Microbial Transformation of a Mixture of Argentatin A and Incanilin

Galal T. Maatooq[a,*] and Joseph J. Hoffmann[b]

[a] University of Mansoura, Faculty of Pharmacy, Department of Pharmacognosy, Mansoura 35516, Egypt. E-Mail: galaltm@mans.edu.eg

[b] University of Arizona, College of Agriculture, Office of Arid Lands Studies, Bioresources Research Facility, 250 E. Valencia Road, Tucson, AZ. 85706

* Author for correspondence and reprint requests

Z. Naturforsch. **57 c,** 489–495 (2002); received November 5, 2001/February 12, 2002

Argentatin A, Incanilin, Biotransformation

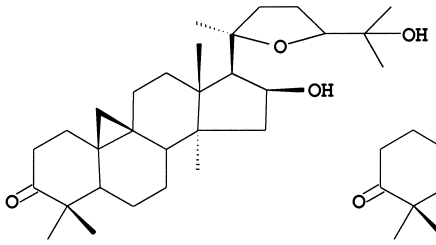
The biotransformation of a mixture of argentatin A (20%) **1** and incanilin (80%) **2** by *Gibberella suabinetti* ATCC 20193 and *Septomyxa affinis* ATCC 6737 demonstrated the conversion of incanilin to 16β-hydroxylanosta-2, 8, 23-triene, while argentatin A did not react. The acetate of this triterpenoid mixture was biotransformed by *Septomyxa affinis* ATCC 6737 to give five metabolites. Argentatin A acetate was transformed to 3β, 16β,30-trihydroxy-cycloart-20, 24-diene, 20*R*, 24*R*-epoxy-16β, 25-dihydroxy-3, 4-seco-cycloart-4(28)-en-3-oic acid acetate and 20*R*, 24*R*-epoxy-16β, 25-dihydroxy-3, 4-seco-cycloart-4(28)-en-3-oic acid. Incanilin acetate was converted to 16β-hydroxylanosta-2, 8, 23-triene and 20*R*, 24*R*-epoxy-16β, 25-dihydroxy-3, 4-seco-lanost-1, 4(28), 8-trien-3-oic acid acetate. The structural elucidations of these metabolites were achieved by different spectroscopic methods.

Introduction

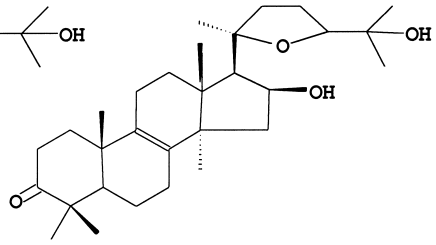
Argentatin A **1** and incanilin **2** are abundant tetracyclic triterpenes isolated from the rubber plant, *Parthenium argentatum,* Gray (guayule) (Rodriguez-Hahn *et al*., 1970; Komoroski *et al*., 1986; Romo De Vivar *et al*., 1990). This triterpenes mixture was obtained during the isolation of the antifungal agents from guayule resin (Maatooq *et al*., 1996). The biotransformation of these secondary metabolites can lead to a biologically active compounds or synthons which will increase the potential industrial importance of guayule. The abilities of some bacteria, fungi and yeast to convert argentatin A and incanilin mixture were investigated. This article describes the biotransformation methods, the isolation of the metabolites and determination of their molecular structure.

Results and Discussion

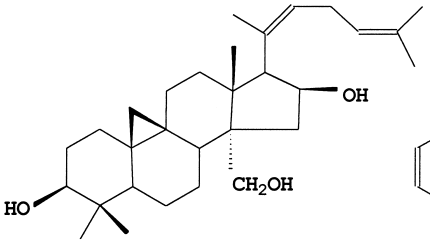
The biotransformation of argentatin A (20%) **1** and incanilin (80%) **2** mixture by *Gibberella saubinetti* ATCC 20193 and *Septomyxa affinis* ATCC 6737 gave the same metabolite, **3**. The spectroscopic data analysis proved that **3** is an incanilin metabolite, since the upfield protons signals characteristic for the cyclopropyl ring in argentatin A were not detected. Metabolite **3** was assigned to be 16β-hydroxylanosta-2, 8, 23-triene. The ^{13}C-NMR spectrum displayed six olefinic carbons at 116.4, 119.6, 132.1, 135.6, 139.9 and 141.3 ppm. Two of these olefinics (132.1 and 135.6 ppm) are consistent with 8-position double bond in incanilin skeleton. The other four signals have to be assigned to four methine groups, since the proton spectrum showed four signals at 5.55 (dd, J = 3, 6 Hz), 5.35 (m), 5.20 (br d) and 5.19 (m) ppm. This indicated that **3** has two extra double bonds compared to incanilin. The locations of these two double bonds were assigned to 2 and 23-positions easly. The 3-position oxo group was disappeared (^{13}C-NMR), which suggest its reduction to a hydroxyl group followed by a dehyration to give the 2-position double bond (139.3 and 141.3 ppm). The 21-methyl group proton signal appear as doublet at 0.92 ppm (J = 8 Hz) and the oxygenations at 21 and 24-position were lost, since the carbon signals around 82.0–87.0 ppm were disappeared. For these reasons, the other two carbon signals at 119.6 and 116.4 ppm and the protons signals at 5.19 (m) and 5.55 ppm (dd, J = 3, 6 Hz) were assigned to another double bond at 23-position. The low value-coupling constant indicated the possible *cis* orientation of these two protons. In addition, the 25-position hydroxyl group was disappeared. This is further confirmed by the appearance of the 26 and 27-methyl groups proton signals each as a doublet at 0.80 and 0.83 ppm. The 16-position



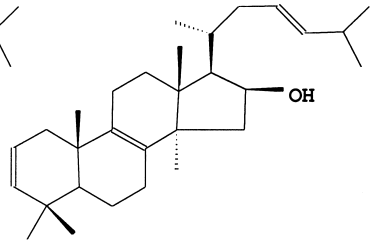
Argentatin A, 1



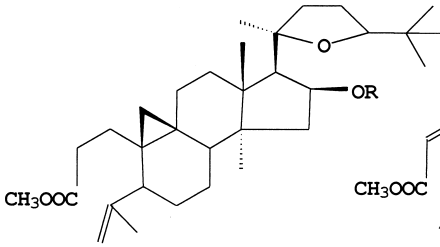
Incanilin, 2



3β, 16β, 30-trihydroxycycloart-20, 24-diene, 5

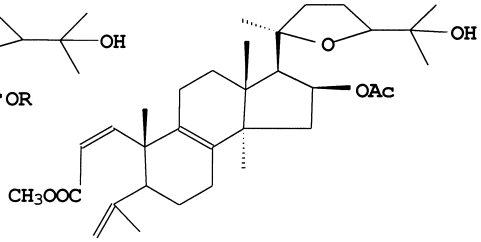


16β-hydroxylanosta-2, 8, 23-triene, 3

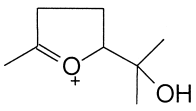


20R, 24R-epoxy-16β, 25-dihydroxy-3, 4-seco-cycloart-4(28)-en-3-oic acid methyl ester acetate, 6 = R = Ac

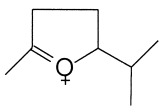
20R, 24R-epoxy-16β, 25-dihydroxy-3, 4-seco-cycloart-4(28)-en-3-oic acid methyl ester, 8 = R = H



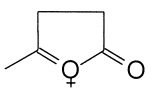
20R, 24R-epoxy-16β, 25-dihydroxy-3, 4-seco-lanost-1, 4(28), 8-trien-3-oic acid methyl ester acetate, 7



m/z 143



m/z 125



m/z 99

m/z 59

hydroxyl group still intact, since a carbon signal at 70.5 ppm and the charcteristic proton multiplet at 3.61 ppm were detected. Based on these evidences, metabolite **3** should have a molecular formula $C_{30}H_{48}O$, requiring *m/z* 424, which was seen as a parent ion peak in the EIMS. Therefore, the structure of **3** should be 16β-hydroxylanosta-2, 8, 23-triene.

Compound **5** demonstrated two upfield proton signals at 0.37 and 1.00 ppm, characteristic to the cyclopropyl protons of argentatin A metabolite. The ^{13}C-spectrum indicated the loss of the THF ring of the side chain, since the carbon signals at 84.7 and 87.2 ppm were absent. The proton and carbon signals characteristic to the oxygented 24-position were also absent. The downfield methyls protons singlets at 1.43 ppm (3H, s, H-21) and 1.65 ppm (6H, s, H-26, H-27), indicated the loss of 21-oxygenation and the 25-hydroxylation and a possible presence of two double bonds in the vicinity of these methyls. The proton broad triplet at 5.68 ppm was assigned to 22-position inferring a double bond at 20-position, while the broad triplet at 5.58 ppm assigned to 24-position and infers a double bond at this place. The multiplet proton signal at 4.22 ppm and the carbon signal at 72.7 ppm were consistent with 16-position. The broad triplet at 3.73 ppm was linked to the carbon signal at 75.5 ppm and assigned to hydroxylated 3-position. The appearance of this proton signal as a broad triplet indicated the possible presence of an axial proton and infers a beta configuration to the hydroxyl group. The new carbon signal at 66.8 ppm and the proton double doublet at 3.98 ppm (J = 10 Hz), integrated for two protons, were assigned to 30-position. This is confirmed by the loss of one methyl signal in ^{1}H and ^{13}C-spectra. The location of this CH_2OH at 30-position was confirmed by the β and γ-effect observed at this vicinity. The β-effect is clearly evidenced at 14-position by 6.6 ppm downfield shift, while the γ-effect is demonstrated by an upfield shift of 8, 13 and 15-positions by 5.3, 3.9 and 5.5 ppm, respectively. These observations and clues conclude **5** to have a molecular formula of $C_{30}H_{52}O_3$. The EIMS gave *m/z* 412 $[M-H_2O-2CH_3]^+$, 394 $[M-2H_2O-2CH_3]^+$ and 379 $[M-2H_2O-3CH_3]^+$, which are consistent with 3β, 16β, 30-trihydroxycycloart-20, 24-diene as a structure of **5**.

The analysis of the spectral data of **6** indicated that it is an argentatin A metabolite. It was obtained by oxidative opening of ring A at 3–4 position, where a carboxylic acid was found at 3-position and an exomethylene is formed at 4(28)-position. It was obtained as methyl ester, after methylation with CH_3I. The MS-spectrum gave *m/z* 484 as a parent peak, which was assigned to $[M-HOAc]^+$. This infers a molecular formula of $C_{33}H_{52}O_6$ for **6**. The characteristic fragments of the side chain with a THF ring at *m/z* 143, 125, 99 and 59 in mass spectrum (Govindachari *et al.*, 1994), and the shielded protons signals at 0.42 and 0.77 ppm for the cyclopropyl CH_2 proton, are indicating that **6** is argentatin A metabolite. In ^{13}C-NMR spectrum an acetate group (170.7 and 20.8 ppm) was detected and a new signal at 174.2 ppm was assigned to the carboxylic carbon at 3-position. This was confirmed by its methylation, since a methyl ester proton singlet at 3.65 ppm and a carbon signal at 51.5 ppm were found. The carbon signals at 111.6 and 149.1 ppm together with the two proton doublets at 4.73 and 4.82 ppm (J = 1 Hz each), were assigned to the

Table I. ^{13}C NMR spectral data of compounds **3**, and **5–8***.

C#	**3**	**5**	**6**	**7**	**8**
1	39.2	36.6	28.7	118.8	28.8
2	139.3	32.7	31.3	120.1	31.4
3	141.3	75.5	174.2	175.2	174.3
4	40.9	40.1	149.1	149.1	149.3
5	54.6	38.6	45.3	50.8	46.1
6	21.1	19.1	19.7	28.4	19.7
7	28.2	27.6	25.5	35.8	25.1
8	135.6	42.5	46.7	140.9	46.7
9	132.1	20.6	20.7	137.6	20.9
10	38.5	22.5	25.1	39.1	23.6
11	21.2	21.6	20.1	28.7	20.3
12	33.2	32.3	33.1	37.6	33.3
13	42.9	42.7	46.5	45.4	47.5
14	46.3	54.3	47.3	48.7	48.5
15	42.8	43.2	45.7	41.4	45.9
16	70.5	72.7	75.2	75.5	73.0
17	55.9	55.6	57.6	56.5	55.5
18	17.6	17.1	21.6	22.4	25.4
19	16.3	30.0	30.5	20.2	30.6
20	32.1	135.2	84.8	85.2	86.9
21	19.9	17.7	28.1	25.3	27.3
22	37.1	117.1	36.6	38.9	37.2
23	119.6	38.9	20.7	29.7	20.9
24	116.4	131.6	82.6	83.4	84.4
25	40.3	142.7	70.6	71.2	70.7
26$^+$	29.7	29.3	28.3	28.3	27.6
27$^+$	29.7	29.2	27.4	26.3	27.1
28	12.0	11.8	111.6	112.5	111.5
29	19.6	19.4	26.6	22.4	26.7
30	23.1	66.8	24.6	25.1	26.1
Ac	–	–	170.7	171.5	–
	–	–	20.8	22.1	–
Others	–	–	51.5	52.0	51.4

* At 62.5 MHz, using $CDCl_3$ as a solvent, TMS is the internal standard and chemical shifts (δ) are expressed in ppm.

$^+$ Assignments may be interchangeable.

new exomethylene group at 4(28)-position. This location was further confirmed by the strong deshielding of 29-methyl group protons signal (1.67 ppm). Therefore the structure of **6** has to be 20*R*, 24*R*-epoxy-16β, 25-dihydroxy-3, 4-seco-cycloart-4(28)-en-3-oic acid methyl ester acetate.

The EIMS of **7** gave *m/z* 542 for $C_{33}H_{50}O_6$. The characteristic fragments *m/z* 143, 125, 99, 59 and 43 are consistent with the side chain having the THF ring (Govindachari *et al*., 1994). The absence of the shielded cyclopropyl protons signals indicated the possible presence of incanilin metabolite. Further analysis of the ^1H, ^{13}C and DEPT spectra indicated that **7** was produced from incanilin same way as production of **6** from argentatin A, but with one more double bond at 1-position. The ^{13}C-spectrum demonstrated 33 carbon signals, including the methyl ester signal at 52.0 ppm and acetate (171.5 and 22.1 ppm). The olefinic region of the spectrum showed the presence of six signals for three double bonds. The two-non protonated olefinics at 137.6 and 140.9 ppm were assigned to 8-position double bond. The carbon signals at 112.5 ppm (CH_2) and 149.1 ppm (non protonated), together with the two protons doublets at 4.66 and 4.68 ppm (J = 1 Hz, each) confirm the presence of an exomethylene group. The DEPT experiment indicated the presence of nine methyl groups only and supports the conversion of one skeletal methyl group to the exomethylene group. The location of this exomethylene group was assigned to 28-position. Further confirmation came through the observed deshielding of 29-position methyl group protons signal (1.63 ppm). This suggested a possible cleavage in ring A between 3 and 4-positions. Position-3 was found to be oxidized to the corresponding carboxylic acid (methylated with CH_3I), where a carbon signal at 175.2 ppm was assigned to this position. The two methine (DEPT) carbon signals at 118.8 and 120.1 ppm together with the two broad proton doublets at 5.50 and 5.37 ppm, were assigned to 1 and 2-positions, respectively. The resulted α, β-unsaturated carboxylic acid gave UV λ_{max} of 241.3 nm which is consistent with the proposed structure for **7** to be 20*R*, 24*R*-epoxy-16β, 25-dihydroxy-3, 4-seco-lanost-1,4(28), 8-trien-3-oic acid methyl ester acetate.

The spectral data of metabolite **8** are similar to those of **6**, with few differences. The mass spectrum gave *m/z* 502 for $[M]^+$, which requires $C_{31}H_{50}O_5$. This is pointing to the possible presence of deacetylated **6**. The absence of the acetate proton and carbon signals supports this conclusion. Therefore **8** should be deacetylated **6** or 20*R*, 24*R*-epoxy-16β, 25-dihydroxy-3, 4-seco-cycloart-4(28)-en-3-oic acid methyl ester.

In conclusion, the biotransformation of argentatin A and incanilin mixture by *Gibberella saubinetti* ATCC 20193 and *Septomyxa affinis* ATCC 6737 gave one metabolite **3**. By spectroscopic analysis, this metabolite was found to be of incanilin substrate, formed by THF ring opening, reduction of the 3-oxo group and formation of new double bonds at 2 and 23-positions. Under these conditions argentatin A did not react. Acetylation of this triterpenoid mixture leads an interesting biotransformation with *Septomyxa affinis* ATCC 6737. It was found that the acetate mixture is better substrate, which fit the enzymatic system of *Septomyxa affinis* compared to the normal triterpenes mixture. In this reaction, argentatin A was converted to three major metabolites **5**, **6** and **8**. A THF ring opening, 3-oxo group reduction and a new double bond formation at 20(22) and 24-positions were observed, **5**. An oxidative ring A opening was observed, **6**, where a carboxylic acid at 3-position and an exomethylene group at 4(28)-position were evidenced. This oxidative opening of ring A could be explained through Bayer-Villeger oxidation by insertion of oxygen function between 3 and 4-positions to form an ester, then hydrolysis followed by a dehydration at 4(28)-position. Esterase enzyme seems to play a role in getting metabolite **8**, where the 16-position acetate group, was found to be hydrolyzed. Incanilin in this acetate mixture was transformed to metabolite **3** and **7**. Metabolite **7** formed from incanilin same way as **6**, in addition to dehydrogenation of 1 and 2-positions.

Experimental

Instrumentation

Melting points are uncorrected. ^1H-NMR and ^{13}C-NMR were measured on a Bruker WM 250 NMR spectrometer, at 250 MHz and 62.5 MHz, respectively, with $CDCl_3$ as a solvent and TMS as the internal standard. The chemical shifts are e×pressed in δ (ppm). DEPT and HETCOR were measured on a Bruker WM 300 NMR spectrome-

ter, at 300 MHz. EIMS (70 eV) was conducted on a Hewlett Packard 5988A spectrometer, equipped with a Hewlett Packard RTE-6/VM data system. IR was conducted on Beckman Acculab I IR spectrometer. ORDs were measured on Autopole III Automatic Polarimeter (Rudolph Scientific, New Jersey). UV data was obtained from Hitachi L-4500 photo-diode-array HPLC.

Substrate material

Argentatin A and incanilin were isolated from *Parthenium argentatum* Gray and were characterized by ^{1}H-, ^{13}C-NMR and mass spectrometry (Rodriguez-Hahn *et al*., 1970; Komoroski *et al*., 1986; Romo De Vivar *et al*., 1990). A mixture of argentatin A-incanilin (20:80) and their acetate mixture were used in these biotransformation reactions. The relative percentage of both compounds was determined by HPLC.

These mixtures were used for those biotransformation reactions because a mixture of argentatin A and incanilin was obtained in large quantities, during our search for anti-fungal agent in guayule resin and their separation is a tedious job.

Fermentation methods

Screening experiments were done in 125 ml DeLong culture flasks, while preparative scale were conducted in either 0.5, one or two-liter flasks. The microorganisms were grown according to the standard two-stages fermentation protocol (Betts *et al*., 1974)

The culture flasks held one fifth of their volume of the following medium; 2% glucose, 0.5% soybean meal, 0.5% yeast extract, 0.5% NaCl and 0.5% K_2HPO_4. The pH of the medium adjusted to 7.0 using 6 N HCl before autoclaving for 20 min at 121° and 15 psi. After inoculation with stage I cultures (in which the microorganism was transferred from the slants to the sterile medium), incubate at 27° and 250 rpm for 72 h before being used to inoculate stage II culture flasks (in which, 10% inoculum volumes of stage I culture was used to inoculate another sterile medium and leave for 24 h to give stage II culture). For screening scale experiments 10 mg of the triterpene mixture in 0.1 ml of DMSO was added to 24-hour- old stage II cultures, which were incubated again and sampled periodically for analysis.

Sampling

Samples of 1 ml each were taken after 12, 24, 36 and 48 h and every other day for 2 weeks following substrate addition. Each sample was extracted by shaking with 0.5 ml EtOAc and spun at $3000\times g$ for 1 m in a desk-top centrifuge. All the EtOAc extract samples were spotted on Si gel GF_{254} TLC plates, and developed in a suitable percentage of $EtOAc/C_6H_{14}$ or Me_2CO/CH_2Cl_2, and visualized after spraying with 0.01% vanillin/H_2SO_4, followed by heating for 5–10 seconds with a heat gun.

It was found that *Gibberella saubinetti* ATCC 20193 and *Septomyxa affinis* ATCC 6737 were able to achieve the goal, especially the latter with the acetylated substrates.

Preparative scale conversion of the triterpenes mixture with Gibberella saubinetti ATCC 20193 (Reaction-A)

Seven 2-liter stage II cultures received 3.5 g of the triterpenes mixture in 10.5 ml of DMSO (1.2 mg substrate per ml of culture medium). After incubation for four weeks under the usual condition, the cultures were combined and exhaustively extracted with 3×3 liter of 10% MeOH/EtOAc. The extract was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield 4.5 g residue.

Preparative scale conversion of the triterpenes mixture with Septomyxa affinis ATCC 6737 (Reaction-B)

Same way as reaction A, to give 4.2 g residue.

Preparative scale conversion of the triterpenes acetate mixture with Septomyxa affinis ATCC 6737 (Reaction-C)

Six 2-liter, eight 1-liter and five 0.5-liter stage II cultures received 4.5 g of the triterpenes acetate mixture in 18 ml of DMSO (1 mg substrate per ml of culture medium). After incubation for three weeks at 200 rpm and 27°, the cultures were combined and exhaustively extracted with 3 × 6 liter of 10% MeOH/EtOAc (v/v). The extract was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to yield 6.4 g residue.

Isolation and purification of reaction A and B metabolites

The TLC indicated the presence of one major spot less polar than the substrate, in both reactions, at R_f = 0.83 (Si gel GF_{254}, C_6H_{14}–EtOAc; 30:70). The crude extract of *Gibberella suabinetti* reaction (4.5 g) and *Septomyxa affinis* reaction (4.2 g) were, separately flash chromatographed, 300 g silica gel, 63–200 μ, 3.5×45 cm. The elution was adopted using acetone/hexane one liter each of 5%, 10%, 15%, 20%, 25%, 35%, 50%, 75% and 100% Me_2CO. Fractions, 300 ml each, were collected and TLC investigated. The chromatograms were visualized after vanillin/sulfuric acid spraying followed by heating with a heat gun for 5–10 seconds. Similar fractions were pooled together. Fractions eluted with 10% in both reactions were separately subjected to prep TLC on 1 mm-thick Si gel plates using C_6H_{14}–EtOAc (4:6, v/v) to give 44 mg and 24 mg, of **3**, from reaction A and B respectively, as white needles.

Isolation and purification of reaction C metabolites

The TLC displayed four new spots at R_f = 0.81, 0.3, 0.15 and 0.5 (Si gel GF_{254}, C_6H_{14}–EtOAc; 7:3). All gave reddish-brown color after spraying with vanillin/H_2SO_4 spray reagent followed by heating with a heat gun for 5–10 s. The crude reaction mixture (6.4 g) was subjected to flash chromatography, silica gel, 400 g, 63–200 μ, 3.5×45 cm. The elution profile was EtOAc/hexane 10%, 15%, 20%, 30%, and 50% then 100% acetone, 1-liter portions each. Eleven fractions were collected 500–700 ml each and TLC investigated. The chromatograms were visualized after vanillin/sulfuric acid spraying followed by heating with a heat gun for 5–10 seconds. Similar fractions were pooled together. Fractions 1 and 2, eluted with 10% were purified on prep. TLC, 1 mm-thick Si gel GF_{254} plates using hexane–EtOAc (1:1, v/v) to give 35 mg of **3** as white needles.

Frs. 4–7 eluted with 15–20% gave 4.1 g of recovered crude substrate material.

Fr. 8 eluted with 30% EtOAc/hexane was subjected to prep. TLC on 1 mm-thick Si gel GF_{254} plates, in C_6H_{14}–EtOAc (1:1). This gave 23 mg of **4** and 21 mg of **5**, both as amorphous powders. The spectroscopic data of **4** indicated that it is a complex mixture and no longer pursued at this time.

Frs. 9–11 afforded 1.2 gm residue. It was subjected to methylation using CH_3I/Me_2CO + K_2CO_3. After the usual work-up, it was subjected to MPLC, Si gel, 140 g, 15–25 μ, 2.6×46 cm. The eluent was 250 ml CH_2Cl_2, 250 ml 0.5% MeOH/CH_2Cl_2, 500 ml 0.75% MeOH/CH_2Cl_2, 300 ml 1%, 600 ml 1.5%, 500 ml 2% and 500 ml 5%. Twenty fractions were collected. Frs. 10–14 eluted with 1% and 1.5% MeOH/CH_2Cl_2, were subjected to acetylation, followed by multiple development (4 times) prep. TLC, on 1 mm-thick Si gel GF_{254} plates using CH_2Cl_2–Me_2CO–*iso*-PrOH (96:4:1, v/v/v) as a solvent. This afforded 188 mg of **6** and 90 mg of **7**, both as a transparent solid mass. Metabolite **7** was further purified on prep. HPLC, using a C18 column and adopting isocatic elution with 80% CH_3CN/H_2O to yield 44 mg of **7** as a transparent solid mass. Frs.16–17 were subjected to prep.TLC on 1 mm-thick Si gel GF_{254} plates using CH_2Cl_2–Me_2CO–*iso*-PrOH (95:3:2, v/v/v) as a solvent, adopting a multiple development technique (4 times). This gave 128 mg of **8** as a solid mass.

Compound **3***; 16β-hydroxylanosta-2, 8, 23-triene*

Needles, m.p. 128–130°, $\alpha[D]^{25}$, –14.92 (CH_2Cl_2; c. 2.0). IR $\upsilon_{max}^{cm^{-1}}$; 3450, 2955, 2860, 1635, 1450, 1220, 1030, 940, 800 and 760. EIMS, 70 eV, *m/z* (rel. int.); 424 $[M]^+$ (1), 414 (2), 399 (1), 398 (4), 397 (12), 396 (38), 381 (5), 363 (58), 337 (33), 271 (20), 253 (40), 211 (39), 157 (51), 143 (58), 91 (42), 82 (40), 69 (92), 55 (100) and 42 (60). ^{1}H-NMR (250 MHz, $CDCl_3$, δ ppm, *J* = Hz); 5.55 (1H, dd, *J* = 3, 6, H-24), 5.35 (1H, m, H-2), 5.20 (1H, br d, H-3),5.19 (1H, m, H-23), 3.61 (1H, m, H-16), 1.03 (3H, s, H-29), 1.01 (3H, s, H-28), 0.92 (3H, d, *J* = 8 Hz, H-21), 0.89 (3H, s, H-30), 0.83 (3H, d, *J* = 7.5 Hz, H-27), 0.82 (3H, s, H-18), 0.80 (3H, d, *J* = 7.5 Hz, H-26) and 0.62 (3H, s, H-19). ^{13}C-NMR data of this compound and the following ones are listed in Table I.

Compound **5***; 3β, 16β, 30-trihydroxycycloart-20, 24-diene*

Amorphous powder. $\alpha[D]^{25}$, –0.44 (CH_2Cl_2; c. 1.0). IR $\upsilon_{max}^{cm^{-1}}$; 3440, 2960. 2840, 1630,1430, 1320, 1220, 1060, 870, and 800. EIMS, 70 eV, *m/z* (rel. int.); 412 $[M-H_2O-2CH_3]^+$ (8), 394 $[M-2H_2O-2CH_3]^+$ (8), 379 $[M-2H_2O-3CH_3]^+$ (18), 337 (4), 311 (3), 369 (19), 251 (29), 215 (12), 175 (18), 159

(22), 121 (20), 95 (23), 81 (41), 69 (100), 55 (88) and 42 (70). ^{1}H-NMR (250 MHz, $CDCl_3$, δ ppm, *J* = Hz); 5.68 (1H, br t, H-22), 5.58 (1H, br t, H-24), 4.22 (1H, m, H-16), 3.98 (2H, dd, *J* = 10 Hz, H-30), 3.73 (1H, br t, H-3), 1.65 (6H, s, H-26, H-27), 1.43 (3H, s, H-21), 1.29 (3H, s, H-28), 1.21 (3H, s, H-29), 1.20 (3H,s, H-18),1.00 (1H, d, *J* = 7 Hz, H-19) and 0.37 (1H, d, *J* = 7 Hz, H-19′).

Compound **6***; 20R, 24R-epoxy-16β, 25-dihydroxy-3, 4-seco-cycloart-4(28)-en-3-oic acid methyl ester acetate*

Solid gum, $\alpha[D]^{25}$, +16.30 (CH_2Cl_2; c. 2.0). IR υ_{max}^{cm-1}; 3490, 2980, 2840, 1735, 1640, 1450, 1260, 1070, 1020, 890 and 800. EIMS, 70 eV, *m/z* (rel. int.); 484 $[M-HOAc]^+$ (1), 469 $[M-HOAc-CH_3]^+$ (3), 425 $[M-HOAc-C_3H_7O]^+$ (3), 383 (2), 342 (3), 301 (2), 275 (1), 241 (2), 143 (100), 125 (32), 99 (5), 84 (12),71 (22), 59 (70) and 43 (90). ^{1}H-NMR (250 MHz, $CDCl_3$, δ ppm, *J* = Hz); 5.47 (1H, m, H-16), 4.82 (1H, d, *J* = 1 Hz, H-28), 4.73 (1H, d, *J* = 1 Hz, H-28′), 3.68 (1H, dd, *J* = 11 Hz, H-24), 3.65 (3H, s, H–Me ester), 2.04 (3H, s, H–Ac), 1.67 (3H, s, H-29), 1.38 (3H, s, H-27), 1.29 (3H, s, H-26), 1.25 (3H, s, H-21), 1.14 (3H,s, H-30), 0.98 (3H, s, H-18), 0.77 (1H, d, *J* = 7 Hz, H-19) and 0.42 (1H, d, *J* = 7 Hz, H-19′).

Compound **7***; 20R, 24R-epoxy-16β, 25-dihydroxy-3, 4-seco-lanost-1, 4(28), 8-trien-3-oic acid methyl ester acetate*

Solid gum, $\alpha[D]^{25}$, +2.61 (CH_2Cl_2; c. 0.5). IR υ_{max}^{cm-1}; 3510, 2960, 2920, 2860, 1730, 1640, 1460, 1340, 1370, 1240 and 890. UV λ_{max} nm; 241.3. EIMS, 70 eV, *m/z* (rel. int.); 542 $[M]^+$ (2), 484 $[M-HOAc]^+$ (1), 469 $[M-HOAc-CH_3]^+$ (3), 455 (3), 425 $[M-HOAc-C_3H_7O]^+$ (3), 397 (2), 301 (3), 227 (4), 211 (6), 159 (12),143 (50), 125 (27), 99 (14), 84 (11),71 (22), 59 (60) and 43 (100). ^{1}H-NMR (250 MHz, $CDCl_3$, δ ppm, *J* = Hz); 5.61 (1H, m, H-16), 5.37 (1H, br d, H-2), 5.30 (1H, br d, H-1), 4.68 (1H, d, *J* = 1 Hz, H-28), 4.66 (1H, d, *J* = 1 Hz, H-28′), 3.68 (1H, dd, *J* = 11 Hz, H-24), 3.64 (3H, s, H–Me ester), 2.06 (3H, s, H–Ac), 1.63 (3H, s, H-29), 1.31 (3H, s, H-27), 1.24 (3H, s, H-26), 1.14 (3H, s, H-21), 1.01 (3H,s, H-30), 0.98 (3H, s, H-18) and 0.90 (3H, s, H-19).

Compound **8***; 20R, 24R-epoxy-16β, 25-dihydroxy-3, 4-seco-cycloart-4(28)-en-3-oic acid methyl ester*

Solid gum, $\alpha[D]^{25}$, +5.84 (CH_2Cl_2; c. 1.5). IR υ_{max}^{cm-1}; 3480, 2970, 2830, 1725, 1640, 1440, 1240, 1060, 1010, 880 and 780. EIMS, 70 eV, *m/z* (rel. int.); 502 $[M]^+$ (1), 469 $[M-H_2O-CH_3]^+$ (1), 443 (2), 425 $[M-H_2O-C_3H_7O]^+$ (1), 383, (1), 327 (1), 301 (1), 273 (1), 233 (1), 199 (2), 143 (100), 125 (24), 99 (5), 84 (9),71 (15), 59 (26) and 43 (25). ^{1}H-NMR (250 MHz, $CDCl_3$, δ ppm, *J* = Hz); 4.81 (1H, d, *J* = 1 Hz, H-28), 4.74 (1H, d, *J* = 1 Hz, H-28′), 3.83 (1H, dd, *J* = 11 Hz, H-24), 3.64 (3H, s, H–Me ester), 3.58 (1H, m, H-16), 1.67 (3H, s, H-29), 1.43 (3H, s, H-21), 1.27 (3H, s, H-27), 1.23 (3H, s, H-26), 1.12 (3H, s, H-30), 0.92 (3H, s, H-18), 0.77 (1H, d, *J* = 7 Hz, H-19) and 0.43 (1H, d, *J* = 7 Hz, H-19′).

Betts R. E., Walters D. E. and Rosazza J. P. N. (1974), Microbial transformation of antitumor compounds. 1. Conversion of acronycine to 9-hydroxyacronycine by *Cunninghamella echinulata*, J. Med. Chem. **17**, 599–602.

Govindachari, T. R., Suresh, G. and Kumari, G. N. K. (1994), Triterpenoids from *Dysoxylum malabaricum*, Phytochemistry **37**, 1127–1129.

Komoroski, R. A., Gregg, E. C., Shockcer, J. P.and Geckle, J. M. (1986), Identification of guayule triterpenes by two-dimensional and multipulse NMR techniques, Magn. Res. Chem. **24**, 534–543.

Maatooq, G. T., Stumpf, D. K., Hoffmann, J. J., Hutter, L. K. and Timmermann, B. N. (1996), Antifungal eudesmanoids from *Parthenium argentatum* × *P. tomentosa,* Phytochemistry **41**, 519–524.

Rodriguez-Hahn, L., Romo de Vivitar, A., Ortega, A., Aguilar, M. and Romo, J. (1970), Determinacion de las estructuras de las argentatinas A, B y C del guayule, Rev. Latinoam. Quim **1**, 24–38.

Romo De Vivar A., Martinez-Vazquez, M., Mustubara C., Perez-Sanchez G. and Joseph-Nathan P. (1990), Triterpenes in *Parthenium argentatum*, Structure of argentatins C and D. Phytochemistry **29**, 915–918 and references therein.