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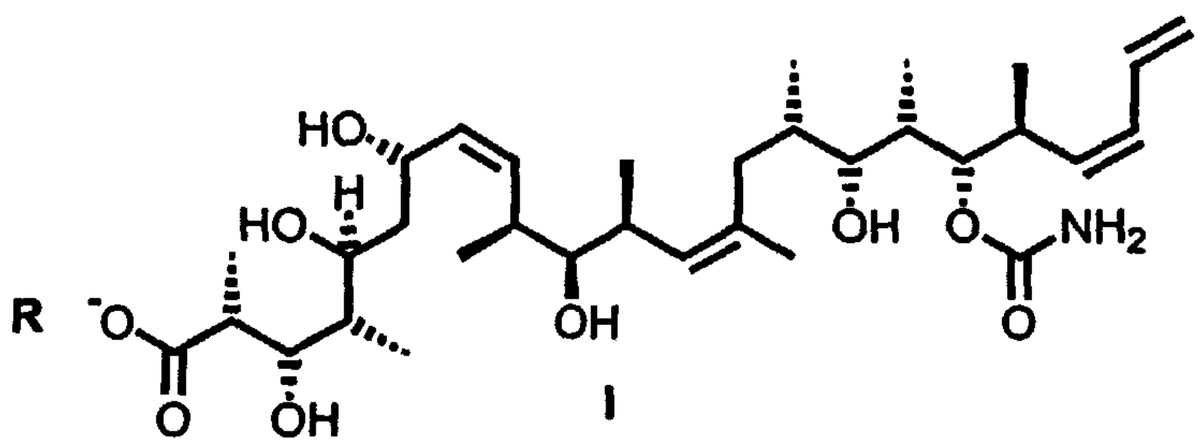
(54) Titre : CERTAINS SELS DE L'ACIDE DISCODERMOLIDE, COMPOSITIONS PHARMACEUTIQUES LES CONTENANT ET UTILISATION DE CES DERNIERS DANS LE TRAITEMENT DES TUMEURS

(54) Title: CERTAIN SALTS OF DISCODERMOLIDE ACID, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN TREATING TUMORS



I (I)

(57) Abrégé/Abstract:
The present invention relates to discodermolidate acid salts of formula (I) where R is as defined herein, pharmaceutical compositions containing said salts, the use of said salts alone or in combination with other therapeutic agents in treating tumors and to a process for making said salts.

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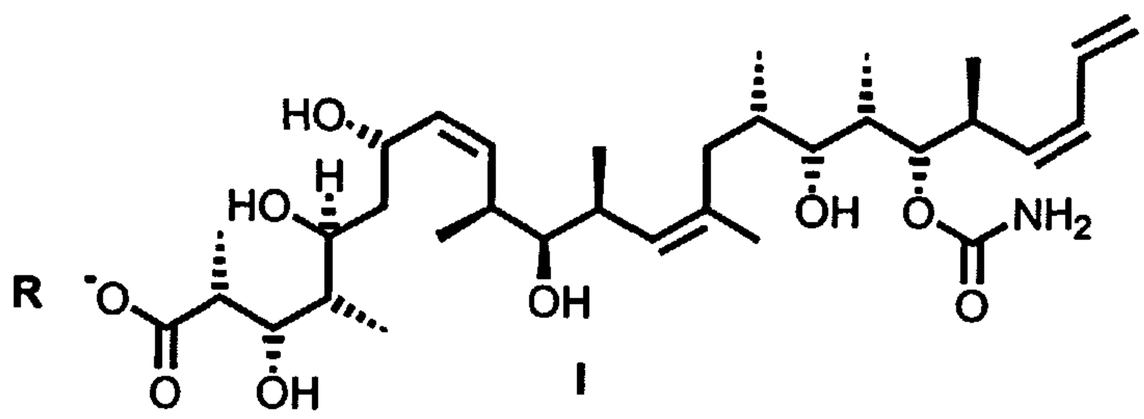
(54) Title: CERTAIN SALTS OF DISCODERMOLIDE ACID, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN TREATING TUMORS



(I)

(57) Abstract: The present invention relates to discodermolidate acid salts of formula (I) where R is as defined herein, pharmaceutical compositions containing said salts, the use of said salts alone or in combination with other therapeutic agents in treating tumors and to a process for making said salts.

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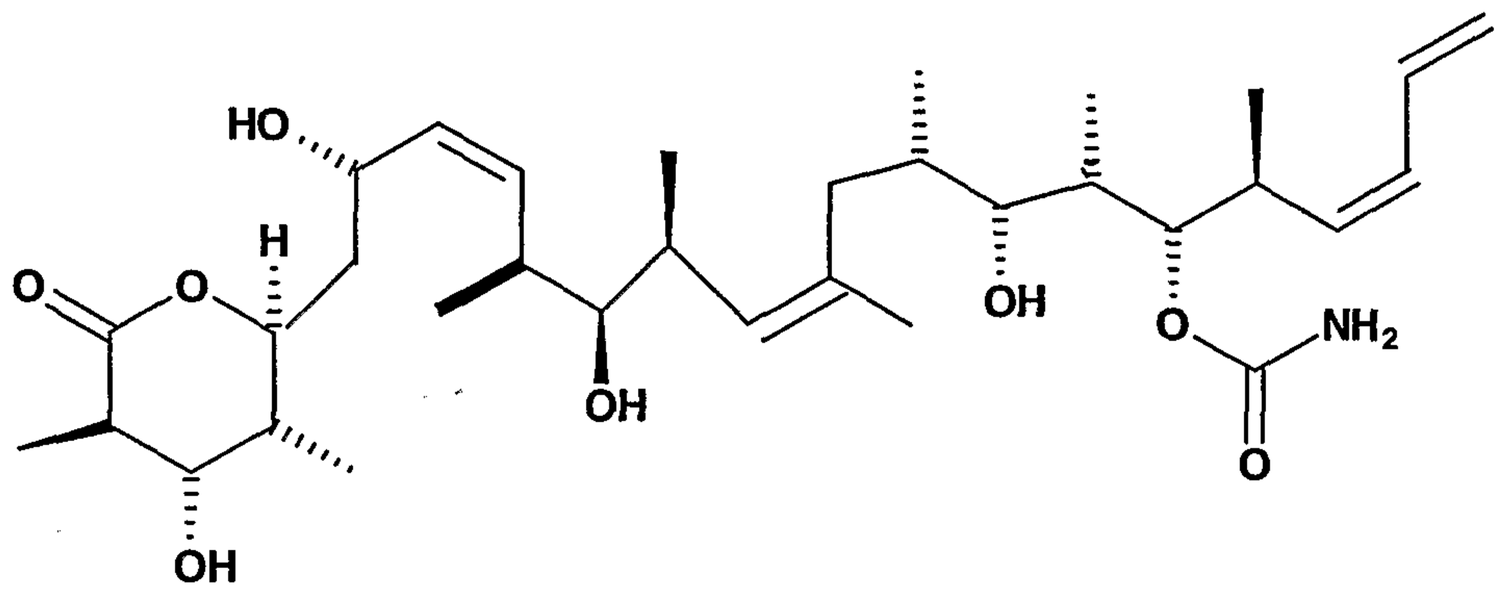
CERTAIN SALTS OF DISCODERMOLIDE ACID, PHARMACEUTICAL COMPOSITIONS CONTAINING THEM AND THEIR USE IN TREATING TUMORS

The present invention relates to the area of chemotherapeutic agents and, more particularly, relates to certain salts of discodermolide acid, and the use of said discodermolide acid salts in treating tumors.

BACKGROUND OF THE INVENTION



1

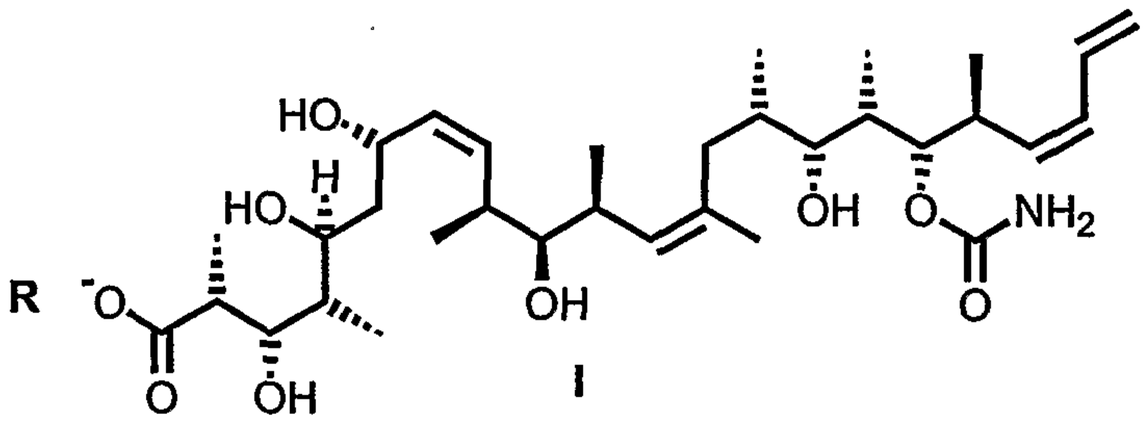
Discodermolide (**1**) is a novel polyketide natural product that was isolated from extracts of the marine sponge *Discodermia dissoluta* by researchers at the Harbor Branch Oceanographic Institution (HBOI) (Gunasekera SP, Gunasekera M, Longley RE, Schulte GK. Discodermolide: a new bioactive polyhydroxylated lactone from the marine sponge *Discodermia dissoluta*. [published erratum appears in J. Org. Chem. 1991;56:1346]. J. Org. Chem. 1990;55:4912-15.). Discodermolide lacks obvious structural resemblance to palitaxel, yet it shares with paclitaxel (the active substance in the drug Taxol) the ability to stabilize microtubules. In mechanism-based assays, discodermolide is more effective than paclitaxel. Discodermolide binds to tubulin competitively with paclitaxel. Since paclitaxel has proven to be useful in treating some cancers, other compounds of the same mechanistic class may have utility against hyperproliferative disorders.

SUMMARY OF THE INVENTION

The present invention provides new anti-tumor agents which are effective against a variety of cancer cells. More particularly, the present invention relates to certain discodermolide acid salts which exhibit a higher degree of selectivity in killing cancer cells. In addition, the present invention provides pharmaceutical compositions useful in treating tumors comprising a therapeutically effective amount of certain discodermolide acid salts. Moreover, the present invention provides a method of treating tumors comprising administering to a mammal afflicted therewith a therapeutically effective amount of certain discodermolide acid salts. Furthermore, the present invention relates to a process for preparing certain discodermolide acid salts.

DETAILED DESCRIPTION OF THE INVENTION

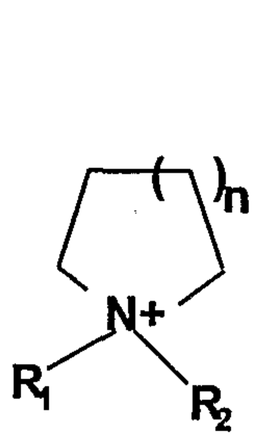
The essence of the instant invention is the discovery that certain discodermolide acid salts are useful in treating tumors. In one embodiment, the instant invention provides new anti-tumor agents of formula I



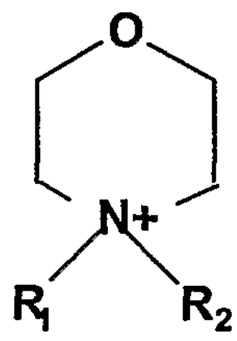
I

where R is Li^+, Na^+, K^+, $1/2Ca^{++}$, $1/2Mg^{++}$, $1/2Mn^{++}$, $R_1R_2R_3R_4N^+$,

or a nitrogen containing ring selected from

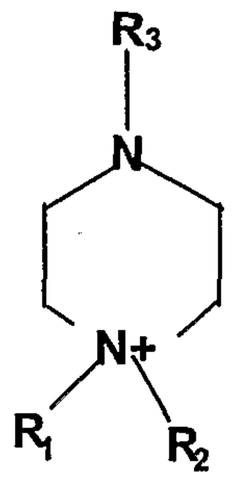


II



III

and



IV

where each of R_1, R_2, R_3 and R_4, independently, is hydrogen, (C_{1-12})alkyl, hydroxy(C_{2-8})alkyl or benzyl; and n is 1-5.

Preferred compounds are those of formula I where

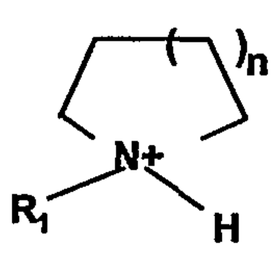
R is Li^+, Na^+, K^+, $1/2Ca^{++}$, $R_1R_2R_3R_4N^+$, or a nitrogen containing ring selected from II, III and IV
where each of R_1, R_2, R_3 and R_4, independently, is hydrogen, (C_{1-8})alkyl, hydroxy(C_{2-6})alkyl or benzyl; and n is 1-3.

More preferred compounds are those of formula I where

R is Na^+, K^+, $1/2Ca^{++}$, $R_1R_2R_3R_4N^+$, or a nitrogen containing ring selected from II and III;
where each of R_1, R_2, R_3 and R_4, independently, is hydrogen, (C_{1-6})alkyl, hydroxy(C_{2-4})alkyl or benzyl; and n is 1 or 2.

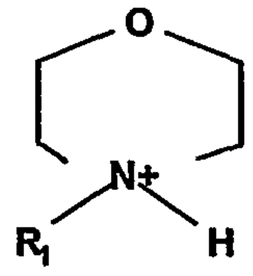
Even more preferred compounds are those of formula I where

R is Na^+, $1/2Ca^{++}$, $R_1R_2R_3HN^+$, or a nitrogen containing ring selected from



IIa

and



IIIa

where each of R_1, R_2 and R_3, independently, is hydrogen, (C_{1-6})alkyl, hydroxyethyl or benzyl; and n is 1 or 2.

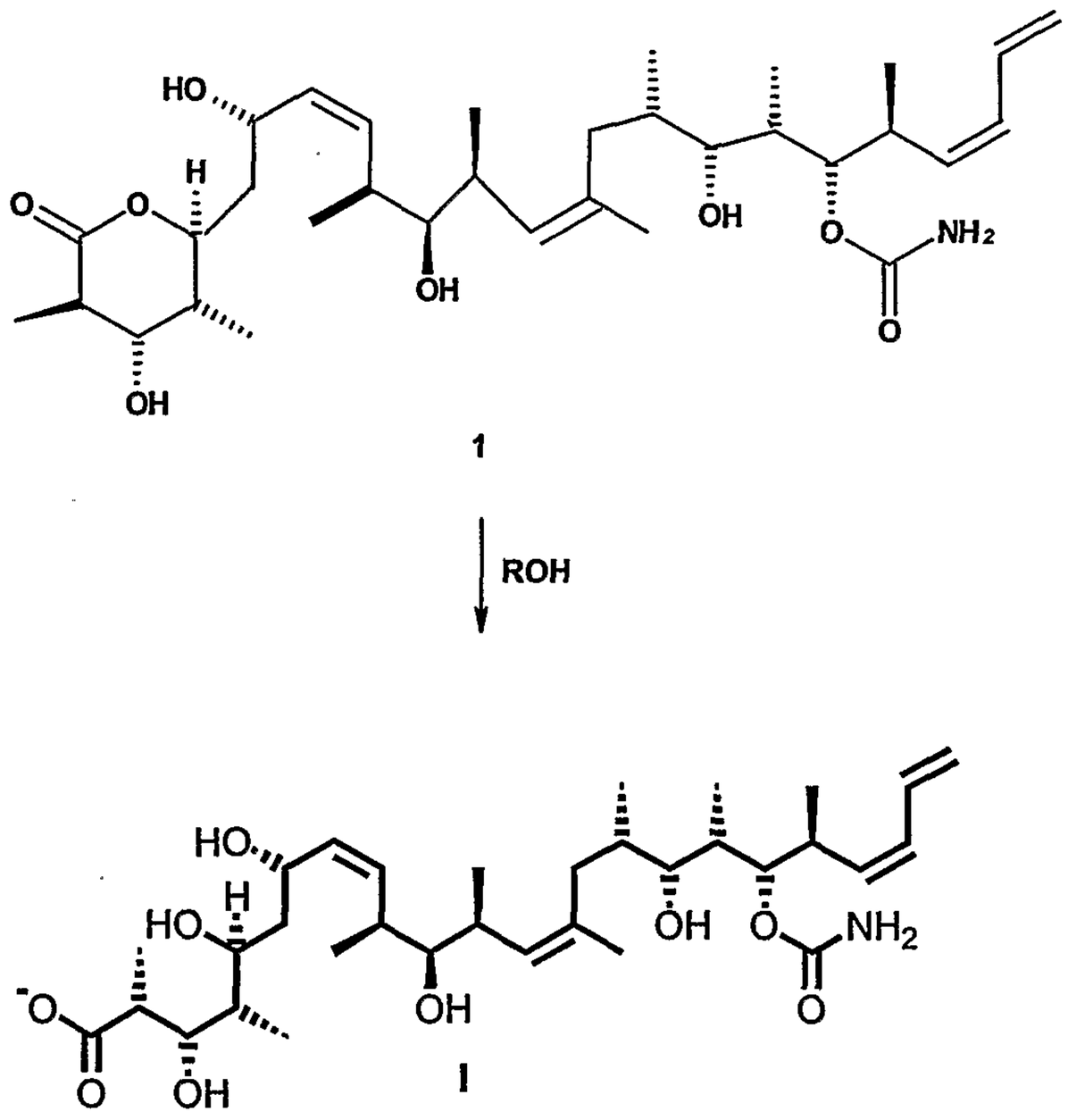
In another embodiment, the instant invention provides pharmaceutical compositions useful in treating tumors comprising a pharmaceutically acceptable carrier or diluent and a therapeutically effective amount of a compound of formula I above.

In still another embodiment, the instant invention provides a method for treating tumors comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound of formula I above.

Furthermore, the present invention relates to the use of a compound of formula I for the therapeutic treatment of the human or animal body and, in particular, in the preparation of a pharmaceutical composition for use in the chemotherapy of tumors.

In the above definitions: the term "(C_{1-12})alkyl" as used herein refers to a straight, branched, or cycloalkyl group consisting solely of carbon and hydrogen and having from 1 to 12 carbon atoms. Examples of "alkyl" groups include methyl, ethyl, propyl, butyl, pentyl, 3-methypentyl, cyclohexyl, cyclopentylbutyl, etc. The "(C_{1-8})alkyl" portion of hydroxy(C_{1-8})alkyl as used herein refers to a straight or branched group consisting solely of carbon and hydrogen and having from 1 to 8 carbon atoms.

Discodermolide acid salts may be prepared from discodermolide of formula 1 as depicted below:



where each R is as defined above.

The preparation of the discodermolide acid salt involves the hydrolysis of 1. The hydrolysis requires between 1 and 100 equivalents of 1 relative to ROH, preferably between 1 and 5 equivalents of I relative to ROH. The hydrolysis is conducted in the presence of a polar organic solvent, preferably an ether, more preferably tetrahydrofuran, at a temperature of between 0°C and 20°C, preferably between 0°C and 10°C, for a period of between 5 minutes and 2 hours, preferably for a period between 15 and 30 minutes.

The total synthesis of discodermolide (1) is described in several publications, e.g., by A.B. Smith et al, J. Am. Chem. Soc. (1995), 117(48), 12011-12; J. Golec et al in the patent application GB 94-15399; or I. Paterson et al, Angew. Chem., Int. Ed. (2000), 39(2), 377-380.

All of the compounds ROH are either known and disclosed in the literature or may be prepared analogous to procedures disclosed in the literature.

If desired, the discodermolide acid salt obtained may be purified by conventional techniques such as chromatography or, in particular, recrystallization (if a solid).

As is evident to those skilled in the art, compounds of formula I contain asymmetric carbon atoms. It should be understood, therefore, that the individual stereoisomers are contemplated as being included within the scope of this invention.

As indicated above, all of the compounds of formula I are anti-tumor agents and are, therefore, useful in inhibiting the growth of various lymphomas, sarcomas, carcinomas, myelomas, and leukemia cell lines. The anti-tumor activity of the compounds of formula I may be demonstrated employing the Anchorage Dependent Growth Monolayer Assay (ADGMA) which measures the growth inhibitory effects of test compounds on proliferation of adherent cell monolayers. This assay was adapted from the 60 cell line assay used by the National Cancer Institute (NCI) with the following modifications:
1) four cell lines representative for the important tumor types, *viz.*, MIP 101 colon carcinoma, HCT 116 colon carcinoma, 1A9 ovarian carcinoma and 1A9PTX22 ovarian carcinoma were utilized; and 2) a tetrazolium derivative, *viz.*, MTT, was utilized to determine cell density.

The ADGMA compares the number of viable cells following a 3-day exposure to a test compound relative to a number of cells present at the time the test compound was added. Cell viability is measured using a tetrazolium derivative, *viz.*, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) that is metabolically reduced in the presence of an electron coupling agent (PMS; phenazine methosulfate) by viable cells to a water-soluble formazan derivative. The absorbence at 540 nm (A540) of the formazan derivative is proportional to the number of viable cells. The IC_{50} for a test compound is the concentration of compound required to reduce the final cell number to 50% of the final control cell number. If cell proliferation is inhibited, the assay further defines compounds as cytostatic (cell number after 3-day compound incubation >cell number at time of compound addition) or cytotoxic (cell number after 3-day compound incubation <cell number at time of compound addition).

The HCT 116 colon carcinoma cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD). The MIP 101 colon carcinoma was obtained from Dr. Robert Kramer (Bristol Meyers Squibb) and was previously described (Niles RM, Wilhelm

SA, Steele GD JR, Burke B, Christensen T, Dexter D, O'Brien MJ, Thomas P, Zamcheck N. Isolation and characterization of an undifferentiated human colon carcinoma cell line (MIP-101). Cancer Invest. 1987;5(6):545-52.). The 1A9 and the 1A9PTX22 ovarian tumor cell lines were obtained from Dr. Tito Fojo, Medicine Branch, Division of Clinical Sciences, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892. The 1A9 is a clone of the ovarian carcinoma cell line, A2780 (Giannakakou P, Sackett, DL, .Kang Y-K, Zhan Z, ButersJTM, Fojo T, Poruchynsky MS. Paclitaxel-resistant human ovarian cancer cells have mutant ß-tubulins that impaired paclitaxel-driven polymerization. J. Biol. Chem. 1997, 272(4):17118-17125). The 1A9PTX22 subline is isolated as an individual clone from the 1A9 cell line in a single step selection by exposure to 5 ng/mL paclitaxel in the presence of 5 µg/mL of verapamil. All cell lines are used between passages 4-20 following thawing. MIP 101 colon carcinoma, HCT 116 colon carcinoma, 1A9 ovarian carcinoma and 1A9PTX22 ovarian carcinoma cell lines are maintained and plated in RPMI 1640 medium containing 10% fetal bovine serum..

Cells are trypsinized and counted using hemacytometer to determine cell concentrations. Cells are then plated in their respective maintenance media (200 µL/well) in 96-well plates at the following concentrations: MIP 101, 2000 cells/well, HCT 116, 2000 cells/well, 1A9, 10000 cells/well, and 1A9PTX22, 10000 cells/well. The number of cells/well is determined in preliminary experiments, and resulted in 75-90% of confluency by day 4 after plating. Initial cell densities, assayed one day after plating, are roughly 0.10-0.20 A540 absorbence units greater than the media blank. 96 well plates are seeded on day 0 and the test compounds are added on day 1. A "time 0" plate is created that received media only in row A and one cell line/row in rows B-E. The "time 0" plate is processed 24 hours after plating (at the time when drugs are added to experimental plates), as follows: To each well 5 µL of the MTT stock solution (0.5 mg/ml in PBS) is added to each well and then incubated for three hours at 37 ° C, 5% CO2, in a humidified environment. Media is then carefully and completely removed. Plates are allowed to dry in the dark. DMSO (dimethylsulfoxide) is added to each well (100 µl/well) and plates are placed on an orbital shaker for 2 hours. Plates are read in the 96-well plate reader at 540 nm in a Molecular Devices plate reader utilizing Softmax Version 2.35 in absorbence mode-endpoint L-1, using DMSO as a blank. One day following plating, test compounds are added (in a final 1:10 dilution) to the test plates and subsequently serial diluted 10 times. The control plate receives 1:10 dilution of the solvent (10% DMSO/90% RPMI 1640) only. Three days after addition of test compounds all the

experimental plates and the control plate are processed as described above for the "time 0" plate. IC_{50} values are determined from graphs of percent net growth as a function of compound concentration. Percent net growth is calculated as (Cell + Drug A_{540} – Initial 540/Cell + Drug Vehicle 540 – Initial 540).

The following IC_{50} values (average ± S.E.M.) in µM can be obtained:

Compound	MIP101	HCT116	1A9	1A9PTX22
Ex. 1	0.3 ± 0.01	0.05 ± 0.02	0.1 ± 0.04	0.2 ± 0.09
paclitaxel	0.2 ± 0.06	0.0003 ± 0.0002	0.047 ± 0.007	0.001 ± 0.001

The anti-tumor activity of the compounds of formula I may further be demonstrated employing the hollow fiber *in vivo* tumor cell cultivation model in athymic (T cell deficient) nude mice. Utilizing this model, one can measure the ability of test compounds to inhibit the growth of human tumor cells in hollow fibers growing subcutaneously (s.c.) in athymic nude mice. The histologic tumor types employed are MIP 101 colon carcinoma, HCT-116 colon carcinoma, 1A9PTX22 ovarian carcinoma, and 1A9 ovarian carcinoma.

Compound	Cell Line	Experiment # 1 % T/C ± SEM	Experiment # 2 % T/C ± SEM	Average % T/C	Δ Mean % Body Weight
Paclitaxel	HCT 116	2±1**	1±2**	1	Exp. 1:None Exp. 2:None
Paclitaxel	MIP 101	113±10	112±11	113	
Paclitaxel	1A9	1±3**	53% Reg. **	26% Reg.	
Paclitaxel	PTX22	109±16	84±8	97	
Ex. 1	HCT 116	15±4**	10±3**	13	Exp. 1:None Exp. 2:None
Ex. 1	MIP 101	50±9**	90±9	70	

Ex. 1	1A922	14±4**	23±8**	19
Ex. 1	1A9PTX22	6±2**	16±7**	11

** P = < 0.01

Paclitaxel dosed daily for 5 days at 15 mg/kg, i.v.
Ex. 1 dosed once at 45 mg/kg, i.v.
Reg. = regression

The cell lines are obtained as described above. As mentioned above, the 1A9PTX22 subline is isolated as an individual clone from the 1A9 cell line in a single step selection by exposure to 5 ng/mL paclitaxel in the presence of 5 µg/mL of verapamil. The 1A9PTX22 cell line is found to be 24-fold more resistant to paclitaxel than the parental 1A9. Resistance to paclitaxel is maintained after two years of culturing in drug-free media, and is attributed to the $Ala^{364} \rightarrow$ Thr mutation in β-tubulin that is found in the 1A9PTX22 cell line.

All cell lines are propagated and expanded in RPMI 1640 medium containing 10% heat-inactivated FBS (Life Technologies, Grand Island, NY) in a tissue culture incubator (37 °C, controlled, humidified atmosphere containing 5% CO_2). Cell expansions are performed in T75 tissue culture flasks (Costar®, Corning, NY). For hollow fiber preparations, cells are harvested at 70-90% confluency using 0.25% Trypsin-EDTA (Life Technologies, Grand Island, NY).

PVDF hollow fibers (Spectrum, Gardena, CA) are soaked in 70% EtOH for 72 hours before use. Individual fibers are flushed with 3 mL of ice-cold tissue culture media using a syringe. Next, each fiber is filled with an appropriate cell suspension ($1x10^6$ cells/mL for the 1A9 and 1A9PTX22 cells, and $0.3x10^6$ cells/mL for the HCT 116 and MIP 101 cells), and both ends of the fiber are sealed. The entire length of the fiber is then sealed into 1.5 cm microcapsules (further called "hollow fibers"), each containing approximately 15 µL of the appropriate cell suspension. After separation, individual hollow fibers are placed in well plates incubated overnight at 37 °C.

Outbred athymic (*nu/nu*) female mice ("Chrls:Athymic Nude-nu", Charles River Laboratories, Wilmington, MA) are anesthetized. For the subcutaneous implantation an 11-gauge trocar

containing one or two hollow fibers is inserted through an incision made with scissors at the nape of the neck of an animal.

One day after the implantation (4 hollow fibers/animal, each hollow fiber containing one cell line: HCT 116, MIP 101, 1A9, and 1A9PTX22) animals are randomly sorted into five groups of six mice/group. The first group is sacrificed, hollow fibers are retrieved, and processed according to a published procedure, to determine the number of viable cells in each fiber (T_0.)

The remaining groups are treated as follows:

Group 1: Example 1, 45 mg/kg, iv, once.

Group 2: Vehicle for Example 1 (16.7% Cremophor EL, 8.3% ethanol, 75% D5W), iv, once.

Group 3: Paclitaxel, 15 mg/kg, iv, daily for 5 days.

Group 4: Vehicle for paclitaxel (12.5% Cremophor EL, 12.5% ethanol, 75% D5W) iv, daily for 5 days.

On day 7 all animals are sacrificed, and hollow fibers are retrieved and processed to determine the number of viable cells in each fiber (T- for fibers from animals treated with experimental compounds, C - for fibers from animals treated with corresponding vehicles). Antitumor activity is expressed as % Mean ΔT / Mean ΔC [comparing cell growth for treatment group to vehicle control group, where % Mean ΔT / Mean ΔC = (Mean T - Mean T_0 / Mean C – Mean T_0) x 100%]. Regressions are calculated using the formula: (1-Mean T / Mean T_0) x 100%. Statistical significance of the results is uniformly evaluated using a two-tailed Student's t-test.

The compounds of formula I can be used alone or in combination with other therapeutic agents. Therapeutic agents for possible combination are especially one or more cytostatic or cytotoxic compounds, for example, a chemotherapeutic agent selected from the group which includes, but is not limited to, an inhibitor of polyamine biosynthesis, an inhibitor of a protein kinase, especially of a serine/threonine protein kinase, such as protein kinase C, or of a tyrosine protein kinase, such as the EGF receptor tyrosine kinase, e.g. PKI166, the VEGF receptor tyrosine kinase, e.g. PTK787, or the PDGF receptor tyrosine kinase, e.g. STI571, a cytokine, a negative growth regulator, such as TGF-ß or IFN-ß, an aromatase inhibitor, e.g.

letrozole, an inhibitor of the interaction of an SH2 domain with a phosphorylated protein, antiestrogens, topoisomerase I inhibitors, topoisomerase II inhibitors, microtubule active agents, e.g. paclitaxel or an epothilone, alkylating agents, antineoplastic antimetabolites, platin compounds, anti-angiogenic compounds, gonadorelin agonists, anti-androgens, bisphosphonates, e.g. AREDIA® or ZOMETA®, and trastuzumab. The structure of the active agents identified by code nos., generic or trade names may be taken from the actual edition of the standard compendium "The Merck Index" or from databases, e.g. Patents International (e.g. IMS World Publications). The corresponding content thereof is hereby incorporated by reference.

The precise dosage of the compounds of formula I to be employed for inhibiting tumors depends upon several factors including the host, the nature and the severity of the condition being treated, the mode of administration and the particular compound and combination partner, if any, employed. However, in general, satisfactory inhibition of tumors is achieved when a compound of formula I is administered parenterally, e.g., intraperitoneally, intravenously, intramuscularly, subcutaneously, intratumorally, or rectally, or enterally, e.g., orally, preferably intravenously or orally, more preferably intravenously at a single dosage of 1-300 mg/kg body weight per cycle (cycle =3-6 weeks) or, for most larger primates, a single dosage of 50-5000 mg per treatment cycle. A preferred intravenous single dosage per 3-6 week treatment cycle is 1-75 mg/kg body weight or, for most larger primates, a daily dosage of 50-1500 mg. A typical intravenous dosage is 45 mg/kg, once every three weeks.

Usually, a small dose is administered initially and the dosage is gradually increased until the optimal dosage for the host under treatment is determined. The upper limit of dosage is that imposed by side effects and can be determined by trial for the host being treated.

The compounds of formula I may be combined with one or more pharmaceutically acceptable carriers and, optionally, one or more other conventional pharmaceutical adjuvants and administered enterally, e.g. orally, in the form of tablets, capsules, caplets, etc. or parenterally, e.g., intraperitoneally or intravenously, in the form of sterile injectable solutions or suspensions. The enteral and parenteral compositions may be prepared by conventional means.

The compounds of formula I may be formulated into enteral and parenteral pharmaceutical compositions containing an amount of the active substance that is effective for inhibiting tumors, such compositions in unit dosage form and such compositions comprising a pharmaceutically acceptable carrier.

The following example shows a representative compound encompassed by this invention and its synthesis. However, it should be clearly understood that it is for purposes of illustration only.

EXAMPLE

Synthesis of (2*R*,3*S*,4*S*,5*S*,7*S*,8*Z*,10*S*,11*S*,12*S*,13*Z*,16*S*,17*S*,18*S*,19*S*,20*S*,21*Z*)-19-[(aminocarbonyl)oxy]-3,5,7,11,17-pentahydroxy-2,4,10,12,14,16,18,20-octamethyl-8,13,21,23-tetracosatetraenoic acid, sodium salt.

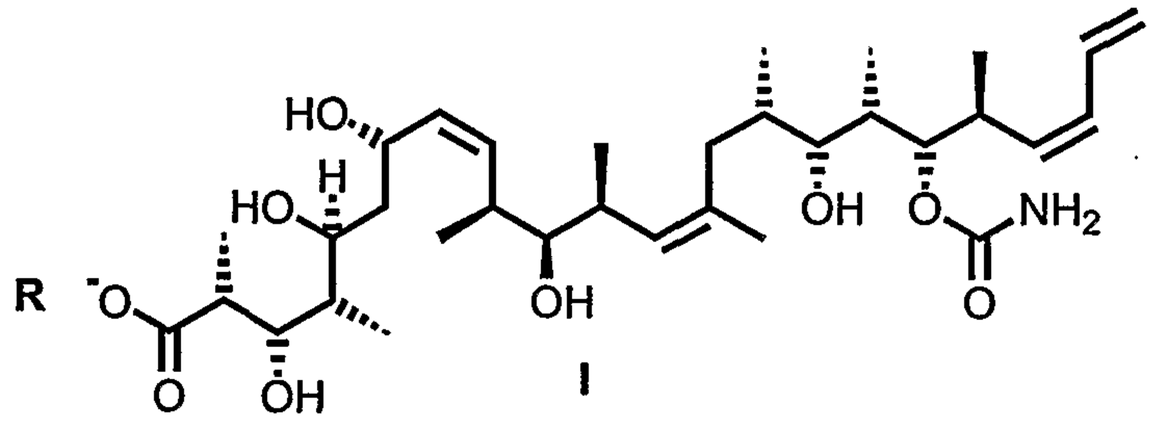
A 25 mL round-bottomed flask is fitted with magnetic stirring and a septum, and maintained under a nitrogen atmosphere. The flask is charged with 95 mg (0.16 mmol) 6-[(2*S*,3*Z*,5*S*,6*S*,7*S*,8*Z*,11*S*,12*R*,13*S*,14*S*,15*S*,16*Z*)-14-[(aminocarbonyl)oxy]-2,6,12-trihydroxy-5,7,9,11,13,15-hexamethyl-3,8,16,18-nonadecatetraenyl]tetrahydro-4-hydroxy-3,5-dimethyl-(3*R*,4*S*,5*R*,6*S*)-2*H*-pyran-2-one [Chemical Abstracts number: 127943-53-7] and 6 mL of THF. After stirring and dissolving the solid, the flask is cooled in an ice bath. 1.6 mL of 0.10 *M* NaOH (0.16 mmol) is added with a syringe over about 25 minutes. After allowing the mixture to warm to room temperature, TLC showed the reaction is complete. TLC conditions: Eluent, 100% EtOAc; Visualization, Vanillan/H_2SO_4/EtOH + heat; R_f = 0.5.). The mixture is partitioned between *t*-BuOMe (30 mL) and water (30 mL). The layers are separated and the product containing aqueous layer is washed with another 30 mL portion of *t*-BuOMe. The aqueous layer is filtered and the solvent evaporated to obtain a white residue. The residue is reconstituted in about 30 mL water and freeze dried to afford the desired product as a white solid.

^{1}H NMR (300MHz, D_2O) δ 6.75 (1H, dd, *J* = 16.8, 11.0 Hz), 6.22 (1H, t, *J* = 11.2, Hz), 5.65 (1H, t, *J* = 9.4 Hz), 5.51 (2H, m), 5.38 (1H, d, *J* = 16.8 Hz), 5.29 (1H, d, *J* = 10.1 Hz), 5.02 (1H, d, *J* = 10.1 Hz), 4.75 (1H, dd, *J* = 10.3, 2.2 Hz), 4.53 (1H, t, *J* = 9.0 Hz), 4.11 (1H, q, *J*= 4.9 Hz), 3.82 (1H, q, *J* = 4.5 Hz), 3.34-3.23 (2H, m), 3.16 (1H, m), 2.71 (1H, m), 2.51 (1H,

m), 2.32 (1H, m), 1.94-1.72 (4H, m), 1.71-1.55 (5H, m), 1.32 (1H, m), 1.11 (3H, d, *J* = 6.7 Hz), 1.07 (3H, d, *J* = 6.7 Hz), 1.02 (3H, d, *J* = 6.7 Hz), 0.97 (3H, d, *J* = 6.7 Hz), 0.88 (3H, d, *J* = 6.7 Hz), 0.87 (3H, d, *J* = 6.7 Hz), 0.80 (3H, d, *J* = 4.5 Hz).

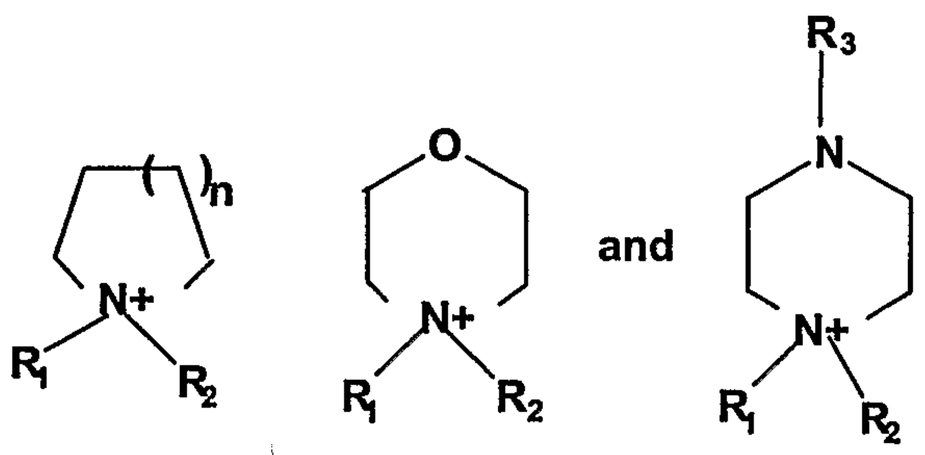
WHAT IS CLAIMED IS:

1. A compound of formula I



I

where R is Li^+, Na^+, K^+, $1/2Ca^{++}$, $1/2Mg^{++}$, $1/2Mn^{++}$, $R_1R_2R_3R_4N^+$, or a nitrogen containing ring selected from



II III IV

where each of R_1, R_2, R_3 and R_4, independently, is hydrogen, (C_{1-12})alkyl, hydroxy(C_{2-8})alkyl or benzyl; and n is 1-5.

2. A compound according to Claim 1 of formula I

where R is Li^+, Na^+, K^+, $1/2Ca^{++}$, $R_1R_2R_3R_4N^+$, or a nitrogen containing ring selected from II, III, and IV
where each of R_1, R_2, R_3 and R_4, independently, is hydrogen, (C_{1-8})alkyl, hydroxy(C_{2-6})alkyl or benzyl; and n is 1-3.

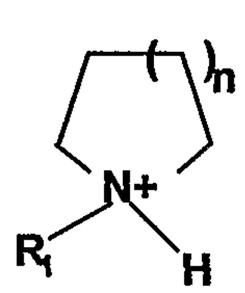
3. A compound according to Claim 2 of formula I

where R is Na^+,K^+, $1/2Ca^{++}$, $R_1R_2R_3R_4N^+$, or a nitrogen containing ring selected from II and III

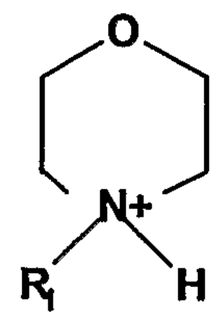
where each of R_1, R_2, R_3 and R_4, independently, is hydrogen, (C_{1-6})alkyl, hydroxy(C_{2-4})alkyl or benzyl; and n is 1 or 2.

4. A compound according to Claim 3 of formula I

where R is Na^+, $1/2Ca^{++}$, $R_1R_2R_3HN^+$, or a nitrogen containing ring selected from



and



IIa IIIa

where each of R_1, R_2 and R_3, independently, is hydrogen, (C_{1-6})alkyl, hydroxyethyl or benzyl; and n is 1 or 2.

5. A compound according to Claim 1 which is (2*R*,3*S*,4*S*,5*S*,7*S*,8*Z*,10*S*,11*S*,12*S*,13*Z*,16*S*, 17*S*,18*S*,19*S*,20*S*,21*Z*)-19-[(aminocarbonyl)oxy]-3,5,7,11,17-pentahydroxy-2,4,10,12,14,16,18,20-octamethyl-8,13,21,23-tetracosatetraenoic acid, sodium salt.

6. A pharmaceutical composition comprising a pharmaceutical acceptable carrier or diluent and a therapeutically effective amount of a compound according to any one of Claims 1 to 4.

7. A pharmaceutical composition according to Claim 6 comprising a pharmaceutical carrier or diluent and a therapeutcially effective amount of (2*R*,3*S*,4*S*,5*S*,7*S*,8*Z*,10*S*,11*S*,

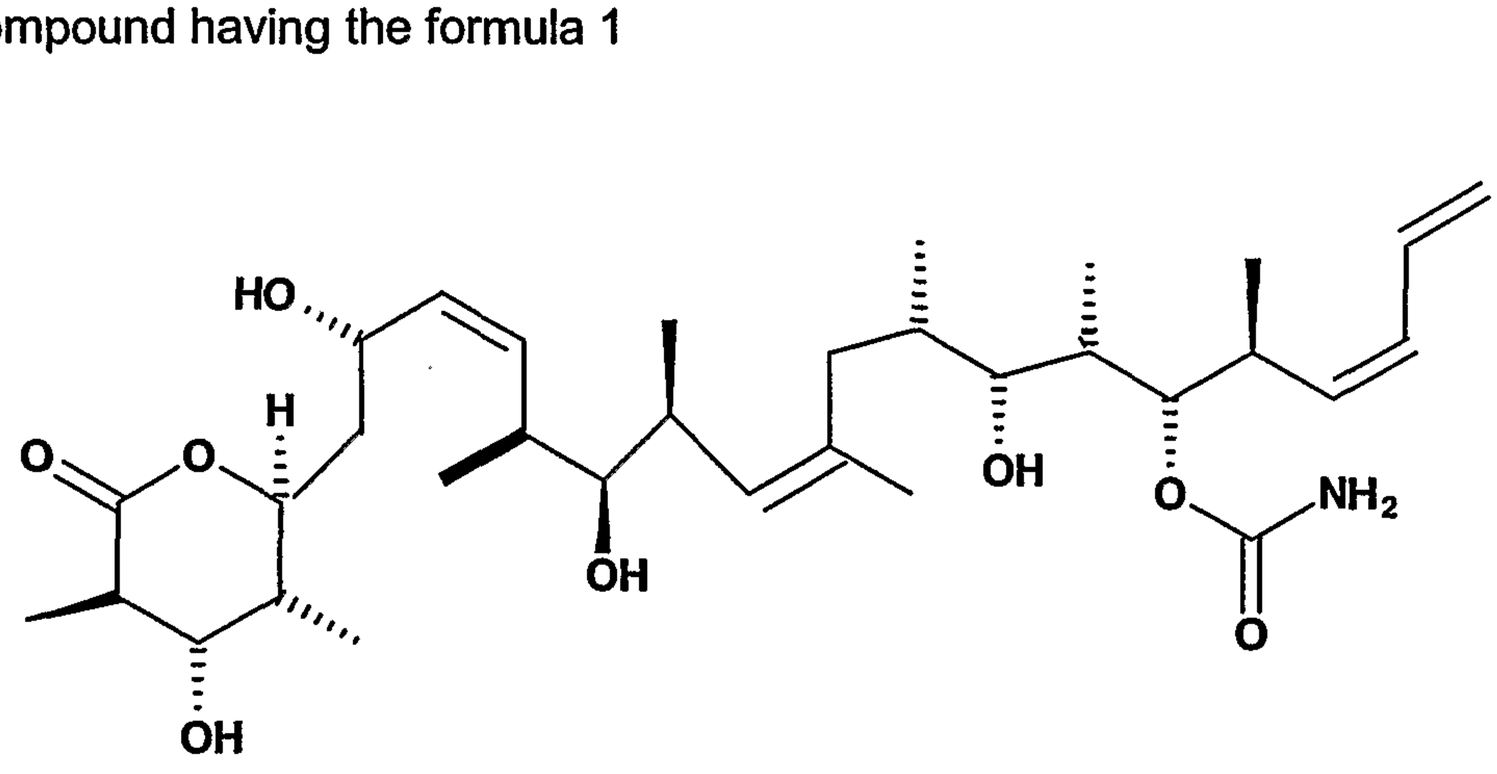
12*S*,13*Z*,16*S*,17*S*,18*S*,19*S*,20*S*,21*Z*)-19-[(aminocarbonyl)oxy]-3,5,7,11,17-pentahydroxy-2,4,10,12,14,16,18,20-octamethyl-8,13,21,23-tetracosatetraenoic acid, sodium salt.

8. A method of treating tumors comprising administering to a mammal in need of such treatment a therapeutically effective amount of a compound according to any one of Claims 1 to 5.

9. A compound of formula I according to any one of Claims 1 to 5 for use for the therapeutic treatment of the human or animal body.

10. The use of a compound of formula I according to any one of Claims 1 to 5 in the preparation of a pharmaceutical composition for use in the chemotherapy of tumors.

11. A process for preparing a compound according to Claim 1 which comprises hydrolyzing a compound having the formula 1



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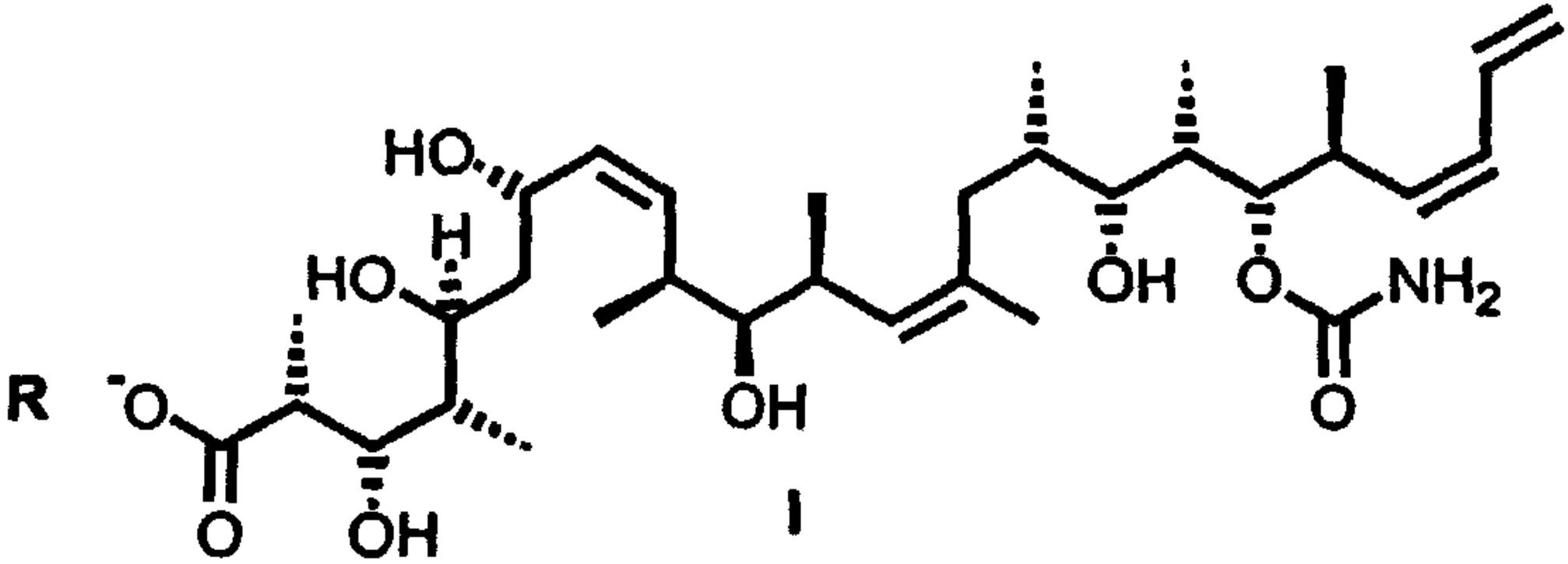
with ROH, where R is as defined in Claim 1, in the presence of a polar organic solvent.

12. A process according to Claim 11 wherein the solvent is an ether and the hydrolysis is carried out at a temperature of between 0°C and 20°C.



I

(I)