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(54) Title: NOVEL GLYCOSIDE COMPOUNDS AND PRODUCTION AND USE THEREOF

(57) Abrégé/Abstract:
Novel glycosides, especially steroidal and non-steroidal glycosides, and a novel method of their production, are provided. For the novel method of producing novel glycosides, aglycon compounds are glycosylated with tri-O-acyl glucal using molecular or ionized halogen as a reaction catalyst. In this method an aglycon, which may be a nonsteroid or a steroid having a reactive functional group (e.g., -OH, -SH, -COOH, $-NH_2$, $-NHR_1$) such as a cholesterol, is glycosylated, such that the glycosylation is performed in a single step. For example, if a steroid, the resulting steryl pyranoside, is by oxidation converted to the corresponding 7-ketosteryl di-O-acyl-pyranoside. The latter pyranoside is selectively reduced to provide the corresponding 7-β-hydroxysteryl 2,3-dideoxy-α-D-erythro-hex-2-enopyranoside. The steroidal and non-steroidal glycosides obtained in this way possess valuable pharmacological properties. In particular, the cholesterol glycoside in vivo exhibits a selective cell-destructive activity on malignant cells which activity is substantially free of side effects on normal cells. The glycosides possess useful properties, especially pharmacological properties which are the same as their respective unglycosylated aglycons. The properties may include a drive-enhancing (stimulating) activity and an anti-inflammatory (immunosuppressive or immunoregulatory) activity.

Abstract

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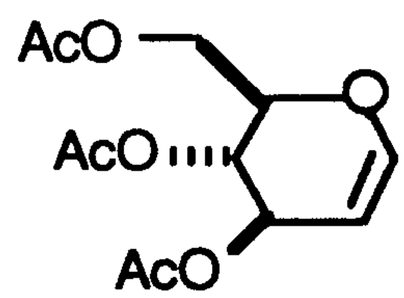
NOVEL GLYCOSIDE COMPOUNDS AND PRODUCTION AND USE THEREOF

Field of the Invention

This invention relates to a surprisingly novel method for the production of a broadly novel type of glycoside. The method comprises glycosylation (i.e., glycosidation) of an aglycon compound having a functional group, e.g., a hydroxy compound such as a hydroxy-steroid or hydroxy-non-steroid. The invention importantly relates to the resulting glycosides as novel compounds of diverse application having desired properties including pharmacodynamic properties; and to medicaments containing the compounds.

It has been surprisingly found according to the invention that an aglycon compound -- in a preferred embodiment, a hydroxy-steroid to be understood as a steroidal alcohol or steroidal phenol -- can be reacted in one step with a glycosidic vinyl ether 3,4,6-tri-O-acyl-D-glucal of formula



where Ac is a lower acyl group, preferably a C_{1-4} acyl group, in the presence of molecular halogen as a catalyst, such as iodine I_2, chlorine Cl_2, bromine Br_2 and fluorine F_2, preferably iodine, to provide the corresponding glycoside in high yield. Thus there is no need for expensive and/or toxic

reagents in this reaction step. Further, as a preferred aspect of the invention, a steroidal glycoside -- a 3-β-ol cholesterol pyranoside which is cholesteryl 4,6-di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside, obtainable by this method -- has been found to be applicable as a pharmacologically active agent for use as a medicament, especially as an anti-neoplastic agent, or in geriatric medicine, or as a sedative or activity-enhancing agent. For convenience in describing the invention, the 4,6-di-O-acyl (or acetyl)-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside will sometimes be referred to herein simply as a DDH pyranoside.

In the accompanying drawings with reference to preferred examples of the invention:

FIGURE 1 is an infrared spectrum of the glucal used in the reaction of Example 1;

FIGURE 2 is an infrared spectrum of the glycosylation product of Example 1;

FIGURE 3 is an NMR spectrum of the same glycosylation product of Example 1;

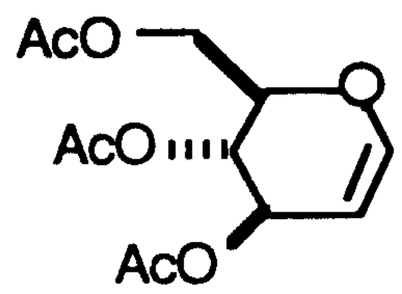
FIGURES 4 and 5 are the IR-spectrum and the NMR-spectrum, respectively of the ketone product of Example 2;

FIGURES 6 and 7 are the IR-spectrum and the NMR-spectrum, respectively, of the 7β-OHC product of Example 3;

FIGURE 8 is a plot showing the tumor cell growth inhibition by selected concentrations of 7β-OH cholesterol in cell culture fluid;

According to one preferred embodiment, the invention concerns a method for the production of a pyranoside compound, wherein an aglycon compound selected from aliphatic, alicyclic, aliphatic-aromatic or aromatic

compounds having a primary, secondary or tertiary functional group preferably selected from -OH, -SH, -COOH, $-NH_2$, and $-NHR_1$, where R_1 is C_{1-4} lower alkyl or phenyl, is glycosylated, characterized in that the aglycon compound is reacted in a solvent with 3,4,6-tri-O-acyl-glucal of formula



in the presence of molecular or ionized halogen as a catalyst, such as iodine I_2, chlorine Cl_2, bromine Br_2, and fluorine F_2, preferably iodine, to produce the corresponding 4,6-di-O-acyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside of said aglycon compound, where acyl is a lower acyl group. In another preferred embodiment a hydroxysteryl compound, preferably a 3β-ol sterol compound, more preferably a delta^5-3β-ol steroid compound such as a cholesterol, (e.g., delta^5-cholesten-3β-ol) is glycosylated by reaction with 3,4,6-tri-O-acyl-D-glucal in a solvent in the presence of molecular or ionized halogen, preferably iodine, as a catalyst. Alternatively, a non-steroidal compound can be thus glycosylated as detailed herein. The reaction is achieved in a single step and in high yield. Thus a double bond which is strongly hindered by the C_4, C_6-acyl groups and thus being inert, is introduced between C_2=C_3 of the glycosidic part of the molecule, whereby as to the hydroxysteryl compound the delta^5 double bond of the cyclopentano-perhydro-phenanthrene skeleton is stabilized and

remains unchanged.

Furthermore, the invention comprises the use of the resulting unsaturated glycoside obtained as an end product or as an intermediate in further reactions to provide functional derivatives which may be steroidal, e.g., cholesterol, derivatives. Thus, in the case of cholesterol, functional groups can be introduced into the perhydro-cyclopentano-phenanthrene skeleton of the unsaturated acylglycoside, wherein the α-bond of the acylglycoside at the same time functions as a protecting group for the original OH-group at C_3 of the phenanthrene skeleton.

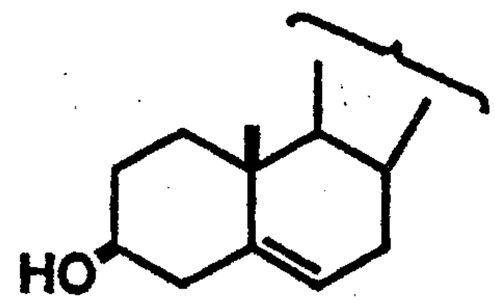
The present method makes use of iodine which is molecularly dissolved in inert solvents. These inert solvents, for example, comprise CH_2Cl_2 dichloromethane, $CHCl_3$ chloroform, CCl_4 carbon tetrachloride, $C_6H_4(CH_3)_2$ xylene, $C_6H_3(CH_3)_3$ mesitylene, $C_6H_5CH(CH_3)_2$ cymene, C_6H_{12} cyclohexane and methyl derivatives thereof, as well as ligroin, petroleum ether and saturated hydrocarbons, such as for example n-pentane or n-heptane, preferably C_6H_6 benzene or $C_6H_5CH_3$ toluene. Using the alternative solvent nitromethane CH_3NO_2, the glycosylation reaction catalyzed by iodine runs quantitatively at room temperature in 2 hours. The method of the invention for the preparation of the DDH pyranoside also makes use of iodine dissolved in ionizing solvents. Using a ketone such as acetone, methyl ethyl ketone (2-butanone), and cyclohexanone as an alternative solvent, the iodine catalyzed reaction between 3,4,6-Tri-O-acetyl-D-glucal and cholesterol runs quantitatively at 20°C in only 60 to 90 minutes. As a preferred embodiment, one can also use tetrahydrofuran (THF) ethers and the like.

Using a lower alcohol such as methanol, ethanol, propanol and the like, the iodine catalyzed reaction between 3,4,6-Tri-O-acetyl-D-glucal and the aglycon runs during 30 minutes time quantitatively at 20°C as well.

The glycosylation method according to the mentioned preferred embodiment is directed to the reaction of the vinyl ether of 3,4,6-tri-O-acyl-D-glucal with a cholesterol such as delta^5-cholesten-3β-ol, with molecularly dissolved halogen, i.e. iodine, as catalyst in one of the aforementioned solvents. The reaction thereby introduces a double bond between C-atoms 2 and 3 while eliminating the acyl group sited at C_3, instead of introducing an iodine atom at C_2 in the glycosidic part of the resulting cholesterylglycoside. This reaction is conveniently followed by IR-spectroscopy, and is complete only when the peak of the glucal at 1650 cm^{-1} has disappeared. The iodine being utilized as catalyst is quantitatively titrated back by a suitable back-titrant reagent such as 0.1 N aqueous sodium thiosulphate ($Na_2S_2O_3$). The method for providing the corresponding di-O-acyl glycoside as exemplified hereinafter for cholesterol compounds is applicable to the glycosylation of not only steroid compounds and cholesterol compounds and precursors but also aglycon compounds in general, i.e., aliphatic, alicyclic, aliphatic-aromatic and aromatic compounds having a primary, secondary or tertiary functional group of the type mentioned. Aglycon compounds that are useful for their pharmacological properties are preferred, since their respective glycosides produced according to the invention have the same utility and useful properties and art-recognized dosage regimens with the possible enhancement

of bioavailability, e.g., at cell membranes, due to the presence of the sugar residue. Preferred hydroxy compounds for glycosylation comprise cholesterols, bile salts, steroid hormones, and vitamin D compounds and precursors as described in Stryer's Biochemistry, 3rd Ed. pp. 559-570, Freeman and Company, New York, 1988.

Examples of such compounds are cholic acid and derivatives, 25-hydroxy-cholesterol, 25-hydroxy-calciferol, pregnenolone, 17α-hydroxy-progesterone, 17α-hydroxy-pregnenolone, 11-desoxy-corticosterone, 11-desoxy-cortisol, corticosterone, cortisol, cortisone, dolichol, androsterone, testosterone, estrone, 17β-estradiol, estratriol-3,16α,17β, 3α,5β-tetrahydro-corticosterone, serotonin, urocortisol, and allocortolone, and the like, preferably cyclopentano-perhydrophenanthrene compounds having the delta^5-3β-OH steryl moiety



in which the delta^5 double bond is stabilized as described by 3β-OH glycosylation resulting in the pyranoside.

Other preferred aglycon compounds for glycosylation include

a) morphine and morphine derivatives such as codeine, ethyl morphine, dihydrocodeine, hydromorphone, oxycodone, nalorphine, levorphan, and the like;

b) α- and β-sympathomimetic (adrenergic) compounds such as p-hydroxyphenylethanolamine, norfenefrine, synephrine, etilefrin, phenylephrine, octapamine, isoprenaline, dichloroisoproterenol, metaproterenol,

terbutaline, buphenine, and the like;

c) antihypertensive compounds such as methyldopa and the like;

d) vasoconstrictor compounds such as α-methylnoradrenaline, and the like;

e) anticholinergic (parasympatholytic) and antispasmodic compounds such as atropine, homatropine, scopolamine and its methobromide and butyl bromide quaternary compounds, podine methyl sulfate, tropine benzilate, and the like;

f) antipsychotic compounds such as acetophenazine, fluphenazine, dixyrazine, perphenazine, hydroxyzine, pericyazine, haloperidol, trifluperidol, moperone, and the like;

g) cardiotonic compounds such as digitoxigenin, digoxigenin, diginatigenin, and the like;

h) estrogen and contraceptive compounds such as ethinylestradiol, mestranol, quinestrol, and the like;

i) antibacterial compounds such as amoxicillin, chloramphenicol, thiamphenicol, tetracycline, chlortetracycline, oxytetracycline, and the like;

j) antitussive compounds such as chlophedianol, clobutinol, zipeprol, and the like;

k) analgesic compounds such as levorphanol, ciramadol, pentazocine, carbetidine, glafenine, salicylamide, and the like;

l) oxytocic compounds such as ergonovine, prostaglandin $F_{2\alpha}$, and the like;

m) antineoplastic compounds such as fluorouracil, and the like;

n) antimalarial compounds such as quinine, and the like;

o) fungicidal compounds such as cycloheximide, and the like;

p) antirheumatic compounds such as oxyphenbutazone, and the like;

q) anticholelithogenic compounds such as chenodiol, and the like;

r) choleretic compounds such as hymecromone, and the like;

s) anti-gout compounds such as allopurinol;

t) anti-parkinsonian compounds such as levodopa, carbidopa, droxidopa, and the like;

u) anti-spasmodic compounds such as ephedrine, and the like;

v) nasal decongestant compounds such as cafaminol, and the like;

w) muscle relaxant compounds such as phenprobamate, guaiacol glycerol ether, and the like;

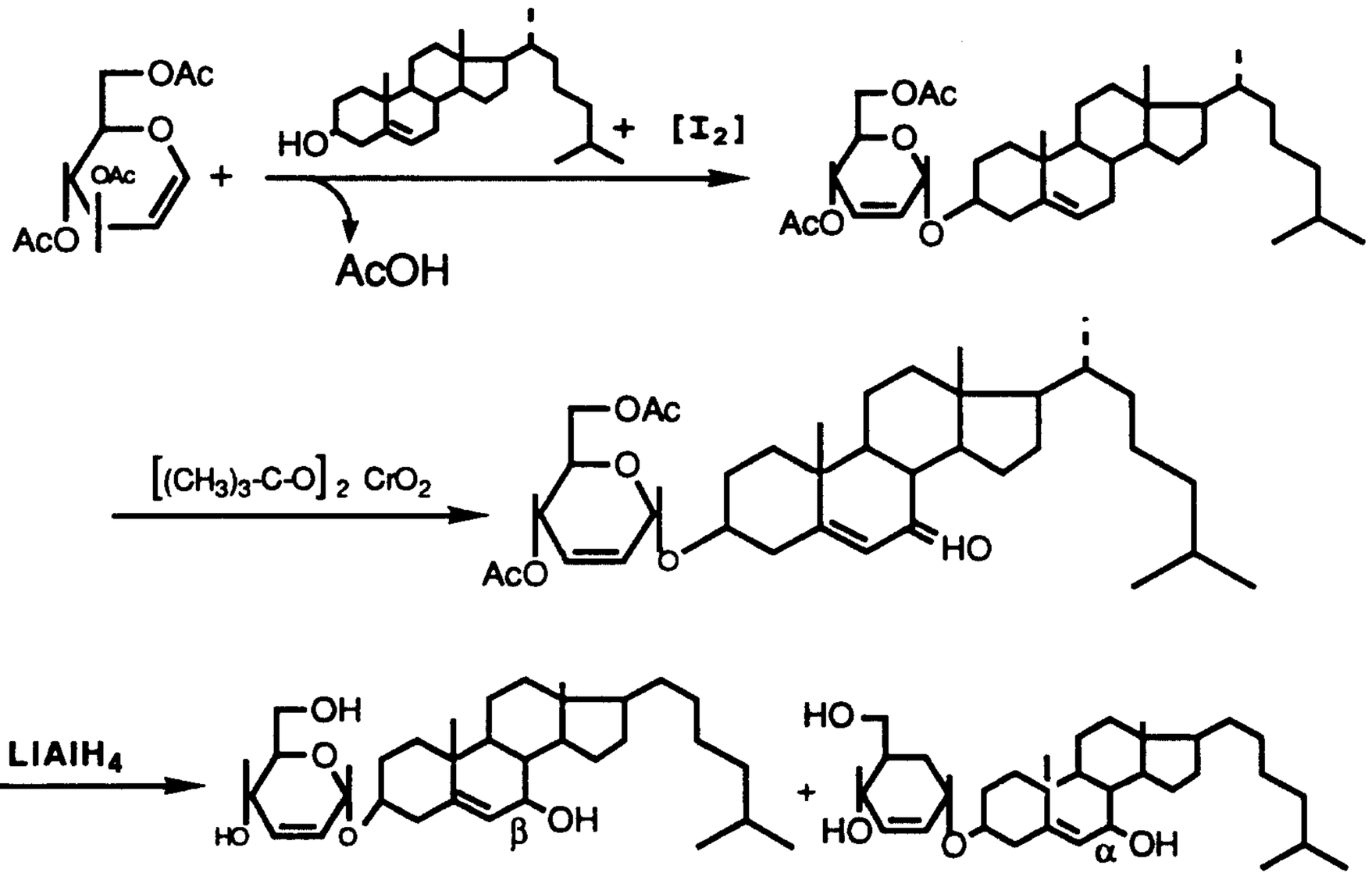
x) anti-inflammatory compounds such as dexamethasone, beclomethasone, and the like; and

y) vitamin and vitamin related compounds such as provitamin D, xanthophyll, vitamin A, vitamin E, thiamin, ascorbic acid, and the like.

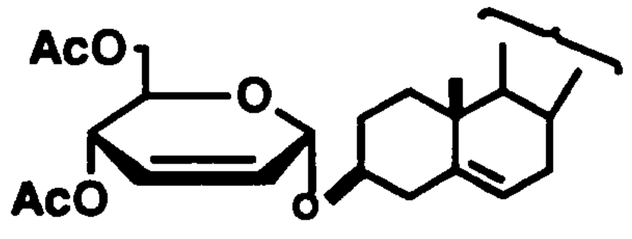
Other preferred aglycon compounds for glycosylation according to the invention include those listed in the "Therapeutic Category and Biological Activity Index" of The Merck Index XI, pp. THER-5 to THER-29, Merck & Co., Inc., Rahway, NJ.

The glycosylation method and related oxidation and

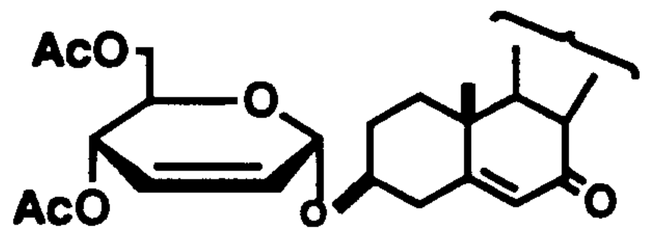
reduction methods described hereinafter may be illustrated by a preferred embodiment employing the starting material cholesterol, as follows:



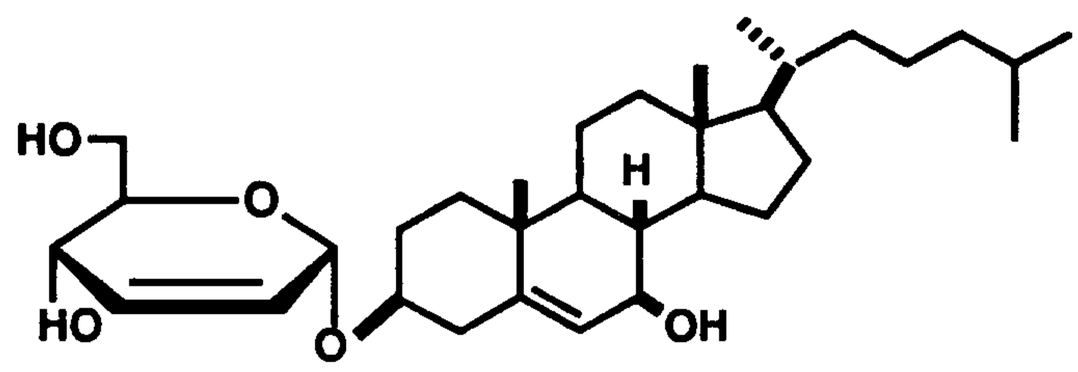
In another method aspect of the invention, the steryl DDH pyranoside product obtained by the glycosylation method can be converted by oxidation of the steroid part into an α-glycosylated 7-keto-sterol such as α-glycosylated 7-keto-cholesterol. The method is applicable to the oxidation of sterol compounds broadly, preferably cyclopentano-perhydro-phenanthrene compounds having the delta^5-3β-OH steryl 4,6-di-O-acyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside moiety



to provide the corresponding 7-keto sterols having the corresponding moiety



The oxidation is accomplished with an oxidizing agent, which preferably contains chromium, with pyridine-chromium trioxide $(C_5H_5N)_2CrO_3$ or pyridine-chlorochromate $(C_5H_5NHCrO_3)Cl$ being preferred and t-butyl chromate being especially preferred. The inert glycosidic double bond between C_2=C_3 thereby remains intact as it is shielded by the C_4, C_6 acyl (e.g., acetyl) groups. The reduction of this 7-ketone with a suitable reducing agent, preferably a complex metal hydride, such as one or more of $LiAlH_4$, $NaBH_4$, and KBH_4, more preferably $LiAlH_4$, leads to a steroidal glycoside according to the invention. In a preferred embodiment, the method importantly provides 7β-hydroxycholesteryl 2,3-dideoxy-α-D-erythro-hex-2-enopyranoside (7β-OHC) of formula



which like cholesterol is systemically biocompatible. The

product is obtained after workup of the reaction mixture, e.g. by chromatographic separation of the C_7 β-hydroxy isomer from the C_7 α-hydroxy isomer, in a suitable solvent mixture, preferably a mixture comprising dichloromethane:acetone preferably in 1:1 mixture.

In a preferred aspect, the invention comprises the novel pyranoside compounds having the above formulas. The novel 7β-hydroxycholesterol DDH pyranoside in particular and its 7-keto precursor possess valuable pharmacological properties. For example, a preferred parenteral dosage regimen in treating the proliferative phase growth of the kind described, allowing for ethical considerations and practices exercised in the clinician's judgment, calls for administration of about 10 to about 40 mg. of 7β-OHC DDH pyranoside per 70 Kg. of body weight, once a day or less often while analysis is made of tumor markers such as CEA, TPA, etc. so that the dosage can be adjusted from time to time to normalize the tumor marker level. Whereas the alpha-isomer, delta^5-cholesten-3β,7α-diol, is formed in the liver as the first degradation product of cholesterol and possesses no physiological activity, the beta-isomer, delta^5-cholesten-3β,7β-diol (as well as its 7-keto analog), is formed in the thymus gland of all mammals as a universal signal substance of the mammalian immune defense. It owes its activity, which is solely directed to malignant cell surfaces, to the fact that it is bound unspecifically by LDL (low density lipoproteins).

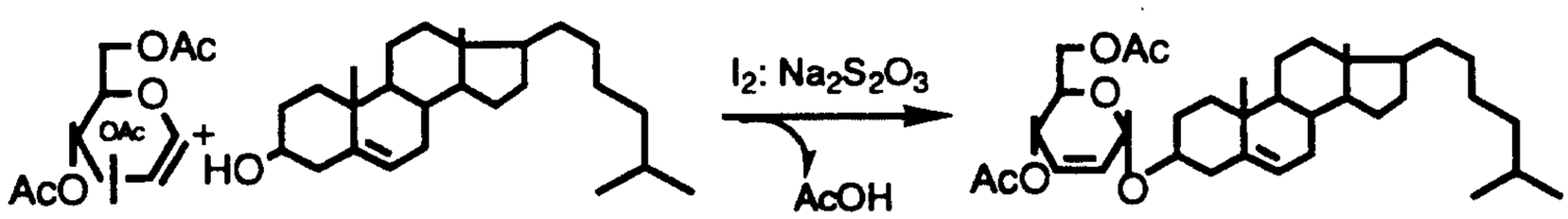
The novel steroid and non-steroid compounds of the invention can be used in the form of pharmaceutical preparations comprising each such compound in a

pharmacogically effective amount in admixture with a pharmaceutically acceptable carrier which may be conventional per se. These preparations may be formulated by well known procedures. In these respects, see for example *Remington's Pharmaceutical Sciences*, Chapter 43, 14th Ed., Mack Publishing Co., Easton, PA 18042, USA. These preparations can be administered in any suitable way such as orally, e.g. in the form of tablets, dragees, gelatin capsules, soft capsules, solutions, emulsions or suspensions or parenterally, e.g. in the form of injectable solutions at suitable pH, e.g. ca. 7.5, or topically, e.g. in the form of a cream.

The invention and the best mode for practicing the same are illustrated by the following examples.

Example 1

Preparation of Cholesteryl 4,6-Di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside



5.0 g (=0.02 mole) molecular iodine were dissolved with stirring in 300 ml benzene in a 2-litre three-necked flask fitted with stirrer, reflux condenser and thermometer. To the wine-red solution thus obtained was added the solution of 27.2 g (=0.10 mole) 3,4,6-tri-O-acetyl-D-glucal and 38.6 g (=0.10 mole) cholesterol (delta^5-cholesten-3β-ol) in 700 ml of benzene. In the course of 2 hours the mixture was heated to 70-75°C. The reaction was monitored by IR-spectroscopy; it was terminated only when the peak of the glucal at 1650 cm^{-1} (Figure 1) had disappeared. The red color of the reaction solution is not significant. After removal of the flask heater the reaction solution is rapidly cooled in a water-bath to about 20-30°C. After transfer into a 2-litre separatory funnel the cooled wine-red reaction solution was thoroughly shaken until complete discoloration with 500 ml + 10% of 0.1 N = 12.5 g + 10% = 13.8 g aqueous solution of $Na_2S_2O_3$, washed twice with water, treated with activated carbon, dried over anhydrous Na_2SO_4 and the solvent distilled off, finally in vacuo.

Crude yield: 58.3 g (= 97.4% th.)

The product, cholesteryl 4,6-di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside, is recrystallized from 2 litres of CH_3OH.

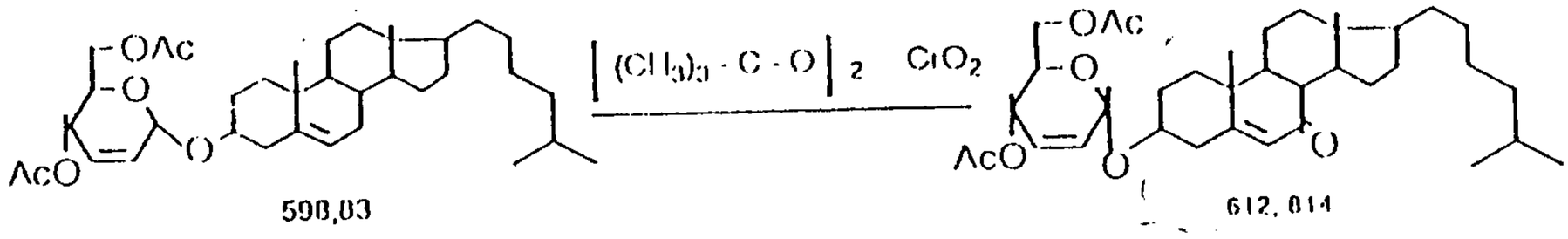
Yield: 56.95 g (= 95.1% th.)
Mp: 118-120°C
IR-spectrum: Figure 2
NMR-spectrum: Figure 3

The invention comprises not only the above glycoside product but also other glycoside products made by the above procedure using as starting materials equivalent amounts of the glucal (or other suitable glucal) and the aglycon selected for glycosidation. Suitable aglycon compounds are selected (for their known use as aglycons) from the aforementioned aliphatic, alicyclic, aliphatic-aromatic or aromatic compounds having a primary, secondary or tertiary functional group (i.e., -OH, -SH, $-NH_2$, $-NHR_1$), preferably a compound listed in the foregoing paragraphs a) to y) or in pages THER-5 to THER-29 of the Merck Index XI, supra. Especially preferred glycoside products made by the above procedure are the respective 4,6-di-O-acyl or acetyl-2,3-dideoxy-α-D-erythro-hex-2-eno-pyranosides of the following aglycon compounds which as DDH pyranoside products are characterized by known IR and NMR spectroscopy data available from the referenced literature.

Hydroxy Aglycon	The Merck Index IX Monograph No.	Use
Cholic acid	2206	choleretic
Clavulanic acid	2342	antibacterial
Amoxicillin	610	antibacterial
Daunorubicin	2825	antineoplastic
Lovastatin	5460	antihyper cholesterolemic
Mevastatin	6088	antihyper cholesterolemic
Simvastatin	8491	antihyperlipidemic

Example 2

Preparation of 7-ketocholesteryl 4,6-Di-O-acetyl-2,3-dideoxy-a-D-erythro-hex-2-enopyranoside



598,03 612, 014

In a 250 ml three-necked flask fitted with reflux condensor, dropping funnel, thermometer and magnetic stirrer 6.00 g (= 0.01 mole) of the unsaturated glycoside title product from Example 1 of mp 118-120°C were dissolved in 45 ml of CCl_4 and heated to boiling (80°C). In the course of 30 minutes a mixture of 10 ml Ac_2O (acetic anhydride) and 40 ml t-butyl chromate solution, prepared according to the Annex, was slowly added dropwise to the boiling solution and stirred for another 10 hours at the boiling point. After cooling, a solution of 6.0 g oxalic acid in 60 ml water was added dropwise in the course of 45 minutes at 5°C to 10°C in an ice-bath followed by 4.2 g solid oxalic acid. Stirring was then continued for another 2 hours. Thereafter separation took place in the separating funnel, the upper dark aqueous phase being extracted twice with CCl_4, the combined CCl_4-solutions extracted with water, saturated solution of $NaHCO_3$ and then with water again, in this order, and dried over Na_2SO_4. Finally the solution was decolorized with activated carbon. After concentration in vacuo, the straw-yellow residue was dissolved in 25 ml of a mixture consisting of cyclohexane 40 : ethyl acetate 10 : chloroform 1 and chromatographed on a silica gel column (diameter 2.5 cm; height 25 cm), charged with 60 g of silica gel 40 (Merck Article 10180) and the same solvent mixture.

Yield: Fraction 1: 1.8 g (= 30.1% of theory) unchanged starting material.

Fraction: 2: 4.2 g (= 68.5% of theory) 7-keto-compound

Mp: 113-115°C

IR-spectrum: Figure 4

NMR-spectrum: Figure 5

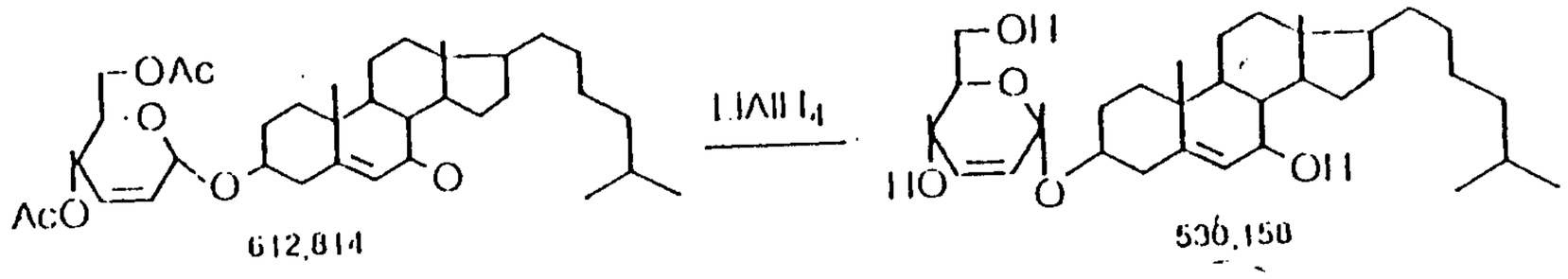
Annex:

Preparation of t-butyl chromate

In a 500 ml beaker, 187.2 g (= 2.5 mole) t-butanol of mp 24.5°C were warmed to 28°C and melted. To this melt, 74 g (= 0.74 mole) of CrO_3 were added by using a thermometer as a stirring bar. In order to keep the reaction temperature below 30°C, occasional cooling with ice-water was necessary. The liquid reaction product was diluted in a separating funnel with 520 ml of CCl_4 and left to stand overnight. This standing is important to allow clarification of the solution. The following morning, the upper dark layer was separated. The clear CCl_4-solution was dried with 50 g of anhydrous Na_2SO_4, filtered and the Na_2SO_4 washed with 320 ml of CCl_4. Thereafter, the combined CCl_4-solutions were concentrated to 400 ml in vacuo in a water-bath at a temperature of 40°C to 45°C, wherein excess t-butanol and CCl_4 were both distilled azeotropically. The solution thus obtained is relatively storage stable as it may be kept unchanged in the refrigerator at -1°C for at least one month.

Example 3

Preparation of 7-β-Hydroxycholesteryl 2,3-Dideoxy-a-D-erythro-hex-2-enopyranoside



6.13 g (=0.01 mole) of pure compound from Example 2 with mp 113-115°C were dissolved by heating in 100 ml peroxide-free ether which has been dried with metallic sodium and cooled to room temperature. A solution of 0.8-1.0 g (= 0.021 mole) $LiAlH_4$ in 100 ml absolute ether was added to a 500 ml three-necked flask with magnetic stirrer, reflux condensor and thermometer. The ethereal solution of the unsaturated aceto-7-keto-glucoside was then added dropwise with sufficient stirring to assure that the reaction temperature did not substantially exceed 20°C. After addition had been terminated, which may take up to two hours, stirring was continued for another two hours.

Afterwards, the reaction mixture was cooled in ice-water and treated drop by drop with H_2O until all H_2 (conducted to the outlet of the hood by means of a tube) had evolved. H_2O-consumption was about 5.0 ml. On a larger scale, the use of $CH_3COOC_2H_5$ is recommended. In order to dissolve the $LiAlO_2$ formed, the solution was stirred with 16 ml of 10% H_2SO_4 and, after transfer to a 500-ml separating funnel, diluted with 100 ml of ether and shaken thoroughly. Thereby, the reaction product, comprising a mixture of the title 7β-OH compound and its 7α-OH isomer, which has separated as crystals, goes completely into solution. The separated acidic aqueous solution was extracted once with ether and the combined

ethereal solution was washed with 100 ml of a saturated NaCl-solution in two portions of 50 ml each. After drying over anhydrous Na_2SO_4, the filtrate was kept in the refrigerator at -1°C for nine hours. The crystals thus obtained are collected by suction over a G4-suction filter and weighed.

Crude yield: 5.10 g (= 96.23% of theory)

Mp: 165-167°C

The product comprising a mixture of the title 7β-OH compound and its 7α-OH isomer was dissolved in 25 ml of dioxane (or THF) by heating and the resulting solution was chromatographed on a column of silica gel (diameter 5.0 cm; height 70 cm) charged with 300 g of silica gel 40 (Merck Article 10180) using a solvent mixture consisting of dichloromethane 1 : acetone 1.

Yield:

Fraction 1: 0.35 g (= 6.8%) 7a-OH-compound, mp: 159-161oC

Fraction 2: 4.60 g (= 90.2%) 7b-OH-compound, mp: 181-183oC

IR-spectrum: Figure 6

NMR-spectrum: Figure 7

The above glycosylation reaction carried out in benzene at 70°C is selective for the desired alpha isomer rather than the beta isomer. Thus the reaction typically produces a ratio of alpha to beta cholesteryl glycosylate isomers (α:β) of greater than 20:1. Similarly, in THF at 67°C and at room temperature the reaction in either case typically produces an α:β ratio of about 9:1.

Another general procedure was used in which, instead of cholesterol, a starting material (i.e., a substrate) having at least one functional group (e.g., OH, SH, and/or -COOH) was used. The procedure is detailed as follows:

GENERAL PROCEDURE

The substrate (1 equiv) was added to a stirring solution of glucal (0.5-1.0 g) and iodine (20 mol%) in THF at room temperature. The reaction was monitored by thin-layer chromatography. Reaction times are listed for each compound. Upon completion the solution was diluted with ether and washed with 10% aqueous $Na_2S_2O_3$ solution. The layers were separated and the aqueous layer was extracted with additional ether. The combined organic layers were dried over $Na_2S_2O_4$, concentrated, and purified by column chromatography on 230-400 mesh SiO_2. All products were recovered as viscous oils except for those compounds for which a melting point is given.

Note: The thiols were added at lower temperatures as indicated for each adduct.

ILLUSTRATIVE SUBSTRATES

ALCOHOLS (all over 90% yield; α-isomer:β-isomer = 5-8:1)

- methanol
- ethanol
- isopropanol
- *tert*-butyl alcohol
- benzyl alcohol
- allyl alcohol

PHENOLS (α-isomer:β-isomer = 6 - 8:1)

- phenol (70%)
- *p*-methoxyphenol (ca. 65%)
- *p*-nitrophenol (ca. 60%)

THIOLS (highly reactive)

- ethyl mercaptan (-78°C to -55°C) (35%) — α-isomer:β-isomer = 16:1
- isopropyl mercaptan (-78°C to 0°C) (77%) — α-isomer:β-isomer = 15:1

tert-butyl mercaptan (-25°C) (68%)	α-isomer only
thiophenol (room temperature) (50%)	α-isomer:β-isomer = 20:1
CARBOXYLIC ACID	
acetic acid (catalytic) (80%)	α-isomer:β-isomer = 10:1

SPECTROSCOPIC DATA FOR ALCOHOL (ROH) PHENOL (ArOH) AND CARBOXYLIC (RC00H) ADDUCTS

R = Me (Reaction Time = 30 min. Yield = 87%)

^{1}H NMR ($CDCl_3$; 300 MHz) δ 2.087 (s,3H), 2.111 (s,3H), 3.457 (s,3H), 4.05-4.13 (m,1H), 4.188 (1H) and 4.266 (1H) (ABq, J_{AB}=12.1 Hz; the 4.188 peaks are further split into d with J=2.5 Hz; the 4.266 peaks are further broken down into d with J=5.3 Hz), 4.934 (s,1H), 5.322 (dd,1H,J=9.7, 1.4 Hz), 5.834 (1H) and 5.894 ppm (1H) (ABq, J_{AB}=10.6 Hz; the 5.834 peaks are further split into dd with J=2.0, 2.0 Hz).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.56, 55.70, 63.26, 65.75, 67.35, 95.60, 128.15, 129.38, 170.08, 170.36 ppm.

IR (KBr) 1048, 1072, 1107, 1137, 1148, 1188, 1233, 1372, 1438, 1449, 1456, 1744, 2910, 2939, 2956 cm^{-1}.

R = Et (Reaction Time = 50 min. Yield = 97%) m.p. (hexanes = 76-77.5°C.

^{1}H NMR ($CDCl_3$; 300 MHz) δ 1.256 (d,J=7.1 Hz,3H), 2.084 (s,3H), 2.105 (s,3H), 3.584 (1H) and 3.837 (1H) (ABq, J_{AB}=9.7 Hz; both sets of peaks are further split into q with J=7.1 Hz), 4.09-4.14 (m,1H), 4.174 (1H) and 4.261 (1H) (ABq, J_{AB}=12.2 Hz; the 4.174 peaks are further split into dd with J=2.8, 2.8 Hz; the 4.261 peaks are further split into d with J=5.3 Hz), 5.050 (s,1H), 5.319 (dd,J=9.6,1.4 Hz, 1H), 5.837 (1H) and 5,891 ppm (1H) (ABq, J_{AB}=10.2 Hz; the 5.837 peaks are further split into dd with J=1.7, 1.4 Hz).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 15.31, 20.66, 20.83, 63.30, 64.25, 65.75, 67.25, 94.40, 128.37, 129.13, 170.17, 170.53 ppm.

IR (KBr) 1019, 1052, 1084, 1108, 1120, 1133, 1137, 1189, 1229, 1239, 1257, 1274, 1372, 1382, 1738, 2902, 2980

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cm^{-1}.

R = i-Pr (Reaction Time = 65 min. Yield = 96%).

^{1}H NMR ($CDCl_3$; 300 MHz) δ 1.186 (d,J=6.2 Hz,3H), 1.258 (d,J=6.2 Hz,3H), 2.081 (s,3H), 2.096 (s,3H), 3.988 (dq,J=6.2, 6.2 Hz,1H), 4.12-4.28 (m,3H), 5.133 (s,1H), 5.301 (dd,J=9.5, 1.4 Hz,1H), 5.804 (1H) and 5.875 ppm (1H) (ABq, J_{AB}=10.3 Hz; the 5.804 peaks are further split into dd with J=2.6, 2.5 Hz,1H).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.59, 20.83, 22.06, 23.54, 63.42, 65.87, 67.22, 70.83, 93.06, 128.82, 128.91, 170.18, 170.55 ppm.

IR (KBr) 1035, 1101, 1125, 1185, 1231, 1372, 1457, 1744, 2933, 2973 cm^{-1}.

R = t-Bu (Reaction Time = 2 hr. Yield = 74%)

^{1}H NMR ($CDCl_3$; 300 MHz) δ 1.292 (s,9H), 2.075 9s,3H), 2.085 (s,3H), 4.12-4.28 (m,3H), 5.271 (d,1H,J=8.0 Hz), 5.321 (dd,1H,J=1.3, 1.3 Hz), 5.744 (1H), and 5.844 ppm (1H) (ABq,J_{AB}=10.2 Hz; the 5.744 peaks are further split into dd with J=2.8, 2.8 Hz).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.72, 20.93, 28.79, 63.35, 65.49, 66.72, 75.23, 89.02, 128.13, 129.67, 170.17, 170.57 ppm.

IR (KBr) 1025, 1044, 1100, 1183, 1196, 1235, 1370, 1392, 1746, 2935, 2976 cm^{-1}.

R = Ph (Reaction Time = 8 hr. Yield = 70%)

^{1}H NMR ($CDCl_3$; 300 MHz) δ 1.976 (s,3H), 2.017 (s,3H), 4.10-4.30 (m,3H), 5.387 (d,J=11.4 Hz,1H), 5.698 (s,2H), 7.00-7.33 ppm (m,5H).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.54, 20.84, 62.66, 65.13, 67.85, 92.12, 117.10, 122.42, 127.14, 129.40, 130.06, 157.08, 170.06, 1740.46 ppm.

IR (KBr) 1005, 1029, 1047, 1076, 1096, 1187, 1222, 1371, 1491, 1589, 1598, 1744 cm^{-1}.

R = p-MeO-Ph (Reaction Time = 12 hr. Yield = 75%) m.p. (hexanes) = 77-77.5°C.

^{1}H NMR ($CDCl_3$; 300 MHz) δ 2.023 (s,3H), 2.109 (s,3H), 3.774 (s,3H), 4.14-4.30 (m,3H), 5.376 (d,J=9.1 Hz,1H), 5.567 (s,1H), 6.007 (s,1H), 6.830 (2H) and 7.047 ppm (2H) (ABq,J_{AB}=9.0 Hz).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.61, 20.85, 55.77, 63.03, 65.60, 67.94, 94.23, 114.85, 115.11, 116.21, 118.90, 127.51, 130.08, 170.32, 170.75 ppm.

IR (KBr) 1010, 1037, 1053, 1184, 1227, 1243, 1255, 1270, 1380, 1507, 1741, 2922, 2934, 2958 cm^{-1}.

R = CH_2Ph (Reaction Time = 80 min. Yield = 95%)

^1H NMR ($CDCl_3$; 300 MHz) δ 2.081 (s,3H), 2,102 (s,3H), 4.09-4.29 (m,3H), 4.601 (1H) and 4.809 (1H) (ABq, J_{AB}=11.7 Hz), 5.139 (s,1H), 5.334 (dd,J=9.4, 1.3 Hz,1H) 5.875 (ABq,2H), 7.29-7.40 ppm (m,5H).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.63, 20.81, 63.19, 65.74, 67.50, 70.35, 93.83, 127.00, 128.56, 128.56, 129.46, 170.17, 170.59 ppm.

IR (KBr) 1026, 1038, 1101, 1137, 1151, 1186, 1227, 1370, 1405, 1436, 1454, 1743, 2904, 2937, 2953, 3032 cm^{-1}.

R = $CH_2CH=CH_2$ (Reaction Time = 80 min. Yield = 88%)

^1H NMR ($CDCl_3$; 300 MHz) δ 2.086 (s,3H), 2.106 (s,3H), 4.05-4.30 (m,5H), 5.084 (s,1H), 5.212 (d,J=10.3 Hz, 1H), 5.28-5.35 (m,2H), 5.848 (1H) and 5.899 (1H) (ABq, J_{AB}=10.3 Hz), 5.92-5.99 ppm (m,1H).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.73, 20.91, 63.00, 65.35, 67.05, 69.22, 93.65, 117.35, 127.86, 129.27, 134.27, 170.18, 170.62 ppm.

R = $CH_2C(CH_3)_3$ (Reaction Time = 30 min. Yield = 97%)

^1H NMR ($CDCl_3$; 300 MHz) δ 0.931 (s,9H), 2.086 (s,3H), 3.133 and 3.481 (ABq, J_{AB}=8.8 Hz, 2H), 4.07-4.27 (m,3H), 4.985 (s,1H) 5.305 (d,J=9.5 Hz,1H), 5.850 and 5.859 (ABq, J_{AB}=10.5 Hz, 2H; the 5.850 ppm peaks are further split into dd with J=2.7 and 2.6 Hz).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.73, 20.91, 63.00, 65.35, 67.05, 69.22, 93.65, 117.35, 127.86, 129.27, 134.27, 170.18, 170.62 ppm.

R = OAc (Reaction time 24 h. Yield = 81%) (20 mol% of acetic acid was used).

^1H NMR ($CDCl_3$; 300 MHz) δ 2.082 (s,3H), 2.095 (s,3H), 2.098 (s,3H), 4.06-4.28 (m,3H), 5.368 (d,J=10.5 Hz, 1H), 5.850 and 6.011 (ABq, J_{AB}=10.6 Hz, 2H; the 5.850 ppm peaks are further split into dd with J=2.7 and 2.2 Hz), 6.294 ppm (s,1H).

SPECTROSCOPIC DATA FOR THIOL (RSH) ADDUCTS

R = Et (-78 to -55°C, Reaction Time = 3.5 hr. Yield = 35%)

^1H NMR ($CDCl_3$; 300 MHz) δ 1.252 (t,J=7.1 Hz, 3H), 2.080

(s,3H), 2.099 (s,3H), 3.581 (1H) and 3.832 (1H) (ABq, J_{AB}=9.7 Hz; both sets of peaks are further split into dq with J=7.1, 7.1 Hz), 4.08-4.29 (m,3H), 5.043 (s,1H), 5.315 (dd,J=9.6, 1.3 Hz, 1H), 5.831 (1H) and 5.883 ppm (1H) (ABq, J_{AB}=10.3 Hz).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 15.31, 20.72, 20.92, 63.15, 64.30, 65.52, 67.02, 94.30, 128.09, 129.03, 170.20, 170.66 ppm.

IR (KBr) 1049, 1079, 1182, 1232, 1371, 1437, 1451, 1743, 2930, 2964 cm^{-1}.

R = i-Pr (-78 to 0°C, Reaction Time = 6 hr. Yield = 77%)

^{1}H NMR ($CDCl_3$; 300 MHz) δ 1.337 (d,3H,J=6.8 Hz), 1.342 (d,3H,J=6.8 Hz), 2.085 (s,6H), 3.145 (dq,1H,J=6.8, 6.8 Hz), 4.15-4.38 (m,3H), 5.349 (d,1H,J=9.6 Hz), 5.638 (s,1H), 5.761 (1H) and 5.935 ppm (1H) (ABq, J_{AB}=10.1 Hz).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.66, 20.92, 23.88, 36.3, 63.14, 65.25, 66.93, 79,61, 126.74, 129.39, 170.21, 170.60 ppm.

IR (KBr) 1050, 1078, 1122, 1157, 1181, 1229, 1369, 1419, 1452, 1743, 2868, 2927, 2960 cm^{-1}.

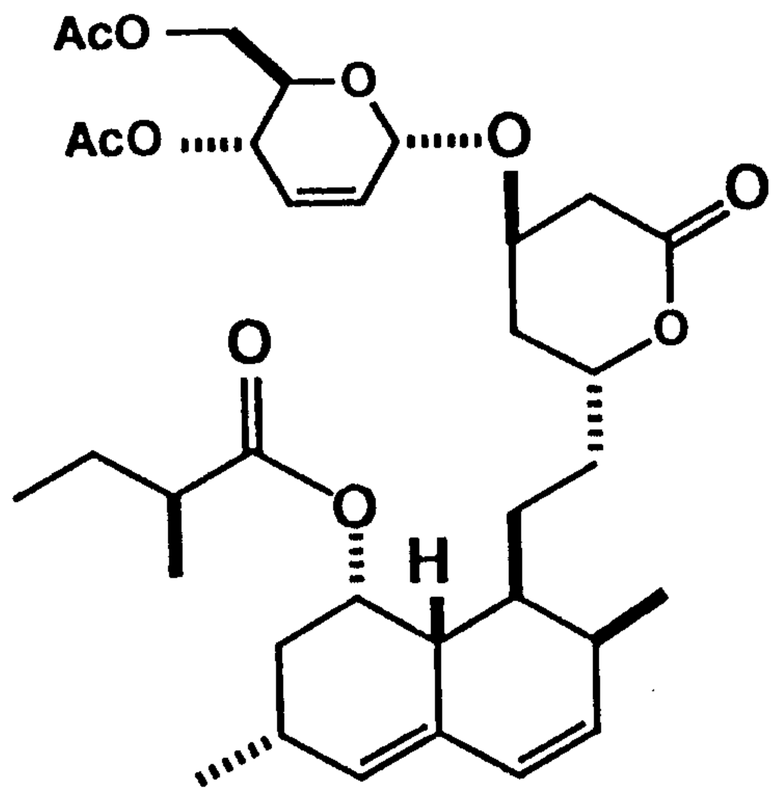
R = t-Bu (-25 to 0°C, Reaction Time = 2 hr. Yield = 68%)

^{1}H NMR ($CDCl_3$; 300 MHz) δ 1.404 (s,9H), 2.070 (s,3H), 2.080 (s,3H), 4.13-4.37 (m,3H), 5.325 (dd,1H,J=9.3, 1.8 Hz), 5.739 (d,1H,J=1.7 Hz), 5.753 (1H) and 5.902 ppm (1H) (ABq,J=10.9 Hz).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 20.70, 20.94, 31.51, 44.33, 63.22, 65.16, 66.84, 78.17, 126.61, 129,67, 170.30, 170.72 ppm.

IR (KBr) 1054, 1078, 1164, 1236, 1368, 1744, 2962 cm^{-1}.

By the same procedure, but using mevinolin (lovastatin) as a substrate rather than cholesterol, the resulting adduct in about 60% yield is Lovastatin 4,6-di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside having the formula:



The reaction is carried out under mild conditions, e.g., preferably at room temperature, for one hour, using triacetyl D-glucal (1.0 equiv), iodine (20 mol %) in THF as the solvent.

R = Lovastatin (Reaction Time = 1 h. Yield = 55%)

^1H NMR ($CDCl_3$; 300 MHz) δ 0.873 (t,J=7.5 Hz,3H), 0.892 (d,J=7.0 Hz, 3H), 1.074 (d,J=7.4 Hz, 3H), 1.105 (d,J=6.9 Hz, 3H), 2.091 (s,3H), 2.094 (s,3H), 3.99-4.05 (m,1H), 4.14-4.28 (m,3H), 4.29-4.34 (m,1H), 4.52-4.61 (m,1H), 5.134 (s,1H), 5.323 (dd,J=9.6, 1.3 Hz, 1H), 5.373 (d,J=2.7 Hz, 1H), 5.75-5.91 (m,2H), 5.909 (part of ABq, J_{AB}=11.1 Hz, 1H), 5.992 ppm (d,J=9.7 Hz, 1H).

^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 11.67, 13.92, 16.23, 20.70, 20.88, 22.85, 24.23, 26.79, 27.51, 30.77, 32.77, 33.22, 35.01, 35.73, 36.65, 37.46, 41.45, 62.98, 65.26, 67.38, 67.85, 68.65, 76.70, 93.12, 127.44, 128.44, 129.49, 129.72, 131.65, 132.94, 169.32, 170.07, 170.47, 176.39 ppm.

By the same procedure, but using either mephenesin or capaicin as a substrate rather than mevinolin, the resulting adduct is mephenesin 4,6-di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside and

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capsaicin 4,6-di-O-acetyl-2,3-dideoxy-α-D-erythro-hex-2-enopyranoside, respectively.

R = Mephenesin (Reaction Time = 1 h. Yield = 85%)

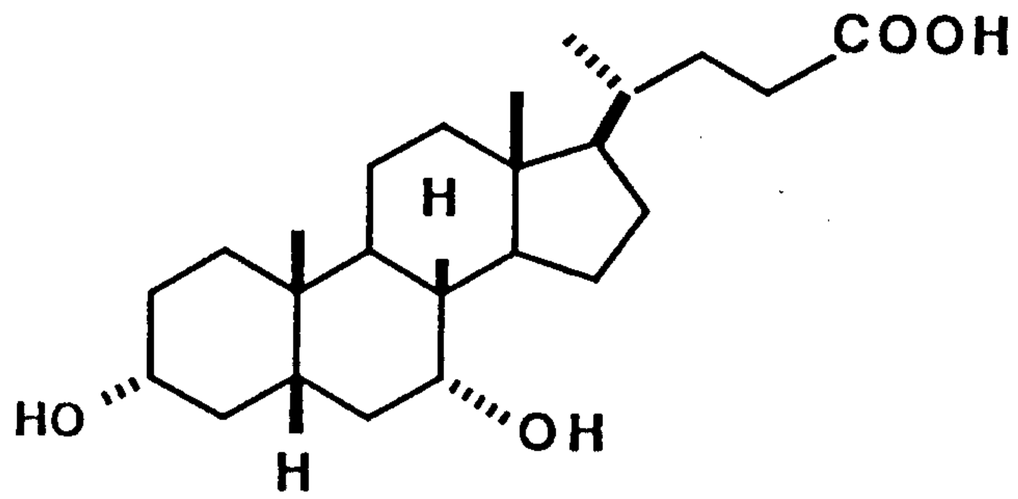
^1H NMR ($CDCl_3$; 300 MHz) δ 2.068 (s,3H), 2.071 (s,3H), 2.077 (s,3H), 2.080 (s,3H), 2.214 (brs, 3H), 3.70-3.82 (m,3H), 3.99-4.40 (m,11H), 5.06-5.12 (m,1H), 5.28-5.39 (m,3H), 5.78-5.99 (m,4H), 6.81-6.91 (m,3H), 7.11-7.19 ppm (m,2H).

R = Capsaicin (Reaction Time = 8 h. reflux in THF. Yield = 60%)

^1H NMR ($CDCl_3$; 300 MHz) δ 0.834 (d,J=6.6 Hz, 3H), 0.948 (d,J=6.7 Hz, 3H), 2.091 (s,3H), 2.093 (s,3H), 3.850 (s), 5.27-5.35 (m,2H), 5.45-5.90 (m,4H), 6.71-6.88 (m,3H).

Adducts with cholic acid or a cholic acid analog by this procedure are as follows:

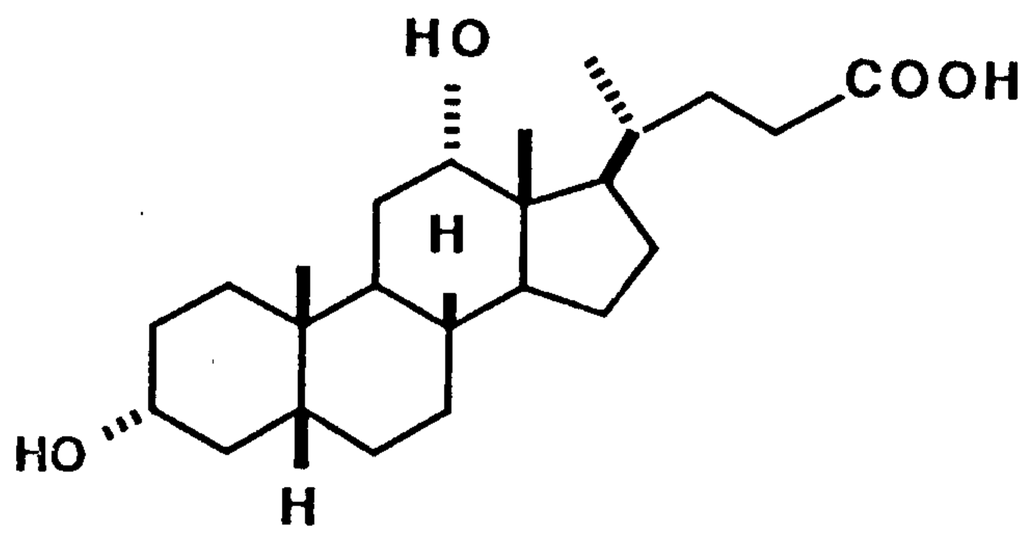
<u>Chenodeoxycholic Acid</u>



<u>Conditions</u>: triacetyl D-glucal (2.0 equiv); iodine (20 mol %); THF, room temperature, 4h

<u>Results</u>: diadduct (89%) chenodeoxycholic acid 3,12-di-(4,6-di-O-acetyl-2,3-deoxy-α-D-erythro-hex-2-enopyranoside).

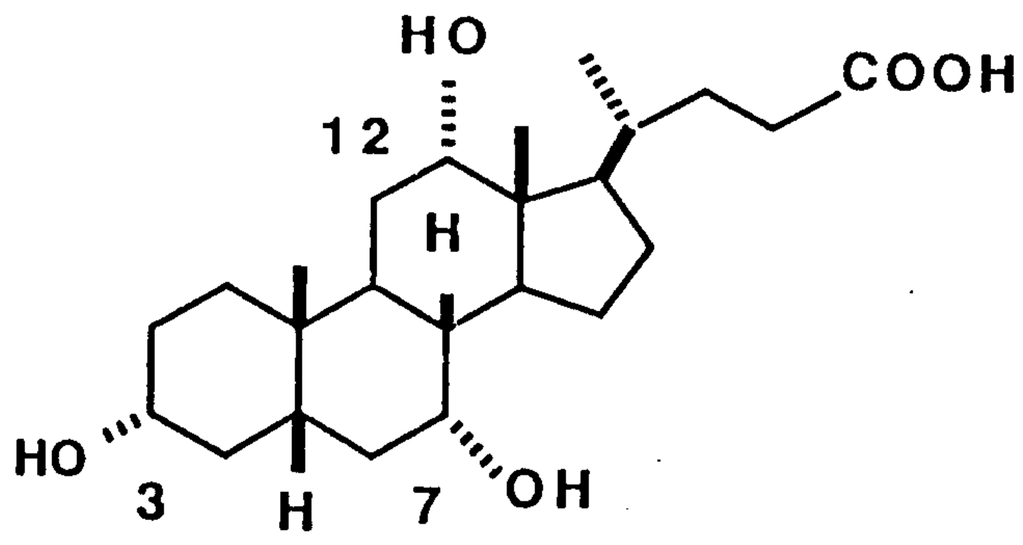
Deoxycholic Acid



Conditions: triacetyl D-glucal (2.0 equiv); iodine (20 mol %); THF, room temperature, 4h

Results: diadduct (91%) chenodeoxycholic acid 3,12-di-(4,6-di-O-acetyl-2,3-deoxy-α-D-erythro-hex-2-enopyranoside).

Cholic Acid



Conditions: triacetyl D-glucal (3.0-5.0 equiv); iodine (20 mol %); THF, reflux, 8h

Results: mixture of di- and triadducts (85%):

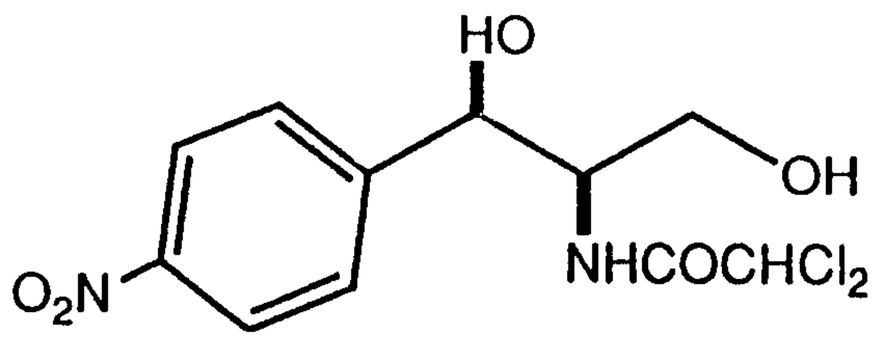
cholic acid 3,7-di-(DDH pyranoside);

cholic acid 3,12-di-(DDH pyranoside); and

cholic acid 3,7,12-tri-(DDH pyranoside).

An adduct with chloramphenicol by this procedure is as follows:

Chloramphenicol



Conditions: triacetyl D-glucal (2.0 equiv); iodine (20 mol %); THF, room temperature, 6h

Results: diadduct (85%) chloramphenicol 1,3-di-(DDH pyranoside).

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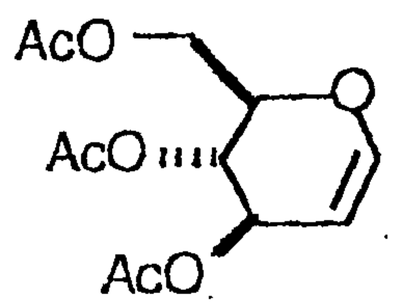
Having described the invention, the embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

Claims

1. Method for the production of a pyranoside compound, wherein a aglycon compound selected from aliphatic, alicyclic, aliphatic-aromatic and aromatic compounds having a primary, secondary or tertiary functional group selected from -OH, -SH and -COOH is glycosylated, characterized in that the aglycon compound is reacted in a solvent with 3,4,6-tri-O-acyl-glucal of formula



in the presence of molecular iodine as a catalyst to produce the corresponding 4,6-di-O-acyl-2,3-dideoxy-alpha-D-erythro-hex-2-enopyranoside of said aglycon compound, where Ac is a lower acyl group.

2. Method according to claim 1, characterized in that the aglycon compound is a delta^{5}-3beta-ol steryl compound.

3. Method according to claim 2, characterized in that a cholesterol compound is reacted with 3,4,6-tri-O-acetyl-D-glucal in an inert solvent to yield the corresponding 4,6-di-O-acetyl-2,3-dideoxy- alpha-D-erythro-hex-2-enopyranoside.

4. Method according to claim 3, characterized in that tetrahydrofuran is used as a solvent.

5. Method according to claim 3, characterized in that the 4,6-di-O-acetyl-2,3-dideoxy-alpha-D-erythro-hex-2-enopyranoside is oxidized with an oxidizing agent to produce 7-ketocholesteryl 4-6-di-O-acetyl-2-3-dideoxy-alpha-D-erythro-hex-2-enopyranoside which in turn is reduced with a metal hydride as a reducing agent to produce 7-beta-hydroxycholesteryl 2,3-dideoxy-alpha-D-erythro-hex-2-enopyranoside.

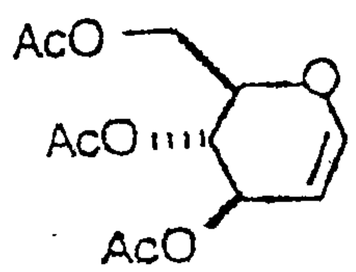
6. Method according to claim 5, characterized in that the oxidizing agent is selected from the group consisting essentially of t-butyl chromate, pyridine-chromium trioxide, and pyridine chlorochromate.

7. Method according to claim 5, characterized in that one or more of $LiAlH_4$, $NaBH_4$, and KBH_4 is used as a reducing agent.

8. Method according to claim 5, characterized in that the 7beta-hydroxycholesteryl pyranoside is isolated by chromatography.

9. Method according to claim 8, characterized in that the isolation is carried out by chromatography using a solvent mixture which comprises dichloromethane and acetone.

10. Method for the production of a pyranoside compound, wherein an aglycon compound selected from aliphatic, alicyclic, aliphatic-aromatic and aromatic compounds having a primary, secondary or tertiary functional group selected from -SH and -COOH is glycosylated, characterized in that the aglycon compound is reacted in a solvent with 3,4,6-tri-O-acyl-glucal of formula



in the presence of molecular iodine as a catalyst to produce the corresponding 4,6-di-O-acyl-2,3-dideoxy-alpha-D-erythro-hex-2-enopyranoside of said aglycon compound, where Ac is a lower acyl group.

11. Method according to claim 10, characterized in that benzene or toluene is used as a solvent.

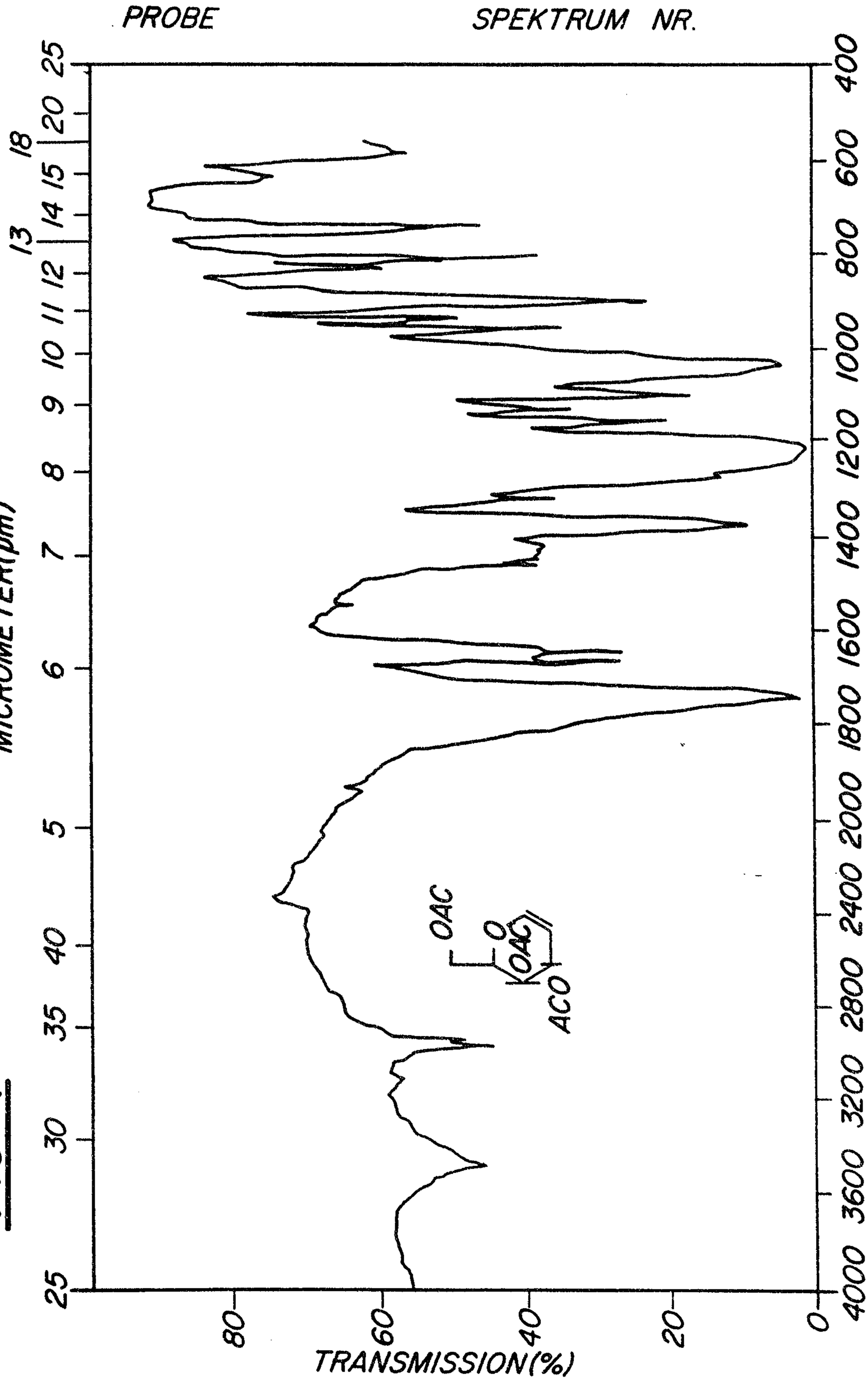


FIG-1

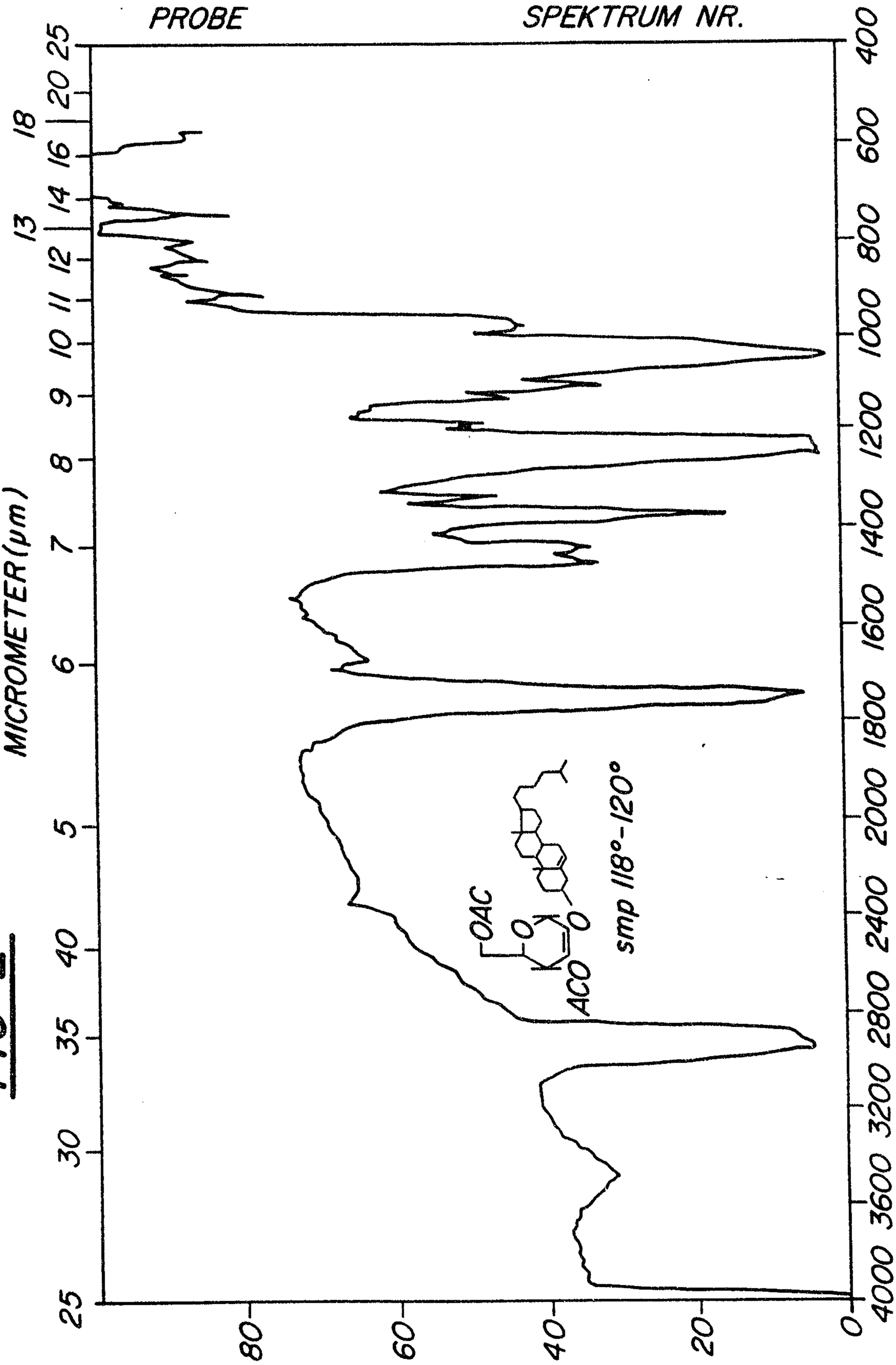
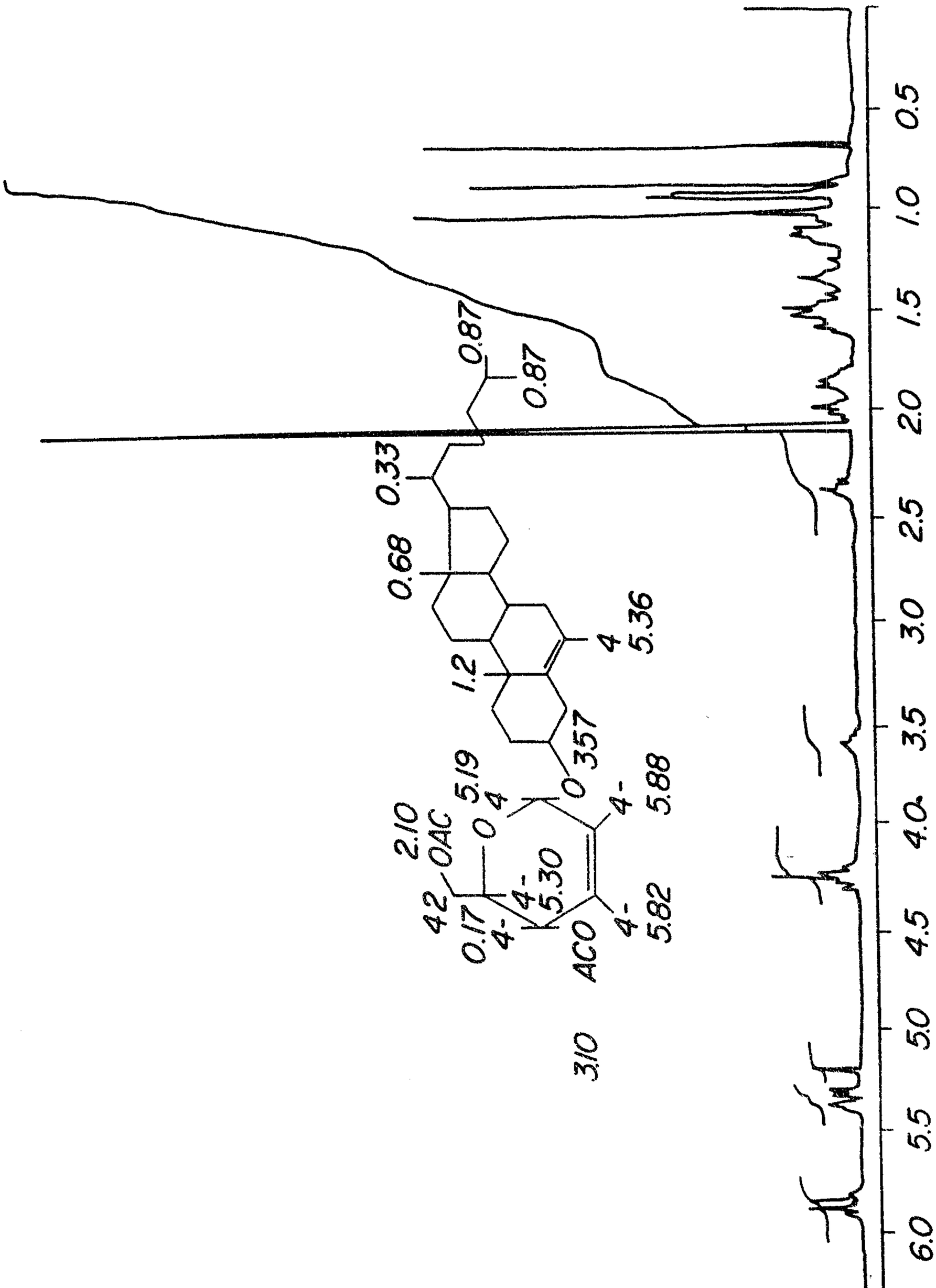


FIG-2

FIG-3


0.87
0.87
0.33
0.68
1.2
4
5.36
2.10
OAC
5.19
4
0
357
4-
42
0.17
4-
5.30
4-
5.82
3.10
ACO
5.88
2.5
2.0
1.5
1.0
0.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0

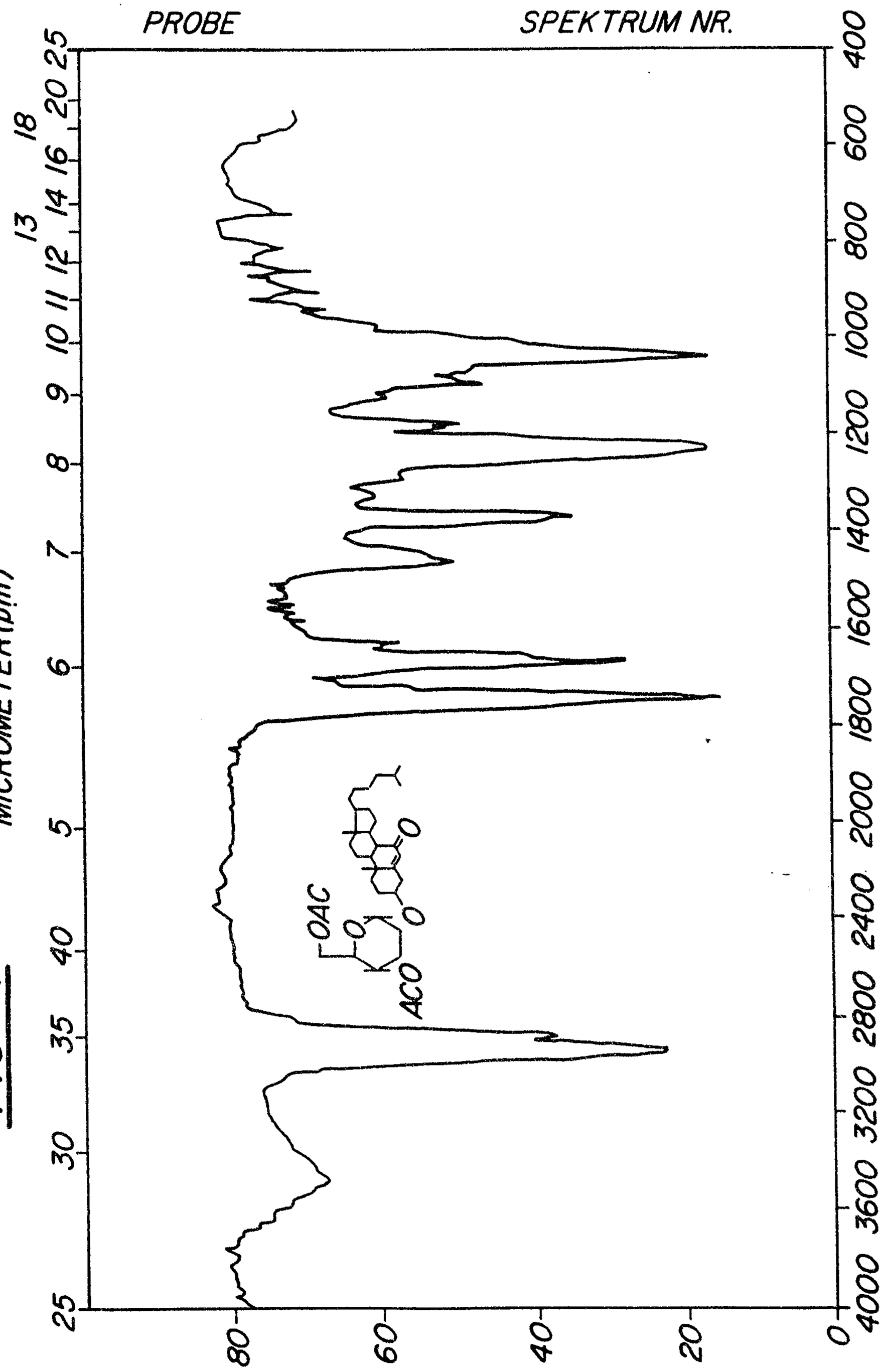
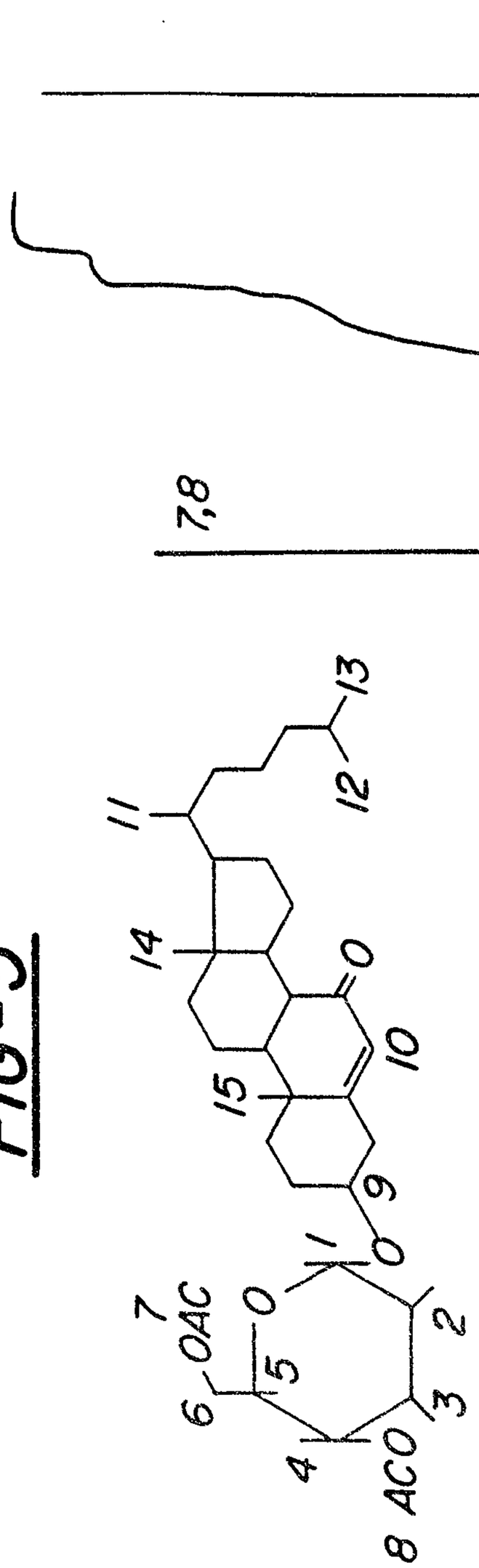


FIG-4

2113819

FIG-5



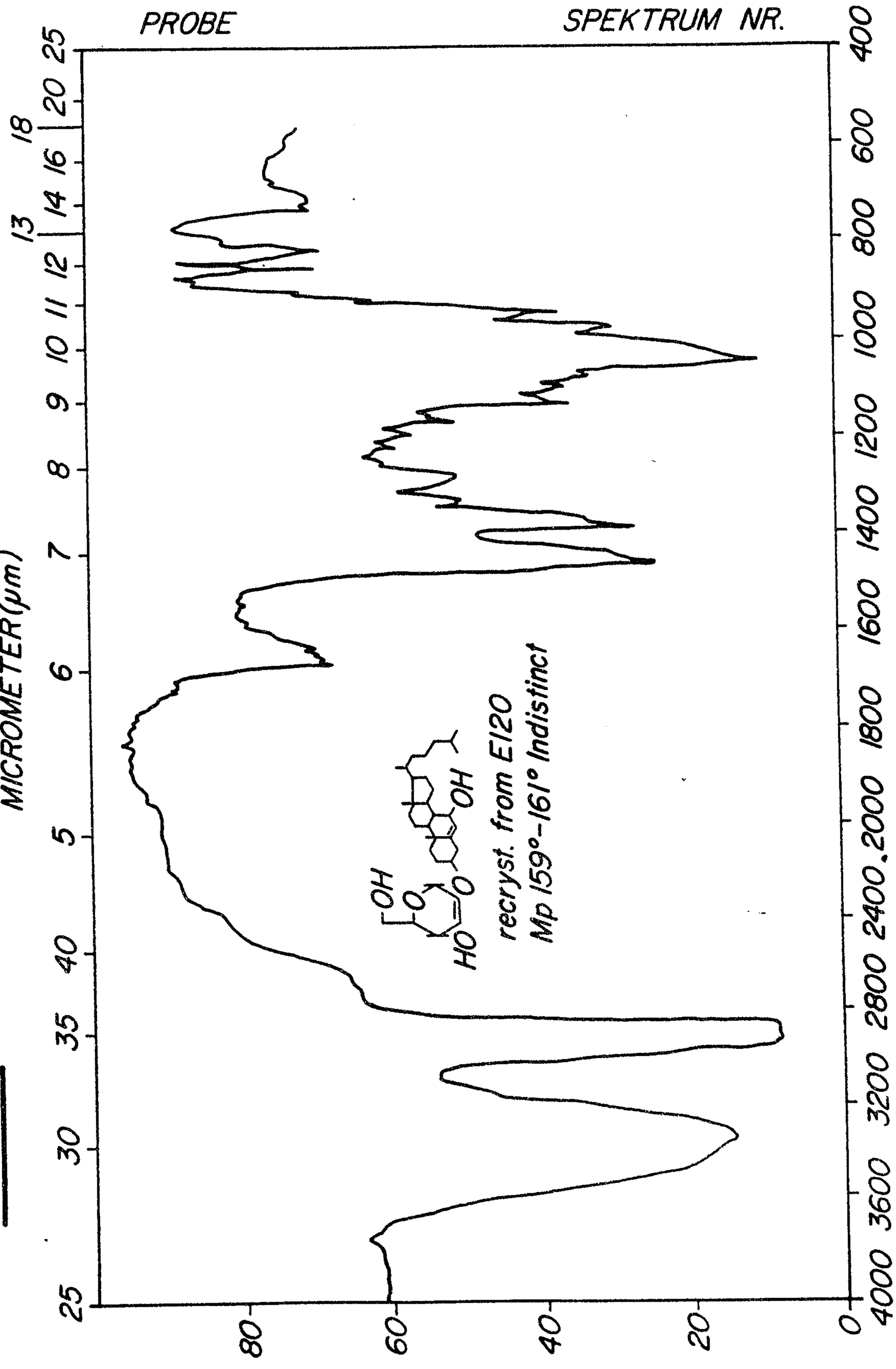

PROBE
SPEKTRUM NR.
400
600
800
1000
1200
1400
1600
1800
2000
2400
2800
3200
3600
4000
WAVENUMBER(CM⁻¹)
MICROMETER(μm)
25
20
18
16
14
13
12
11
10
9
8
7
6
5
40
35
30
25
80
60
40
20
0
TRANSMISSION (%)
OH
O
HO
O
OH
recryst. from E120
Mp 159°-161° Indistinct

FIG-6

FIG-7

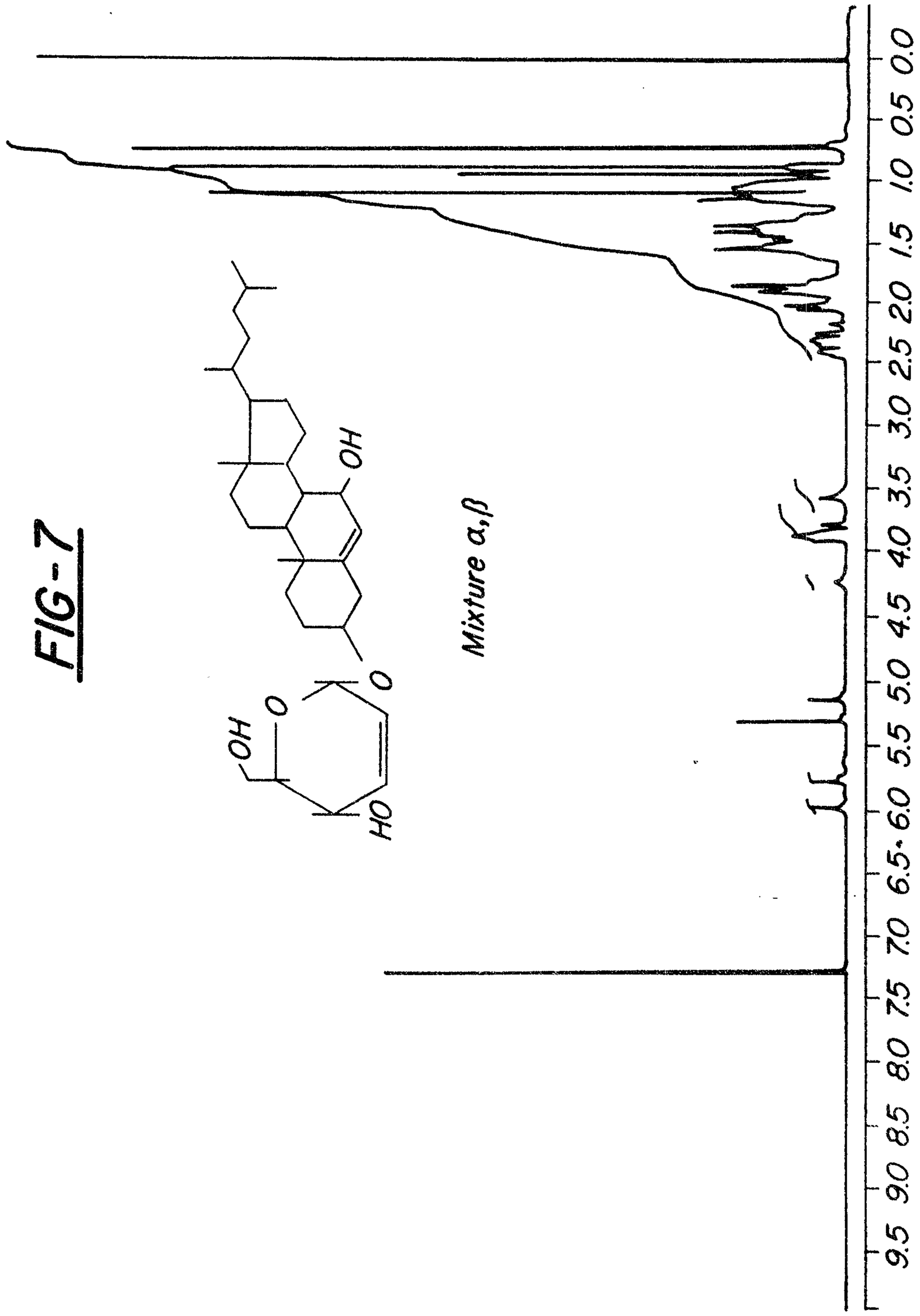
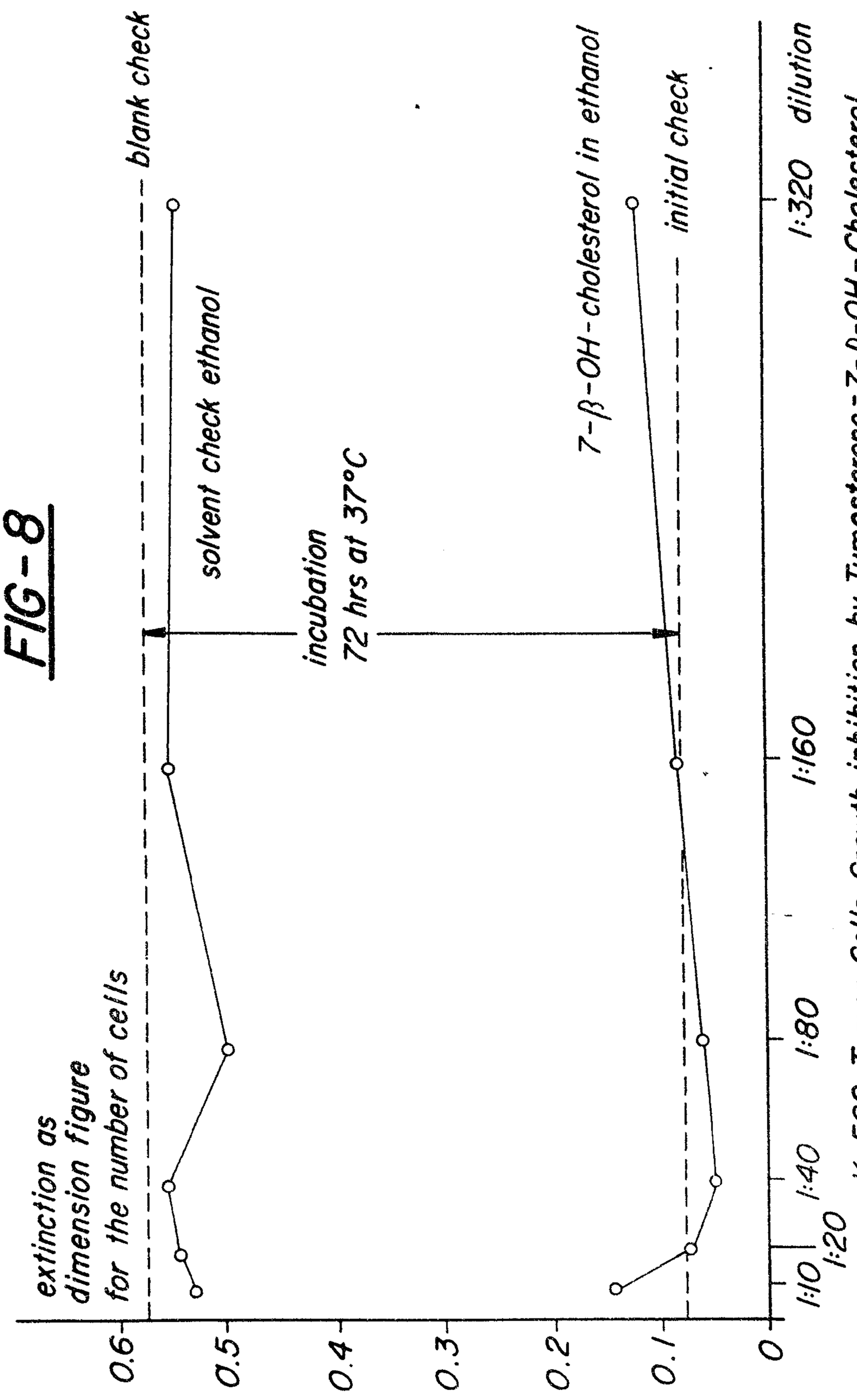


FIG-8


blank check
solvent check ethanol
incubation
72 hrs at 37°C
7-β-OH-cholesterol in ethanol
initial check
dilution
1:320
1:160
1:80
1:40
1:20
1:10
0
0.1
0.2
0.3
0.4
0.5
0.6
extinction as
dimension figure
for the number of cells
K-562 Tumor Cells Growth inhibition by Tumosterone = 7-β-OH-Cholesterol
(points represents average values from 3 single measured values)