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(54) Titre : MEDICAMENT POUR INHIBER LE FACTEUR DE TRANSMISSION NF-κB
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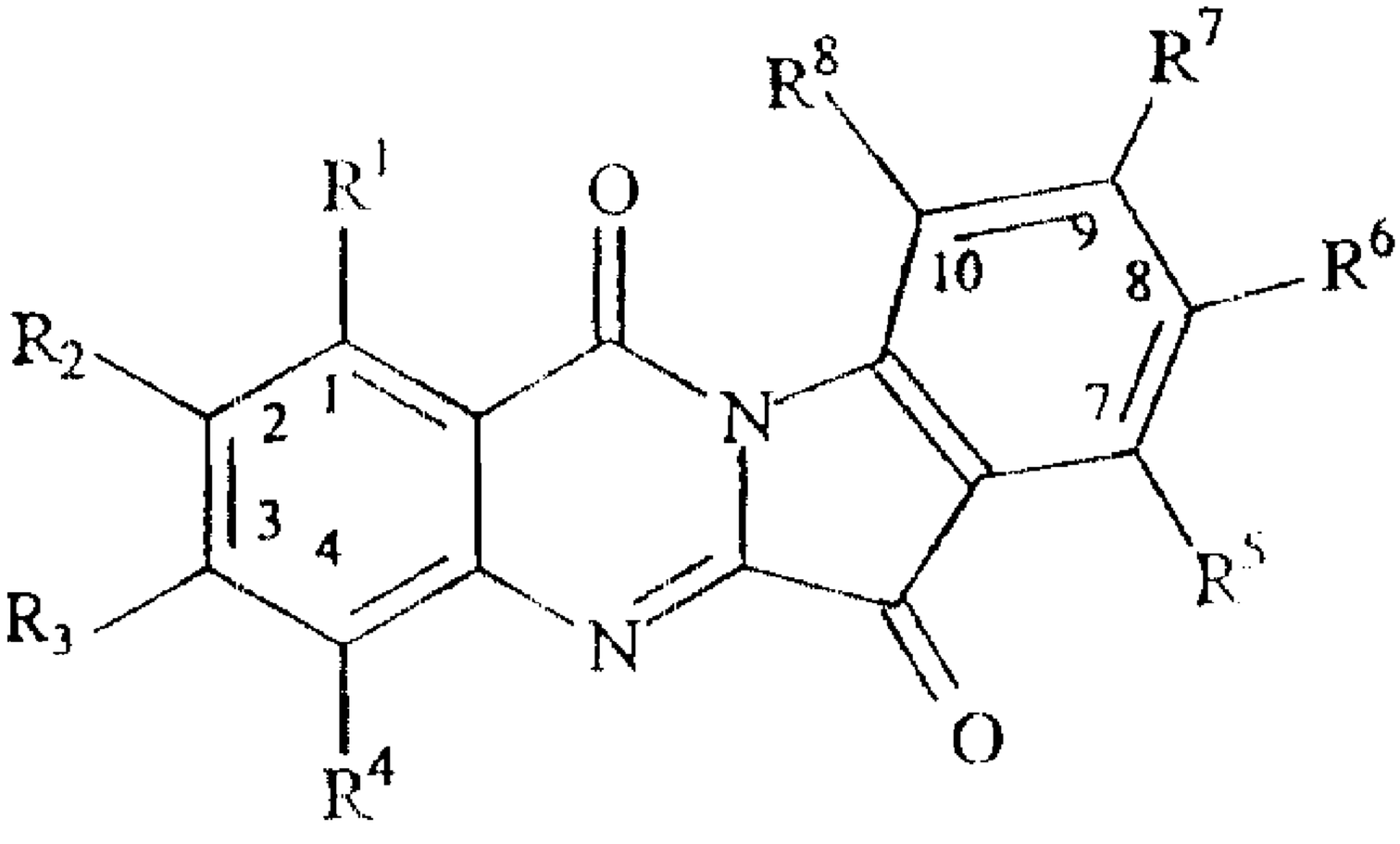
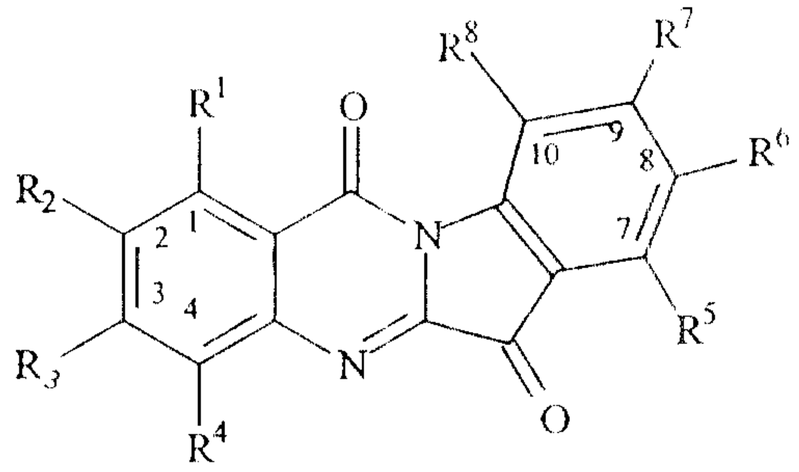


(I)

(57) Abrégé/Abstract:
Compounds of formula (I) (see formula I) wherein R^1 through R^8 are selected independently from hydrogen atoms and pharmaceutically acceptable substituents, as well as pharmaceutically acceptable salts of compounds of formula (I) and mixtures of substances containing at least one compound of formula (I) or a pharmaceutically acceptable salt thereof are used for preparation of medicaments for inhibiting transcription factor NF-κB and/or for inhibiting COX with a preferential inhibitory effect for COX-2.

ABSTRACT OF THE INVENTION

Compounds of formula (I)



(I)

wherein R^1 through R^8 are selected independently from hydrogen atoms and pharmaceutically acceptable substituents, as well as pharmaceutically acceptable salts of compounds of formula (I) and mixtures of substances containing at least one compound of formula (I) or a pharmaceutically acceptable salt thereof are used for preparation of medicaments for inhibiting transcription factor NF-κB and/or for inhibiting COX with a preferential inhibitory effect for COX-2.

PHARMACEUTICALS FOR INHIBITION OF NF-κB

The present invention relates to the use of certain indolo-[2,1-b]quinazoline-6,12-diketones and of mixtures of substances, notably in the form of vegetable extracts, containing such compounds, for the production of medicaments or pharmaceutical compositions for inhibition of the transcription factor NF-κB (NF-κB for brevity herein) and/or for inhibition of cyclooxygenase compounds with preferential inhibition of cyclooxygenase-2 (referred to herein as COX-2 for short).

Transcription factors are proteins capable of inducing reading of coded genetic information by a specific bond to specific DNA-segments. Transcription factor NF-κB plays an important role in various inflammatory processes because many inflammation-promoting inducers lead to its activation. Once activated, the transcription factor causes expression of various gene products which, in turn, catalyze synthesis of inflammation promoting substances. COX-2 is of particular importance as the key enzyme in the synthesis of inflammation-promoting prostaglandins. In addition to the classic acute or chronic inflammatory processes, NF-κB is also implicated, according to present knowledge, in other types of diseases.

In recent years, various substances effecting inhibition of NF-κB have been disclosed. These include the curcuminoids and derivatives of caffeic acid, acetylsalicylic acid and sodium salt of salicylic acid, the alkaloid tetrandrine, as well as some sesquiterpene lactones. The inhibiting effect of curcuminoids and derivatives of caffeic acid is weak and does appear not to be caused by the radical-scavenging properties of the compounds. The inhibiting effect of the salicylates is weak as well. The NF-κB inhibiting effect of sesquiterpene lactones is connected with the presence of an exocyclic α–positioned methylene group on a γ–lactone ring. Because of its reactivity the methylene group enters easily into covalent bonds with nucleophilic groups (e.g. SH- groups of cysteine).

Active agents having an inhibiting effect on NF-κB provide a novel approach, however, to influence inflammatory processes and could be used to inhibit acute and chronic inflammatory processes, or used as antirheumatic agents, as well as for prevention and treatment of neurodegenerative diseases.

Cyclooxygenase compounds, in turn, can be said to be among the key enzymes in biosynthesis of eicosanoides, and they catalyze reaction of arachidonic acid with short lived prostaglandins G and H which react by spontaneous decomposition and further enzymatic reactions to form

a plurality of physiologically effective prostanoids. The eicosanoids are paracrinic hormones derived from C_{20}-fatty acids (arachidonic acids). Important acyclic eicosanoids are the leucotrienes and lipoxines; cyclic derivatives are prostaglandins and thromboxane. Generally, compounds derived from PGH_2 (prostaglandin H_2) are grouped under the generic term "prostanoids"; PGH_2, in turn, is formed from arachidonic acid by cyclooxygenase. The eicosanoids have many biological effects, the physiological basis of which has not been fully understood in view of the multiplicity of structures and because of effects which, in part, depend upon tissue.

Prostaglandins and thromboxanes play a role as tissue hormones in a plurality of physiological and pathological processes, among others as mediators for inflammatory processes. Consequently, an inhibition of cyclooxygenases leads to reduction of the pro-inflammatory effective prostanoids. This mechanism is responsible for anti-inflammatory and analgesic effects of non-steroidal anti-rheumatic drugs (NSAIDs) but also for numerous side effects for the intestinal tract, as well as for kidneys and blood clotting. Accordingly, the search for improved effective agents continues.

The term "cyclooxygenase" includes – according to present knowledge – two isoenzymes (also termed isozymes): cyclooxygenase-1 which is expressed constitutively in many cells and is responsible for synthesis of prostaglandins and other mediators which play a role in various physiological processes, inter alia, maintenance of an intact mucus membrane of the stomach and normal renal functions, as well as aggregation of blood platelets; cyclooxygenase-2, on the other hand, does not, or only at a low rate, appear constitutively in various tissues but is expressed increasingly in the course of inflammatory processes which is the reason for naming COX-2 as the "inducible cyclooxygenase".

According to present knowledge, COX-2 has physiological functions in the kidneys, in the brain/spinal cord, and in the female reproduction system, and pathological functions in inflammation processes, in pain, Morbus Alzheimer and cancer.

The effect of NSAIDs as anti-rheumatic agents and anti-inflammatory agents, respectively, is based at least in part, upon a general, i.e. non-selective, inhibition of both isozymes of cyclooxygenase. Undesired side effects, notably erosion of mucous membrane of the stomach and the influencing blood clotting are to be ascribed essentially to inhibition of cyclooxygenase-1 and the correlated inhibition of synthesis of physiologically important prostaglandins.

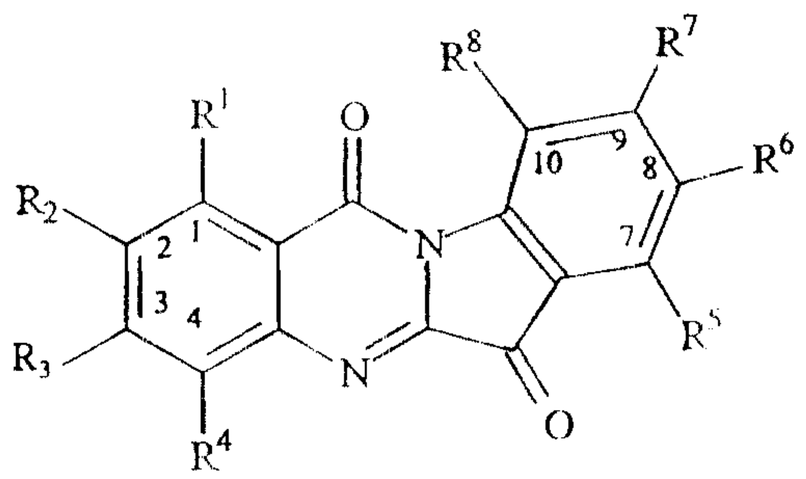
Substances with a selective, that is, preferential inhibitory effect upon inducible cyclooxygenase, i.e. COX-2 , would be expected to have reduced side effects while maintaining a compa-

rable effectiveness as anti-phlogistics and as anti-rheumatic agents because, in connection with such substances, the eicosanoids which are important for homeostasis are not influenced, or influenced only to a substantially lesser degree. The term "preferential inhibition" is intended herein to indicate a relatively stronger inhibition to the extent that change becomes physiologically significant.

Accordingly, it is a first objective of the invention to provide for new medicaments, pharmaceutical compositions or agents for NF-κB inhibition and/or for direct or indirect inhibition of cyclooxygenase compounds with preference for inhibition of cyclooxygenase-2.

It has been found that this objective can be achieved by naturally occurring as well synthetically accessible tryptanthrin (formula I, in which R^1 through R^8 are hydrogen atoms (systematic name is indolo-[2,1-b]quinazoline-6,12-dione) - disclosed e.g. in WO 95/13807 for controlling Mycobacter tuberculosis - and by suitably substituted derivatives of this compound and its salts, as well as by mixtures of compounds containing such substances.

Accordingly, the invention concerns indolo-[2,1-b]quinazoline-6,12-diones, i.e. compounds of formula (I)



(I)

wherein R^1 through R^8 are selected independently from hydrogen atoms and pharmaceutically acceptable substituents, as well as pharmaceutically acceptable salts of compounds of formula (I) .

It is believed that the inhibitory effect upon transcription factor NF-κB is in context with an inhibition of the cyclooxygenase compounds with preferential inhibition of cyclooxygenase-2. As will be explained in more detail below, mixtures or compositions used preferably according to the invention - namely specific vegetable extracts – inhibit release of histamine and/or serotonin - in addition to the above mentioned inhibition of transcription factor NF-κB with preferential inhibi-

tion of cyclooxygenase-2 apparently due to active agents of formula (I) contained therein – and can therefore be used for treatment of diseases connected with such release.

Serotonin (5-hydroxy tryptamine) and histamine belong to the group of biogenic amines formed from precursors (5-hydroxy tryptophan and histidine, respectively) by decarboxylation. Both substances are mediators (endogenic agents) which are released in the blood due to different stimuli from tissues or neoplasms. Histamine and serotonin have pathological significance in connection with inflammatory processes, allergies, and shock states. In addition and depending upon the metabolic state, they cause vasodilatation or vasoconstriction, increased permeability of blood vessels, constriction of uterine or bronchial musculature, serve as neurotransmitters in the central nervous system and, in the case of serotonin, cause an increased aggregation of thrombocytes.

Various inflammatory and allergic processes are connected with an increased release of histamine from tissue mast cells, notably in the case of type 1 allergies (early state) which may become manifest in connection with anaphylactic shock, urticaria, asthma, rhinitis or conjunctivitis. In the human body, cell-connected IgE-globulines serve as antibodies which (in a simplified representation) are connected in a bridge-like manner by (bivalent) antigens and, as a consequence, increase permeability of tissue mast cells for histamine. The consequence is an increased release of histamine with the above described physiological effects.

Histamine receptors belong to the family of receptors coupled to G-protein and may be divided into various sub-types (H_1, H_2, H_3). So called H_1 anti-histaminics are predominantly used in connection with treatment of allergic processes and also as antiemetics or hypnotics.

Serotonin in the human body appears predominantly in the entero chromaffinic cells of the intestinal mucous membranes, in thromobcytes and in the central nervous system. To date, numerous serotonin receptors, so called 5-HT-receptors (5-HT_1 through 5-HT_4 and various sub-types) have been identified.

According to present knowledge, 5-HT-receptors (5-HT_{2B}) play a particularly important role in the area of the trigemino-vascular system of cerebral tissue in the symptomology of migraine.

Generally, selective inhibition of individual receptors causes a selective pharmacological effect of corresponding antagonists. 5-HT-receptor antagonists are used as psychopharmacological agents, as anti-hypertensive agents, and as antiemetics.

Serotonin and histamine released from tissue are also capable to stimulate the so called nociceptors, that is, pain receptors. Inhibition of release of such mediators from tissue mast cells and tissue provides a rational approach to development of anti-inflammatory and analgetically effective substances.

The exact physiological context of the different inhibitory effects set forth above has not been fully understood, and no satisfactory interpretation of such context has been found so far. On the other hand, the teaching of the present invention, namely that compounds of formula (I) and notably mixtures of substances of such compounds in the form of extracts of Isatis tinctoria have the inhibitory effects mentioned above, has been well-established, and such compounds and mixtures are suitable for preparation of corresponding pharmaceutical agents as well as for an effective treatment of such chronic or acute states as will be influenced positively by the inhibitory effects explained above.

Tryptanthrin (or couropitine A, or indolo-[2,1-b]quinazoline-6,12-dione; the formula (I) compound in which R^1 through R^8 are hydrogen) is an alkaloid that has been known for many decades and has been found in various plants (Strobilanthus cusia, Polygonum tinctoria, Isatis tinctoria, Courcoupita guianensis, Wrightia tinctoria, Calanthe spec.), and in microorganisms (Candida lipolytica); it can be isolated from such sources. Naturally occurring or synthetically obtained tryptanthrin can be used according to the invention as such or as a starting material for synthesis of pharmaceutically acceptable derivatives and salts thereof.

Tryptanthrin and its derivatives have been assessed for anti-microbial activity, and have been suggested for inhibiting Mycobacterium tuberculosis as mentioned above.

According to the best knowledge of applicant, effectiveness of these substances for inhibition of the transcription factor NF-κB and/or of the cyclooxygenase compounds with preferential inhibition of cyclooxygenase-2 is novel and was not to be expected in view of its known properties. This is also true for mixtures of substances described herein as well as for extracts exhibiting such inhibition effectiveness, and which additionally show an inhibitory effect upon release of histamine and/or serotonin.

According to a preferred embodiment, compounds of the above formula (I) for use according to the invention can be applied, in the form of naturally occurring tryptanthrin (formula I wherein all R^1 through R^8 are hydrogen atoms), if desired in the form of a pharmaceutically acceptable salt.

One or more of R^1 through R^8 may represent pharmaceutically acceptable substituents, preferably selected from the following groups: halogen (F, Cl, Br); C_1-C_{10} alkyl (methyl through decyl; linear or non-linear and optionally substituted by hydroxyl and/or halogen and/or with one or more oxygen atoms interrupting the chain); C_5-C_6 cycloalkyl; optionally ring-substituted heterocyclic compounds having from 6 to 10 carbon atoms; amino; imino; nitro; C_1-C_{10} alkylsulfonyl; C_6-C_{10} aryl; phenyl; arylalkyl (wherein the alkyl and aryl groups correspond to the above definition), arylalkylaryl (wherein the alkyl and aryl groups are as defined above), C_6-C_{10} aryloxy; C_6-C_{10} arylamino; acylamino having 1-10 carbon atoms in the acyl group; acyloxyamino having 1-10 carbon atoms in the acyl group, alkylamino acylamino with a total of 1-10 carbon atoms; C_1-C_{10} alkylamino sulfonylamino, C_1-C_{10} alkylamino, C_1-C_{10} alkenylamino; C_1-C_{10} dialkyl amino; C_1-C_{10} alkoxyalkyl amino; cyano; formyl; $COOR_{11}$, wherein R_{11} is selected from hydrogen, C_1-C_{10} alkyl, C_6-C_{10} aryl, heterocyclic groups including up to 12 carbon atoms, or mono- or disaccharide; and $COONR_{12}R_{13}$, wherein R_{12} and R_{13} are selected independently from the above substituents, or from amino acids or peptides.

Pharmaceutically acceptable salts of compounds of formula (I) are salts formed with inorganic or organic acids, such as typically hydrochloric acid, sulfuric acid, phosphoric acid, citric acid, lactic acid and other pharmaceutically acceptable acids.

The indolo-[2,1-b]quinazoline-6,12-diones of formula (I) define a novel class of anti-inflammatory agents with a specific dual mechanism of effectiveness, namely both in the area of transcription as well as in the area of eicosanoid synthesis.

Several indolo-[2,1-b]quinazoline-6,12-diones, such as the preferred compounds of formula (I) in which all R_1 through R_8 are hydrogen atoms, as well as preferred compounds of formula (I) in which R_1, $R_2 = R_4$, $R_5 = R_7$, $R_8 = H$, R_3 is a nitrogen-containing substituent, such as 4 M, and R_6 is halogen, preferably Cl, provide advantageous candidate agents for treatment of diseases where the connection with activation of NF-κB is known and/or where induction of COX-2 formation has been established, or is suspected, or is to be established yet, notably as antiphlogistic and antirheumatic agents, for preventive or adjuvant treatment of Morbus Alzheimer and of carcinogenic diseases, as well as for preventive or adjunctive permanent treatment of old-age ailments.

Further included among preferred compounds of formula (I) are compounds which (in the tests described in the examples below) yield NF-κB values of not more than about 10 μM/liter, preferably not more than about 5 μM/liter, and which (in the COX-2 test system) yield IC_{50} values

of not more than about 1 μM/liter, notably not more than about 0,05 μM/liter, and wherein COX-1 values are at least twice as high as the COX-2 values, preferably at least about ten times higher.

Compounds of formula (I) and pharmaceutically acceptable salts thereof, respectively, can be processed to yield liquid or solid drug preparations with the conventional adjuvants, extenders, carriers, auxiliary agents for compression, aroma substances, and the like. Typical dosages will be in the range of 1-100 mg/day, but it is to be expected that no critical upper limit exists since, to date, no toxicity for mammals has been detected.

Preferred mixtures of substances for use according to the invention are plant extracts or essences, notably such extracts from Isatis tinctoria that contain at least one effective compound of formula (I), specifically tryptanthrin. Such extracts can be used as such, i.e. without isolation of active ingredients but after controlling the content of active ingredient, or can be transformed into pharmaceutical agents for treatment of diseases that are influenced advantageously by the inhibitory effects caused by the active ingredients of formula (I).

Isatis tinctoria (dyer's woad) suitable for obtaining a preferred extract of the type mentioned above is a well known plant, the leaves of which (Isatidis folium) are used as a blue coloring agent because of their content of glycosides of indican and isatan B, respectively, which, in turn, are transformed by hydrolysis into indoxyl that oxydizes to form indigo dye; this dye can also be produced synthetically.

The production of indigo dye from Isatis tinctoria (dyer's woad) has been known for some time. For example, German patent application DE-A-4 211 719 discloses production of a commercially pure dye while other plant constituents, notably tryptophane compounds other than the dye, such as anthranilic acid derivatives, glycosides of mustard seed oil, flavonoides, terpenes, and lignanes were considered to be waste products and are discarded in the known process.

Further, Traditional Chinese Medicine contains numerous disclosures about combinations of various drug plants with differing indications and without effectiveness data, or with indications that cannot be verified; sometimes, reference is made to the plant Isatis tinctoria which has been known as a haemostatic agent since Pliny.

For example, US patent No.5 665 393 discloses a composition consisting of differing medicinal plants of Traditional Chinese Medicine suggesting use of this combination for treatment of cancer of the prostate gland. In addition to Panax (pseudo-Ginseng) the composition contains Ganoderma, Dentrathema, Glycyrrhiza, Scutellaria, Rabdiosa and Serenoa in addition to Isatis. Inhibitory effects upon specific cells of prostate cancer are cited as a proof of pharmaceutical

activity and two individual cases are reported in which the mixture of extracts was used in combination with classic chemotherapy.

US patent No. 5 837 257 again is based upon pharmacology according to Traditional Chinese Medicine and relates to a method for treating hepatitis, leukemia and HIV virus infections using one of the medicinal plants Solanum, Lespedeza, Senecio and Ligustrum, optionally in combination with fourteen other medicinal plants, inter alia Isatis sp. Proof of effectiveness is based upon an in vitro test of an anti-HIV effect of aqueous extracts and upon use with hepatitis patients. The antiviral effect is not ascribed to any particular plant but only to their various combinations.

Further examples of this type can be found in Traditional Chinese Medicine; which discloses a medicated tooth paste for treatment of lesions and inflammation of the oral cavity with an additional effect as a hair tonic.

The above prior art about use of Isatis tinctoria can be summarized to the effect that it is al ways used in a multicomponent mixture containing Isatis tinctoria as one of several plant components and ascribing, with one exception, no specific pharmaceutical effects to Isatis tinctoria, not to speak of any specific disclosure of active ingredients of these plants. The effects described are always attributed to specific combinations of medicinal plants.

As mentioned briefly above, extracts of Isatis tinctoria are a preferred embodiment of a composition in the form of a mixture of components used according to the invention and can be obtained via prior art extraction methods by extraction of this plants, as well as of other plants known to contain the active ingredient tryptanthrin.

Various methods are suitable to obtain extracts for use according to the invention. A particularly preferred method is high pressure extraction with a gas in its undercritical liquid or its supercritical fluid state, e.g. lower alkanes, such as propane, butane, or (preferred) CO_2 in pure state, or in mixture with a normally liquid organic solvent.

Generally, extracts for use according to the invention can be obtained by means of solvents and solvent mixtures which are capable of extracting lipophilic and somewhat polar constituents from the extracted plant material. In addition to high-pressure extraction method mentioned

above, extracts can be obtained with normally liquid (liquid at normal temperature) solvents, e.g. alcohols, such as ethanol, halo hydrocarbons, such as dichloromethane, and petroleum ether.

Extract from Isatis tinctoria for use according to the invention is normally obtained from the leaves of the plant but can also be obtained from other plant constituents, notably root material. Such extracts contain, in addition to tryptanthrin, a plurality of differing chemical substances, notably the well known indole compound indican and isatan B, the dyes indirubin and indigo obtainable therefrom, as well as further alkaloids of the indole type, anthranilic acid derivates, glycosides of mustard oil, and further natural substances from the groups of the flavanoides, terpenes, lignanes, glycosides, and organic acids.

Substantially untreated or virgin extract, for example after composition control and packaging, as well as a processed extract, e.g. enriched by specific selection of an extracting agent and/or by an aftertreatment with regard to constituents normally contained in the extract so as to increase or decrease the content of any specific constituent, can be used according the invention, both in liquid as well as in solid form and with our without usual adjuvants, compression aids for tablet production, solubilizing agents, preservatives, aroma substances and other additives of the type used in the art for preparation of medicinal plant extracts.

Research leading to the present invention has shown that extract of Isatis tinctoria obtained with extracting agents of the above mentioned type provide for a significant inhibition of transcription factor NF-κB, notably an inhibition of at least about 50% and preferably at least about 75%.

The invention will now be explained further with reference to the subsequent and non-limiting examples. Reference is made to the enclosed drawings in which:

Figures 1-9 are diagrams of dosage-versus-inhibition of extracts obtained according to the examples and

Figures 10-16 are chromatograms of extracts obtained in the examples.

EXAMPLE 1

The active agent was isolated essentially according to the method disclosed by Honda et al, M. Planta Medica 38 (1980) 275-276, applied for recovery from Isatis tinctoria L. Isolation from other species mentioned above can be carried out in an analogous manner.

One kg of dried pulverized plant material ("drug") was extracted three times with ten liters of methanol (plus 2% acetic acid) during 24 hours to exhaustion; the extract was evaporated to dryness (240 g) and re-dissolved in water (with 1% ammonia added). The resulting material was then shaken with dichloromethane, and the extract obtained was re-evaporated to dryness (30g).

The extract was fractionated stepwise with hexane-chloroform 9:1 - 1:2, chloroform and ethyl acetate in a silica-gel column; the chloroform fraction was treated for chromatographic separation with a column (eluting agent plus chloroform), and the tryptanthrin zone was recovered. Tryptanthrin in the form of yellow crystals (35 mg) was recovered from the fraction obtained after re-crystallization from ethyl acetate/chloroform.

EXAMPLE 2

Tryptanthrin as well as the A- and D- ring substituted indolo-[2,1-b]quinazoline-6,12-diones are obtained synthetically by conventional methods, preferably according to the general synthesis methods as disclosed by Mitscher et al, Heterocycles (1981), 14, 1017-1021. The tryptanthrin derivative is obtained from the correspondingly substituted isatin and isatoic anhydride by addition of NaH in dried TMF so as to yield the targeted tryptanthrin derivative. Synthesis of the substituted isatin derivatives was effected according to the method of Baker and Mitscher, patent WO95/13807. The aniline derivatives required as starting substances are available commercially or can be obtained by conventional synthesis methods (cf. e.g. Furniss et al, Practical Organic Chemistry, 5th edition, 1989, March, J., Advanced Organic Chemistry, 3rd edition 1985).

The isatoic anhydride derivatives were prepared in an analogous manner by the method of Baker and Mitscher. The starting substances used were the corresponding derivatives of 2-amino benzoic acid.

EXAMPLE 3

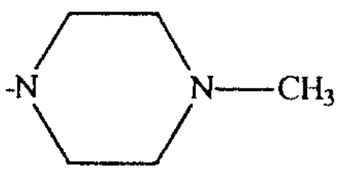
The following indolo-[2,1-b]quinazoline-6,12-diones were synthesized, inter alia, according to the synthesis method described above:

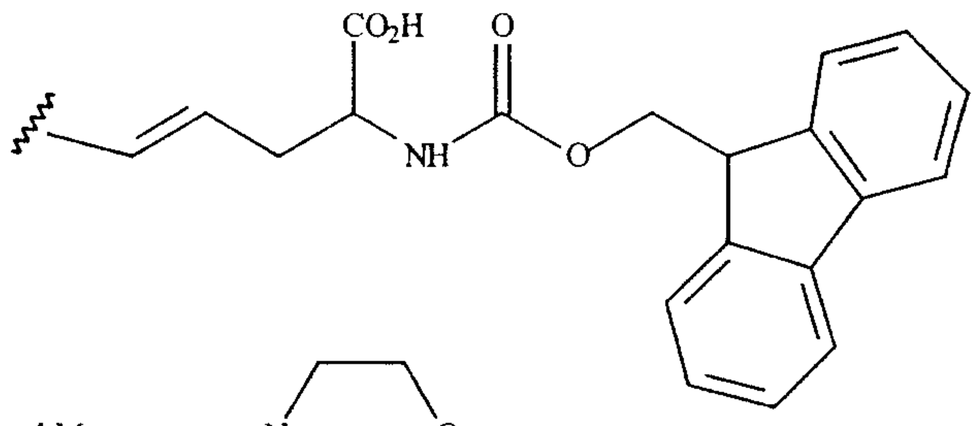
Compd.	Substituents R_1 to R_8							
No.	1	2	3	4	5	6	7	8
1	H	H	H	H	H	H	H	H
2	H	Cl	H	H	H	F	H	H
3	H	Cl	H	H	H	NO_2	H	H
4	H	CH_3O	CH_3O	H	H	H	H	H
5	H	Cl	H	H	H	H	H	H
6	H	NO_2	H	H	H	H	H	H
7	H	H	H	H	H	Br	H	H
8	H	H	H	H	H	NO_2	H	H
9	H	H	H	H	H	F	H	H
10	H	H	H	H	H	Cl	H	H
11	H	H	H	H	H	OCH_3	H	H
12	H	H	H	H	H	CH_3	H	H
13	H	H	H	H	H	I	H	H
14	H	CH_3	H	H	H	H	H	H
15	CH_3	H	H	H	H	H	H	H
16	H	H	H	CH_3	H	H	H	H
17	H	F	H	H	H	F	H	H
18	H	Br	H	H	H	H	H	H
19	H	F	H	H	H	H	H	H
20	H	NH_2	H	H	H	F	H	H
21	H	H	H	H	H	H	Cl	H
22	H	H	H	H	Cl	H	H	H
23	H	H	H	CH_3O	H	F	H	H
24	H	CH_3	H	CH_3	H	F	H	H
25	H	CH_3	H	H	H	F	H	H
26	H	H	F	H	H	F	H	H
27	H	H	H	H	H	H	H	F
28	F	H	H	H	H	F	H	H
29	CH_3	H	H	H	H	F	H	H
30	H	H	H	CH_3	H	F	H	H
31	H	H	H	H	H	F	H	F
32	H	H	NMP	H	H	F	H	H
33	H	H	H	H	NMP	H	H	H
34	H	H	H	H	H	H	NMP	H
35	NMP	H	H	H	H	F	H	H
36	H	H	H	H	H	F	NMP	H
37	H	H	H	H	H	CO_2Et	H	H
38	H	H	H	F	H	F	H	H
39	H	H	H	H	H	F	F	H
40	H	H	F	H	H	F	NMP	H
41	H	H	H	H	H	F	3-MP	H
42	H	H	H	H	H	F	2-MNBP	H
43	H	H	H	H	H	H	NBP	H

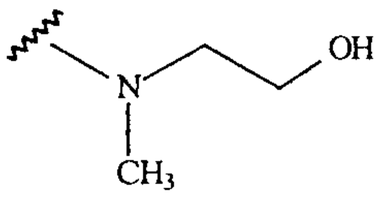
Compd.	Substituents R_1 to R_8							
No.	1	2	3	4	5	6	7	8
44	H	H	H	H	H	F	NMP	F
45	H	H	H	H	H	H	P	H
46	H	F	F	H	H	F	H	H
47	H	F	NMP	H	H	F	H	H
48	Cl	H	Cl	H	H	I	H	H
49	Cl	H	H	Cl	H	I	H	H
50	H	I	H	H	H	CO_2Et	H	H
51	H	I	H	H	H	I	H	H
52	H	H	H	OCH_3	H	I	H	H
53	H	H	H	OCH_3	H	H	H	H
54	H	I	H	I	H	I	H	H
55	H	Br	H	Br	H	I	H	H
56	H	I	H	I	H	CO_2Et	H	H
57	H	H	H	H	H	Cl	H	CH_3
58	H	F	NBP	H	H	I	H	H
59	F	Br	H	Br	H	I	H	H
60	Cl	Br	H	Br	H	I	H	H
61	F	Br	H	Br	H	H	H	H
62	H	F	3-MNBP	H	H	I	H	H
63	H	H	H	H	H	CO_2Bn	H	H
64	H	F	P	H	H	I	H	H
65	H	H	H	OH	H	I	H	H
66	H	H	H	H	H	$CO_2$2'-octyl	H	H
67	H	F	3-MP	H	H	I	H	H
68	H	H	H	OCH_3	H	CO_2Et	H	H
69	H	H	H	OCH_3	H	SO_2n-octyl	H	H
70	H	H	H	OCH_3	H	SO_2NMP	H	H
71	H	H	H	OCH_3	H	$CO_2$2'-octyl	H	H
72	H	H	H	OCH_3	H	CO_2H	H	H
73	Cl	H	Cl	H	H	FmocAG	H	H
74	Cl	H	Cl	H	H	H	H	H
75	Cl	H	Cl	H	H	$AAGOCH_3$	H	H
76	H	H	H	OCH_3	H	1-Octenyl	H	H
77	H	F	3-MP	H	H	CO_2H	H	H
78	H	1-Octenyl	H	H	H	Cl	H	H
79	H	I	H	H	H	Cl	H	H
80	H	Octyl	H	H	H	Cl	H	H
81	H	OPO_3Na_2	H	H	H	H	H	H
82	H	OH	H	H	H	H	H	H
83	H	H	F	H	H	Cl	H	H
84	H	H	H	Obn	H	F	H	H
85	H	H	NME	H	H	Cl	H	H
86	H	H	4-M	H	H	Cl	H	H

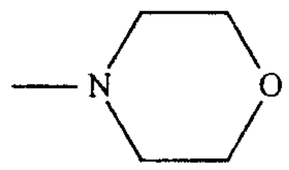
Compd.	Substituents R_1 to R_8							
No.	1	2	3	4	5	6	7	8
87	H	H	piperidine	H	H	Cl	H	H
88	H	octyl	H	H	H	H	H	H
89	H	6-acoctenyl	H	H	H	Cl	H	H
90	H	NEDME	H	H	H	Cl	H	H
91	H	6-acoctyl	H	H	H	Cl	H	H
92	H	H	H	OH	H	F	H	H
93	H	H	DPA	H	H	Cl	H	H
94	H	H	H	OCH_3	H	cis-1-octenyl	H	H
95	H	2-AG	H	H	H	Cl	H	H
96	H	H	H	OCH_3	H	octyl	H	H
97	H	H	H	H	H	CF_3	H	H
98	H	H	NMEP	H	H	Cl	H	H
99	H	H	SAR	H	H	Cl	H	H
100	H	H	NME sacch-ester	H	H	Cl	H	H
101	H	H	H	H	H	OCF_3	H	H
102	H	H	NME octyl-ester	H	H	Cl	H	H
103	H	H	H	H	H	n-octyl	H	H
104	H	H	F	H	H	n-octyl	H	H
105	H	H	n-octyl	H	H	n-octyl	H	H
106	H	H	NME	H	H	F	H	H
107	H	H	NME	H	H	n-octyl	H	H
108	H	H	NMP	H	H	n-octyl	H	H
109	H	H	F	H	H	OCF_3	H	H
110	H	H	NME	H	H	OCF_3	H	H

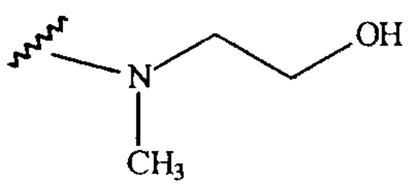
The abbreviations used in table 1 have the following significance:

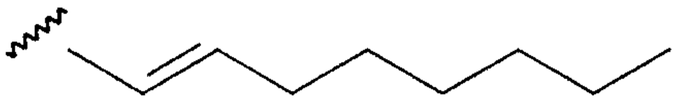
NMP = 

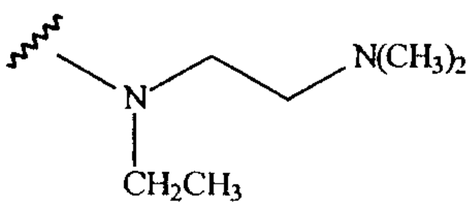
FmocAG = 

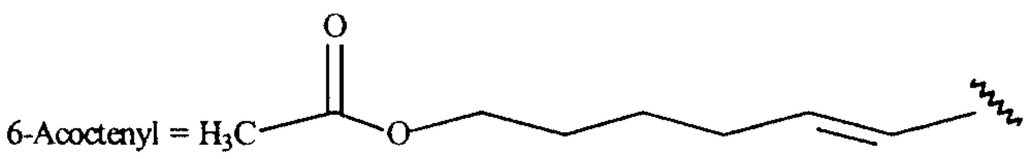
AAGOCH$_3$ = 

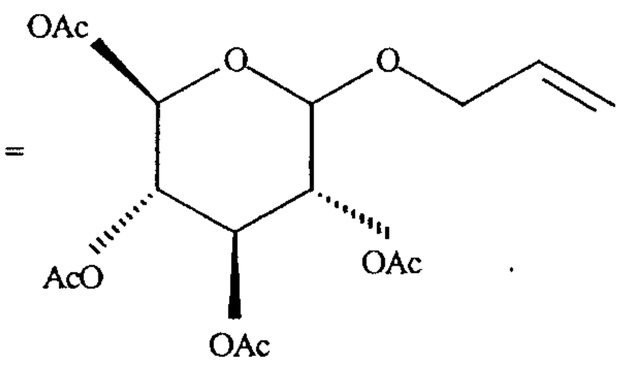
4-M = 

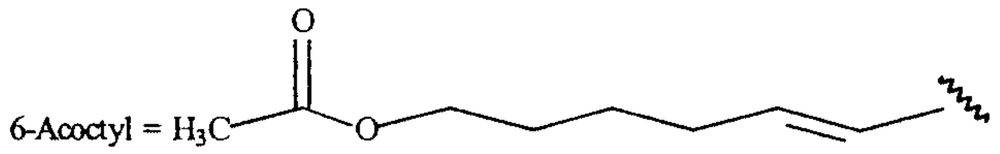
NME = 

1-Octenyl = 

NEDME = 

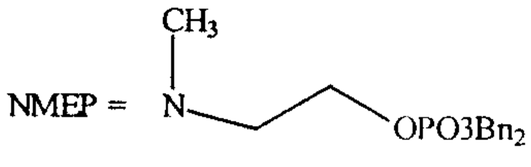
6-Acoctenyl = 

2-AG = 

6-Acoctyl = 

Bn = CH2C6H5

DPA = Dipentylaminio

NMEP = 

NMP =

NBP =

SAR =

3-MP =

P =

2-MNBP =

EXAMPLE 4

a. Inhibition of COX-2

The COX-2 inhibition effect was determined according to the method of Berg et al, J. Pharmacol. Toxicol. Methods 37, 179-186 (1997).

To this end, Mono Mac 6 cells with differing concentrations of the testing substances were incubated for 30 minutes. Subsequently, lipopolysaccharide (LPS, 100ng/ml) was added from a pipette and incubation was repeated for 6 hours. Thereafter, arachidonic acid was added (final concentration 30 μM) during 15 minutes and the Keto-PGF1α was determined by way of ELISA (Enzyme Linked Immuno Sorbent Assay) after incubation.

In an alternative method, test substance and LPS were added simultaneously. Determination of COX-1inhibition was carried out in HEL-cells according to the method of Berg et al., J. Pharmacol. Toxicol. Methods 37, 179-186 (1997). To this end, HEL-cells with differing concentrations of the testing substances were incubated for 30 minutes with subsequent addition of arachidonic acid (final concentration 30μM) and the thromboxane B2 thus formed after another 15 minutes of incubation was measured by way of ELISA .

b. NF-κB –Inhibition

Test substances produced according to Example 2 were tested for their NF-κB inhibiting effect in a cellular test system free of cells, essentially according to the method disclosed by Rüngeler, P. et al, Planta Medica 64, 588-593 (1998). The electrophoretic shift of mobility was measured to determine NF-κB binding to DNA both in-vivo and in-vitro.

In-vivo binding was carried out by means of Jurkat T-cells. The cells were incubated for 1 hour with varying concentrations of the various extracts and were stimulated subsequently for 1 hour with TNK-α. Total protein extracts of the cells were recovered and tested in an electrophoretic mobility shift assay (EMSA) for NF-κB binding activity.

In-vitro binding was also determined with Jurkat T-cells. Cell extracts of stimulated as well as of unstimulated cells were obtained. Equal amounts of protein (10-20μg) were incubated for 2 hours with concentration series of the compounds in the test and then analyzed by way of electrophoretic mobility shift assay (EMSA). Data of the selected compounds are listed in table 2.

Table 2

COMPOUND No.	COX-1	COX-2	NF-κB
1	2	0,04	1
5	10	2	50
10	1	0,5	5
20	5	0,5	5
24	>50	>50	>100
32	1	1	10
37	>50	>50	>100
42	>50	>50	>100
56	)*	)*	)*
60	)*	)*	)*
69	>50	>50	>100
86	5	0,01	0,5
99	5	0,5	1
100	10	0,02	5
110	2	0,5	5

COX-1 and COX-2: IC 50 (μM/liter)
NF-κB : limiting concentration (μM/liter) at which EMSA still indicated inhibition
)* cytotoxic at tested concentrations.

Compounds 1, 10, 20, 32, 86, 99 and 110 form a group of preferred compounds wherein compounds 1 and 86 are even more preferred and show a particularly preferential inhibition of inducible COX-2.

EXAMPLES 5 -10

Plant material (subsequently termed "drug") of Isatis tinctoria in the amounts given in Table 3 and with the extracting agents also specified in Table 3 were treated at room tem-

perature by maceration with agitation. The dried drug was comminuted, extracted 3 times during the period specified in Table 3, and filtered. The filtrates were combined, concentrated under vacuum, freeze dried, and weighed.

When using fresh drug, extraction was carried out in a turbo-mixer at 4500 rotations per minute three times with solvent and without preceding comminution, and the slurry obtained was filtered. The combined filtrates were concentrated under vacuum, freeze dried, and weighed.

Table 3

Example	Drug	State	Sieve (mm)	Weight Drug (g)	Solvent amount (liters)	Extraction period (min)	Weight Extract (g)
5	Leaf	dried	0,7	100	CH_2Cl_2 3x1	3x60	3,61
6	Root	dried	0,7	100	CH_2Cl_2 3x1	3x60	1,12
7	Leaf	dried	0,7	100	EtOH 3x1	3x60	20,71
8	Root	dried	0,7	100	EtOH 3X1	3x60	1,28
9	Leaf	fresh	-	100	EtOH 3x0,75	3x60	3,81
10	Root	fresh	-	100	EtOH 3X0,75	3x60	2,07

EXAMPLES 11 – 15

The dried drug was comminuted (sieve set 0,7 mm) and extracted successively under laboratory conditions during the periods specified in Table 4 with supercritical CO_2, CO_2/ethanol (ethanol amount 20% by volume) as "fluid" at 50 and 90° C, respectively; process parameters were as specified (extraction period = Time, gas flow = Flow, temperature = Tmp, amount of extracted obtained = Extr.). The extract was concentrated in vacuum if required and freeze dried.

Table 4

Exam.	Drug	Weight (g)	Fluid	time (min)	Flow (ml/min)	Tmp (°C)	Pressr. (bar)	Extr. (mg)
11	Leaf	18	CO_2	360	210-290	50	300	264
12	Leaf	18	20%)*	300	200-290	50	300	878
13	Leaf	18	20%)*	300	200-290	90	300	151
14	Leaf	18	CO_2	420	200-290	50	300	307
15	Leaf	18	20%)*	300	200-290	50	300	993

)* a mixture of carbon dioxide (CO_2 and 20% ethanol (EtOH)

HPLC analysis of the extracts of examples 5 - 15 indicated no substantial differences of the compositions. Further, DC analysis indicated close correspondence of the extracts of example 11 and the hexane extract.

EXAMPLES 16-21

Operation in these examples was similar to that used in examples 11 - 15, however using a SFE laboratory extraction plant ISCO™ 2100 equipped with two 1OODX high pressure pumps for CO_2 and the modifier (ethanol); materials, amounts, process conditions and result data are summarized in Table 5 below.

Table 5

Example	Drug	Wght (g)	Fluid	Time (min)	Flow (ml/min)	Temp. (°C)	Pressure (bar)	Extr. (mg)
16	Leaf	18	CO_2	180	0,8-1,0	50	500	68
17	Leaf	18	CO_2	180	0,8-1,0	50	690	144
18	Leaf	18	CO_2^*	180	0,8-1,0	50	350	173
19	Leaf	18	CO_2^{**}	180	0,8-1,0	50	350	55
20	Leaf	18	CO_2^{***}	180	0,8-1,0	50	350	253
21	Leaf	18	CO_2^{**} *	180	0,8-1,0	50	690	313

CO_2^* = CO_2 with 0,5 Vol.% EtOH-H_2O (1:1 Vol.)
CO_2^{**} = CO_2 with 0,5 Vol.% H_2O
CO_2^{***} = CO_2 with 0,5 Vol.% EtOH

As is apparent from Table 5, high pressure extraction with CO_2 in a mixture with a normally liquid solvent is a preferred form of producing the extracts to be used according to the invention.

EXAMPLE 22

For investigation of the pharmacological profile in vitro, extracts prepared according to Examples 5, 6, 7, 11 and 12 were tested in cell-free and cellular test systems according to a prior art method for NF-κB inhibitory effects. The method applied was essentially as defined by Rungeler, P. et al., Planta Medica 64, 588-593 (1998). NF-κB binding to DNA was determined in-vivo and in-vitro by determining the electrophoretic mobility shift.

Binding in-vivo was determined by means of Jurkat T-cells. The cells were incubated with differing concentration of the various extracts during one hour and subsequently stimulated with TNF-α during one hour.

Total protein extracts of the cells were recovered and examined for NF-κB binding activity in an assay of electrophoretic mobility shift (electrophoretic mobility shift assay, EMSA).

Binding in vitro was again tested with Jurkat T-cells. Cell extracts of stimulated and of unstimulated cells were recovered. Equal protein amounts (10 –20 μg) were analyzed after two hours of incubation with concentration series of the various isatis extracts by means of EMSA.

Table 6

Inhibition of Activation of NF-κB in Jurkat T- cells

Conc. (µg/ml)	5	6	7	11	12
100	+	+	+	+	+
50	+	+	+	+	+
25	(+)	(+)	-	-	(+)
10	-	-	-	-	-

+ : inhibition of NF-κB-activation; (reduced activation)
- : no inhibition

EXAMPLE 23

Extracts produced according to the Examples 15, 17 and 20 were examined for their dose-dependant inhibition of COX-2, histamine release, and serotonin release. Determination of the COX-2 inhibitory effect was effected according to the method described in Example 4.

Inhibition of histamine release was determined according to the method of Hakanson et al., Anal. Biochem 47 (1972) 356-370 with rat tissue mast cells after stimulation with the substance designated in that paper as Compound 48/80.

Inhibition of serotonin release was determined according to the method of Theoharides et al., Biochem. Pharmacol 34 (1985) 1389-1398, again on rat tissue mast cells.

Dosage-dependant inhibition diagrams are shown in Figures 1 – 9. Calculation of the IC_{50} values yielded the following results:

Table 7

Example	COX-2	Histamine-Release	Serotonin-Release
15	30	1,8	1,1
17	4,9	4,8	2,4
20	5,1	7,6	2.1

EXAMPLE 24

Extracts were characterized by their chromatograms (chromatographic fingerprint) obtained by high pressure liquid chromatography (HPLC). A binary high pressure gradient apparatus (Hewlett Packard HP 1100) with a diode array detector, autosampler, and column oven was used to this end. Separation conditions were as follow:

Separation column: LiChroCART™ cartridge (4x125 mm), packed with LiChrospher™ 100 RP-18 of 5 μm average particle size;

Solvent program: H_2O-acetonitrile 90:10 during 5 minutes, then H_2O-acetonitrile gradient from 90:10 to 10:90 in 45 minutes, H_2O-acetonitrile 10:90 during 15 minutes, subsequently H_2O-acetonitrile gradient from 10:90 to 90:10 in 15 minutes;

Flow rate: 1 ml/min, detection at 245 nm and 387 nm.

The chromatogram of the extract of example 14 is shown in Figure 10 with detection at 254 nm (top) and 387 nm (bottom). The substances known to be typical for isatis and suitable as indicator substances for characterization of the extract are tryptanthrin (1), indigo (2) and indirubin (3). Identification of these substances was effected by means of their characteristic UV-vis spectra and comparison with reference substances.

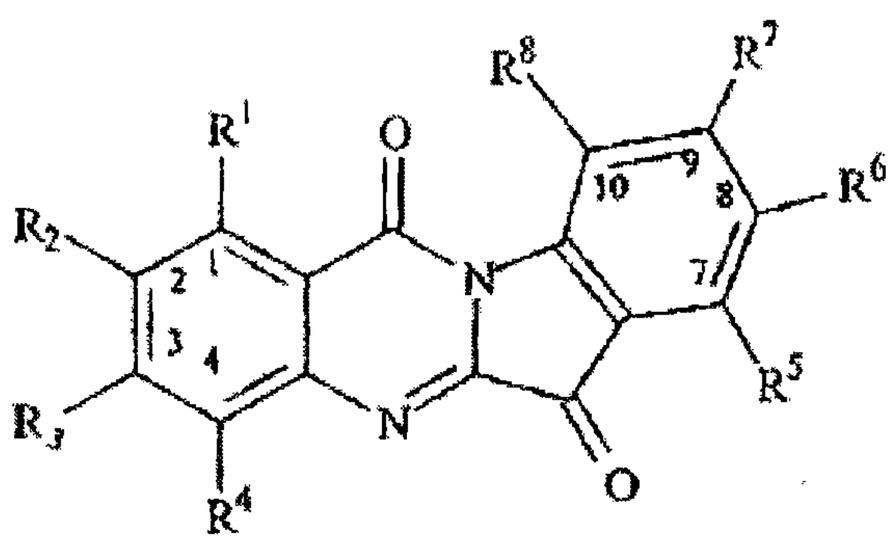
Figure 11 shows the chromatogram of extract of example 15, Figure 12 is the fingerprint of extract according to Example 17, and Figure 13 is for extract of Example 18. Separation of an extract recovered according to Example 19 is shown in Figure 14. An extract according to Example 20 is represented in Figure 15, and an extract according to Example 21 in Figure 16. All SFE extracts contain compounds (1), (2) and (3).

Dosages of Isatis extracts for use according to the invention and characterized by the chromatograms above will generally correspond to conventional dosages of comparable medicinal plant extracts, i.e. will be in the range of from 50 mg to 1000 mg/day. No critical upper limit for toxicity has been found so far. In this context, extracts can be used according to the invention as such, or in mixture with conventional additives and adjuvants for production of solid or liquid pharmaceutical compositions and/or combined with other therapeutically active substances.

In sum, the invention provides for new means and methods for treatment of diseases connected directly or indirectly with NF-κB, notably as anti-phlogistic or anti-rheumatic agents, respectively, instead of or in combination with known antiphlogistic agents for preventive or adjuvant treatment of neurodegenerative diseases, of Morbus Alzheimer, and of cancer disease as well as for preventive permanent medication, notably against old-age ailments.

CLAIMS:

1. A use for treatment of disease by at least one of inhibiting transcription factor NF-$_K$B, inhibiting COX-2, inhibiting release of histamine or inhibiting release of serotonin, of compound of formula (I),



(I)

wherein R^1 through R^8 are independently hydrogen atoms or pharmaceutically acceptable substitutents, said pharmaceutically acceptable substitutents being;

halogen;

C_1-C_{10} alkyl which is unsubstituted or substituted by one or more of hydroxyl or halogen, or with one or more oxygen atoms interrupting the chain, and, wherein the C_1-C_{10} alkyl is linear or non-linear;

C_5-C_6 cycloalkyl;

heterocyclic compound which is unsubstituted, or substituted with methyl or with tert-butoxy carbonyl, and having a total of from 4 to 10 carbon atoms;

amino;

imino;

nitro;

C_1-C_{10} alkylsulfonyl;

C_6-C_{10} aryl;

arylalkyl, wherein the aryl group is as defined above and the alkyl group is C_1-C_{10} alkyl as defined above or C_5-C_6 cycloalkyl;

arylalkylaryl, wherein the aryl groups are as defined above and the alkyl group is C_1-C_{10} alkyl as defined above or C_5-C_6 cycloalkyl;

C_6-C_{10} aryloxy;

C_6-C_{10} arylamino;

acylamino having 1-10 carbon atoms in the acyl group;

acyloxyamino having 1-10 carbon atoms in the acyl group;

alkylamino acylamino with a total of 1-10 carbon atoms;

C_1-C_{10} alkylamino sufonylamino;

C_1-C_{10} alkylamino;

C_1-C_{10} alkenylamino;

C_1-C_{10} dialkyl amino;

alkoxyalkyl amino with a total of 1-10 carbon atoms;

cyano;

formyl;

$COOR_{11}$, wherein R_{11} is hydrogen, C_1-C_{10} alkyl, C_6-C_{10} aryl, heterocyclic groups including up to 12 carbon atoms, monosaccharide or disaccharide;

or

$COONR_{12}R_{13}$, wherein R_{12} and R_{13} are independently a halogen; C_1-C_{10} alkyl which is unsubstituted or substituted by one or more of hydroxyl or halogen, or with one or more oxygen atoms interrupting the chain, and, wherein, wherein the C_1-C_{10} alkyl is linear or non-linear; C_5-C_6 cycloalkyl; heterocyclic compound which is unsubstituted or substituted with methyl having a total of from 4 to 10 carbon atoms; amino; imino; nitro; C_1-C_{10} alkylsulfonyl; C_6-C_{10} aryl; arylalkyl, wherein the aryl group is as defined above and the alkyl group is C_1-C_{10} alkyl as defined above or C_5-C_6 cycloalkyl; arylalkylaryl, wherein the aryl groups are as defined above and the alkyl group is C_1-C_{10} alkyl as defined above or C_5-C_6 cycloalkyl; C_6-C_{10} aryloxy; C_6-C_{10} arylamino; acylamino having 1-10 carbon atoms in the acyl group; acyloxyamino having 1-10 carbon atoms in the acyl group; alkylamino acylamino with a total of 1-10 carbon atoms; C_1-C_{10} alkylamino sufonylamino; C_1-C_{10} alkylamino; C_1-C_{10} alkenylamino; C_1-C_{10} dialkyl amino; alkoxyalkyl amino with a total of 1-10 carbon atoms; cyano; formyl; $COOR_{11}$, wherein R_{11} is selected from hydrogen, C_1-C_{10} alkyl, C_6-

C_{10} aryl, heterocyclic groups including up to 12 carbon atoms, monosaccharide, disaccharide, amino acids or peptides;

or pharmaceutically acceptable salts of a compound of formula (I), or mixtures of substances containing at least one compound of formula (I) or a pharmaceutically acceptable salt thereof.

2. The use according to claim 1 wherein the C_6-C_{10} aryl is phenyl.

3. The use according to claim 1, wherein the halogen is F, Cl, or Br.

4. The use according to claim1, wherein the C_1-C_{10} alkyl is substituted by one or more of hydroxyl or halogen or with one or more oxygen atoms interrupting the chain.

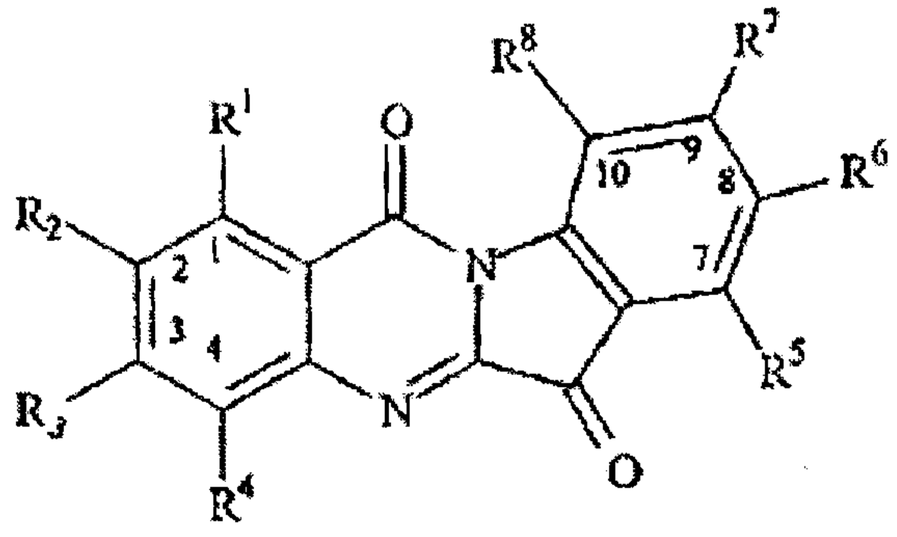
5. The use according to claim 1 for treatment of an inflammatory disease.

6. The use according to claim 5 wherein the inflammatory disease is a rheumatic disease form.

7. The use according to claim 1 for preventive or adjuvant treatment of Morbus Alzheimer.

8. The use according to any one of claims 1 to 7, wherein an extract obtained by extraction of Isatis tinctoria with an extracting agent consisting at least in part of CO_2 is used as said mixture of substances containing at least one compound of formula (I).

9. A use of compound of formula (I),



(I)

wherein R^1 through R^8 are independently hydrogen atoms or pharmaceutically acceptable substitutents, said pharmaceutically acceptable substitutents being;

halogen;

C_1-C_{10} alkyl which is unsubstituted or substituted by one or more of hydroxyl or halogen, or with one or more oxygen atoms interrupting the chain, and, wherein the C_1-C_{10} alkyl is linear or non-linear;

C_5-C_6 cycloalkyl;

heterocyclic compound which is unsubstituted or substituted with methyl and having a total of from 4 to 10 carbon atoms;

amino;

imino;

nitro;

C_1-C_{10} alkylsulfonyl;

C_6-C_{10} aryl;

arylalkyl, wherein the aryl group is as defined above and the alkyl group is C_1-C_{10} alkyl as defined above or C_5-C_6 cycloalkyl;

arylalkylaryl, wherein the aryl groups are as defined above and the alkyl group is C_1-C_{10} alkyl as defined above or C_5-C_6 cycloalkyl;

C_6-C_{10} aryloxy;

C_6-C_{10} arylamino;

acylamino having 1-10 carbon atoms in the acyl group;

acyloxyamino having 1-10 carbon atoms in the acyl group;

alkylamino acylamino with a total of 1-10 carbon atoms;

C_1-C_{10} alkylamino sufonylamino;

C_1-C_{10} alkylamino;

C_1-C_{10} alkenylamino;

C_1-C_{10} dialkyl amino;

alkoxyalkyl amino with a total of 1-10 carbon atoms;

cyano;

formyl;

$COOR_{11}$, wherein R_{11} is hydrogen, C_1-C_{10} alkyl, C_6-C_{10} aryl, heterocyclic groups including up to 12 carbon atoms, monosaccharide or disaccharide; or

$COONR_{12}R_{13}$, wherein R_{12} and R_{13} are independently a halogen; C_1-C_{10} alkyl which is unsubstituted or substituted by one or more of hydroxyl or halogen, or with one or more oxygen atoms interrupting the chain, and, wherein, wherein the C_1-C_{10} alkyl is linear or non-linear; C_5-C_6 cycloalkyl; heterocyclic compound which is unsubstituted or substituted with methyl having a total of from 4 to 10 carbon atoms; amino; imino; nitro; C_1-C_{10} alkylsulfonyl; C_6-C_{10} aryl; arylalkyl, wherein the aryl group is as defined above and the alkyl group is C_1-C_{10} alkyl as defined above or C_5-C_6 cycloalkyl; arylalkylaryl, wherein the aryl groups are as defined above and the alkyl group is C_1-C_{10} alkyl as defined above or C_5-C_6 cycloalkyl; C_6-C_{10} aryloxy; C_6-C_{10} arylamino; acylamino having 1-10 carbon atoms in the acyl group; acyloxyamino having 1-10 carbon atoms in the acyl group; alkylamino acylamino with a total of 1-10 carbon atoms; C_1-C_{10} alkylamino sufonylamino; C_1-C_{10} alkylamino; C_1-C_{10} alkenylamino; C_1-C_{10} dialkyl amino; alkoxyalkyl amino with a total of 1-10 carbon atoms; cyano; formyl; $COOR_{11}$, wherein R_{11} is selected from hydrogen, C_1-C_{10} alkyl, C_6-C_{10} aryl, heterocyclic groups including up to 12 carbon atoms, monosaccharide, disaccharide, amino acids or peptides;

or pharmaceutically acceptable salts of a compound of formula (I), or mixtures of substances containing at least one compound of formula (I) or a pharmaceutically acceptable salt thereof for the preparation of a medicament for treatment of disease by at least one of inhibiting transcription factor NF-$_K$B, inhibiting COX-2, inhibiting release of histamine or inhibiting release of serotonin.

10. The use according to claim 9 wherein the C_6-C_{10} aryl is phenyl.

11. The use according to claim 9, wherein the halogen is F, Cl, or Br.

12. The use according to claim 9, wherein the C_1-C_{10} alkyl is substituted by one or more of hydroxyl or halogen or with one or more oxygen atoms interrupting the chain.

13. The use according to claim 9 for treatment of an inflammatory disease.

14. The use according to claim 13, wherein the inflammatory disease is a rheumatic disease form.

15. The use according to claim 9 for preventive or adjuvant treatment of Morbus Alzheimer.

16. The use according to any one of claims 9 to 15, wherein an extract obtained by extraction of Isatis tinctoria with an extracting agent consisting at least in part of CO_2 is used as said mixture of substances containing at least one compound of formula (I).

17. The use according to claim 9, wherein said medicament has an anti-phlogistic effect for treatment of inflammatory diseases.

18. The use according to claim 9, wherein said medicament has an anti-rheumatic effect for treatment of rheumatic disease forms.

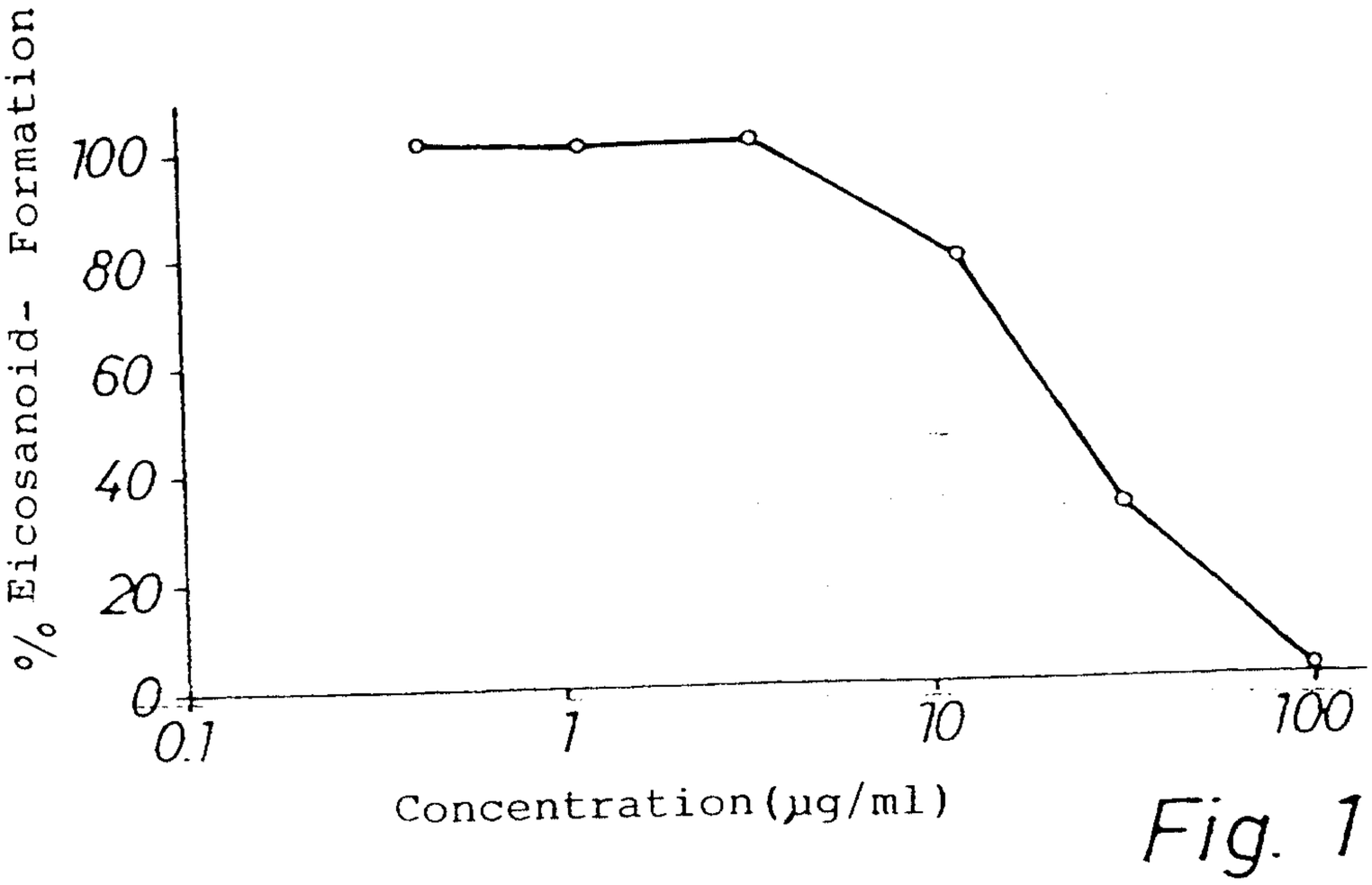


Fig. 1

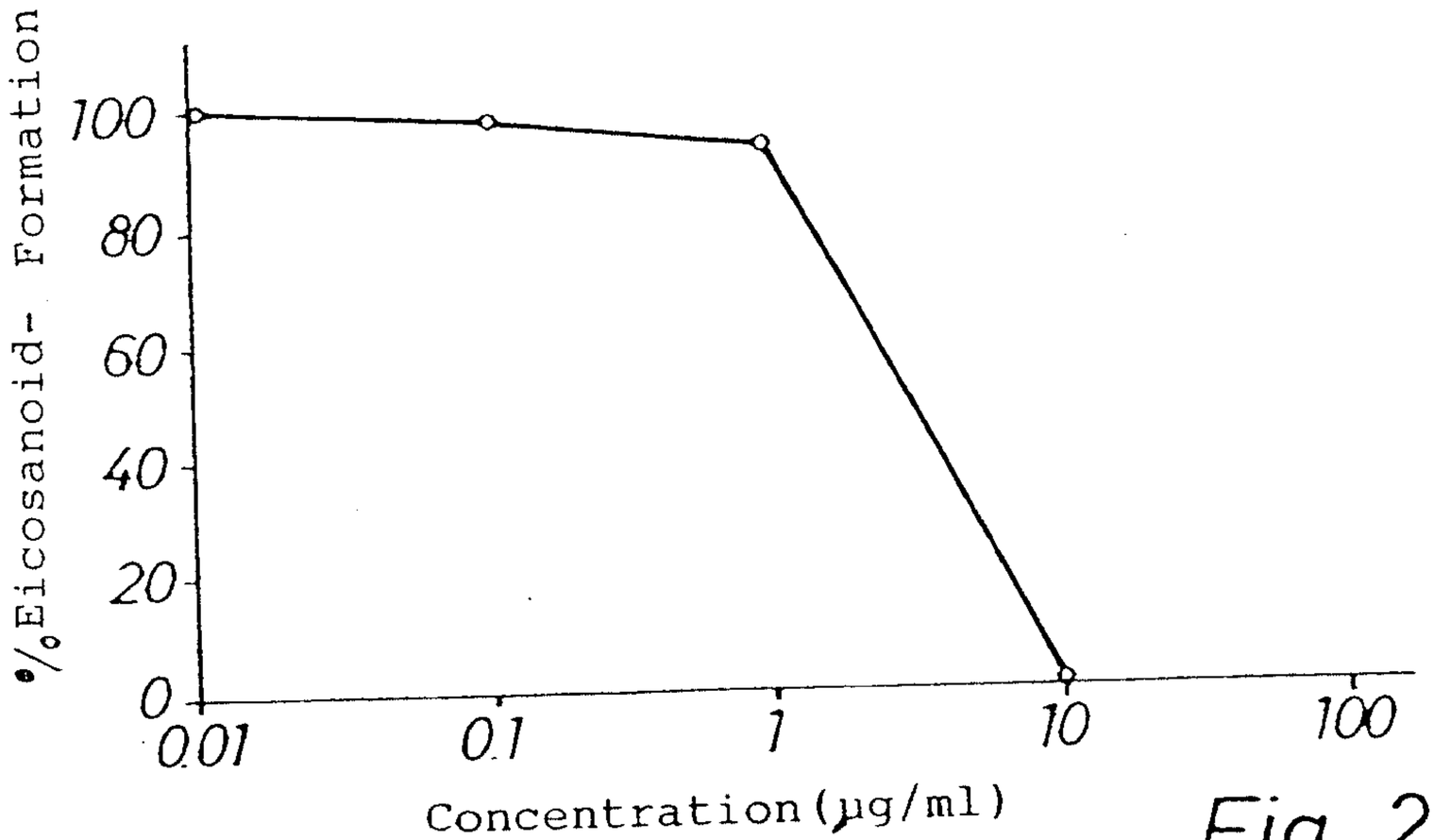


Fig. 2

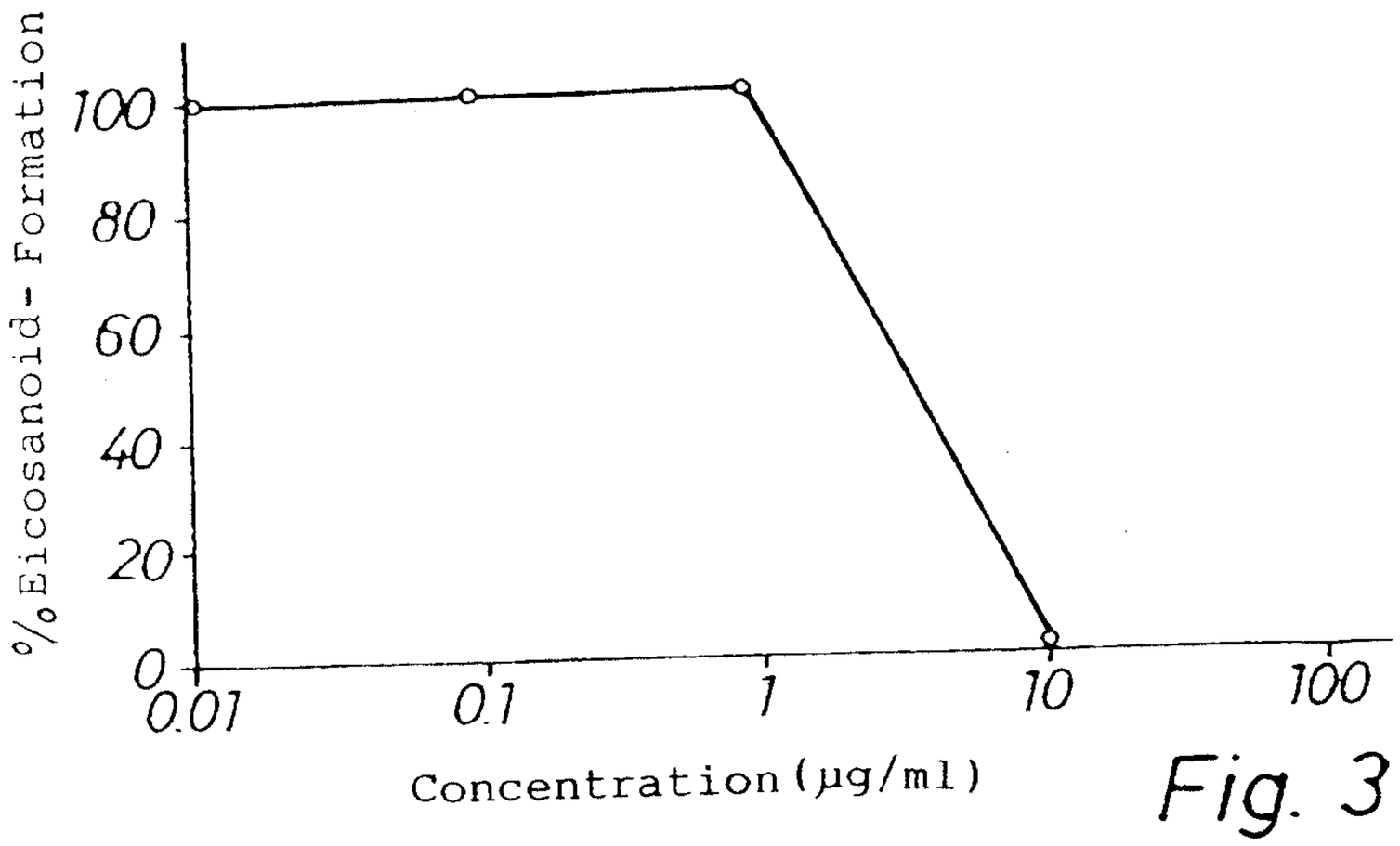


Fig. 3

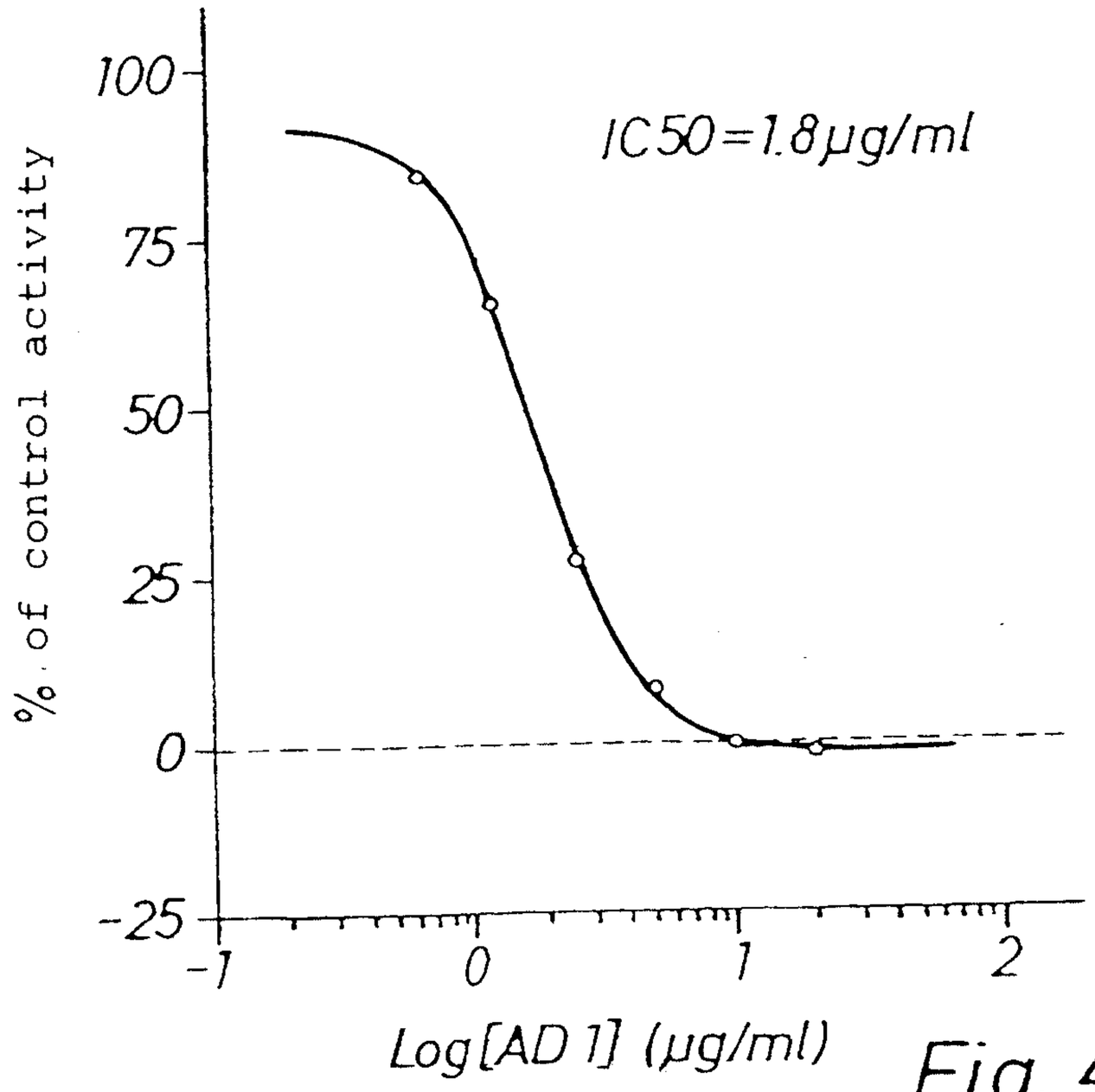
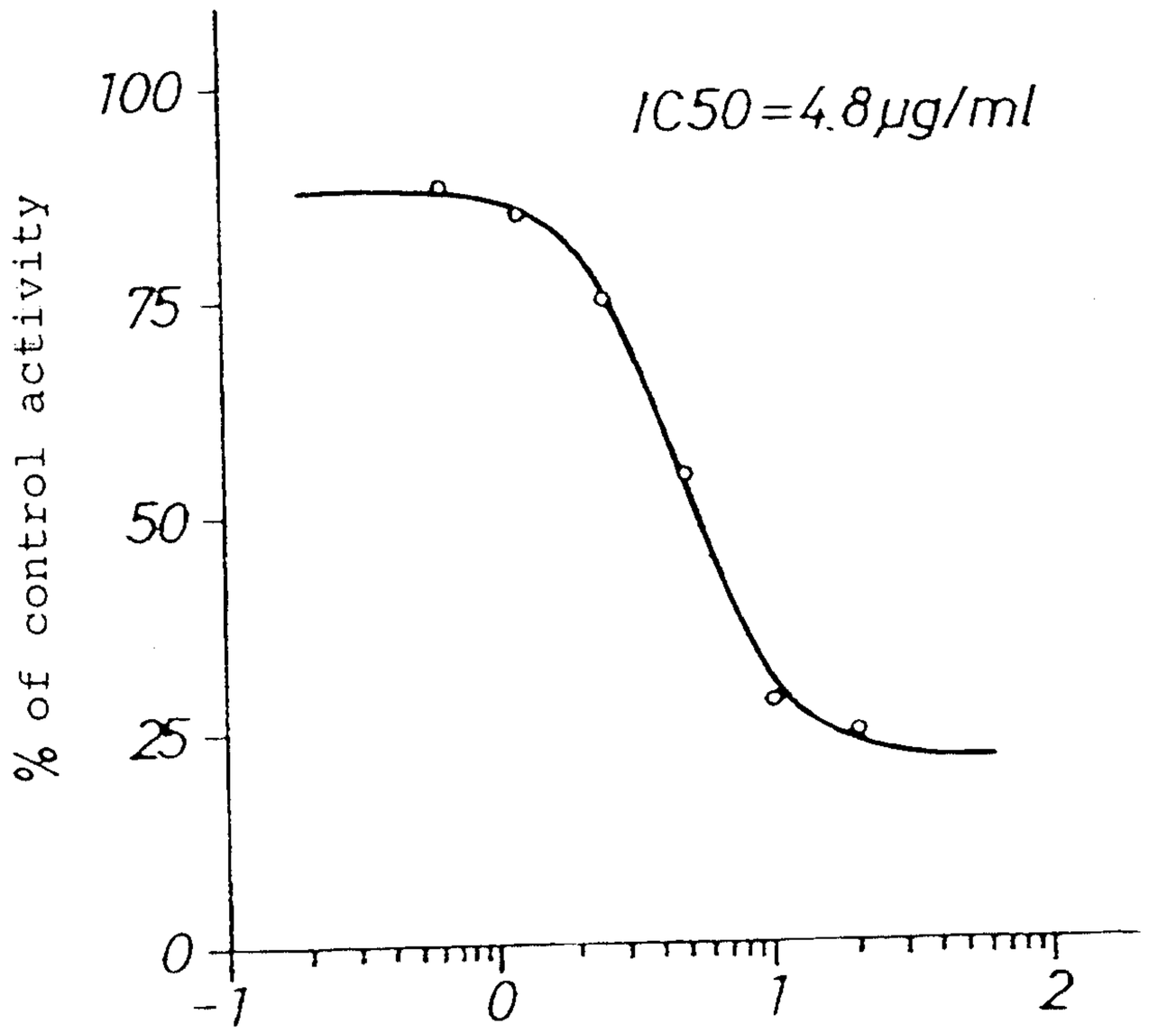

IC50=1.8µg/ml
100
75
50
25
0
-25
% of control activity
-1
0
1
2
Log[AD 1] (µg/ml)

Fig. 4


IC50=4.8µg/ml
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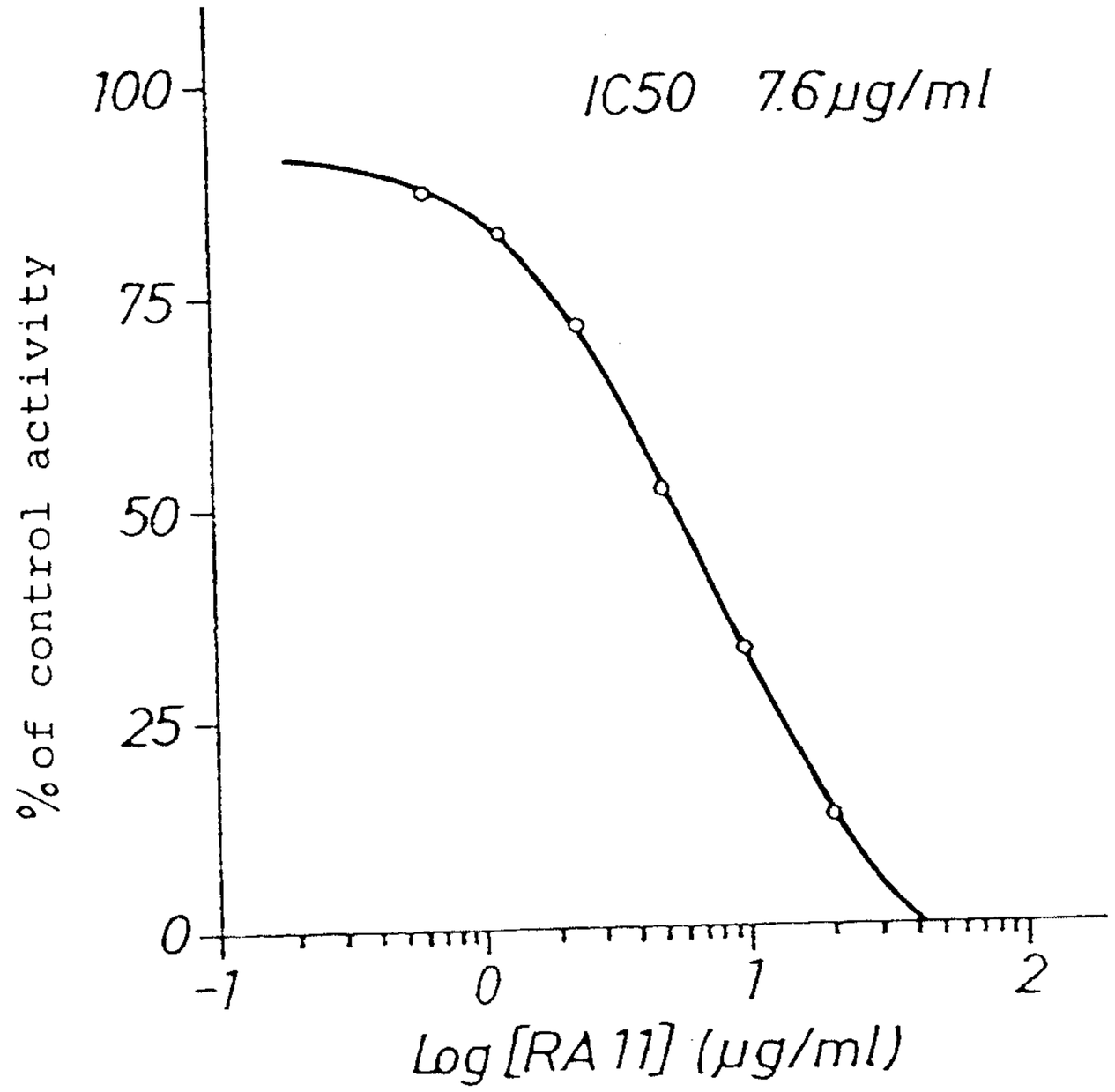
25
0
% of control activity
-1
0
1
2
Log[RA 3] (µg/ml)

Fig. 5


IC50 7.6µg/ml
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50
25
0
% of control activity
-1
0
1
2
Log [RA 11] (µg/ml)

Fig. 6

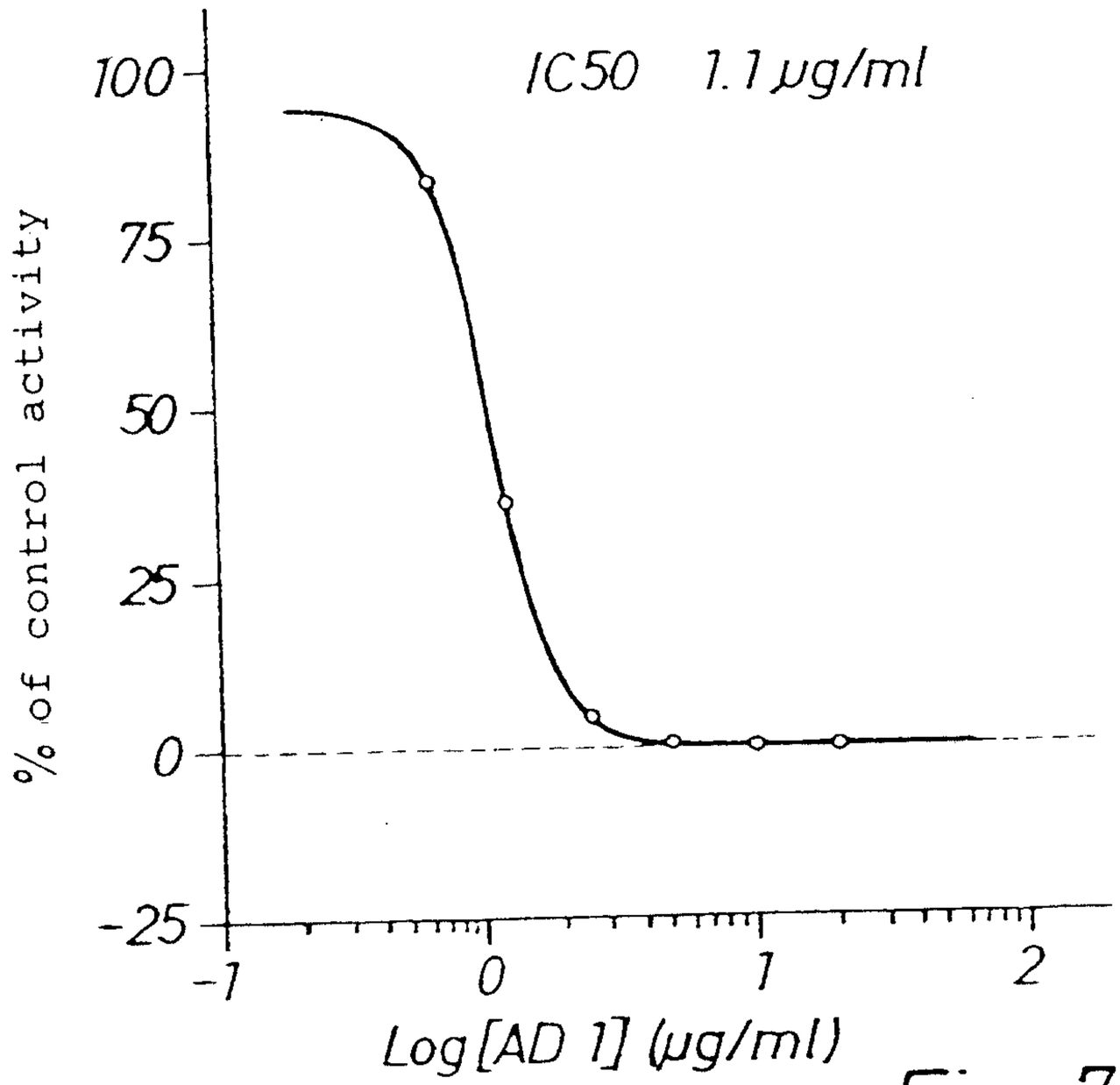
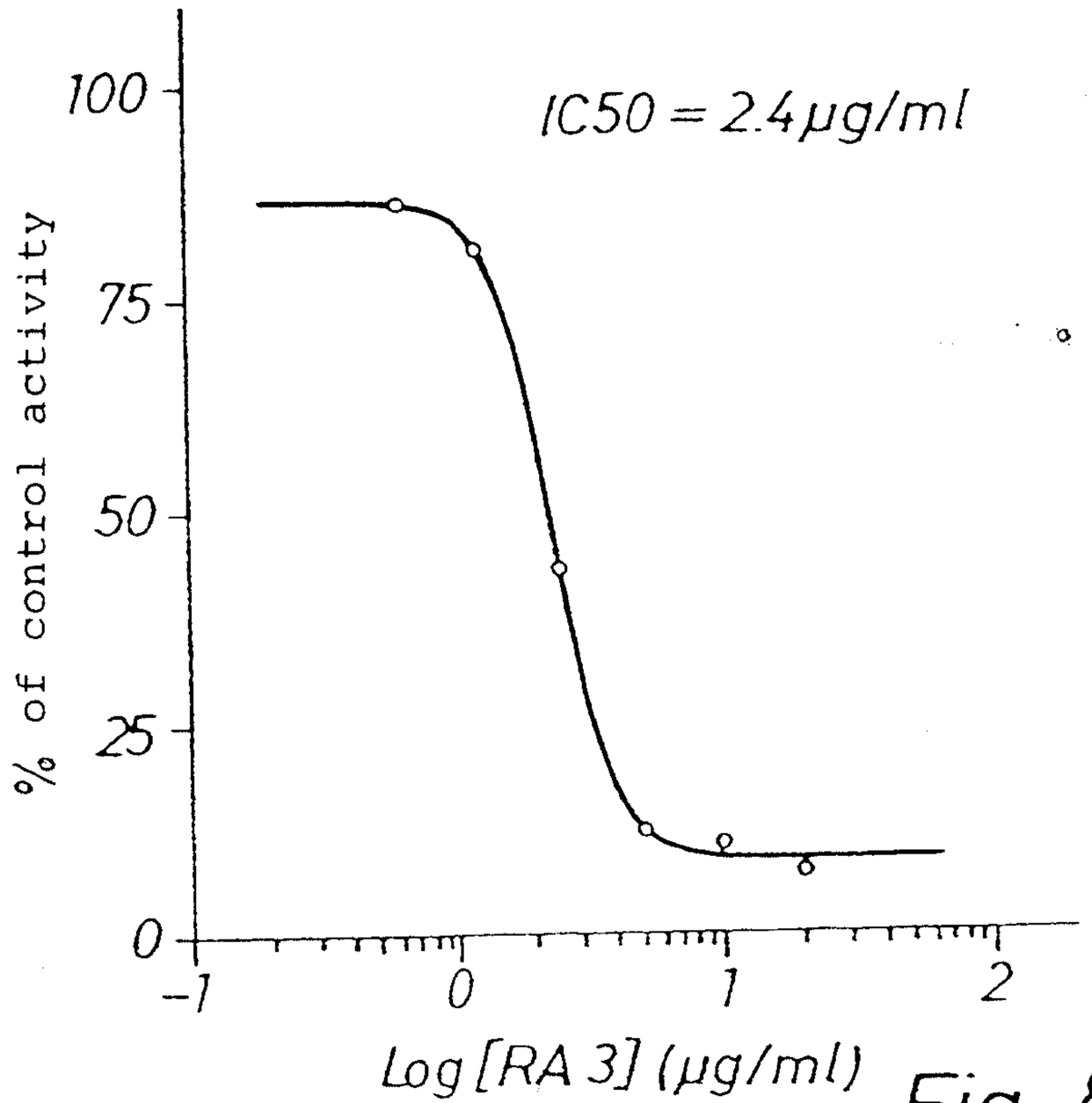

IC50 1.1µg/ml
100
75
50
25
0
-25
% of control activity
-1
0
1
2
Log [AD 1] (µg/ml)

Fig. 7


IC50 = 2.4 µg/ml
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75
50
25
0
% of control activity
-1
0
1
2
Log [RA 3] (µg/ml)

Fig. 8

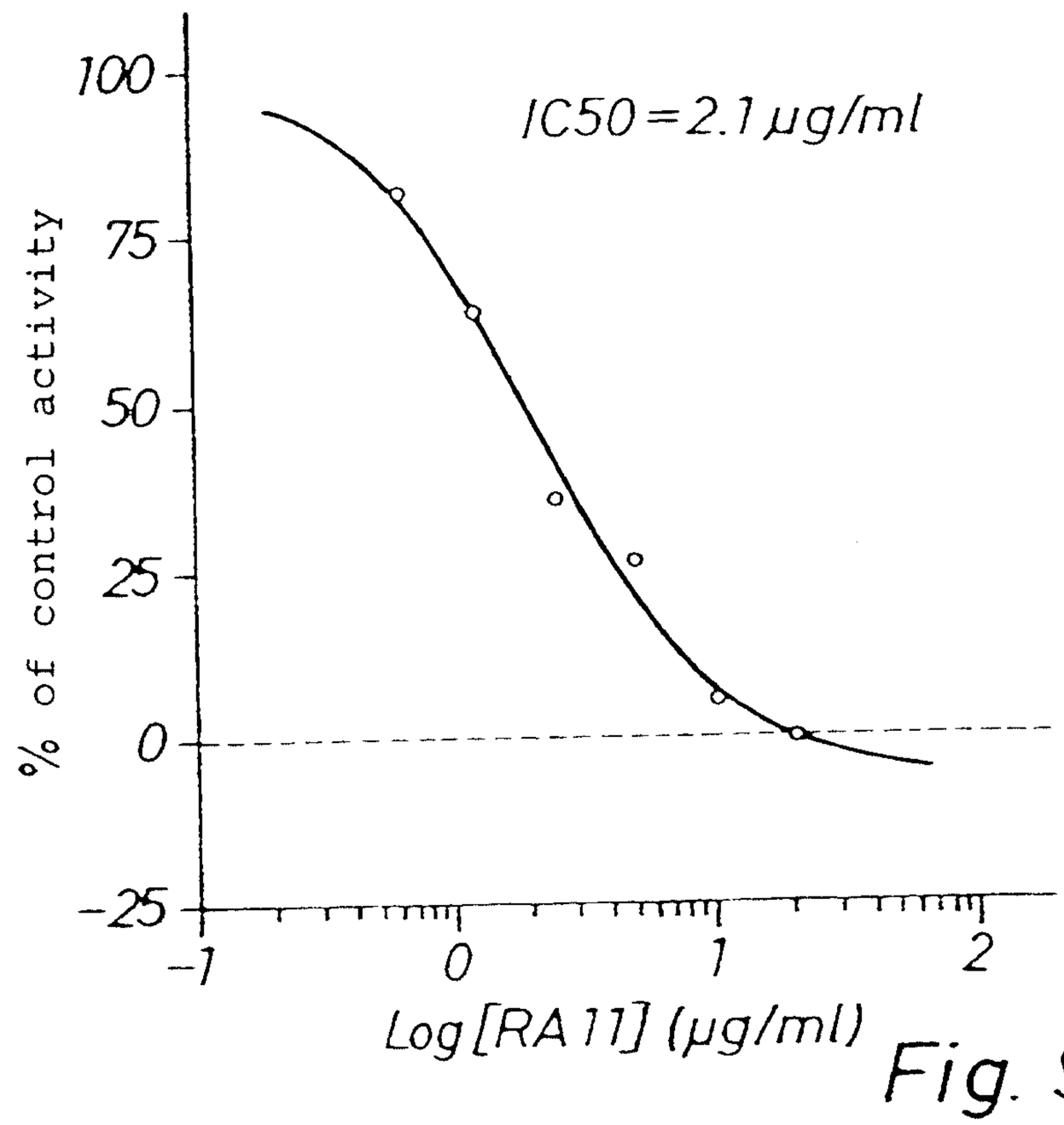
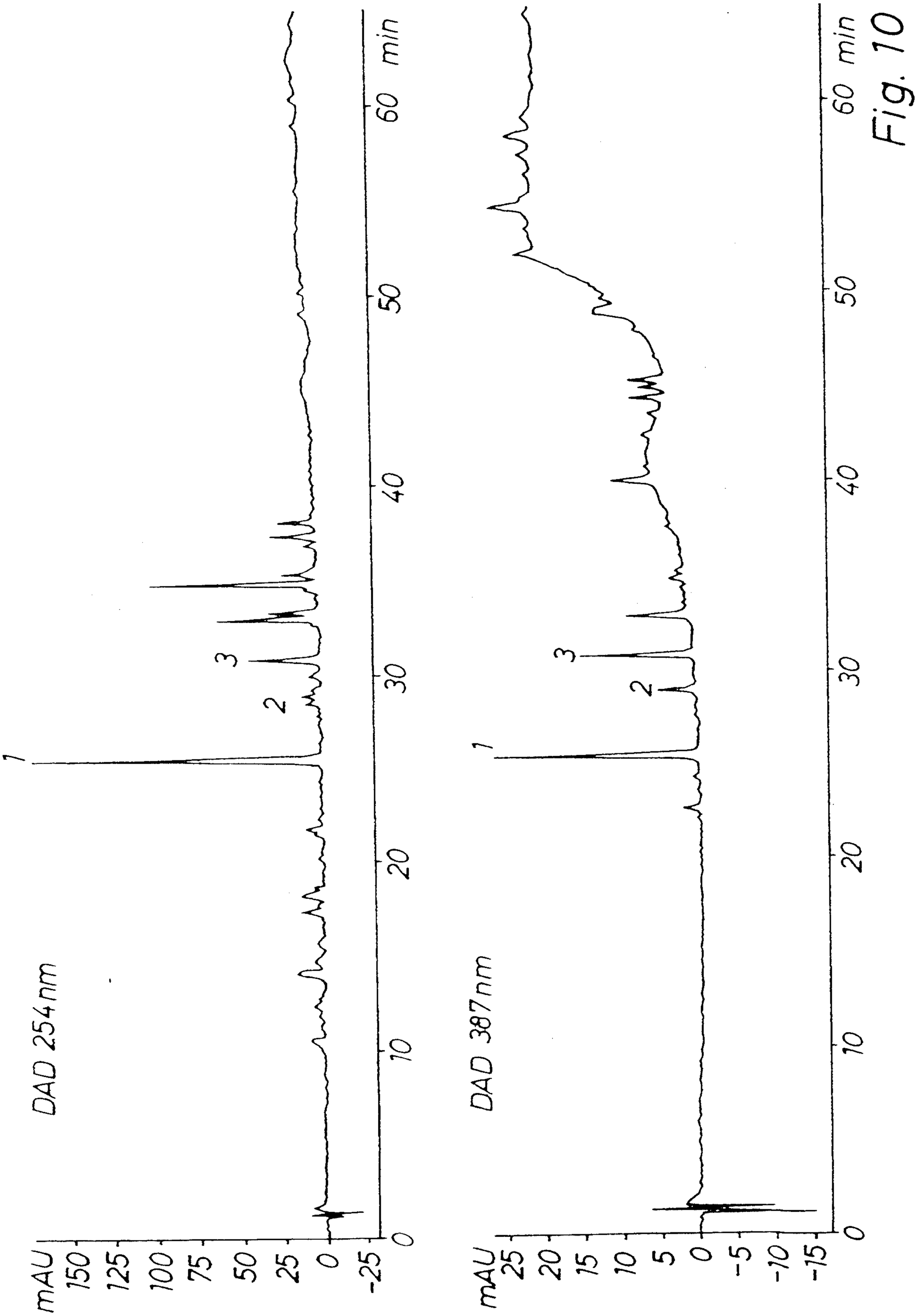

IC50 = 2.1 µg/ml
100
75
50
25
0
-25
% of control activity
-1
0
1
2
Log [RA 11] (µg/ml)

Fig. 9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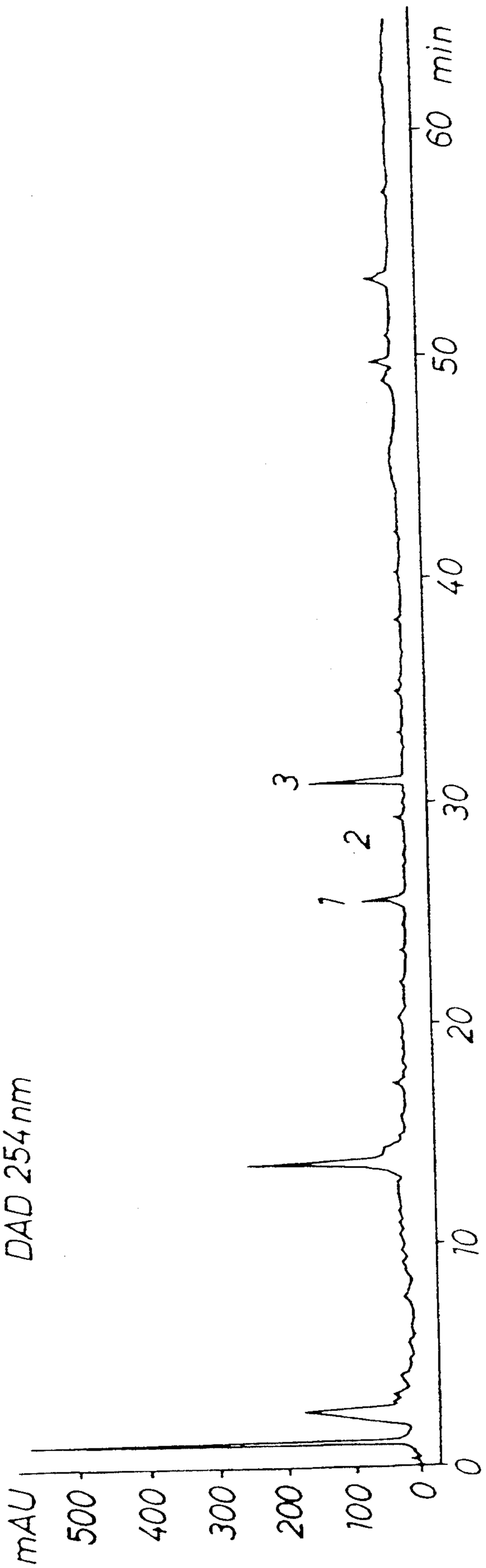
DAD 387nm
mAU
150
125
100
75
50
25
0
-25
25
20
15
10
5
0
-5
-10
-15
1
2
3
10
20
30
40
50
60 min

Fig. 10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mAU
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400
300
200
100
0
10
20
30
40
50
60 min
1
2
3

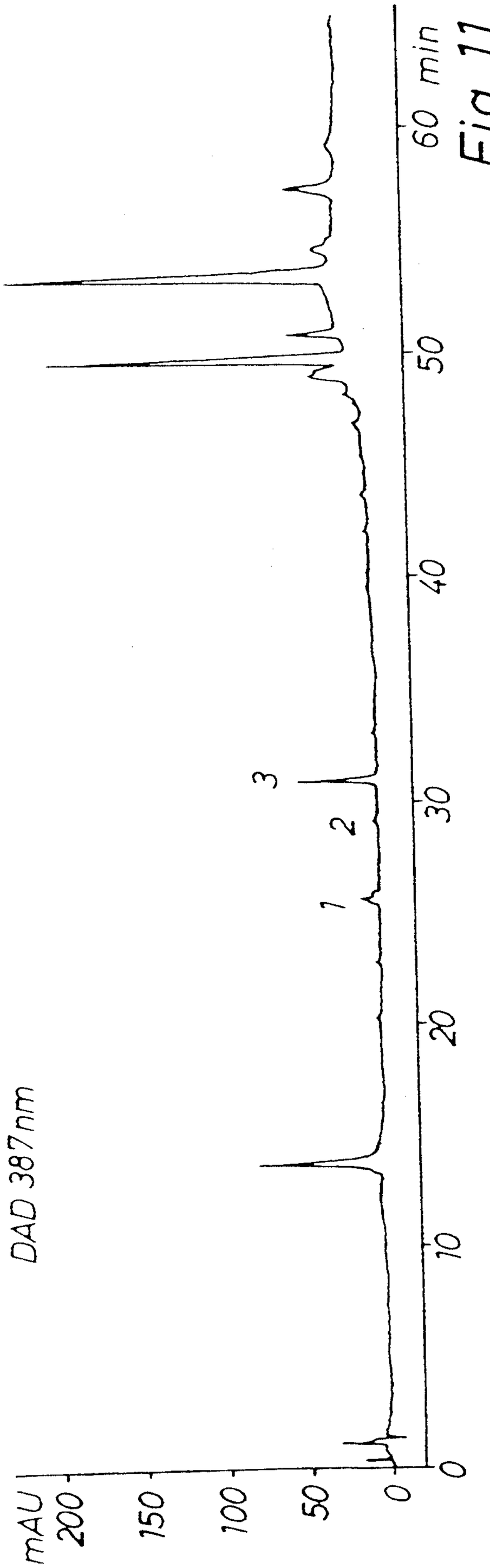

DAD 387 nm
mAU
200
150
100
50
0
10
20
30
40
50
60 min
1
2
3

Fig. 11

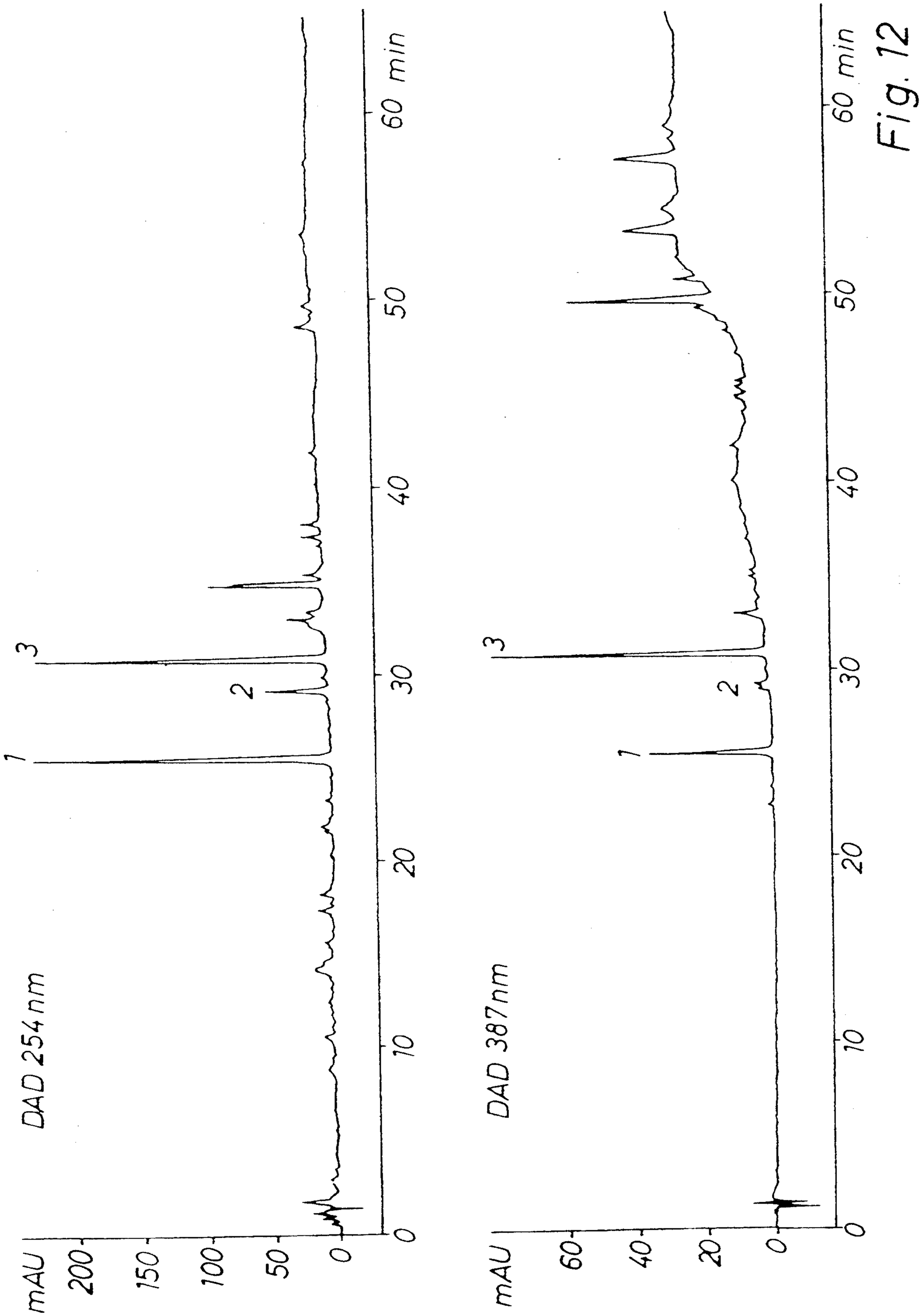

DAD 254 nm
mAU
200
150
100
50
0
10
20
30
40
50
60 min
1
2
3
DAD 387 nm
mAU
60
40
20
0
10
20
30
40
50
60 min
1
2
3

Fig. 12

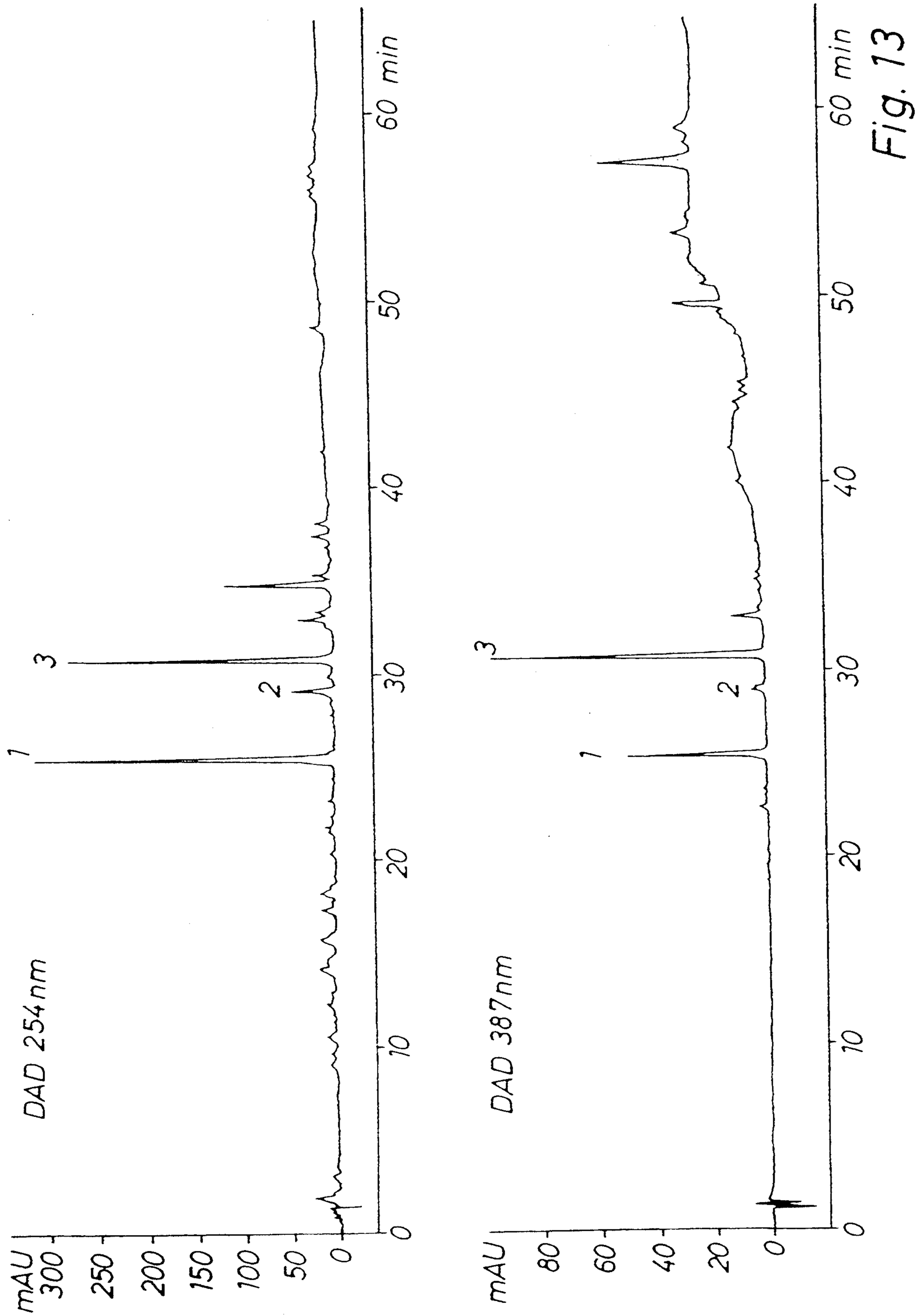

DAD 254nm
DAD 387nm
mAU
300
250
200
150
100
50
0
80
60
40
20
0
1
2
3
10
20
30
40
50
60 min

Fig. 13

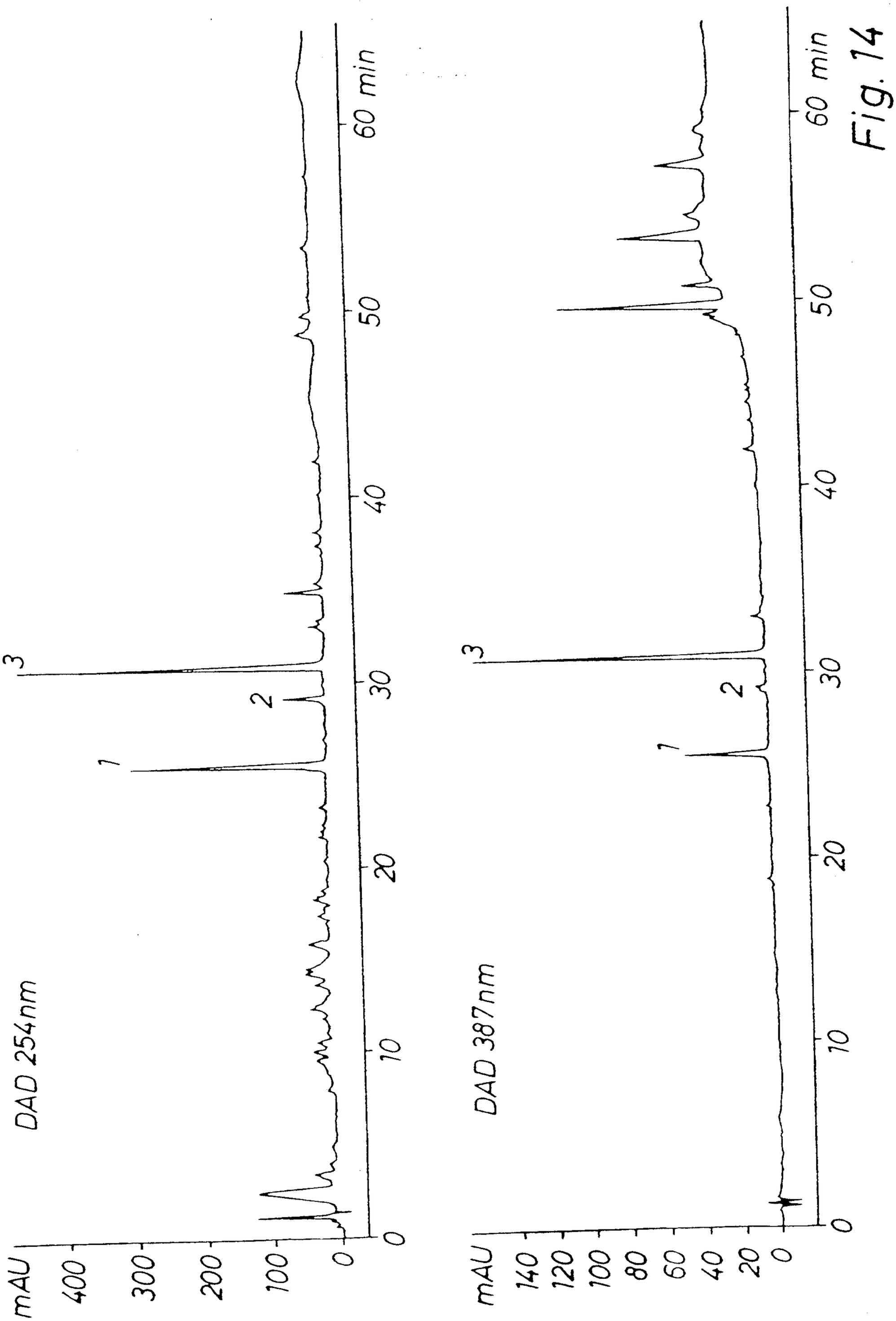

DAD 254nm
mAU
400
300
200
100
0
0
10
20
30
40
50
60 min
1
2
3
DAD 387nm
mAU
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60 min
1
2
3

Fig. 14

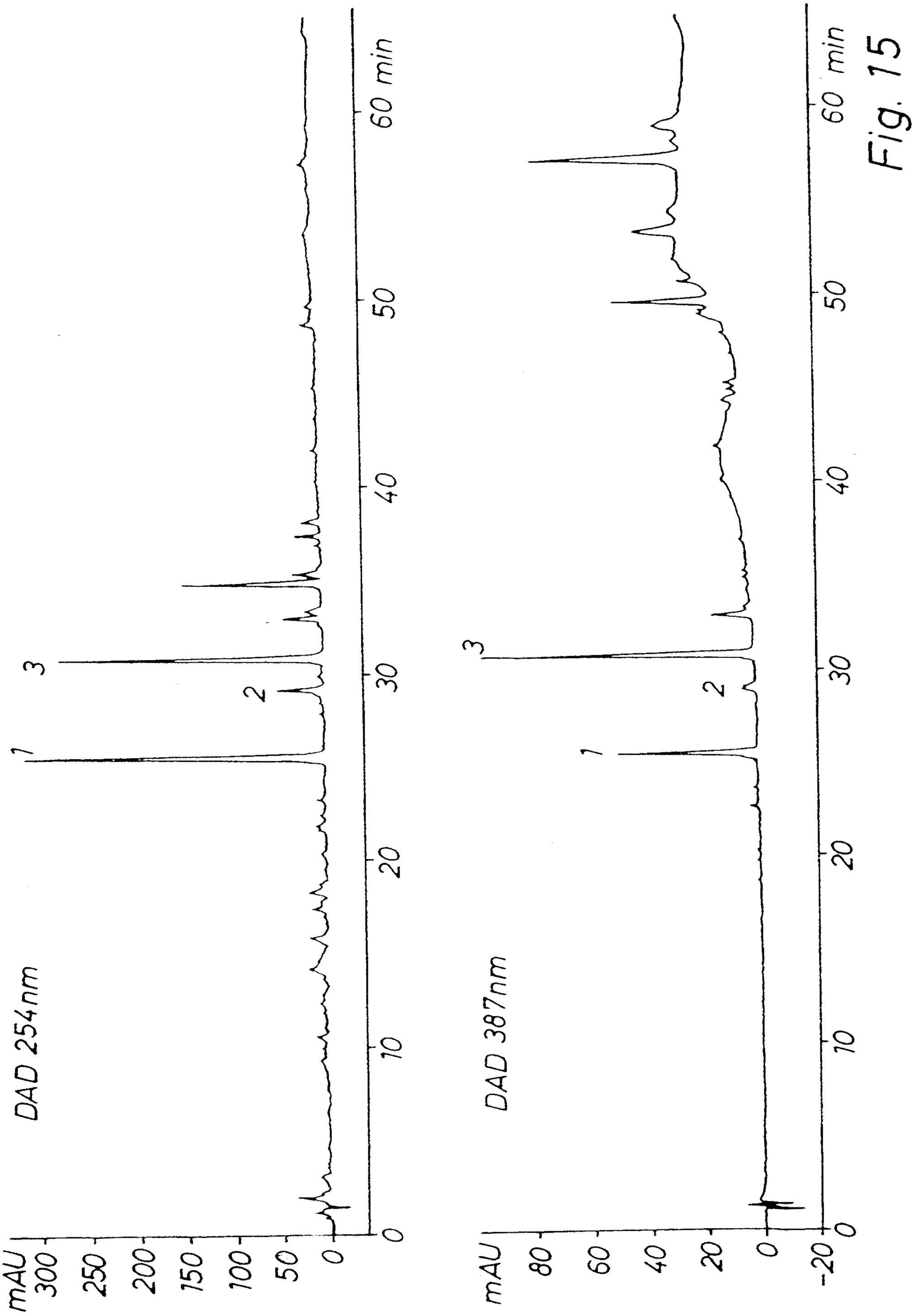

DAD 254nm
mAU
300
250
200
150
100
50
0
1
2
3
10
20
30
40
50
60 min
DAD 387nm
mAU
80
60
40
20
0
-20
10
20
30
40
50
60 min

Fig. 15

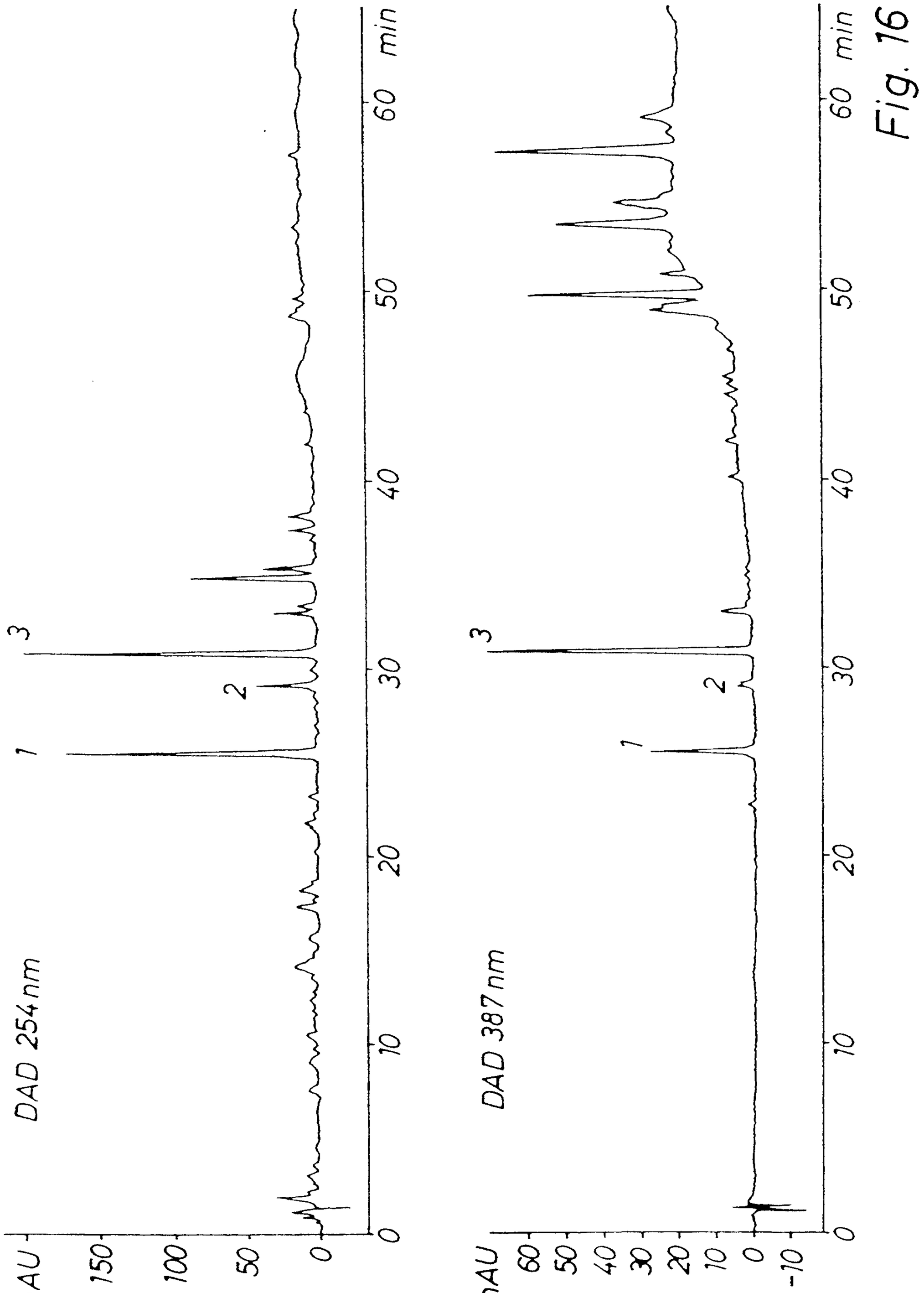
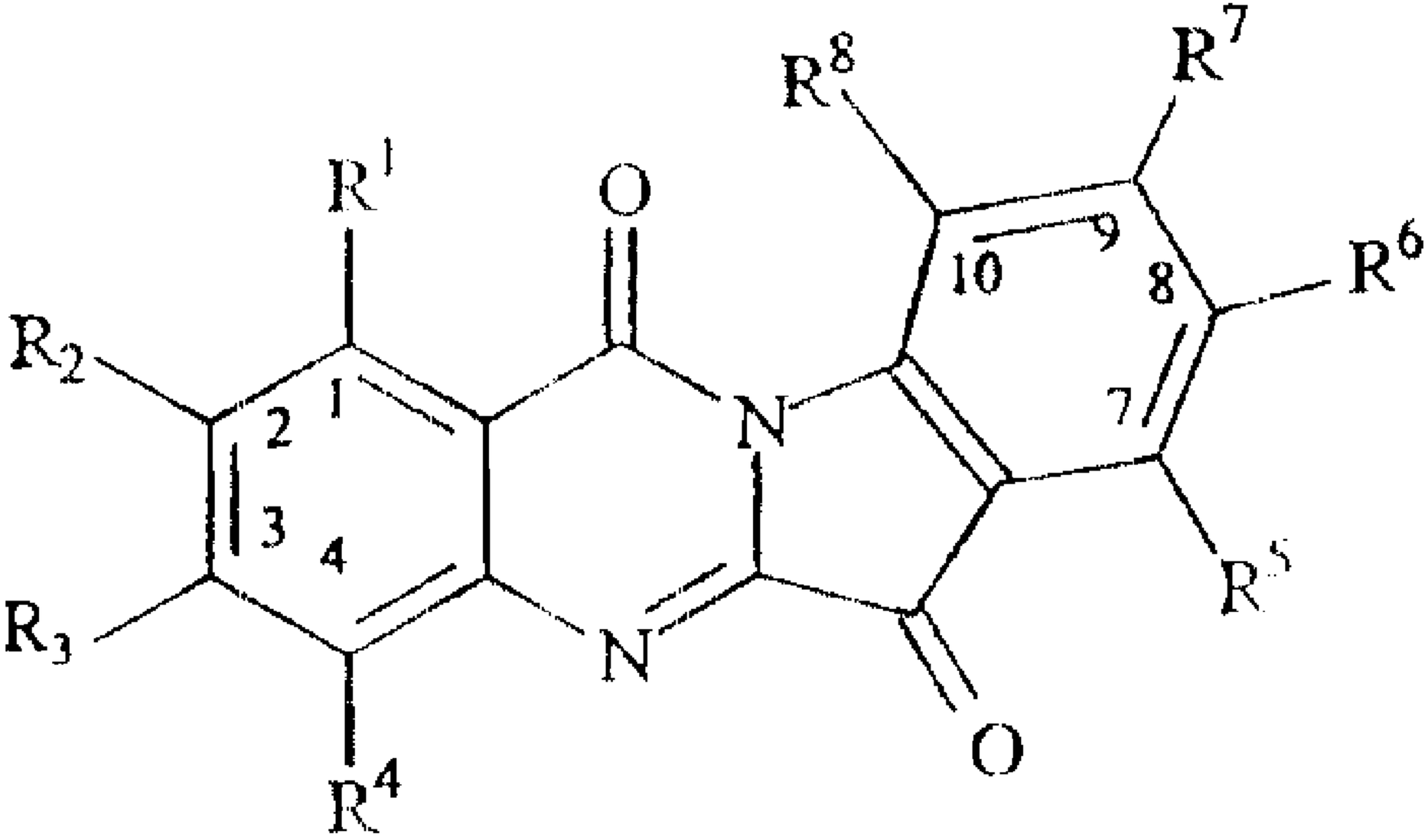

DAD 254 nm
mAU
150
100
50
0
1
2
3
0
10
20
30
40
50
60 min
DAD 387 nm
mAU
60
50
40
30
20
10
0
-10
1
2
3
0
10
20
30
40
50
60 min

Fig. 16



(I)