW	29411.765
SW2	29411.765
HZ/PT	.898
VD	.0080
PW	0.0
RD	0.0
AQ	1.114
RG	100
NS	4096
TE	300
DE	23.8
DR	12
DW	17
FW	36800
O2	10517.000
DP	30H CPD
LB	2.000
GB	0.0
NC	6
CX	20.00
CY	5.00
FI	220.000P
F2	20.001P
MI	.00
DC	1.000
HZ/CM	1.257E3
PPM/CM	10.000
IS	1
SR	-10277.15
DI	1.0000000

SI	30H
P9	110.00
D2	.0050000
S2	20H
PI	5.20
VD	.0080000
P2	10.40
RD	0.0
PW	0.0
DE	23.80
NS	4096
DS	1

FIG. 25D

FIG. 25E



PULPROG	cosygr.ssp
SOLVENT	DMSO
AQ	0.3543240 sec
FIDRES	1.411217 Hz
DW	173.0 usec
RG	128
NUCLEUS	1H
HL1	1 dB
D1	1.0000000 sec
P1	7.5 usec
D0	0.0000030 sec
D31	0.0010000 sec
D20	0.0005000 sec
P0	3.8 sec
DE	247.1 usec
SFO1	360.1372440 MHz
SWH	2890.17 Hz
TD	2048
NS	4
DS	4
INO	0.0003460 sec

F1-ACQUISITION PARAMETERS

NDO	1
TD	256
SFO1	360.1372 MHz
FIDRES	11.289726 Hz
SW	8.025 ppm

F2-PROCESSING PARAMETERS

SI	2048
SF	360.135841 WHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

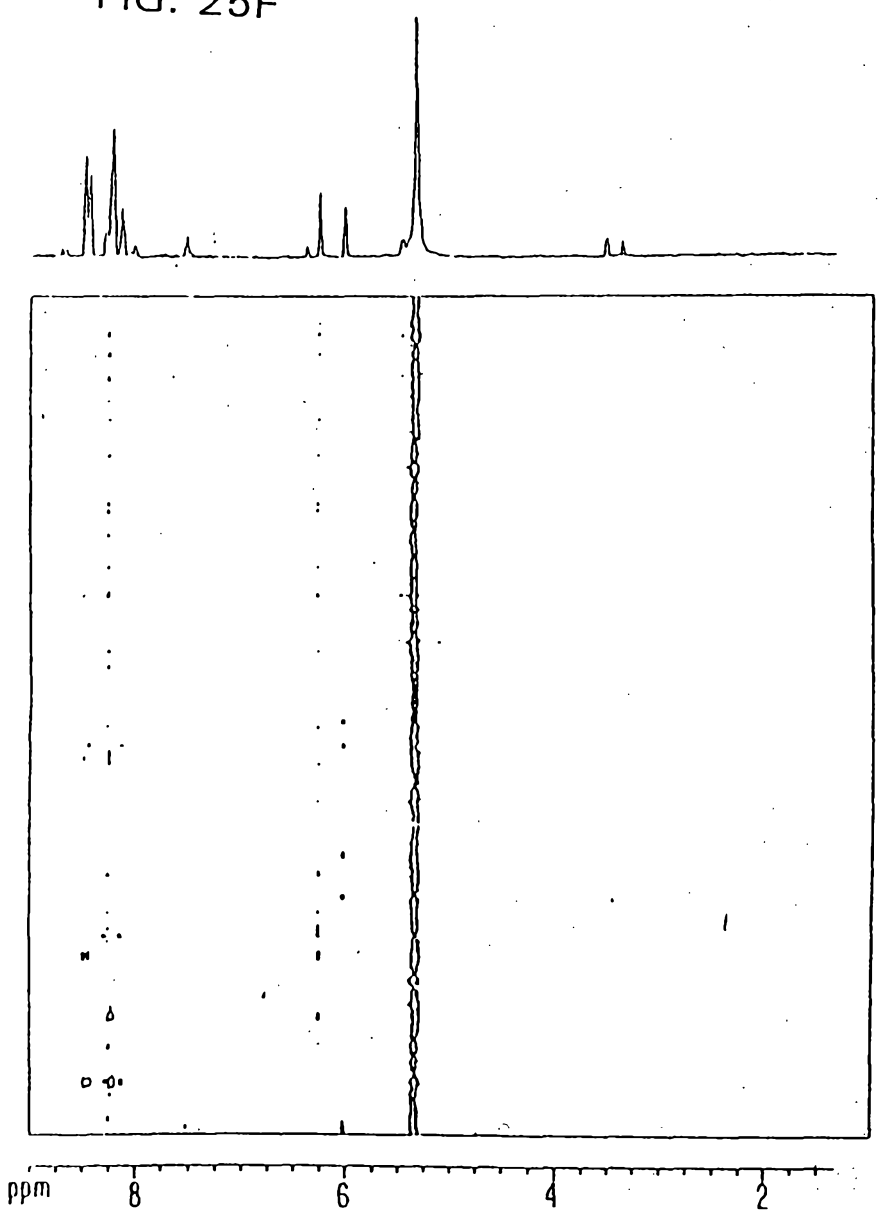
F1-PROCESSING PARAMETERS

SI	1024
MC2	QF
SF	360.1357694 MHz
WDW	SINE
SSB	0
LB	0.00 Hz
GB	0

2D NMR PLOT PARAMETERS

CX2	12.29 cm
CX1	12.29 cm
F2PL0	7.417 ppm
F2L0	2671.25 Hz
F2PH1	1.649 ppm
F2H1	593.94 Hz
F1PL0	7.467 ppm
F1L0	2688.96 Hz
F1PH1	1.683 ppm
F1H1	606.00 Hz
F2PPMCM	0.46924 ppm/cm
F2HZCM	168.99025 Hz/cm
F1PPMCM	0.47051 ppm/cm
F1HZCM	169.44925 Hz/cm

FIG. 25F



PULPROG	inv4ipirnd
SOLVENT	dioxane
AQ	0.3543240 sec
FIDRES	1.411217 Hz
DW	173.0 usec
RG	128
NUCLEUS	1H
P1	7.5 usec
CNST2	145.0000000
HL1	1 dB
D1	1.0000000 sec
D2	0.0034483 sec
P3	13.5 usec
SFO2	90.5625400 MHz
D6	0.0680000
D0	0.0000030 sec
P2	15.0 usec
DE	247.1 usec
SFO1	360.1372440 MHz
SWH	2890.17 Hz
TD	2048
NS	16
DS	4
IN0	0.0000300 sec

F1-ACQUISITION PARAMETERS

ND0	2
TD	256
SFO1	90.56254 MHz
FIDRES	65.091827 Hz
SW	184.000 ppm

F2-PROCESSING PARAMETERS

SI	1024
SF	360.1353214 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.40

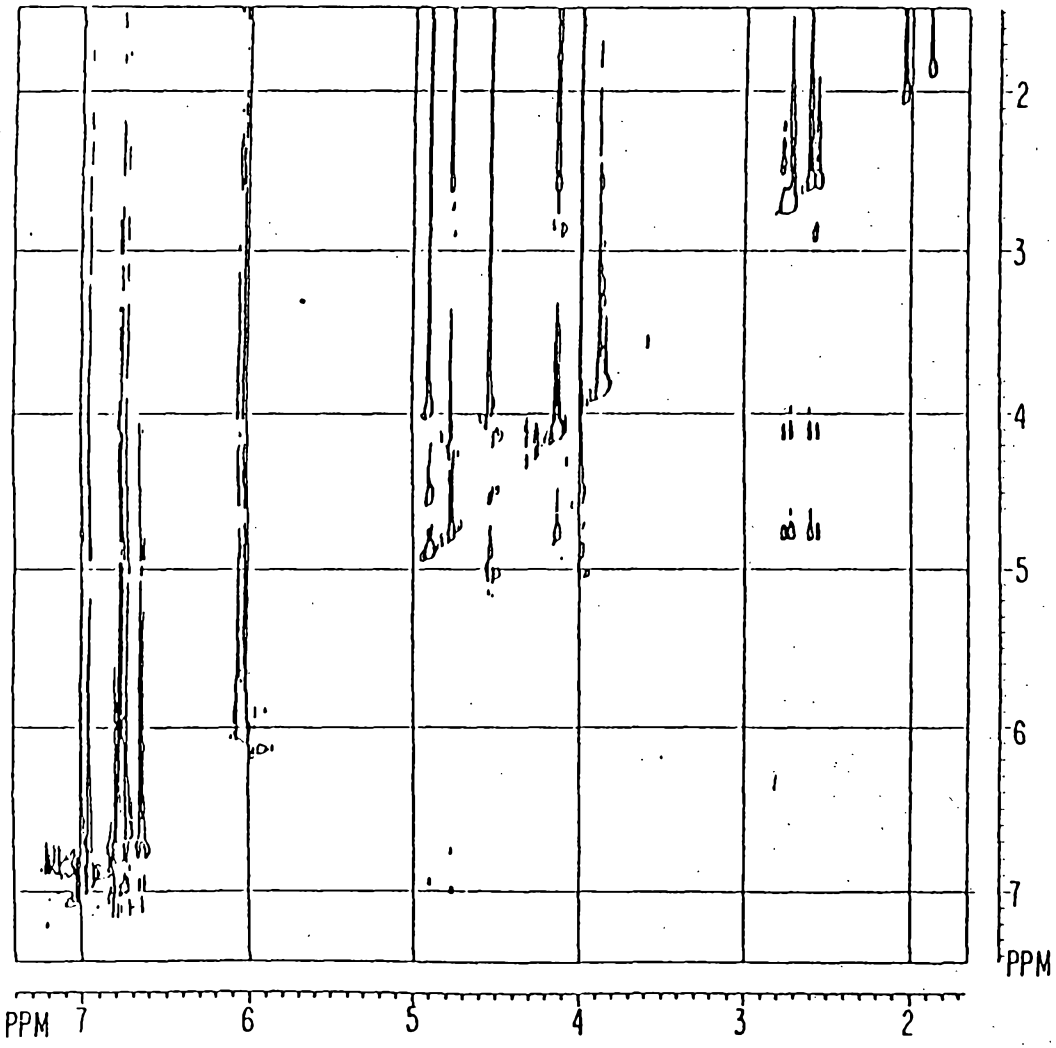
F1-PROCESSING PARAMETERS

SI	1024
MC2	QF
SF	90.5539705 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

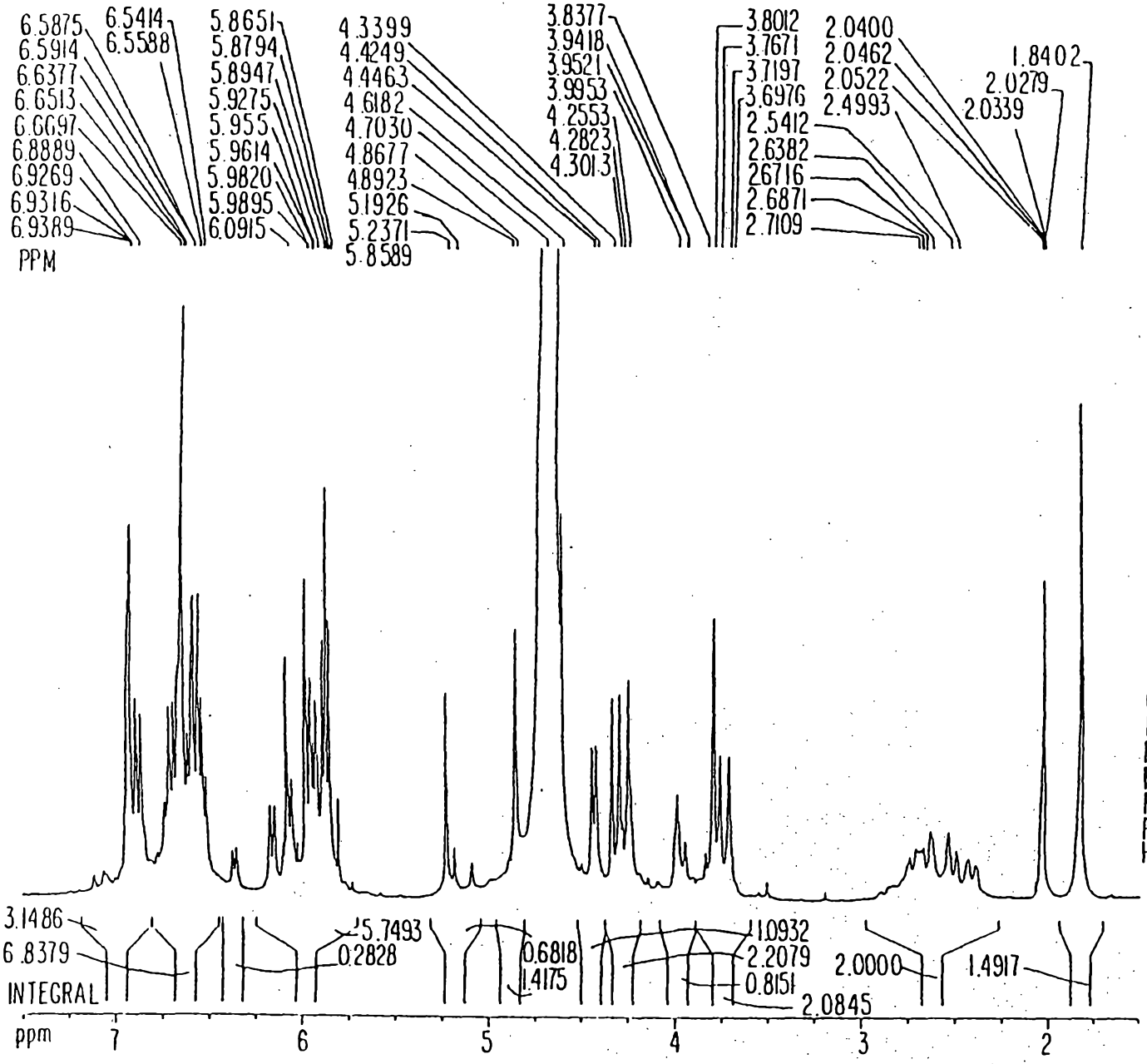
2D NMR PLOT PARAMETERS

CX2	13.19 cm
CX1	13.19 cm
F2PLO	9.000 ppm
F2LO	3241.22 Hz
F2PH1	1.000 ppm
F2H1	359.99 Hz
F1PLO	180.642 ppm
F1LO	16357.88 Hz
F1PH1	8.626 ppm
F1H1	781.12 Hz
F2PPMCM	0.60644 ppm/cm
F2HZCM	218.39912 Hz/cm
F1PPMCM	13.03894 ppm/cm
F1HZCM	1180.72815 Hz/cm

FIG. 25G



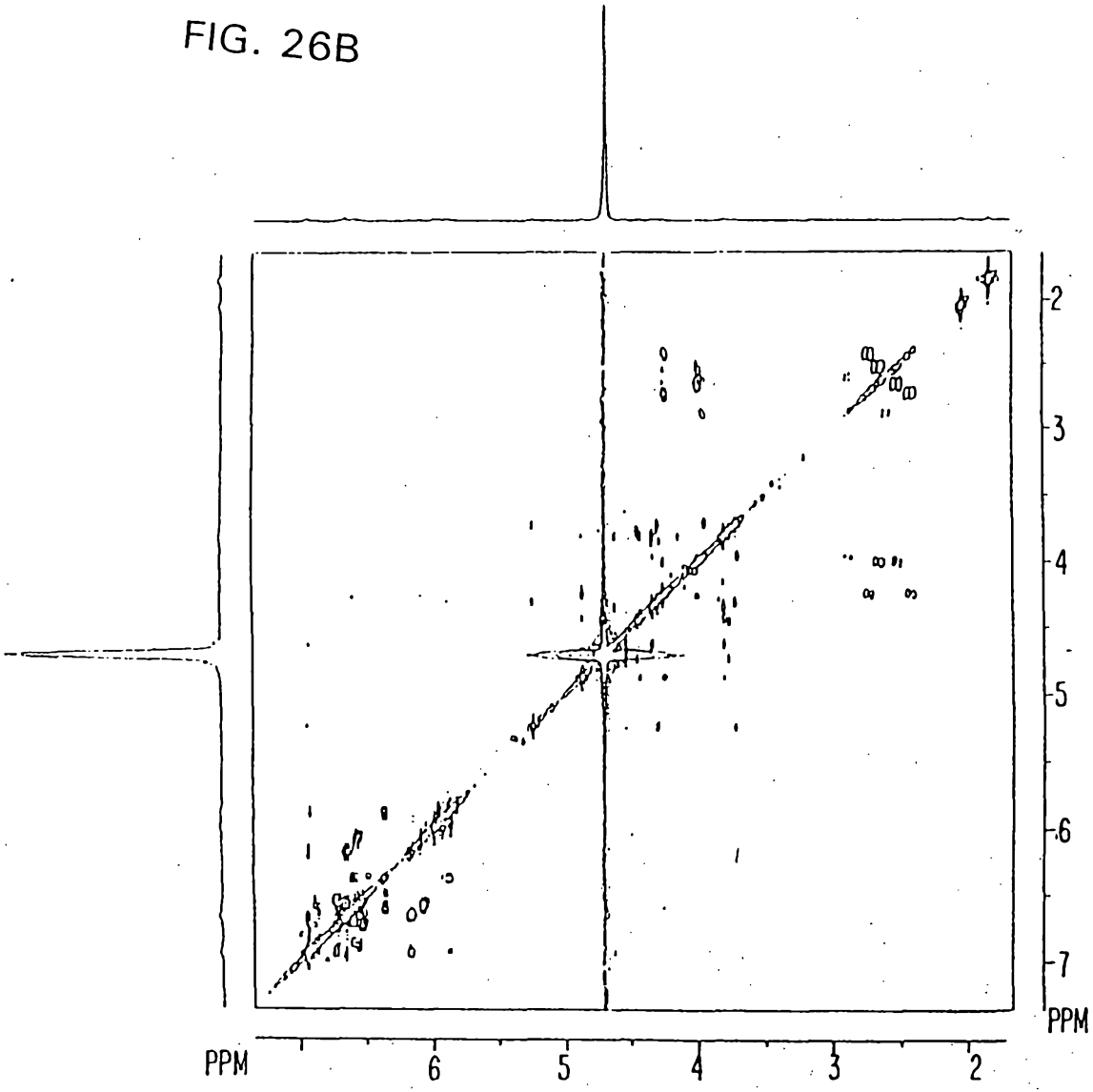
PULPROG	mlevprtp	
SOLVENT	MeOH	
AQ	0.3543240	sec
FIDRES	1.411217	Hz
DW	173.0	usec
RG	256	
NUCLEUS	1H	
P6	52.0	usec
HL1	1	dB
D11	0.0300000	sec
D12	0.0000200	sec
HL2	76	dB
P18	10000000.0	usec
D13	0.0000040	sec
P1	7.5	usec
D0	0.0000030	sec
HL3	17	dB
P17	2500.0	usec
P7	104.0	usec
L1	56	
P5	34.7	usec
DE	247.1	usec
SFO1	360.1372552	MHz
SWH	2890.17	
TD	2048	
NS	8	
DS	4	
INO	0.0001730	sec
F1-ACQUISITION PARAMETERS		
NDO	2	
TD	128	
SFO1	360.1373	MHz
FIDRES	22.579477	Hz
SW	8.025	ppm
F2-PROCESSING PARAMETERS		
SI	1024	
SF	360.1358581	MHz
WDW	SINE	
SSB	4	
LB	0.00	Hz
GB	0	
PC	1.40	
F1-PROCESSING PARAMETERS		
SI	1024	
MC2	TPPI	
SF	360.1358964	MHz
WDW	SINE	
SSB	4	
LB	0.00	Hz
GB	0	
2D NMR PLOT PARAMETERS		
CX2	15.00	cm
CX1	15.00	cm
F2PLO	7.406	ppm
F2LO	2667.20	Hz
F2PHI	1.654	ppm
F2HI	595.53	Hz
F1PLO	7.444	ppm
F1LO	2681.01	Hz
F1PHI	1.473	ppm
F1HI	530.31	Hz
F2PPMCM	0.38350	ppm/cm
F2HZCM	138.11116	Hz cm
F1PPMCM	0.39813	ppm cm
F1HZCM	143.37968	Hz/cm




```
PULPROG          zg
SOLVENT
AQ          3.7683377
FIDRES      0.132685
DW          115.0
RG          64
NUCLEUS     1H
HL1         1
D1          1.0000000
PI          5.0
DE          164.3
SFO1        360.1374401
SWH         4347.83
TD          32768
NS          64
DS          0
F2-PROCESSING PARAMETERS
SI          32768
SF          360.1358584
WDW         no
SSB         0
LB          0.00
GB          0
PC          1.00
1D NMR PLOT PARAMETERS
CX          22.00
F1P         7.500
F1          2701.02
F2P         1.500
F2          540.20
PPMCM       0.27273
HZCM        98.21887
```

FIG. 26A

FIG. 26B



PULPROG	cosygr.ssp	
SOLVENT	DMSO	
AQ	0.3543240	sec
FIDRES	1.411217	Hz
DW	173.0	usec
RG	128	
NUCLEUS	1H	
HL1	1	dB
D1	1.0000000	sec
P1	7.5	usec
D0	0.0000030	sec
D31	0.0010000	sec
D20	0.0005000	sec
P0	3.8	sec
DE	247.1	usec
SFO1	360.1374919	MHz
SWH	2890.17	Hz
TD	2048	
NS	8	
DS	4	
INO	0.0003460	sec

F1-ACQUISITION PARAMETERS

NDO	1	
TD	256	
SFO1	360.1372	MHz
FIDRES	11.289726	Hz
SW	8.025	ppm

F2-PROCESSING PARAMETERS

SI	2048	
SF	360.1358548	WHz
WDW	SINE	
SSB	0	
LB	0.00	Hz
GB	0	
PC	1.40	

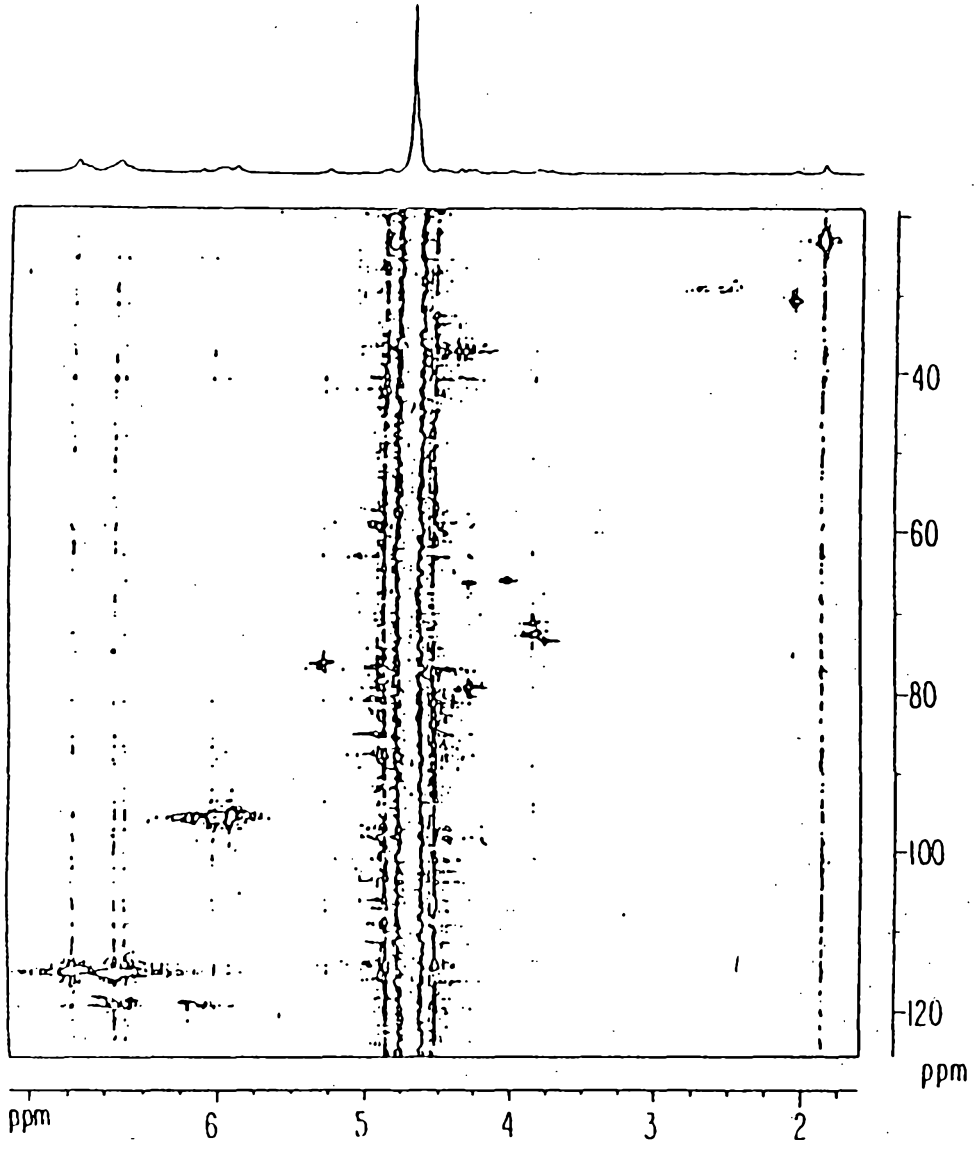
F1-.PROCESSING PARAMETERS

SI	1024	
MC2	QF	
SF	360.1355256	MHz
WDW	SINE	
SSB	0	
LB	0.00	Hz
GB	0	

2D NMR PLOT PARAMETERS

CX2	12.29	cm
CX1	12.29	cm
F2PLO	7.351	ppm
F2LO	2647.53	Hz
F2PH1	1.677	ppm
F2H1	604.09	Hz
F1PLO	7.360	ppm
F1LO	2650.44	Hz
F1PH1	1.638	ppm
F1H1	590.06	Hz
F2PPMCM	0.46159	ppm/cm
F2HZCM	166.23495	Hz/cm
F1PPMCM	0.46541	ppm/cm
F1HZCM	167.61238	Hz/cm

FIG. 26C



PULPROG	inv4	
SOLVENT	dioxane	
AQ	0.3543240	sec
FIDRES	1.411217	Hz
DW	173.0	usec
RG	128	
NUCLEUS	1H	
P1	7.5	usec
CNST2	145.0000000	
HL1	1	dB
D1	1.0000000	sec
D2	0.0034483	sec
P3	13.5	usec
SF02	90.5625400	MHz
D0	0.0000030	sec
P2	15.0	usec
D13	0.0000040	sec
DL0	17.00	dB
DE	247.1	usec
SF01	360.1374901	WHz
SWH	2890.17	Hz
TD	2048	
P31	65.0	usec
NS	16	
DS	4	
IN0	0.0000300	sec
F1-ACQUISITION PARAMETERS		
ND0	2	
TD	512	
SF01	90.56254	WHz
FIDRES	32.545914	Hz
SW	184.000	ppm
F2-PROCESSING PARAMETERS		
SI	2048	
SF	360.1358480	WHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	
PC	1.40	

F1-PROCESSING PARAMETERS		
SI	2048	
MC2	QF	
SF	90.5561565	WHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	
2D NMR PLOT PARAMETERS		
CX2	12.29	cm
CX1	12.29	cm
F2PL0	7.397	ppm
F2L0	2663.83	Hz
F2PH1	1.613	ppm
F2H1	580.87	Hz
F1PL0	125.840	ppm
F1L0	11395.56	Hz
F1PH1	19.098	ppm
F1H1	1729.42	Hz
F2PPMCM	0.47061	ppm/cm
F2HZCM	140.46389	Hz cm
F1PPMCM	6.64526	ppm cm
F1HZCM	786.50421	Hz/cm

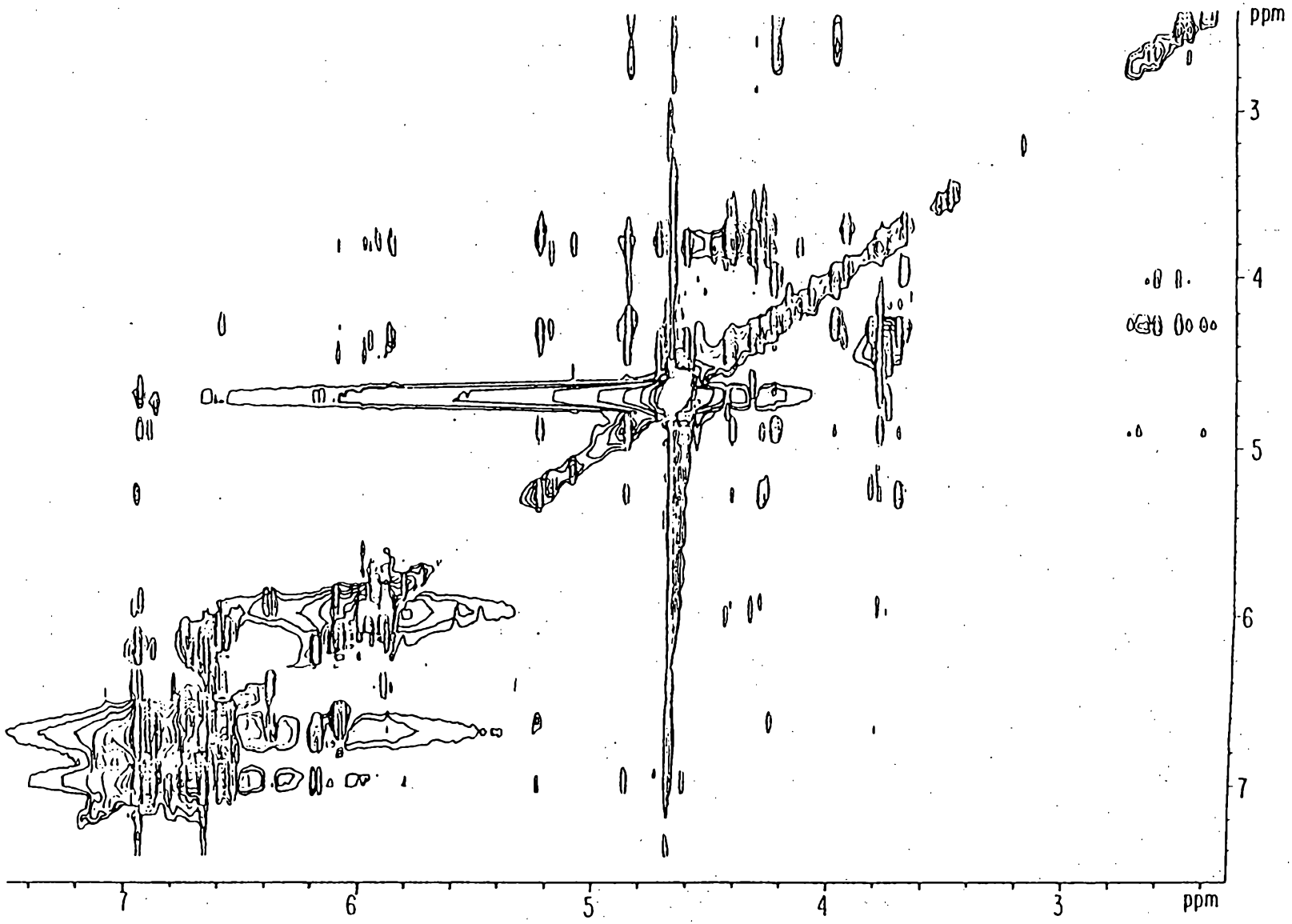
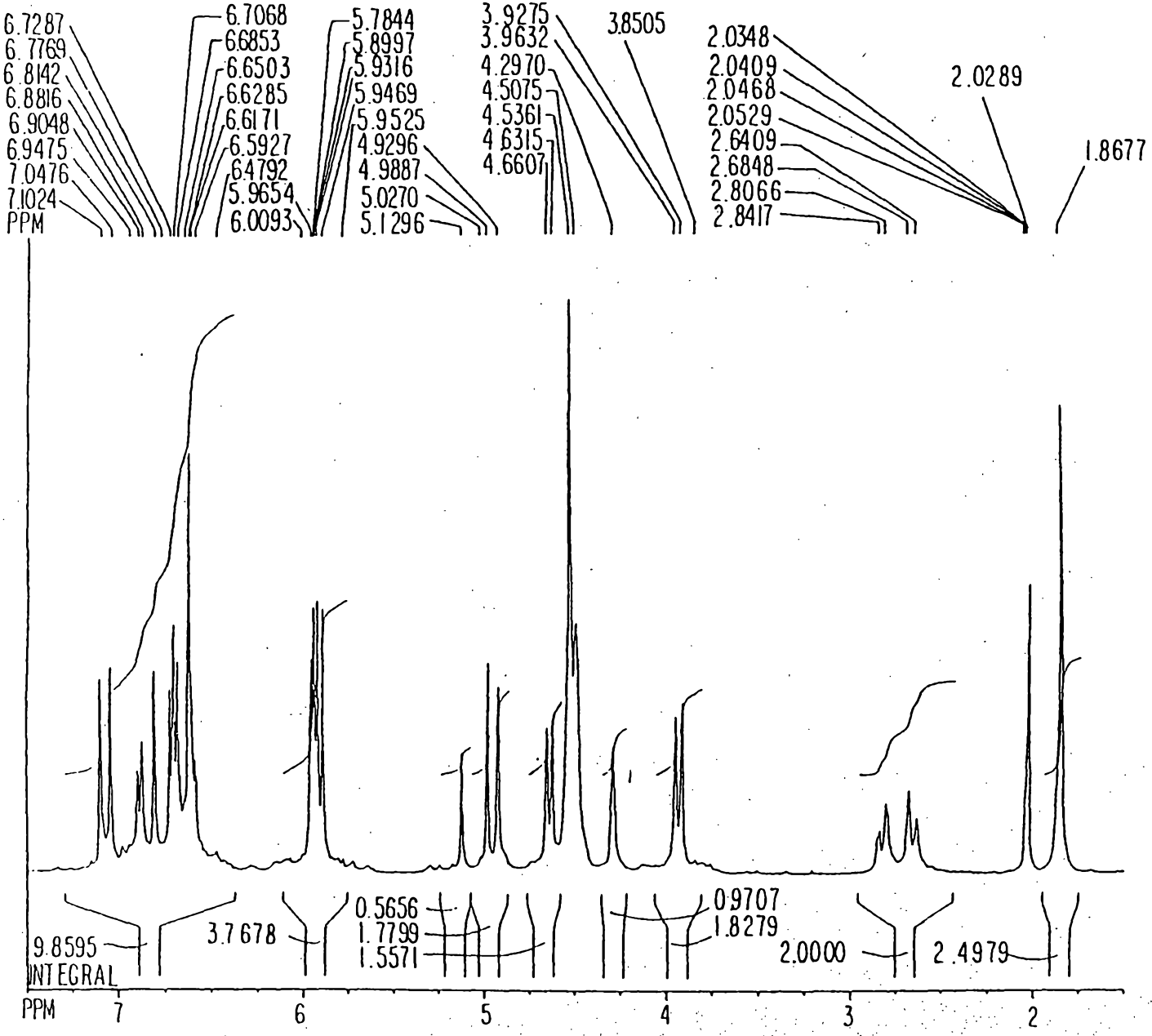


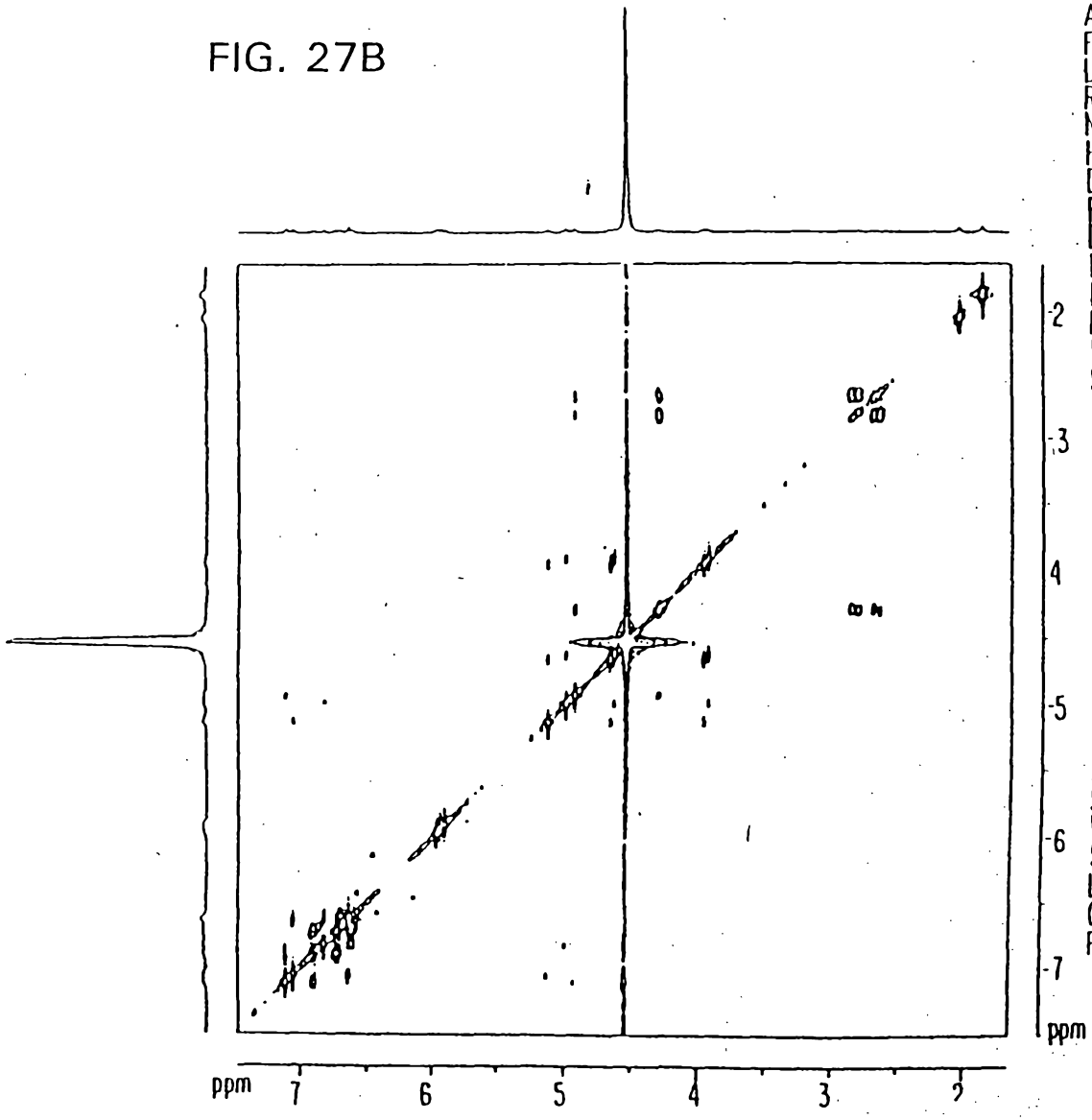
FIG. 26D




```
PULPROG        zgpr
SOLVENT
AQ          3.7683372
FIDRES      0.132685
DW          115.0
RG          128
NUCLEUS     1H
HL1         1
DI2         0.0000200
HL2         76
P18         1000000.0
DI3         0.0000040
P1          5.0
DE          164.3
SFO1        360.1374888
SWH         4347.83
TD          32768
NS          64
DS          0
F2-PROCESSING PARAMETER
SI          32768
SF          360.1358577
WDW         no
SSB         0
LB          0.00
GB          0
PC          1.00
1D NMR PLOT PARAMETERS
CX          22.00
F1P         7.500
F1          2701.02
F2P         1.500
F2          540.20
PPMCM       0.27273
HZCM        98.21887
```

FIG. 27A

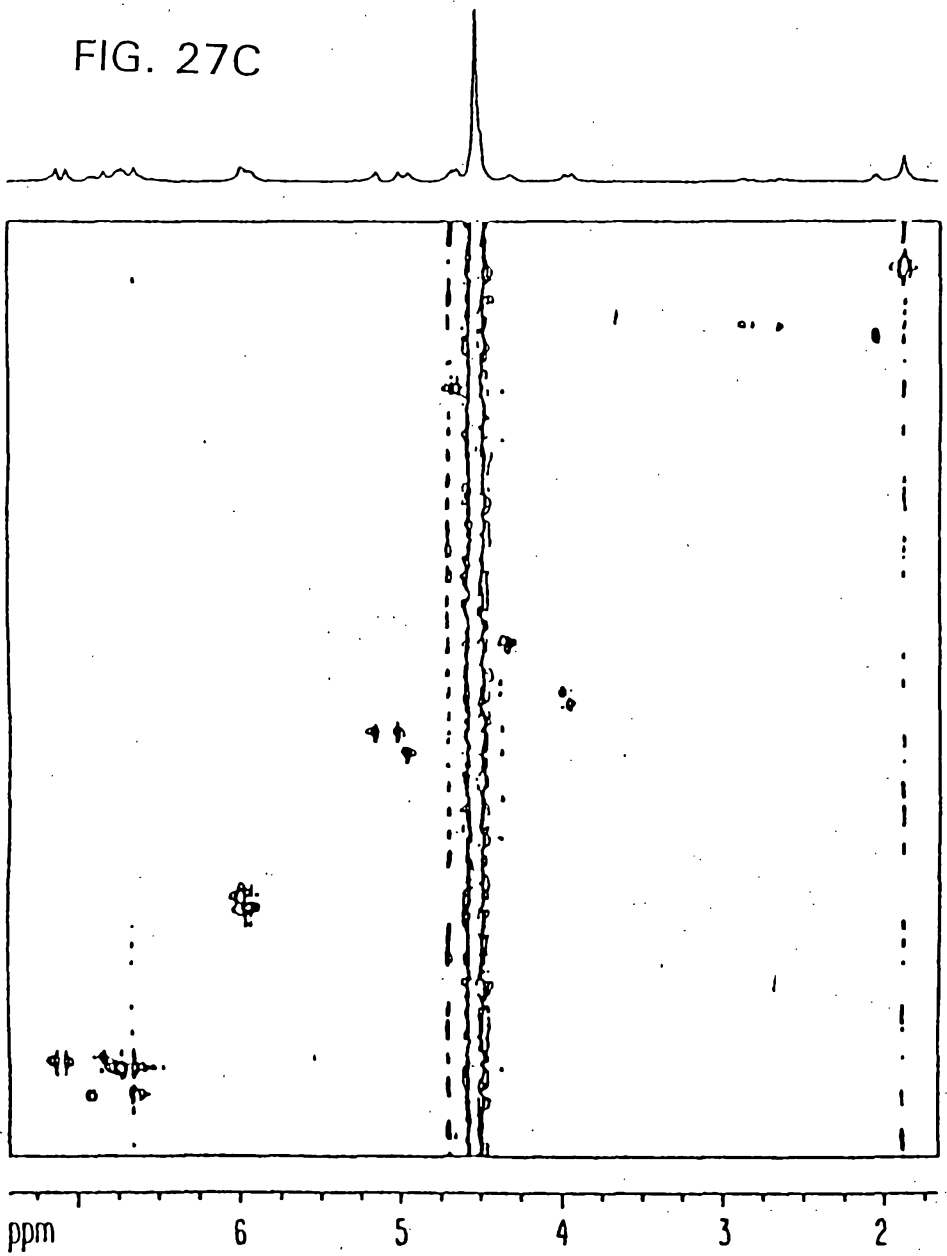
FIG. 27B



PULPROG	cosygr.ssp	
SOLVENT	DMSO	
AQ	0.3543240	sec
FIDRES	1.411217	Hz
DW	173.0	usec
RG	128	
NUCLEUS	1H	
HL1	1	dB
D1	1.0000000	sec
P1	7.5	usec
D0	0.0000030	sec
D31	0.0010000	sec
D20	0.0005000	sec
P0	3.8	sec
DE	247.1	usec
SFO1	360.1374919	MHz
SWH	2890.17	Hz
TD	2048	
NS	8	
DS	4	
INO	0.0003460	sec
F1-ACQUISITION PARAMETERS		
NDO	1	
TD	256	
SFO1	360.1372	MHz
FIDRES	11.289726	Hz
SW	8.025	ppm
F2-PROCESSING PARAMETERS		
SI	2048	
SF	360.1358548	MHz
WDW	SINE	
SSB	0	
LB	0.00	Hz
GB	0	
PC	1.40	

F1-PROCESSING PARAMETERS		
SI	1024	
MC2	QF	
SF	360.1355256	MHz
WDW	SINE	
SSB	0	
LB	0.00	Hz
GB	0	
2D NMR PLOT PARAMETERS		
CX2	12.29	cm
CX1	12.29	cm
F2PLO	7.477	ppm
F2LO	2692.69	Hz
F2PHI	1.662	ppm
F2HI	598.44	Hz
F1PLO	7.501	ppm
F1LO	2701.24	Hz
F1PHI	1.638	ppm
F1HI	590.06	Hz
F2PPMCM	0.47307	ppm/cm
F2HZCM	170.36786	Hz/cm
F1PPMCM	0.47689	ppm/cm
F1HZCM	171.74516	Hz/cm

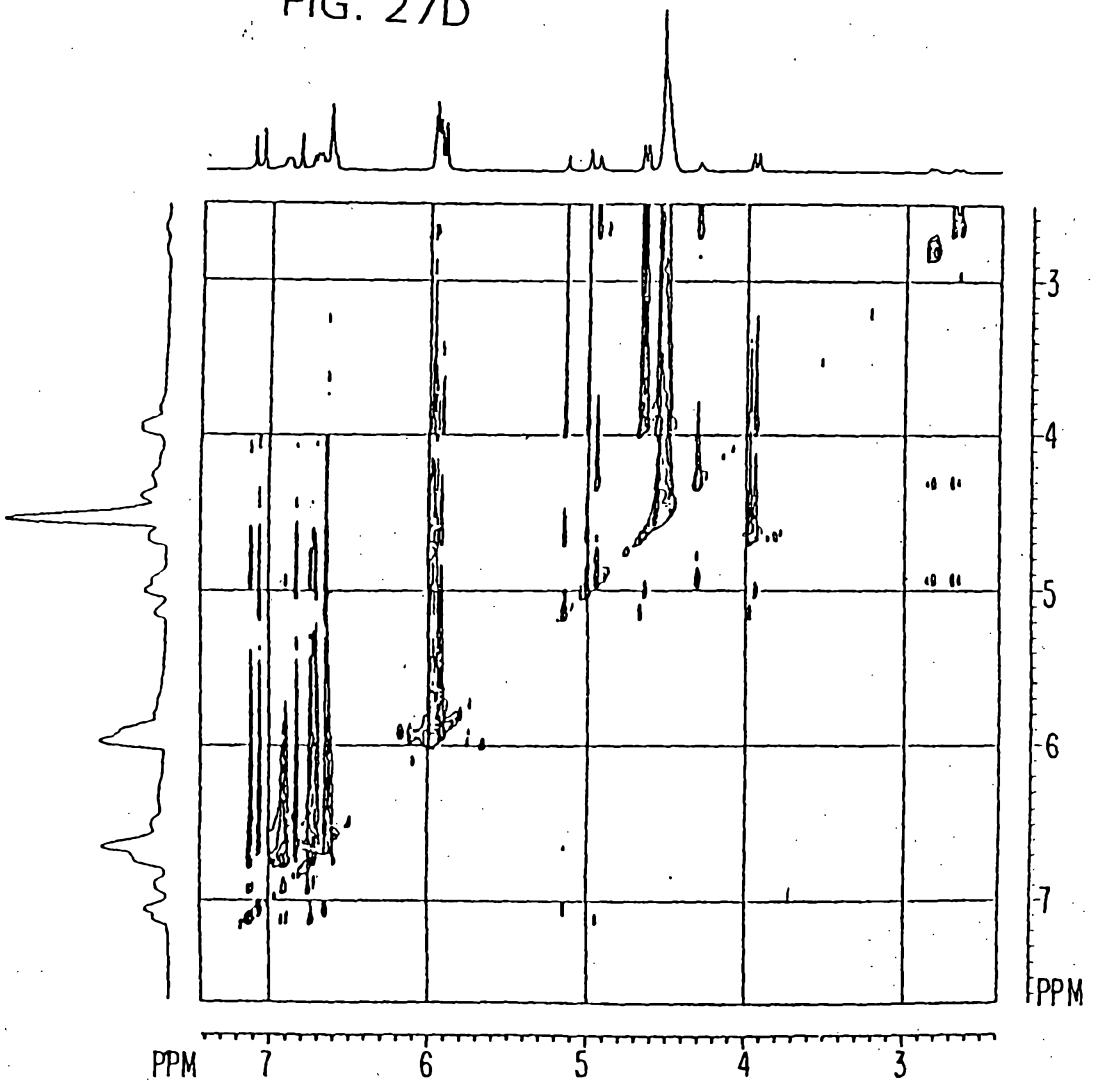
FIG. 27C



PULPROG	inv4				
SOLVENT	dioxane				
AQ	0.3543240	sec			
FIDRES	1.411217	Hz			
DW	173.0	usec			
RG	256				
NUCLEUS	1H				
P1	7.5	usec			
CNST2	145.0000000				
HL1	1	dB			
D1	1.0000000	sec			
D2	0.0034483	sec			
P3	13.5	usec			
SFO2	90.5625400	MHz			
DO	0.0000030	sec			
P2	15.0	usec			
D13	0.0000040	sec			
DLO	17.00	dB			
DE	247.1	usec			
SFO1	360.1374901	MHz			
SWH	2890.17	Hz			
TD	2048				
P31	65.0	usec			
NS	16				
DS	4				
INO	0.0000300	sec			
F1-ACQUISITION PARAMETERS					
NDO	2				
TD	256				
SFO1	90.56254	MHz			
FIDRES	65.09187	Hz			
SW	184.000	ppm			
F2-PROCESSING PARAMETERS					
SI	2048				
SF	360.1358480	MHz			
WDW	QSINE				
SSB	2				
LB	0.00	Hz			
GB	0				
PC	1.40				

F1-PROCESSING PARAMETERS		
SI	1024	
MC2	QF	
SF	90.5561565	MHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	
2D NMR PLOT PARAMETERS		
CX2	12.29	cm
CX1	12.29	cm
F2PLO	7.428	ppm
F2LO	2675.12	Hz
F2PHI	1.675	ppm
F2HI	603.45	Hz
F1PLO	125.840	ppm
F1LO	11395.56	Hz
F1PHI	16.941	ppm
F1HI	1534.15	Hz
F2PPMCM	0.46806	ppm/cm
F2HZCM	168.56526	Hz cm
F1PPMCM	8.86072	ppm cm
F1HZCM	802.39313	Hz/cm

FIG. 27D



PULPROG	mlevprtp
SOLVENT	MeOH
AQ	0.3543240 sec
FIDRES	1.411217 Hz
DW	173.0 usec
RG	128
NUCLEUS	1H
HL1	1 dB
D11	0.0300000 sec
D12	0.0000200 sec
HL2	76 dB
P18	1000000.0 uses
D13	0.0000040 sec
P1	7.5 usec
D0	0.0000030 sec
HL3	17 dB
P17	2500.0 sec
P7	104.0 usec
L1	56
P5	34.7 usec
DE	247.1 usec
SFO1	360.1374901 MHz
SWH	2890.17 Hz
TD	2048
NS	16
DS	4
INO	0.0001730 sec
F1-ACQUISITION PARAMETERS	
NDO	2
TD	128
SFO1	360.1375 MHz
FIDRES	22.579477 Hz
SW	8.025 ppm
F2-PROCESSING PARAMETERS	
SI	1024
SF	360.1358526 MHz
WDW	SINE
SSB	4
LB	0.00 Hz
GB	0

PC	1.40
F1-.PROCESSING PARAMETERS	
SI	1024
MC2	TPPI
SF	360.13585 MHz
WDW	SINE
SSB	4
LB	0.00 Hz
GB	0
2D NMR PLOT PARAMETERS	
CX2	12.29 cm
CX1	12.29 cm
F2PLO	7.428 ppm
F2LO	2675.22 Hz
F2PHI	2.397 ppm
F2HI	863.21 Hz
F1PLO	7.652 ppm
F1LO	2755.84 Hz
F1PHI	2.511 ppm
F1HI	904.32 Hz
F2PPMCM	0.40939 ppm/cm
F2HZCM	170.36786 Hz/cm
F1PPMCM	0.41832 ppm/cm
F1HZCM	150.65236 Hz/cm

FIG. 28

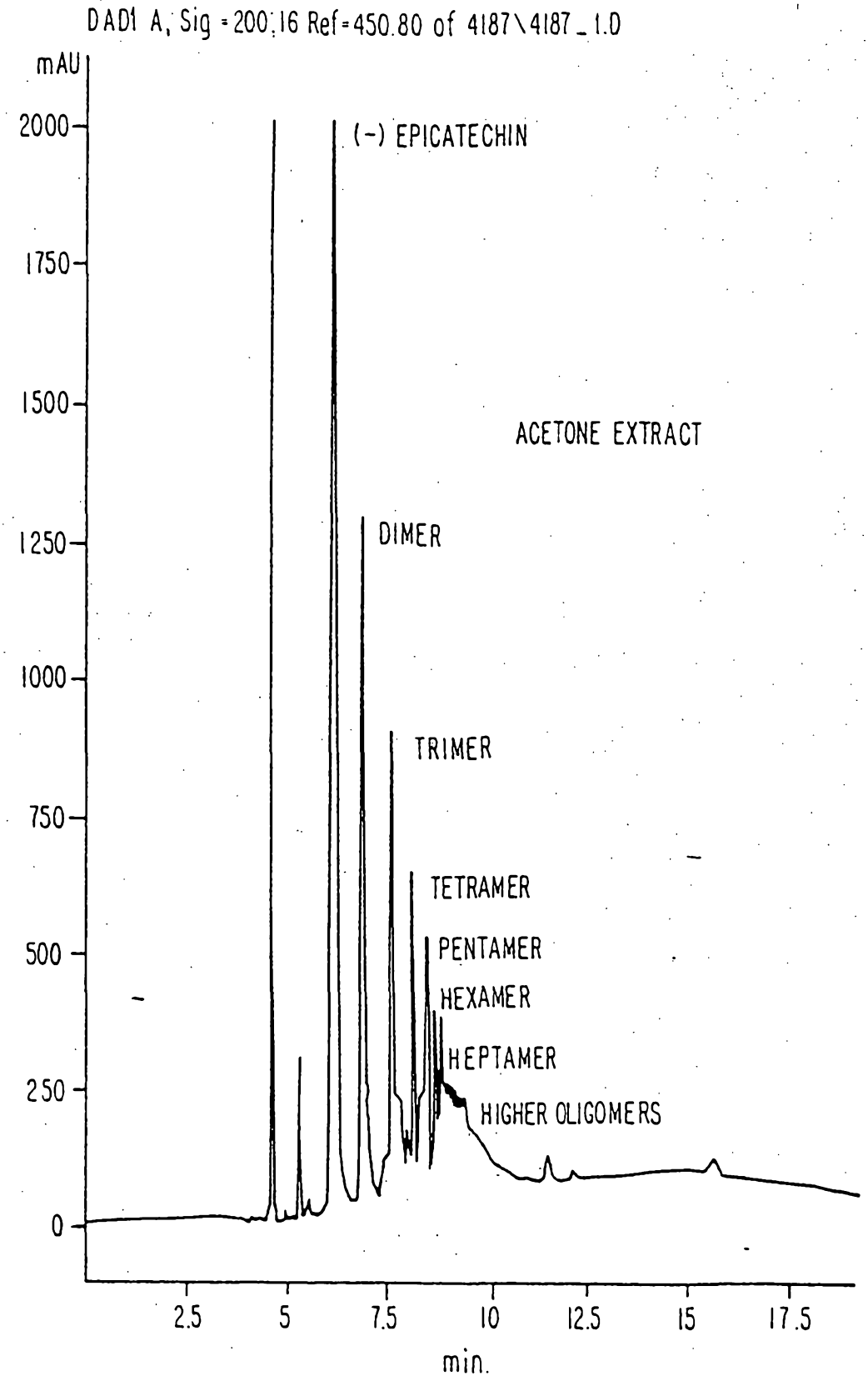

DAD1 A, Sig = 200,16 Ref = 450,80 of 4187\4187_1.0
mAU
2000
1750
1500
1250
1000
750
500
250
0
(-) EPICATECHIN
ACETONE EXTRACT
DIMER
TRIMER
TETRAMER
PENTAMER
HEXAMER
HEPTAMER
HIGHER OLIGOMERS
2.5
5
7.5
10
12.5
15
17.5
min.

FIG. 29A

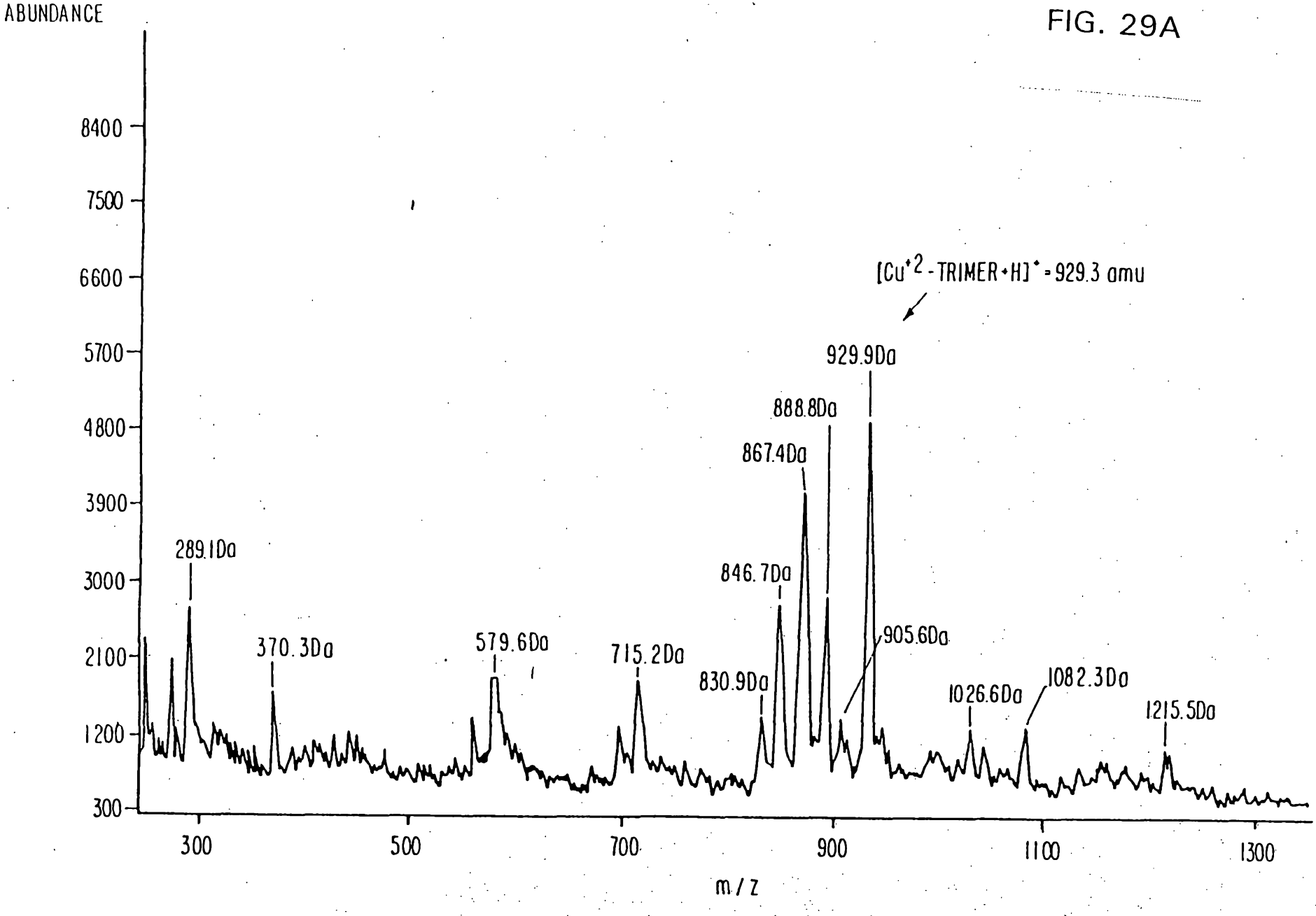

ABUNDANCE
8400
7500
6600
5700
4800
3900
3000
2100
1200
300
[Cu+2 - TRIMER + H]+ = 929.3 amu
929.9Da
888.8Da
867.4Da
846.7Da
905.6Da
830.9Da
289.1Da
370.3Da
579.6Da
715.2Da
1026.6Da
1082.3Da
1215.5Da
300
500
700
900
1100
1300
m/z

FIG. 29B

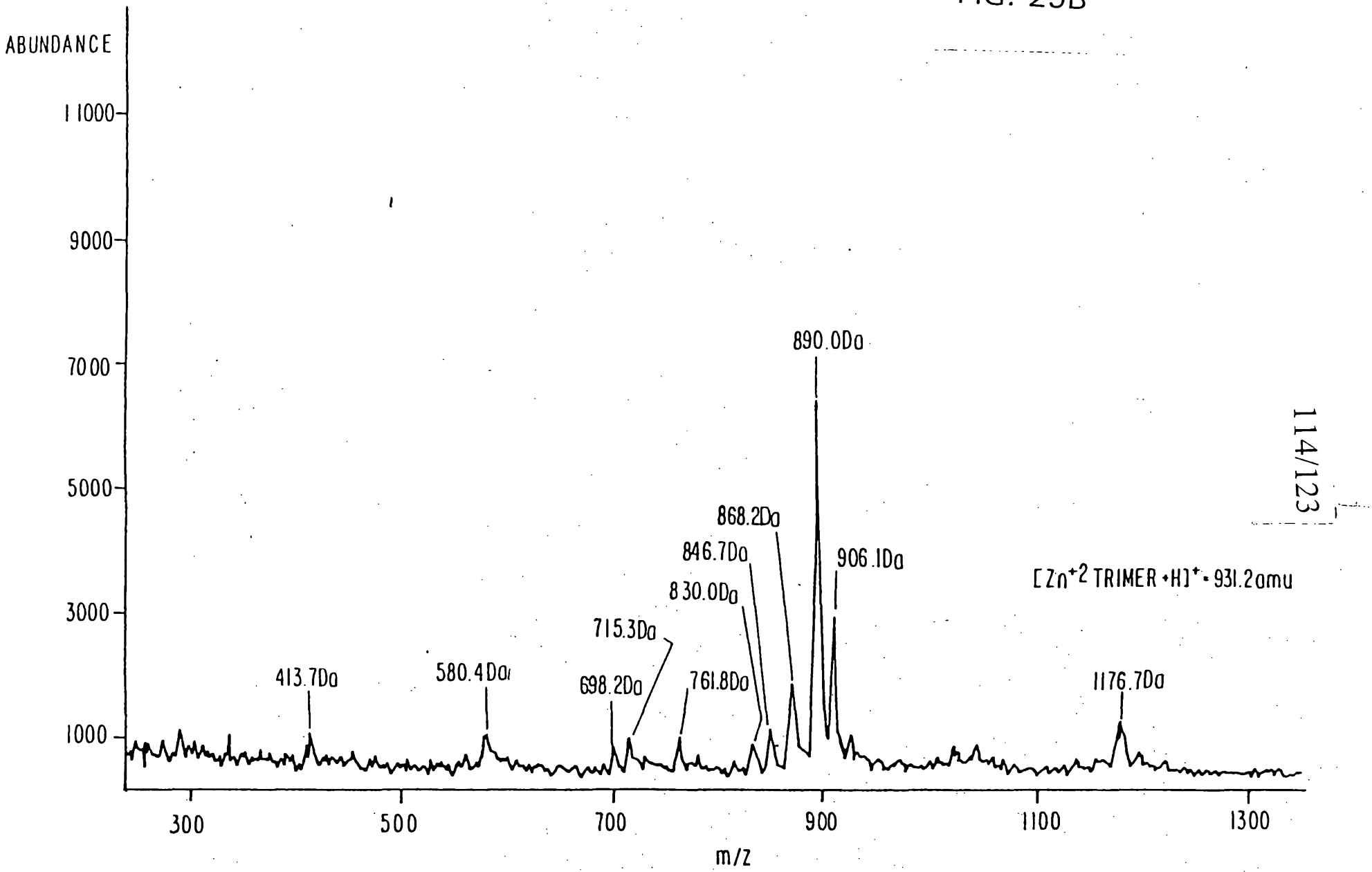

ABUNDANCE
11000
9000
7000
5000
3000
1000
300
500
700
900
1100
1300
m/z
890.0Da
868.2Da
846.7Da
830.0Da
715.3Da
906.1Da
413.7Da
580.4Da
698.2Da
761.8Da
1176.7Da
[Zn+2 TRIMER+H]+=931.2amu

FIG. 29C

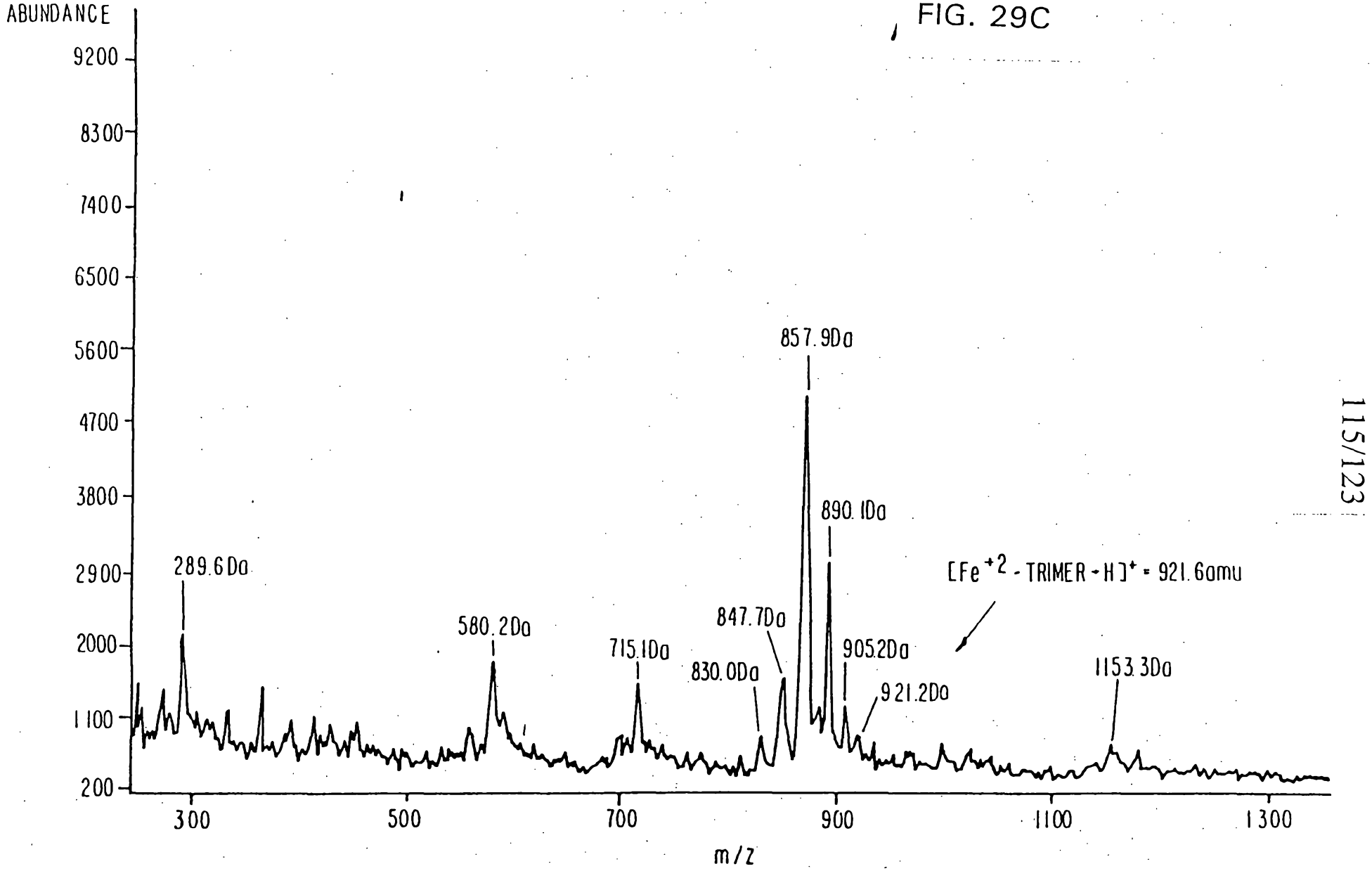

ABUNDANCE
9200
8300
7400
6500
5600
4700
3800
2900
2000
1100
200
289.6Da
580.2Da
715.1Da
830.0Da
847.7Da
857.9Da
890.1Da
905.2Da
921.2Da
1153.3Da
[Fe+2 - TRIMER - H]+ = 921.6amu
300
500
700
900
1100
1300
m/z

FIG. 29D

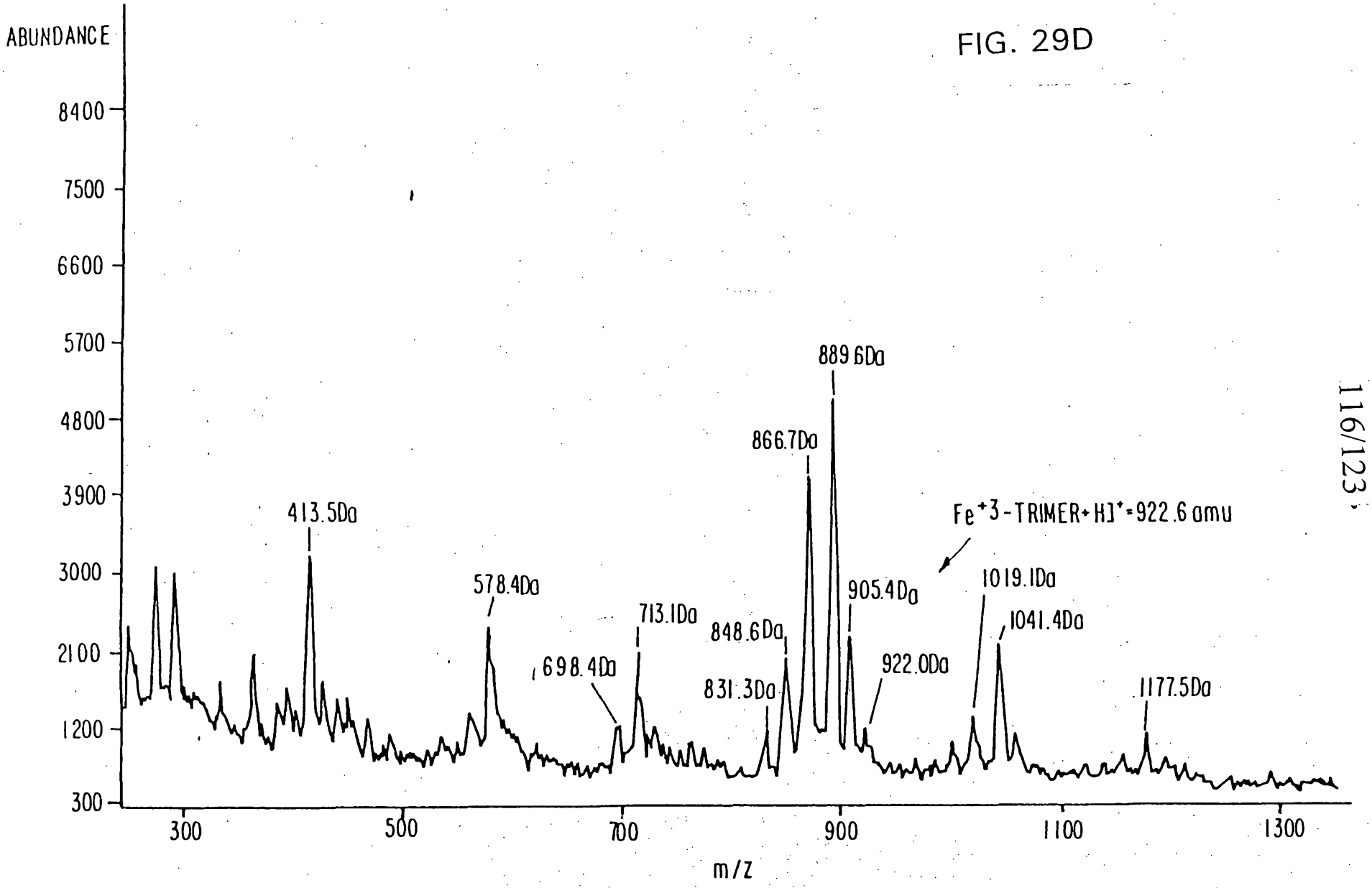

ABUNDANCE
8400
7500
6600
5700
4800
3900
3000
2100
1200
300
413.5Da
578.4Da
698.4Da
713.1Da
831.3Da
848.6Da
866.7Da
889.6Da
905.4Da
922.0Da
Fe+3-TRIMER+H]+=922.6 amu
1019.1Da
1041.4Da
1177.5Da
300
500
700
900
1100
1300
m/z

FIG. 29E

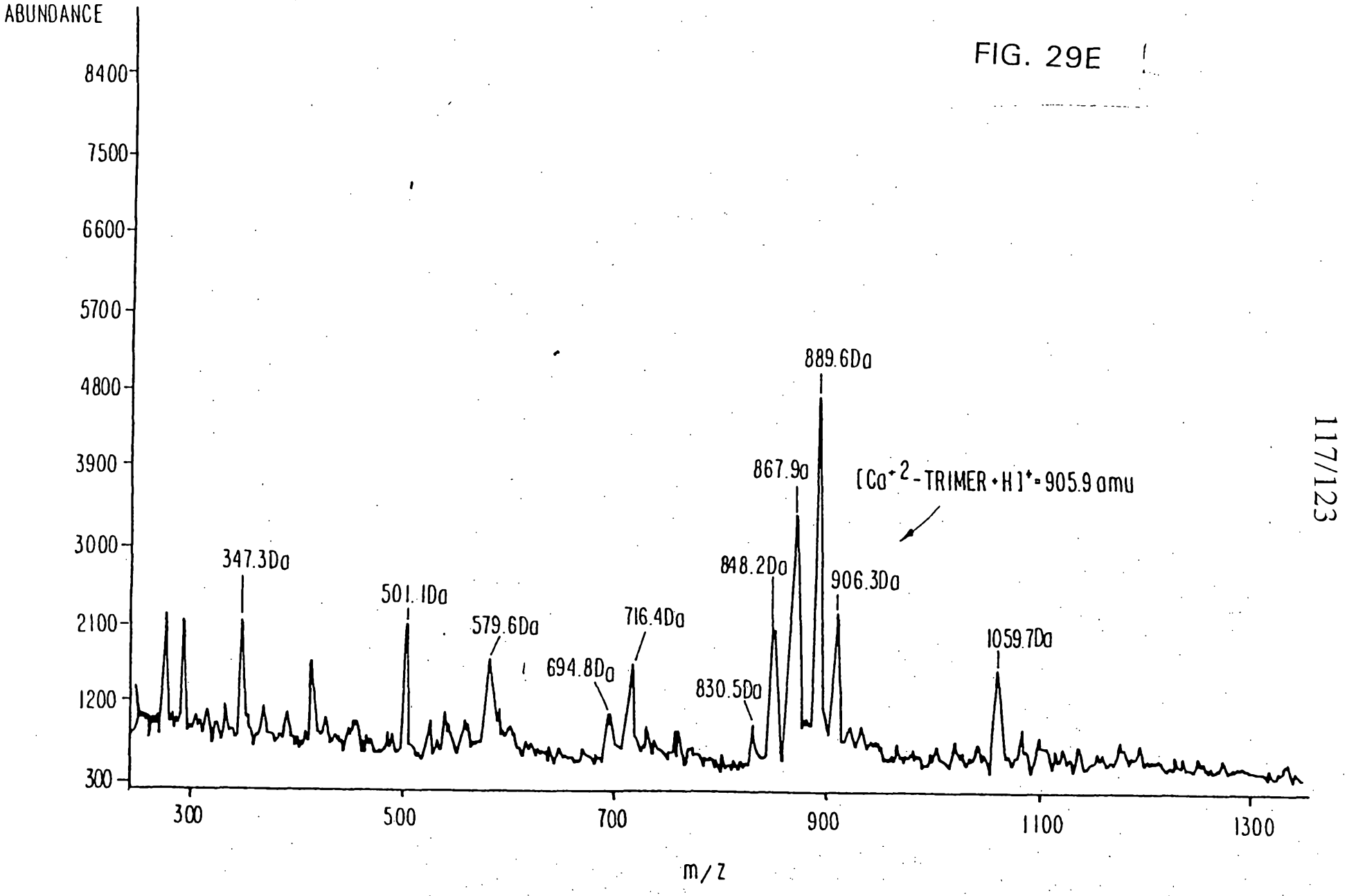

ABUNDANCE
8400
7500
6600
5700
4800
3900
3000
2100
1200
300
347.3Da
501.1Da
579.6Da
694.8Da
716.4Da
830.5Da
848.2Da
867.9a
889.6Da
906.3Da
1059.7Da
[Ca+2-TRIMER+H]+=905.9 amu
300
500
700
900
1100
1300
m/z

FIG. 29F

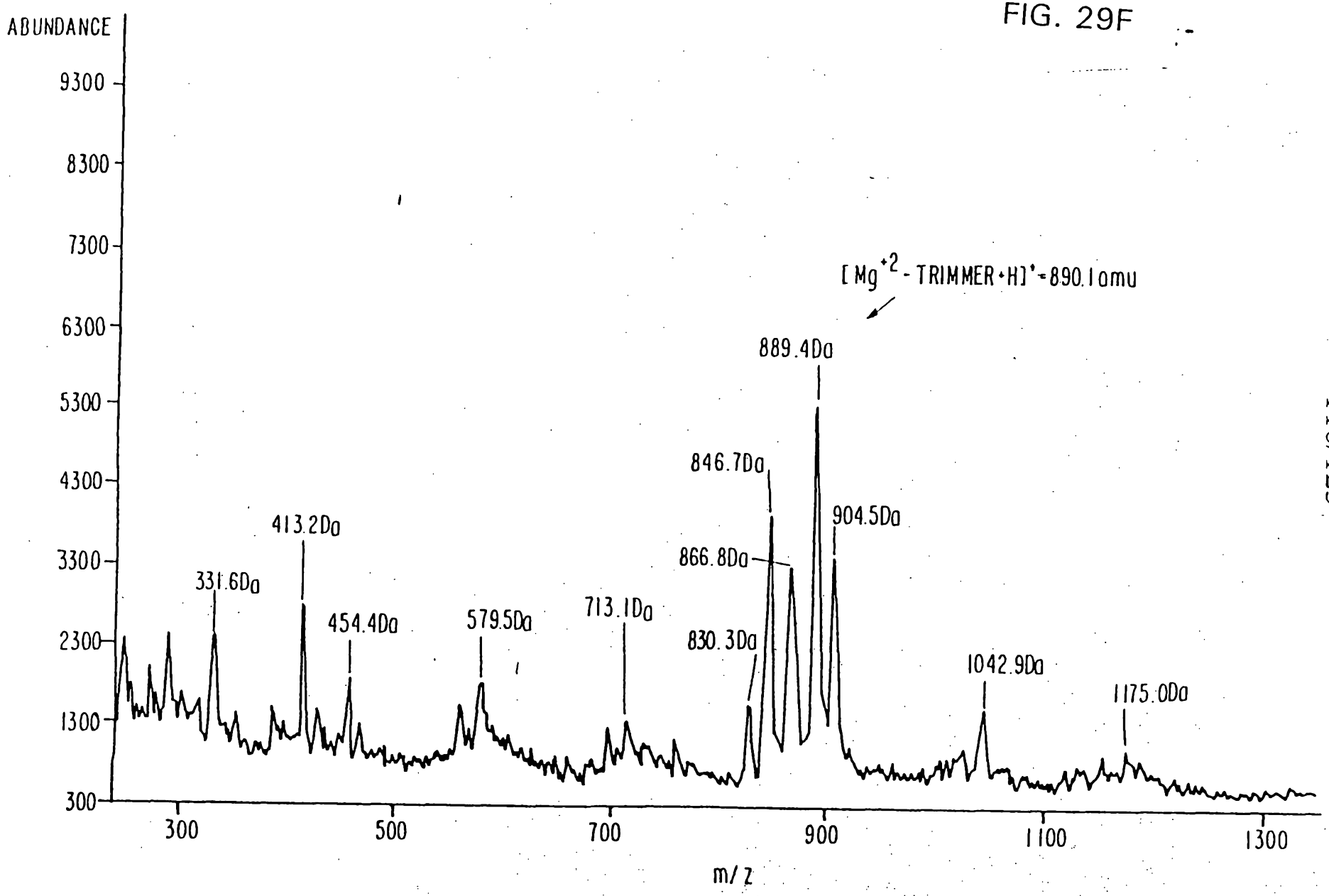

ABUNDANCE
9300
8300
7300
6300
5300
4300
3300
2300
1300
300
[Mg+2 - TRIMMER+H]+=890.1amu
889.4Da
846.7Da
904.5Da
866.8Da
413.2Da
331.6Da
454.4Da
579.5Da
713.1Da
830.3Da
1042.9Da
1175.0Da
300
500
700
900
1100
1300
m/z

FIG. 30

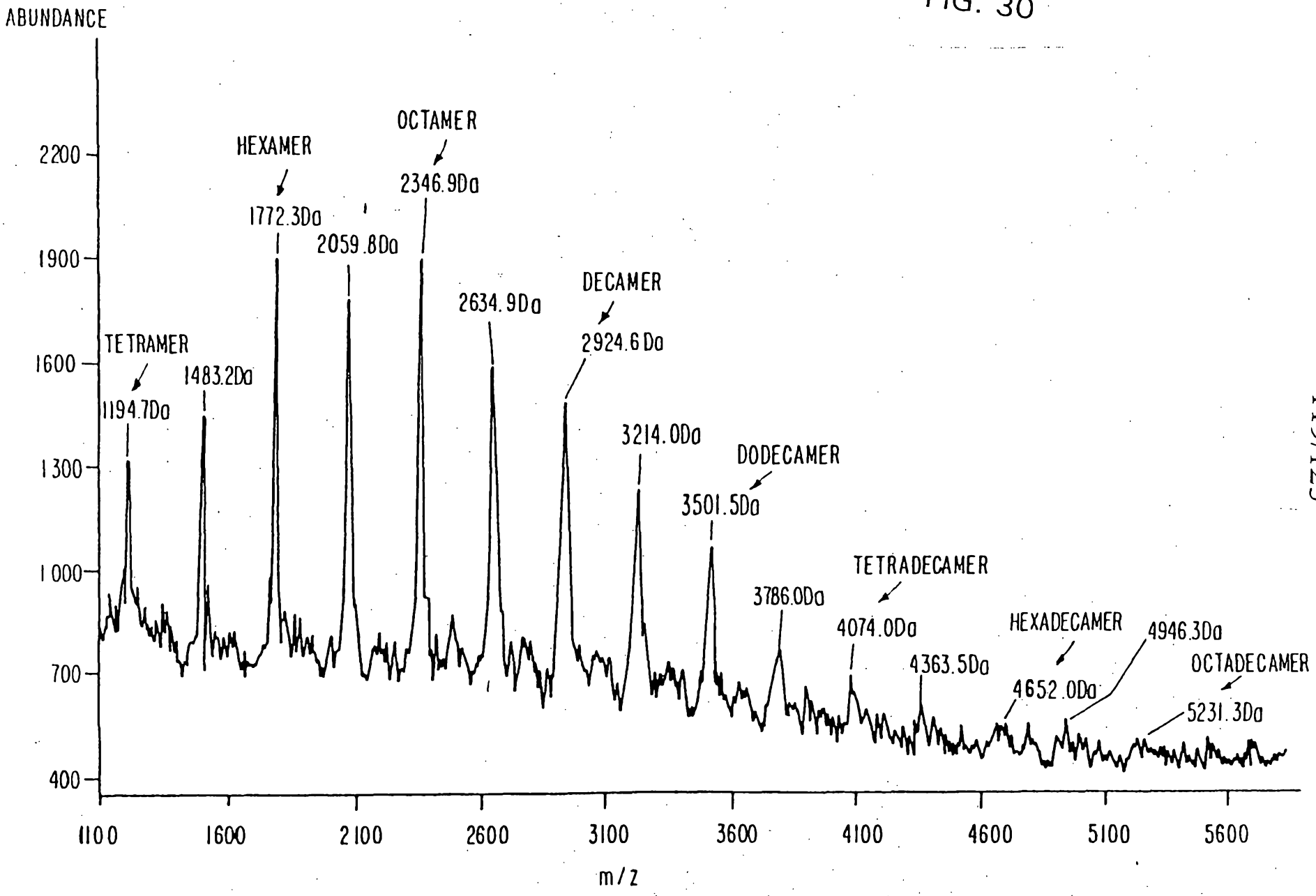

ABUNDANCE
2200
1900
1600
1300
1000
700
400
1100
1600
2100
2600
3100
3600
4100
4600
5100
5600
m/z
TETRAMER
1194.7Da
1483.2Da
HEXAMER
1772.3Da
2059.8Da
OCTAMER
2346.9Da
2634.9Da
DECAMER
2924.6Da
3214.0Da
DODECAMER
3501.5Da
3786.0Da
TETRADECAMER
4074.0Da
4363.5Da
HEXADECAMER
4652.0Da
4946.3Da
OCTADECAMER
5231.3Da

FIG. 31

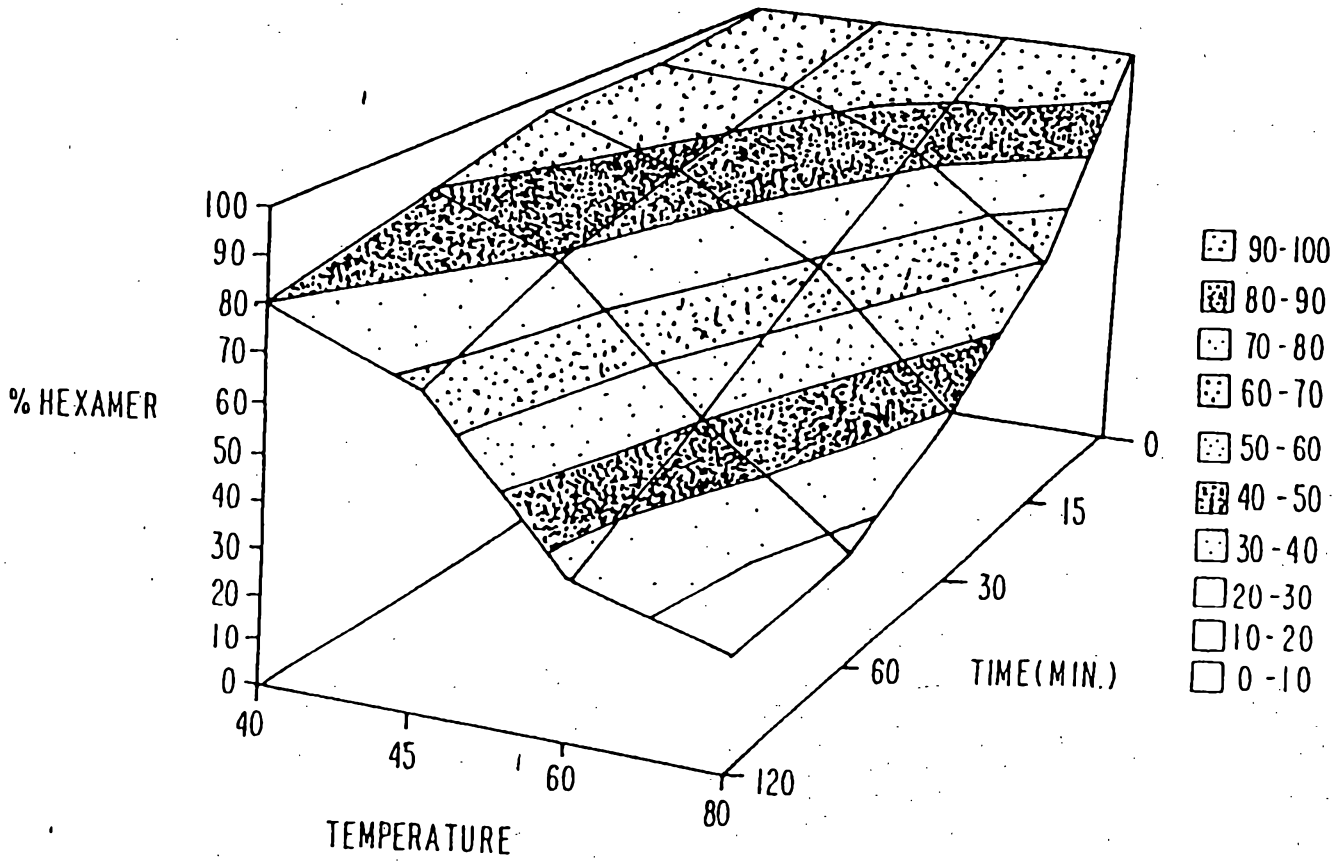

% HEXAMER
100
90
80
70
60
50
40
30
20
10
0
40
45
60
80
TEMPERATURE
0
15
30
60
120
TIME(MIN.)
90-100
80-90
70-80
60-70
50-60
40-50
30-40
20-30
10-20
0-10

FIG. 32

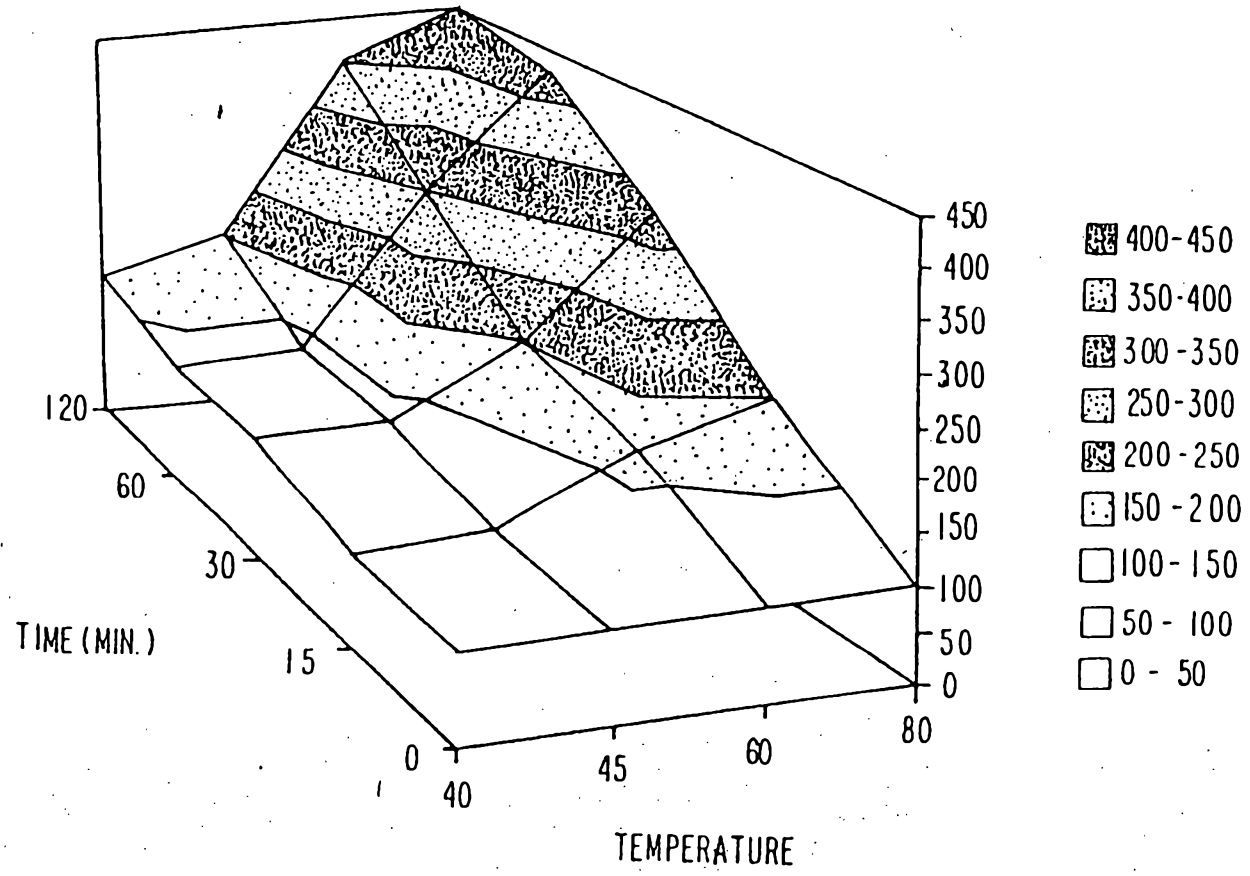

120
60
30
15
0
TIME (MIN.)
40
45
60
80
TEMPERATURE
450
400
350
300
250
200
150
100
50
0
400-450
350-400
300-350
250-300
200-250
150-200
100-150
50-100
0-50

FIG. 33

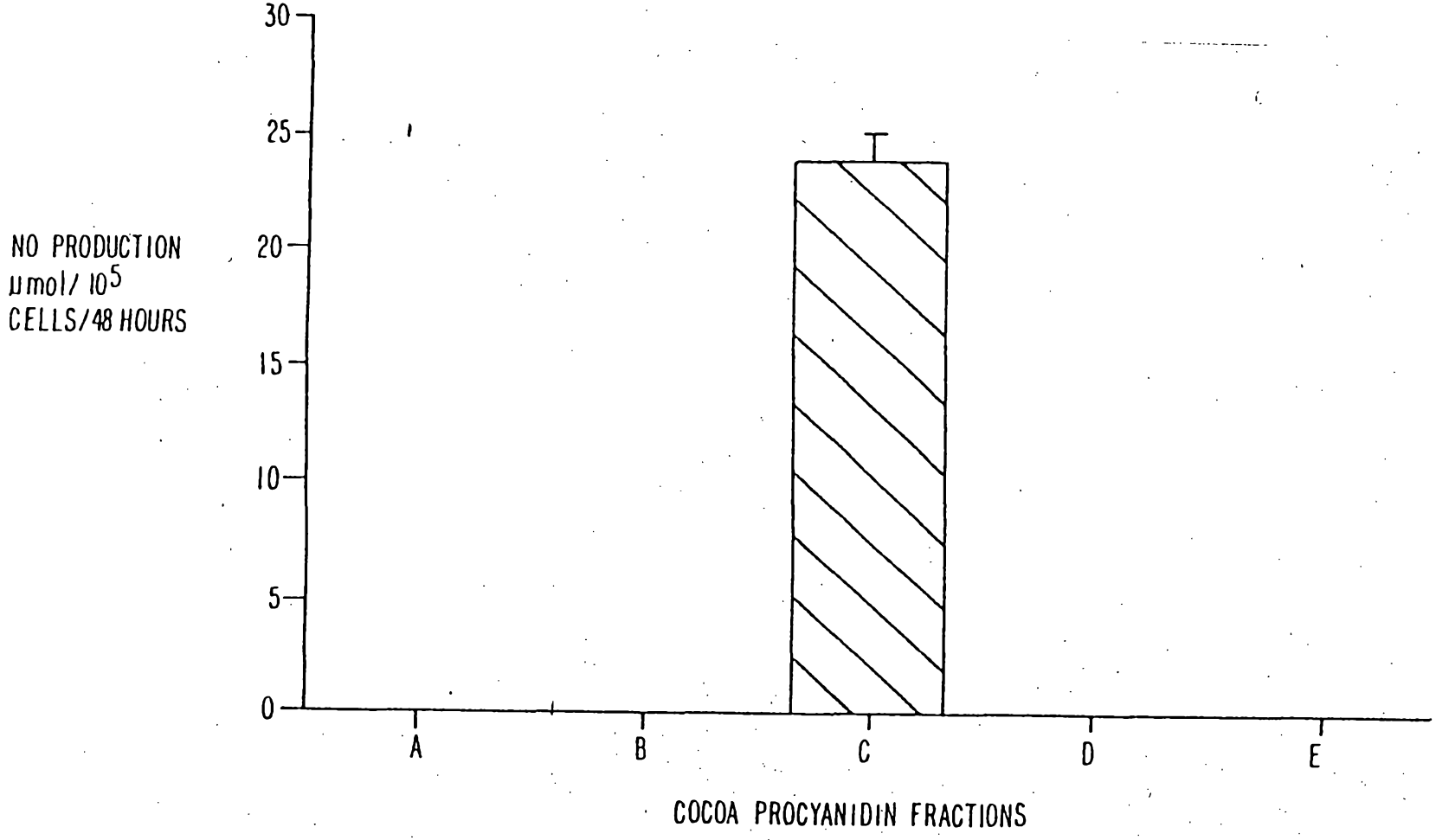
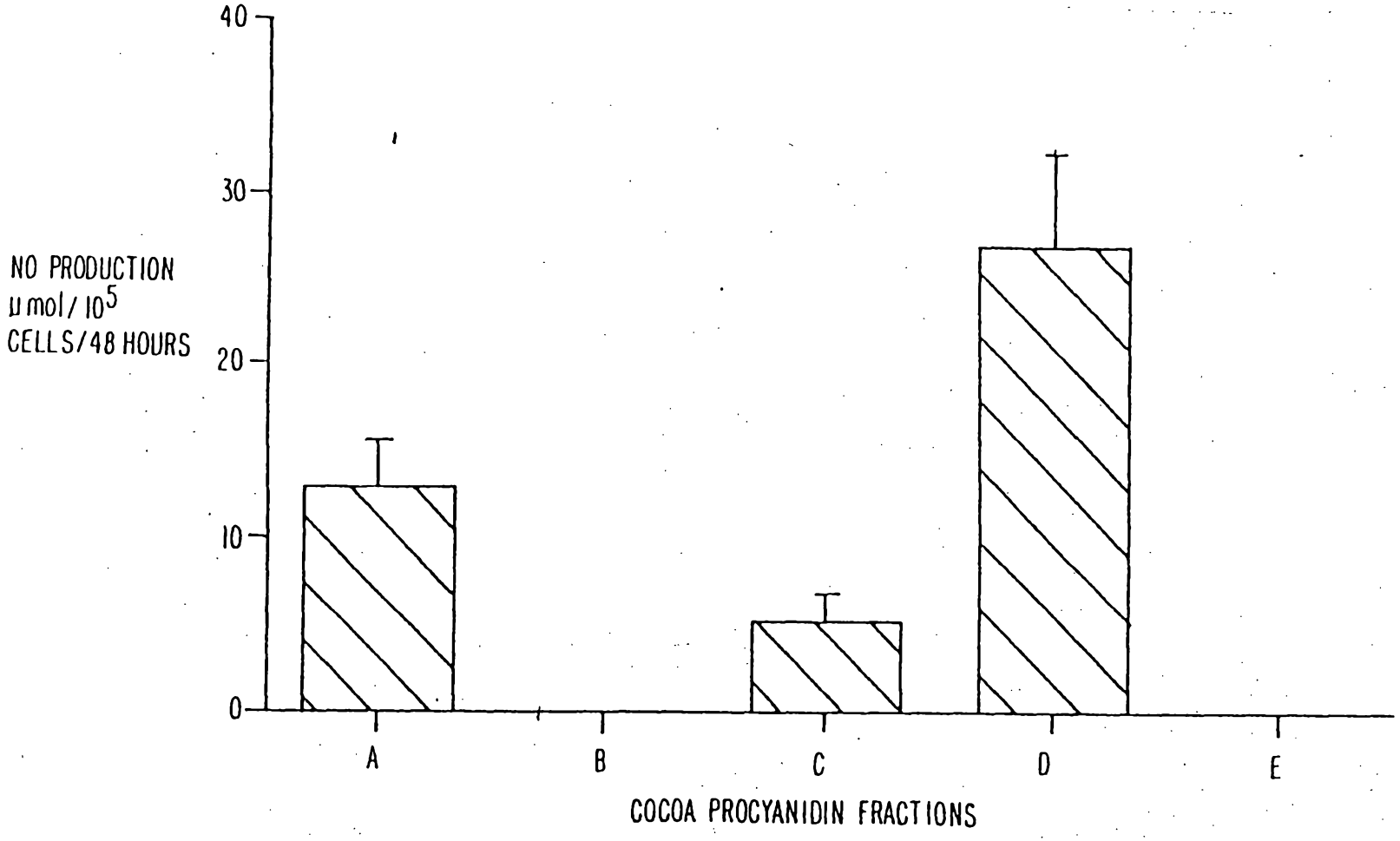

NO PRODUCTION
µmol/ 10^5
CELLS/48 HOURS
30
25
20
15
10
5
0
A
B
C
D
E
COCOA PROCYANIDIN FRACTIONS

FIG. 34


40
30
20
10
0
NO PRODUCTION
μmol/10^5
CELLS/48 HOURS
A
B
C
D
E
COCOA PROCYANIDIN FRACTIONS