

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 January 2001 (11.01.2001)

PCT

(10) International Publication Number
WO 01/02408 A1

(51) International Patent Classification⁷: **C07D 493/22**,
A61K 31/35 // (C07D 493/22, 313:00, 311:00, 307:00,
307:00)

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(21) International Application Number: PCT/EP00/05973

(22) International Filing Date: 27 June 2000 (27.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ,
BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,
DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,
LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT,
TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(30) Priority Data:
99112553.5 1 July 1999 (01.07.1999) EP

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG,
CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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Published:

- With international search report.
- Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/02408 A1

(54) Title: NOVEL XANTHONE COMPOUNDS, THEIR PREPARATION AND USE AS MEDICAMENT

(57) Abstract: This invention relates to noxel xanthone compounds, their preparation and use as medicament. More particularly this invention is directed to the isolation of the novel xanthone natural product *sootpenseone* from *Dasy-mashalon sootpense Craib, Annonaceae*, its identification and derivatization, and the use of *sootpenseone* and its derivatives as anticancer agents.

**Novel xanthone compounds,
their preparation and use as medicament**

Introduction

This invention relates to novel xanthone compounds, their preparation and use as a medicament. More particularly this invention is directed to the isolation of the novel xanthone natural product sootepenseone from *Dasymaschalon sootepense Craib*, *Annonaceae*, its identification and derivatization, and the use of sootepenseone and its derivatives as anticancer agents.

Field of Invention

Cancer is perhaps one of the most active anti-human factor operating in the world today, and efforts are being made all over the scientific world to prevent and eradicate it.

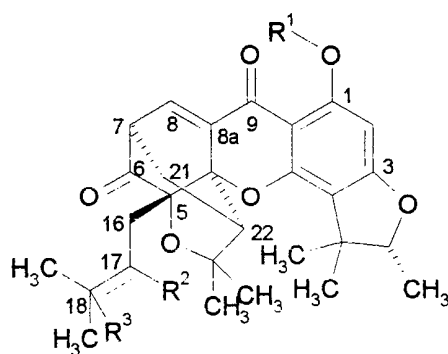
New agents with chemotherapeutic value in the fight against cancer is obviously a medical problem of high importance. But the development of new drugs in the cancer field is a difficult task given that anticancer agents must be lethal to, or incapacitate tumor cells, but they should not cause excessive damage to normal cells. At present the state of knowledge in cancer biology and in medical chemistry does not warrant the designing of new classes of molecules which may be effective antitumor agents. Despite the great progress made in cancer biology, molecular pharmacology, pharmacokinetics, medical chemistry and allied fields, the knowledge sought after, is still elusive.

Since the concept of chemotherapeutic treatment of malignant diseases had come to the forefront during the last decades, plant principles and their derivatives have been intensively investigated by scientists all over the world as new antitumor inhibitors. Examples for important anticancer agents of plant origins are the alkaloids vincalukoblastine (vinblastine) and leurocristine (vincristine), both isolated from

Catharanthus roseus. A comprehensive review on natural products as anticancer agents is given by Shradha Sinha and Audha Jain, in: Progress in Drug Research, Vol. 42, pages 53-132 (1994) Basel (Switzerland).

Summary of invention

In accordance with the present invention there are provided novel cytotoxic xanthone compounds of the general formula (I)



(I)

wherein

R^1 is a hydrogen atom; a methyl group (-CH₃), a C₂-C₆ alkyl residue, a formyl group (-CHO); an acetyl residue (-COCH₃), -CO-C₂₋₆-alkyl, CO-C₃₋₈-cycloalkyl, -CO-C₆₋₁₈-aryl or -CO-C₇₋₂₄-aralkyl residue each having optionally one or more substituents selected from the group consisting of -OH, -SH, -NH₂, -NHC₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -NHC₆₋₁₄-aryl, -N(C₆₋₁₄-aryl)₂, -N(C₁₋₆-alkyl)(C₆₋₁₄-aryl), -NHCOR², -NO₂, -CN, -(CO)R³, -(CS)R⁴, -F, -Cl, -Br, -I, -O-C₁₋₆-alkyl, -O-C₆₋₁₄-aryl, -O-(CO)R⁵, -S-C₁₋₆-alkyl, -S-C₆₋₁₄-aryl, -SOR⁶, and -SO₂R⁷, wherein R² to R⁷ stands independently of each other for a hydrogen atom, -C₁₋₆-alkyl, -O-C₁₋₆-alkyl, -O-C₆₋₁₄-aryl, -NH₂, -NHC₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -NHC₆₋₁₄-aryl, -N(C₆₋₁₄-aryl)₂, -N(C₁₋₆-alkyl)(C₆₋₁₄-aryl), -S-C₁₋₆-alkyl, -S-C₆₋₁₄-aryl residue;

a -COO-C₁₋₆-alkyl residue having optionally one or more substituents selected from the group consisting of -OH, -SH, -NH₂, -NHC₁₋₆-alkyl, -N(C₁₋₆-alkyl)₂, -NHC₆₋₁₄-aryl, -N(C₆₋₁₄-aryl)₂, -N(C₁₋₆-alkyl)(C₆₋₁₄-aryl), -NHCOR⁸, -NO₂, -CN, -(CO)R⁹, -(CS)R¹⁰, -F, -Cl, -Br, -I, -O-C₁₋₆-alkyl, -O-C₆₋₁₄-aryl, -O-(CO)R¹¹, -S-C₁₋₆-alkyl, -S-C₆₋₁₄-aryl, -SOR¹²,

and $-SO_2R^{13}$, wherein R^8 to R^{13} stands independently of each other for a hydrogen atom, $-C_{1-6}$ -alkyl, $-O-C_{1-6}$ -alkyl, $-O-C_{6-14}$ -aryl, $-NH_2$, $-NHC_{1-6}$ -alkyl, $-N(C_{1-6}$ -alkyl) $_2$, $-NHC_{6-14}$ -aryl, $-N(C_{6-14}$ -aryl) $_2$, $-N(C_{1-6}$ -alkyl)(C_{6-14} -aryl), $-S-C_{1-6}$ -alkyl, $-S-C_{6-14}$ -aryl residue;

a $-CONR^{14}R^{15}$ residue wherein R^{14} and R^{15} stand independently of each other for a hydrogen atom, $-C_{1-6}$ -alkyl, $-O-C_{1-6}$ -alkyl, $-O-C_{6-14}$ -aryl, $-NH_2$, $-NHC_{1-6}$ -alkyl, $-N(C_{1-6}$ -alkyl) $_2$, $-NHC_{6-14}$ -aryl, $-N(C_{6-14}$ -aryl) $_2$, $-N(C_{1-6}$ -alkyl)(C_{6-14} -aryl), $-S-C_{1-6}$ -alkyl, $-S-C_{6-14}$ -aryl residue;

or a counter cation selected from the group consisting of an alkali or earth alkali metal such as Li, Na, K, Ca, Mg, $NR^{16}R^{17}R^{18}R^{19}(+)$ wherein R^{16} to R^{19} stands independently of each other for a hydrogen atom or a C_1 - C_6 -alkyl residue;

R^2 and R^3 either form part of the $C^{17}=C^{18}$ -double bond or are each hydrogen, or a tautomer, an enantiomer, an stereoisomer or a physiologically acceptable salt or a solvate thereof or mixtures thereof.

In the case of a compound according to formula 1 above in the form of a phenolate with a di- or multivalent counter cation, the remaining positive charge can be compensated by association with a physiologically acceptable anion such as Cl^- or OH^- .

The novel compound according to formula I, wherein R^1 is a hydrogen atom and R^2 and R^3 form part of the $C^{17}-C^{18}$ -double bond, has been given the name *sootepenseone* (1).

According to another aspect of the invention there is provided a process for manufacturing a compound according to formula I by isolation of *sootepenseone* (1) from the leaves of *Dasymaschalon sootepenseone* Craib, *Annonaceae* and its subsequent derivatization.

The present invention further provides the use of the compounds according to formula (I) as medicament, in particular for the treatment of cancer diseases.

The present invention further provides pharmaceutical formulations, comprising an effective amount of a compound according to formula (I) for treating a patient in need thereof. As used herein, an effective amount of a compound according to formula (I) is defined as the amount of the compound which, upon administration to a patient, inhibits growth of tumor cells, kills malignant cells, reduces the volume or size of the tumors or eliminates the tumor entirely in the treated patient.

Thus, the substantially pure compounds in accordance with the invention can be formulated into dosage forms using pharmaceutically acceptable carriers for oral, topical or parenteral administration to patients in need of oncolytic therapy.

In a preferred embodiment, the patient is a mammal, in particular a human.

The effective amount to be administered to a patient is typically based on body surface area, patient weight, and patient condition. The interrelationship of dosages for animals or humans (based on milligrams per meter squared of body surface) is described by Freireich, E.J. et al., *Cancer Chemother. Rep.*, 50 (4) 219 (1966). Body surface area may be approximately determined from patient height and weight (see e.g. *Scientific Tables*, Geigy Pharmaceuticals, Ardy, N.Y., pages 537-538 (1970)). Preferred dose levels will also depend on the attending physicians assessment of both the nature of the patient's particular cancerous condition and the overall physical condition of the patient. Effective antitumor doses of the present xanthone compounds range from 1 microgram per kilogram to about 5000 micrograms per kilogram of patient body weight, more preferably between 2 micrograms to about 1000 micrograms per kilogram of patient body weight.

Effective doses will also vary, as recognized by those skilled in the art, dependant on route of administration, excipient usage and the possibility of co-usage with other therapeutic treatments including other anti-tumor agents, and radiation therapy.

The present pharmaceutical formulation may be administered intravenous, intramuscular, intradermal, subcutaneous, intraperitoneally, topical, or intravenous in the form of a liposome.

Examples of dosage forms include aqueous solutions of the active agent, in an isotonic saline, 5% glucose or other well-known pharmaceutically acceptable liquid carrier. Additional solubilizing agents well-known to those familiar with the art can be utilized as pharmaceutical excipients for delivery of the active agent. Alternatively, the present compounds can be chemically modified to enhance water solubility, for example, by formation of pharmaceutically acceptable phenolate salts.

The present compounds can also be formulated into dosage forms for other routes of administration utilizing well-known methods. The pharmaceutical compositions can be formulated, for example, in dosage forms for oral administration in a capsule, a gel seal or a tablet. Capsules may comprise any well-known pharmaceutically acceptable material such as gelatin or cellulose derivatives. Tablets may be formulated in accordance with conventional procedure by compressing mixtures of the active agent and solid carriers, and lubricants well-known to those familiar with the art. Examples of solid carriers include starch, sugar, bentonite. The compounds of the present invention can also be administered in a form of a hard shell tablet or capsule containing for example, lactose or mannitol as a binder and a conventional fillers and tableting agents.

The terms „effective amount“ and „effective dose“ as referring to the treatment of animals is defined herein to mean those quantities of alkaloid which will cause remission or inhibition of growth of the cancer disease in the animal to which it is administered, without imparting an intolerable toxic response. The effective amount may vary with the way of administration, the administration schedule, the kind of tumor, and other related factors, all of which may be varied without departing from the scope or operativeness of the invention. Generally an effective dose would be one within the range of about 0.001-100.0 mg/kg of body weight/day.

The terms „cancer“ or „tumor“ as used herein include, but are in no way limited to, adenocarcinomas, glioblastomas (and other brain tumors), breast, cervical, colorectal, endometrial, gastric, liver, lung (small cell and non-small cell), lymphomas (including non-hodgkin's, Burkitt's, diffuse large cell,, follicular and diffuse Hodgkin's), melanoma (metastatic), neuroblastoma, osteogenic sarcoma, ovarian,

retinoblastoma, soft tissue sarcomas, testicular and other tumors which respond to chemotherapy.

Other objects and advantages of the invention will become readily apparent from the ensuing description.

Detailed description of the invention

The inventive compounds according to formula (I) have a pentacyclic xanthone ring system (see for a review: Sultanbawa, M.U.S., Xanthonoids of tropical plants, Tetrahedron 36 (1980) 1465-1506). The following natural compounds are reported as having a similar ring system:

Gambogic acid (2), isolated from *Garcinia hanburyi* (see Amorosa, M. et al., Ann. Chim. (Rome), 1966, 56, 232; Ahmad, A.S. et al., J. Chem. Soc. (C), 1966, 772 (structure); Arnone, A. et al., Tetr. Lett., 1967, 4201 (pmr data, structure), morellin (3) isolated from *Garcinia morella* (see: Rao, B.S., J. Chem. Soc, 1937, 853 (isolation); Kartha, G. et al., Tetr. Lett., 1963, 459 (cryst. structure)); Nair, P.M. et al., Indian J. Chem., 1964, 2, 402 (structure)), hanburin (4) isolated from *Garcinia hanburyi* (see: Asano, J. et al., Phytochemistry, 1996, 41, 815 (isolation, uv, ir, pmr, cmr data) and forbesione (5) isolated from *Garcinia forbesii* (see: Yuan-Wah eong, Leslie J. Harrison, Graham J. Bennett and Hugh T.-W. Tan, J. Chem. Research (S) 1996, 392-393).

These compounds have at C-5 an isoprenyl side chain in common with a hydrogen bonded phenolic hydroxy group. Morellin (3) and gambogic acid (2) have a chromene ring system in common. All compounds (2) to (5) have in common a bicyclo[2.2.2]octene carbon skeleton fused to a 2,2-dimethyl-tetrahydrofuran ring system (see fig. 1).

However, these compounds show significant structural differences as compared to the compounds according to formula I of the present invention:

- 1.) the C-5 isoprenyl side chain is oxidized to an aldehyde as in (3) or to a carboxylic acid as in (2);
- 2.) the condensed dihydrofuran ring in 3,4-position is missing as in (4) or instead a pyranone ring is present in the 2,3-position as in (3)
- 3.) the ring system is substituted with an additional isoprenyl side chain at C-5 as in (3) and (5)

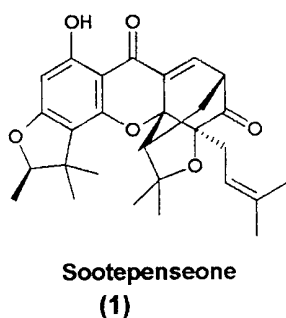
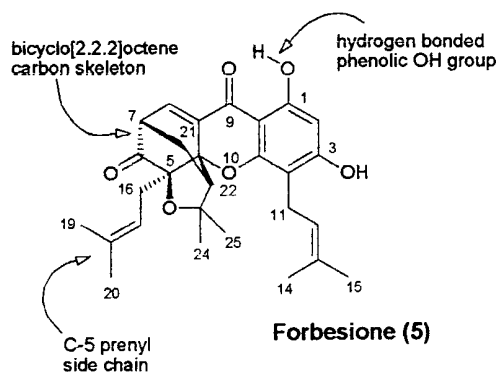
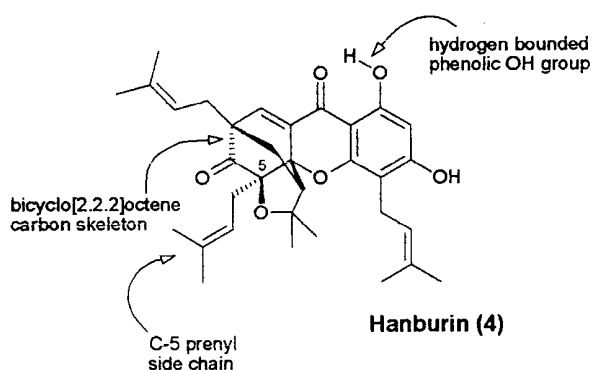
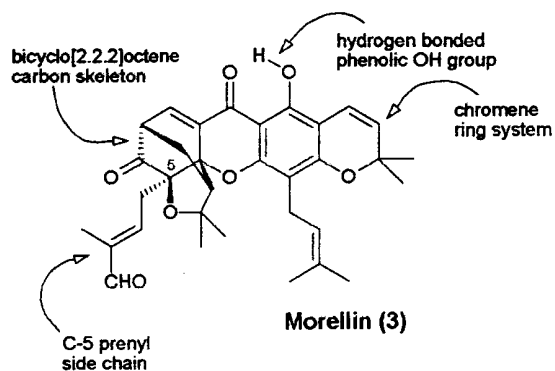
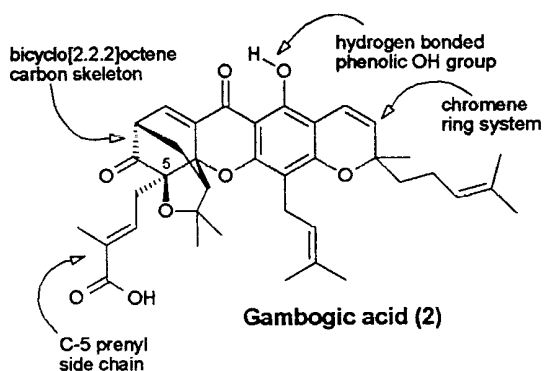
By contrast, the compounds of the present invention contain fully substituted dihydrofuran rings except at carbon 2', fused to the modified xanthone ring system.

For the taxonomy of *Dasymaschalon sootepense* Craib see V.H. Heywood, "Flowering Plants of the World", University Press, Oxford, 1978.

Surprisingly, the compounds of the present invention show remarkable antitumor activity. Moreover, the present compounds have a low toxicity.

Thus the xanthone compounds according to the present invention are new and involve an inventive step.

The structures of (2) to (5) are summarized below:



Example 1

Isolation of sootepenseone (1; VR-3016, D-25637) from *Dasymaschalon sootepense*

Plant material has been collected in November 1994 in Doitung, Chiangrai province, northern Thailand. Leaves were air sun dried (about 30-35°C) for three days and stored in a cloth bag. Dry powdered leaves (6.4 kg) of *D. sootepense* were sequentially extracted with hexane (110 litre), chloroform (107 litre) and methanol (180 litre) to give the corresponding crude extracts in 204.2, 192.7 and 1187.0 gm, respectively. The crude methanol extract was further partitioned with ethyl

acetate(12 litre)-water(4.35 litre) and n-butanol(10 litre)-water to give ethyl acetate, n-butanol and water fractions in 103.6, 388.7 and 401.8 gm, respectively.

The ethyl acetate fraction (100 gm) was chromatographed on silica gel (Merck no. 7734, 1.62 kg, the extract was premixed with 180 g of the silica gel), eluting with mixtures of hexane-ethyl acetate and ethyl acetate-methanol. Fractions (300 ml, each) were combined on the basis of TLC to give a total of 19 fractions (F₁ to F₁₉). Fractions F₇ (1.10 g) and F₈ (0.84 gm) eluting with 7-8 % ethyl acetate-hexane, were repeatedly chromatographed on silica gel employing hexane-ethyl acetate as eluting solvents. The fraction eluted with 30% ethyl acetate-hexane gave a light yellow solid which was further purified by radial chromatography (silica gel, 20% ethyl acetate-hexane) and recrystallization from methylene chloride-methanol to give VR-3016 (0.2373 gm). The mother liquor was purified by HPLC (methylene chloride), followed recrystallization in the same solvent to give additional 0.1103 g of VR-3016. The compound has been identified as a new modified xanthone derivative, which has been given the name sootepenseone 1, on the basis of spectral data and single crystal x-ray diffraction analysis.

Physico-chemical data of sootepenseone (1):

m.p. 192-193°C

$[\alpha]_D^{28}$ -8.00, c=0.075 in CHCl₃

Elemental analysis: Found: C, 72.32; H, 6.89.

C₂₈H₃₂O₆ requires: C, 72.39, H, 6.94.

IR, λ_{\max} CHCl₃ cm⁻¹: 3560, 3033, 3011, 2980, 2932, 1740, 1638, 1590, 1470, 1428, 1382.

UV, λ_{\max} EtOH nm (log e): 213(3.06), 263(2.18), 326(sh)(2.66), 355(2.74).

Mass Spectrum: m/z (70eV) 464(2%), 436(100), 421(45), 367(17), 339(60), 297(40), 281(8), 241(7), 215(28), 69(90).

NMR assignments: ¹H and ¹³C NMR (300 and 400 Mhz, CDCl₃):

see separate page

The described isolation procedure is summarized in scheme 1.

Characterization of the structure of sootepenseone (1)

The identity of sootepenseone was revealed by analysis of its spectral data i.e. the infrared spectrum, ultraviolet spectrum, mass spectrum and particularly the ^1H n.m.r and ^{13}C n.m.r spectra.

Table 1

^1H -n.m.r. data of *sootepenseone* (1) (δ Units, multiplicities)

Protons and assignments	Sootepenseone (1)	long range ^1H - ^{13}C -correlation (correlated C-atoms)
C2-H	6.05 (s)	C1, C4, C9a
C7-H	3.42 (dd)	C6, C8a, C5*, C2''
C8-H	7.50 (d)	C4b, C6, C7
C1'-H	1.40 (d)	C2', C3'
C2'-H	4.40 (q)	-
C4'-H	1.18 (s)	C2', C3', C4'
C5'-H	1.60 (s)	C2', C3', C4'
C1''-CH ₂	2.27 (dd)	-
C1'''-CH ₂	1.37 (dd)	-
C2''-H	2.45 (d)	C4b, C8a, C1'', C3''
C4''-H	1.28 (s)	C2'', C3''
C5''-H	1.73 (s)	C2'', C3''
C1'''-CH ₂	2.58 (bd)	C4b, C5, C3''', C4'''
C1''''-CH ₂	2.50 (dd)	C4b
C2''''-H	4.40 (m)	-
C4''''-H	1.37 (dd)	C2''', C3'''
C5''''-H	1.10 (dd)	C2''', C3'''
C1-OH	13.10 (s)	C1, C2, C9a

*: suggested correlation

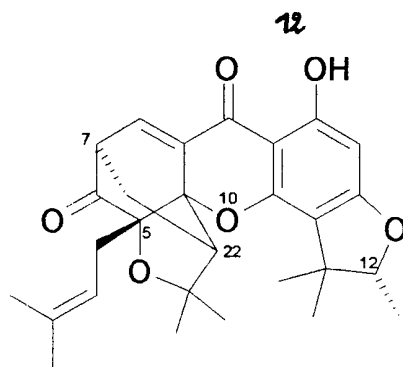
Table 2

¹³C-n.m.r. data of *sootepenseone* (1) (δ Units, multiplicities)

C-atoms and assignments	Sootepenseone (1)
C1	166.2 (s)
C2	92.6 (s)
C3	168.5 (s)
C4	113.6 (d)
C4a	156.0 (s)
C4b	90.9 (s)
C5	84.6 (s)
C6	203.6 (s)
C7	47.1 (d)
C8	134.1 (d)
C8a	133.7 (s)
C9	178.9 (s)
C9a	101.4 (s)
C1'	13.5 (q)
C2'	91.0 (d)
C3'	43.2 (s)
C4'	21.0 (q)
C5'	23.9 (q)
C1''	26.0 (t)
C2''	49.6 (d)
C3''	82.9 (s)
C4''	28.9 (q)
C5''	30.7 (q)
C1'''	29.0 (t)
C2'''	117.8 (d)
C3'''	135.3 (s)
C4'''	25.5 (q)
C5'''	16.9 (q)

Relative stereochemistry:

The relative stereochemistry of *sootepenseone* (1) has been confirmed by single crystal x-ray diffraction analysis. Hence the absolute configuration is either the stereochemistry as depicted below



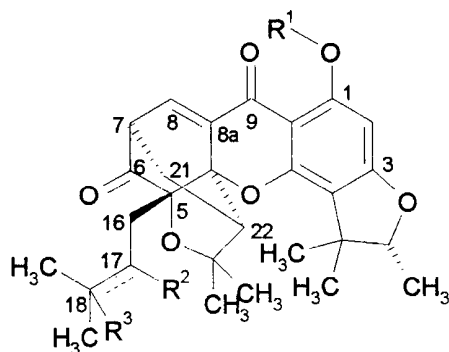
Sootepenseone

(1)

or the corresponding enantiomeric form thereof. The relative stereochemistry as depicted above for *sootepenseone* (1) is also valid for the sootepenseone derivatives according to formula (I), except for those derivatives where inversion or racemization occurred under the selected reaction conditions at one or more of the chiral centers at C-5, C-7, C-10a, C-12 and C-22.

Preparation of derivatives of sootepenseone

Table 3



(I)

Compound No.	R ¹	R ²	R ³
1 (Sootepenseone)	H	R ² and R ³ forming part of the C ¹⁷ -C ¹⁸ double bond	
6	acetyl	ditto	
7	a pharmaceutically acceptable counter ion*	ditto	
8	C ₂ -C ₂₀ -alkyl-carbonyl	ditto	
9	methyl	ditto	
10	C ₂ -C ₂₀ -alkyl	ditto	
11	benzoyl	ditto	
12	benzoyl substituted with -OH or -OCH ₃	ditto	
13	H	H	H

* suitable counter cations for the phenolat anion are, for example, Na⁺, K⁺, ½ Mg⁺⁺, ½ Ca⁺⁺, HN(C₁₋₆-alkyl)₃⁺

Preparation of the compounds 6 to 13 can be accomplished starting from sootepenseone (1) by organic chemistry standard derivatization procedures which are well-known to the person skilled in the art.

Example 2

Preparation of 1-O-Acetyl-sootepenseone (6)

A solution of 1 mg of sootepenseone (1) in 1ml anhydrous acetic acid anhydride was stirred at room temperature for 1 h. The solvent was evaporated in a water bath under reduced pressure. Yield: 1 mg of (6) as a crude residue.

For example, salts of sootepenseone (7) can be prepared according to methods and reagents as described in Houben-Weyl, *Methoden der Organischen Chemie (methods in organic chemistry)*, 4th edition 1963, volume 6/2, "Sauerstoffverbindungen I (oxygen compounds I), part 2", pages 35 ff.

So, for example, one equivalent of a suitable base can be reacted with one equivalent of sootepenseone in a suitable solvent and then evaporating off the solvent or filtrating off the precipitated salt (7). A suitable base, for example, can be selected from the group consisting of alkali or earth alkali hydroxides or an organic amine.

Methylation of the OH-group (compound no. 9) can be accomplished starting from sootepenseone (1) with diazomethan in analogy to the method as described by Mustafa; Hishmat; JOCEAH; J.Org.Chem.; 22; 1957; 1644, 1646.

Acylation of the OH-group (compounds no. 6, 8, 11 and 12) can be accomplished starting from sootepenseone (1) in analogy to the method and reagents as described in Houben-Weyl, *Methoden der Organischen Chemie (methods in organic chemistry)*, 4th edition 1985, volume E5, "Carbonsäuren und Carbonsäure-Derivate (carboxylic acids and their derivatives)", pages 691 ff.

Alkylation of the OH-group at C-1 (compound no. 10) can be accomplished starting from sootepenseone (1) in analogy to the standard procedures and by using standard reagents as described in Houben-Weyl, *Methoden der Organischen*

Chemie (methods in organic chemistry), 4th edition, volume VI/3, "Sauerstoffverbindungen (oxygen compounds), part 3", Georg Thieme Verlag Stuttgart, 1965, pages 49 ff.

Hydrogenation of the isoprenyl C¹⁷-C¹⁸ double bound to the C¹⁷-C¹⁷ single bound (wherein R² and R³ in the formula (I) are each a hydrogen atom; compound no. 13) can be performed by standard procedures as described for example in Houben-Weyl, *Methoden der Organischen Chemie (methods in organic chemistry)*, 4th edition, volume IV/1c, "Reduktion (reduction), part I", Georg Thieme Verlag Stuttgart, 1981, pages 15 ff.

Biological activity:

The compound according to the invention are less toxic than the standard compounds (see table 1). On the other hand, sootepenseone (1; D-25637) is more active in the hollow-fiber test as the standard compounds (see table 3).

Table 1: Toxicity of sootepenseone (D-25637)

Compound	LD50 (mg/kg i.p. mice)
Sootepenseone (1; D-25637)	> 100
Actinomycin D	ca. 1
Vinblastin	ca. 6
Adriamycin	ca. 40
Bleomycin	ca. 80

i.p. intraperitoneal

Discussion of the results:

Sootepenseone is at least 100 times less toxic than Actinomycin D, about 16 times less toxic than Vinblastin, and 60% and 20% less toxic than Adriamycin resp. Bleomycin.

2. *In vitro* antitumor activity (XTT proliferation/cytotoxicity test)

The XTT-assay was carried out as described by D.A. Scudiero et al., *Cancer Res.* 48 (september 1, 1988), pp. 4827-4833. The results of this procedure are expressed as that dose which inhibits growth by 50% as compared to control growth after 45 hours following application of the substance. The dose value as obtained is referred to as ED 50 and activity is indicated for ED 50 levels of $\leq 30 \mu\text{g/ml}$. The smaller the ED 50 level, the more active is the test material. The activities of sootepenseone (1) obtained in Example 1 are reported below in Table 2.

Table 2:

Compound	Cell line	ED 50 μ g/ml
Sootepenseone (1; D-25637)	KB	1.74
	L1210	1.74
	SK-OV-3	1.74
	LNCAP	1.74
Actinomycin D	KB	0.17
	L1210	0.17
	SK-OV-3	1.74
	LNCAP	0.17
Adriamycin	KB	0.17
	L1210	0.017
	SK-OV-3	0.17
	LNCAP	0.17
Bleomycin	KB	0.17
	L1210	0.017
	SK-OV-3	0.17
	LNCAP	0.17
Vinblastin	KB	0.17
	L1210	0.017
	SK-OV-3	0.17
	LNCAP	0.17

KB: epidermal carcinoma of the oral cavity

L1210: mice lymphatic leukemia

LNCaP: lymphoma metastasis of prostate carcinoma

SK-OV-3: human ovarian carcinoma

MCF-7: human breast cancer

Discussion of the results:

D-25637 has a significant anticancer activity against all tested tumor cell lines.

3. *In vivo* antitumor activity of sootepenseone (D-25637) (hollow fiber assay)

The hollow-fiber test was carried out as described by Melinda G. Hollingshead et al. in *Life Sciences*, Vol. 57, No. 2, pp. 131-141, 1995. The results are shown in table 3.

Table 3:

Compound	Dose (mg/kg)	Location	% Inhibition (cell line)	
			KB	MCF-7
Sootepenseone (1; D-25637)	4 x 10 i.p.	s.c.	49	41
Actinomycin D	4 x 0.1 i.p.	s.c.	40	-150
Adriamycin	4 x 4 i.p.	s.c.	52	41
Bleomycin	4 x 8 i.p.	s.c.	53	-67
Vinblastin	4 x 0.65 i.p.	s.c.	13	-165

KB: epidermal carcinoma of the oral cavity

MCF-7: human breast cancer

s.c. subcutaneous

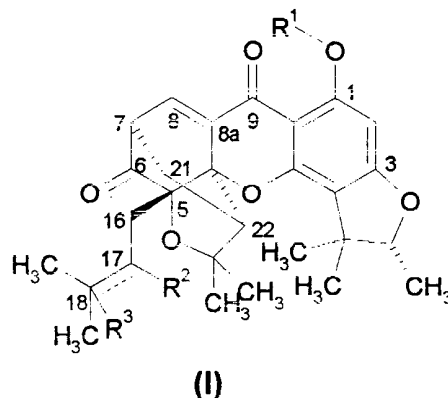
i.p. intraperitoneal

Discussion of the results:

D-25637 is more active (49% inhibition) against the KB tumor cell line than Vinblastin (13%) and Actinomycin D (40%), and nearly as active as Bleomycin (53%). Moreover, against the MCF-7 cell line D-25637 showed an as strong anticancer activity (41%) as adriamycin, while Actinomycin D, Bleomycin and Vinblastin enhanced tumor growth (negative inhibition values indicate increase in cell growth compared to untreated control group).

Claims:

1. A compound according to general formula I



wherein

R^1 is a hydrogen atom; a methyl group ($-\text{CH}_3$), a $\text{C}_2\text{-C}_6$ alkyl residue, a formyl group ($-\text{CHO}$); an acetyl residue ($-\text{COCH}_3$), $-\text{CO-C}_{2-6}$ -alkyl, CO-C_{3-8} -cycloalkyl, $-\text{CO-C}_{6-18}$ -aryl or $-\text{CO-C}_{7-24}$ -aralkyl residue each having optionally one or more substituents selected from the group consisting of $-\text{OH}$, $-\text{SH}$, $-\text{NH}_2$, $-\text{NHC}_{1-6}$ -alkyl, $-\text{N}(\text{C}_{1-6}\text{-alkyl})_2$, $-\text{NHC}_{6-14}$ -aryl, $-\text{N}(\text{C}_{6-14}\text{-aryl})_2$, $-\text{N}(\text{C}_{1-6}\text{-alkyl})(\text{C}_{6-14}\text{-aryl})$, $-\text{NHCOR}^2$, $-\text{NO}_2$, $-\text{CN}$, $-(\text{CO})\text{R}^3$, $-(\text{CS})\text{R}^4$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{O-C}_{1-6}$ -alkyl, $-\text{O-C}_{6-14}$ -aryl, $-\text{O}-(\text{CO})\text{R}^5$, $-\text{S-C}_{1-6}$ -alkyl, $-\text{S-C}_{6-14}$ -aryl, $-\text{SOR}^6$, and $-\text{SO}_2\text{R}^7$, wherein R^2 to R^7 stands independently of each other for a hydrogen atom, $-\text{C}_{1-6}$ -alkyl, $-\text{O-C}_{1-6}$ -alkyl, $-\text{O-C}_{6-14}$ -aryl, $-\text{NH}_2$, $-\text{NHC}_{1-6}$ -alkyl, $-\text{N}(\text{C}_{1-6}\text{-alkyl})_2$, $-\text{NHC}_{6-14}$ -aryl, $-\text{N}(\text{C}_{6-14}\text{-aryl})_2$, $-\text{N}(\text{C}_{1-6}\text{-alkyl})(\text{C}_{6-14}\text{-aryl})$, $-\text{S-C}_{1-6}$ -alkyl, $-\text{S-C}_{6-14}$ -aryl residue;

a $-\text{COO-C}_{1-6}$ -alkyl residue having optionally one or more substituents selected from the group consisting of $-\text{OH}$, $-\text{SH}$, $-\text{NH}_2$, $-\text{NHC}_{1-6}$ -alkyl, $-\text{N}(\text{C}_{1-6}\text{-alkyl})_2$, $-\text{NHC}_{6-14}$ -aryl, $-\text{N}(\text{C}_{6-14}\text{-aryl})_2$, $-\text{N}(\text{C}_{1-6}\text{-alkyl})(\text{C}_{6-14}\text{-aryl})$, $-\text{NHCOR}^8$, $-\text{NO}_2$, $-\text{CN}$, $-(\text{CO})\text{R}^9$, $-(\text{CS})\text{R}^{10}$, $-\text{F}$, $-\text{Cl}$, $-\text{Br}$, $-\text{I}$, $-\text{O-C}_{1-6}$ -alkyl, $-\text{O-C}_{6-14}$ -aryl, $-\text{O}-(\text{CO})\text{R}^{11}$, $-\text{S-C}_{1-6}$ -alkyl, $-\text{S-C}_{6-14}$ -aryl, $-\text{SOR}^{12}$, and $-\text{SO}_2\text{R}^{13}$, wherein R^8 to R^{13} stands independently of each other for a hydrogen atom, $-\text{C}_{1-6}$ -alkyl, $-\text{O-C}_{1-6}$ -alkyl, $-\text{O-C}_{6-14}$ -aryl, $-\text{NH}_2$, $-\text{NHC}_{1-6}$ -alkyl, $-\text{N}(\text{C}_{1-6}\text{-alkyl})_2$, $-\text{NHC}_{6-14}$ -aryl, $-\text{N}(\text{C}_{6-14}\text{-aryl})_2$, $-\text{N}(\text{C}_{1-6}\text{-alkyl})(\text{C}_{6-14}\text{-aryl})$, $-\text{S-C}_{1-6}$ -alkyl, $-\text{S-C}_{6-14}$ -aryl residue;

a $-\text{CONR}^{14}\text{R}^{15}$ residue wherein R^{14} and R^{15} stands independently of each other for a hydrogen atom, $-\text{C}_{1-6}$ -alkyl, $-\text{O-C}_{1-6}$ -alkyl, $-\text{O-C}_{6-14}$ -aryl, $-\text{NH}_2$, $-\text{NHC}_{1-6}$ -alkyl, $-\text{N}(\text{C}_{1-6}$ -

alkyl)₂, -NHC₆₋₁₄-aryl, -N(C₆₋₁₄-aryl)₂, -N(C₁₋₆-alkyl)(C₆₋₁₄-aryl), -S-C₁₋₆-alkyl, -S-C₆₋₁₄-aryl residue;

or a counter cation selected from the group consisting of an alkali or earth alkali metal ion such as Li⁺, Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺, NR¹⁶R¹⁷R¹⁸R¹⁹(⁺) wherein R¹⁶ to R¹⁹ stands independently of each other for a hydrogen atom or a C₁-C₆-alkyl residue;

R² and R³ either form part of the C¹⁷=C¹⁸-double bond or are each hydrogen,

or a tautomer, an enantiomer or a stereoisomer thereof or a physiologically acceptable salt or solvate thereof or mixtures thereof.

2. Compound according to formula I of claim 1, wherein R¹ is a hydrogen atom and R² and R³ form part of the C¹⁷=C¹⁸-double bond (Sootepenseone).

3. A process for the manufacture of a compound according to formula I of claim 1 or claim 2, wherein R¹ is a hydrogen atom and R² and R³ form part of the C¹⁷=C¹⁸ double bond (Sootepenseone), comprising the steps of

- (a) extracting leaves of *Dasymaschalon sootepenseone* Graib, Annonaceae successively with organic solvents in an order of increasing polarity,
- (b) partitioning the crude extract of the highest polarity with a mixture of water and slightly miscible polar organic solvent,
- (c) purification of the organic extract by chromatography with suitable solvents,
- (d) optionally repeating step c) with fractions containing sootepenseone, and
- (e) optionally purification by recrystallization.

4. A process for the manufacture of a compound according to formula I of claim 1, wherein R¹ is a counter cation selected from the group consisting of an alkali or earth alkali metal ion such as Li⁺, Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺, NR¹⁶R¹⁷R¹⁸R¹⁹(⁺), wherein R¹⁶ to R¹⁹ stands independently of each other for a hydrogen atom or a C₁-C₆-alkyl residue a hydrogen atom, and R² and R³ form part of the C¹⁷=C¹⁸ double bond, comprising reacting a compound according to formula 1 of claim 1, wherein R¹ is a hydrogen anion, with at least one equivalent of a suitable inorganic or organic base by methods known *per se* to obtain the corresponding phenolate salt.

5. A process for the manufacture of a compound according to formula I of claim 1, wherein R^1 has the meaning as defined in claim 1 and wherein R^2 and R^3 are each a hydrogen atom, comprising the step of reacting a compound according to claim 1, wherein R^1 has the meaning as defined in claim 1 and wherein R^2 and R^3 form part of the $C^{17}=C^{18}$ double bond, with hydrogen in the presence or absence of a suitable catalyst by a method known *per se*.

6. A process for the manufacture of a compound according to formula I of claim 1, wherein R^1 is a methyl group ($-CH_3$), a C_2 - C_6 alkyl residue, a formyl group ($-CHO$); an acetyl residue ($-COCH_3$), $-CO-C_{2-6}$ -alkyl, $CO-C_{3-8}$ -cycloalkyl, $-CO-C_{6-18}$ -aryl or $-CO-C_{7-24}$ -aralkyl residue each having optionally one or more substituents selected from the group consisting of $-OH$, $-SH$, $-NH_2$, $-NHC_{1-6}$ -alkyl, $-N(C_{1-6}$ -alkyl) $_2$, $-NHC_{6-14}$ -aryl, $-N(C_{6-14}$ -aryl) $_2$, $-N(C_{1-6}$ -alkyl)(C_{6-14} -aryl), $-NHCOR^2$, $-NO_2$, $-CN$, $-(CO)R^3$, $-(CS)R^4$, $-F$, $-Cl$, $-Br$, $-I$, $-O-C_{1-6}$ -alkyl, $-O-C_{6-14}$ -aryl, $-O-(CO)R^5$, $-S-C_{1-6}$ -alkyl, $-S-C_{6-14}$ -aryl, $-SOR^6$, and $-SO_2R^7$, wherein R^2 to R^7 stands independently of each other for a hydrogen atom, $-C_{1-6}$ -alkyl, $-O-C_{1-6}$ -alkyl, $-O-C_{6-14}$ -aryl, $-NH_2$, $-NHC_{1-6}$ -alkyl, $-N(C_{1-6}$ -alkyl) $_2$, $-NHC_{6-14}$ -aryl, $-N(C_{6-14}$ -aryl) $_2$, $-N(C_{1-6}$ -alkyl)(C_{6-14} -aryl), $-S-C_{1-6}$ -alkyl, $-S-C_{6-14}$ -aryl residue;

a $-COO-C_{1-6}$ -alkyl residue having optionally one or more substituents selected from the group consisting of $-OH$, $-SH$, $-NH_2$, $-NHC_{1-6}$ -alkyl, $-N(C_{1-6}$ -alkyl) $_2$, $-NHC_{6-14}$ -aryl, $-N(C_{6-14}$ -aryl) $_2$, $-N(C_{1-6}$ -alkyl)(C_{6-14} -aryl), $-NHCOR^8$, $-NO_2$, $-CN$, $-(CO)R^9$, $-(CS)R^{10}$, $-F$, $-Cl$, $-Br$, $-I$, $-O-C_{1-6}$ -alkyl, $-O-C_{6-14}$ -aryl, $-O-(CO)R^{11}$, $-S-C_{1-6}$ -alkyl, $-S-C_{6-14}$ -aryl, $-SOR^{12}$, and $-SO_2R^{13}$, wherein R^8 to R^{13} stands independently of each other for a hydrogen atom, $-C_{1-6}$ -alkyl, $-O-C_{1-6}$ -alkyl, $-O-C_{6-14}$ -aryl, $-NH_2$, $-NHC_{1-6}$ -alkyl, $-N(C_{1-6}$ -alkyl) $_2$, $-NHC_{6-14}$ -aryl, $-N(C_{6-14}$ -aryl) $_2$, $-N(C_{1-6}$ -alkyl)(C_{6-14} -aryl), $-S-C_{1-6}$ -alkyl, $-S-C_{6-14}$ -aryl residue;

a $-CONR^{14}R^{15}$ residue wherein R^{14} and R^{15} stands independently of each other for a hydrogen atom, $-C_{1-6}$ -alkyl, $-O-C_{1-6}$ -alkyl, $-O-C_{6-14}$ -aryl, $-NH_2$, $-NHC_{1-6}$ -alkyl, $-N(C_{1-6}$ -alkyl) $_2$, $-NHC_{6-14}$ -aryl, $-N(C_{6-14}$ -aryl) $_2$, $-N(C_{1-6}$ -alkyl)(C_{6-14} -aryl), $-S-C_{1-6}$ -alkyl, $-S-C_{6-14}$ -aryl residue and wherein R^2 and R^3 either form part of the $C^{17}=C^{18}$ -double bond or are each a hydrogen atom, comprising the step of reacting a compound according to claim 1, wherein R^1 is a hydrogen atom or a counter cation selected from the group

consisting of an alkali or earth alkali metal ion such as Li⁺, Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺, NR¹⁶R¹⁷R¹⁸R¹⁹(+) wherein R¹⁶ to R¹⁹ stand independently of each other for a hydrogen atom or a C₁-C₆-alkyl residue, with a suitable alkylating or acylating reagent in the presence or absence of a base by a method known *per se*.

7. A compound according to formula I of claim 1 or 2 for use as a medicament.

8. A compound according to formula I of claim 1 or claim 2 for use as an anticancer agent.

9. A medicament for use in the treatment of a human or animal disease, containing at least one compound according to formula I of claim 1 or claim 2, optionally together with commonly used physiologically acceptable carriers, diluents and/or excipients.

10. A medicament for use in the treatment of a human or animal cancer disease, containing at least one compound according to formula I of claim 1 or claim 2, optionally together with commonly used physiologically acceptable carriers, diluents and/or excipients.

11. Use of a compound according to formula I of claim 1 or claim 2 for the manufacture of a medicament in the treatment of a human or animal disease.

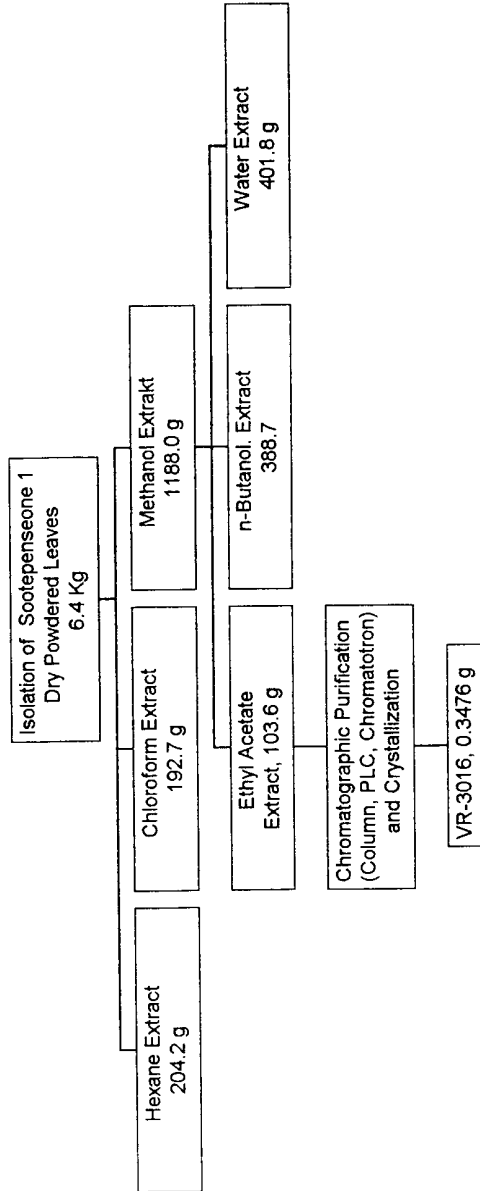
12. Use of a compound according to formula I of claim 1 or claim 2 for the manufacture of a medicament for the treatment of cancer.

13. A process for the manufacture of a medicament according to claim 9 or 10, characterized in that one or more compounds according to formula I of claim 1 or claim 2 are processed, optionally together with commonly used physiologically acceptable carriers, diluents and/or excipients, into an appropriate pharmaceutical form.

14. A method of treatment a tumor by administering an effective dose of at least one compound according to formula I of claim 1 or claim 2 to a human or animal patient in need thereof.

15. A compound according to formula I of claim 1 obtainable by the process according to anyone claim 3 to 6.

Scheme 1



INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/05973

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07D493/22 A61K31/35 //(C07D493/22, 313:00, 311:00, 307:00, 307:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, BEILSTEIN Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CAO S -G ET AL: "Novel Cytotoxic Polyprenylated Xanthonoids from <i>Garcinia gaudichaudii</i> (Guttiferae)" TETRAHEDRON, vol. 54, no. 36, 3 September 1998 (1998-09-03), page 10915-10924 XP004130029 ISSN: 0040-4020 page 10916	1-15
A	ASANO ET AL.: "Cytotoxic Xanthones from <i>Garcinia hanburyi</i> " PHYTOCHEMISTRY, vol. 41, no. 3, 1996, pages 815-820, XP002116431 cited in the application page 816	1-15

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

13 November 2000

Date of mailing of the international search report

21/11/2000

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/05973

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 119, no. 12, 20 September 1993 (1993-09-20) Columbus, Ohio, US; abstract no. 124959, LIN ET AL.: "Isogambonic acid and isomorellinol from Garcinia hanburyi" XP002116433 & Magn. Reson. Chem. (1993), 31(4), 340-347 abstract ---	1-15
A	SULTANBAWA: "Xanthonoids of tropical plants" TETRAHEDRON, vol. 36, 1980, pages 1465-1506, XP002116432 cited in the application the whole document ---	1-15
A	CHEMICAL ABSTRACTS, vol. 129, no. 26, 28 December 1998 (1998-12-28) Columbus, Ohio, US; abstract no. 341671, SINZ ET AL.: "Novel acetogenins from the leaves of Dasymaschalon sootepense" XP002116434 & Helv. Chim. Acta (1998), 81(9), 1608-1615 abstract -----	1-15