Microbiological and Chemical Transformations of Argentatin B

Galal T. Maatooq

Department of Pharmacognosy, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt. E-mail: galaltm@yahoo.com

Z. Naturforsch. **58c**, 249–255 (2003); received October 31/November 14, 2002

Argentatin B is a naturally occurring tetracyclic triterpene isolated from *Parthenium argentatum* x *P. tomentosa.* It was microbiologically transformed to 16, 24-epoxycycloartan-3α, 25-diol, (isoargentatin D), by *Nocardia corallina* var. *taoka* ATCC 31338, *Mycobacterium species* NRRL B3683 and *Septomyxa affinis* ATCC 6737. The later microbe also produced 16, 24-epoxycycloartan-3β, 25-diol (argentatin D) and 1, 2-didehydroargentatin B, (isoargentatin D). Sodium hydroxide converted argentatin B to argentatin D and isoargentatin D. Hydrochloric acid treatment gave cycloartan-25-ol-3, 24-dione. Cerium sulfate/sulfuric acid/aqueous methanol induced scission of the isopropanol moiety and provided an isomeric mixture of 24-methoxy-25–27-trinorargentatin B. Oxidation of this isomeric mixture with pyridinium chlorochromate, selectively, attacked the isomer with the equatorial proton at position-24 to give the corresponding lactone, 24-oxo-25–27-trinorargentatin B. The produced compounds were characterized by spectroscopic methods.

Key words: Argentatin B, Biotransformation, Cerium Sulfate

Introduction

Incanilin, argentatins A, B, C and D and isoargentatin B are naturally occurring, tetracyclic triterpenes which were isolated from the rubber plant *Parthenium argentatum* Gray (guayule), Asteraceae (Rodriguez-Hahn *et al.*, 1970; Komoroski *et al.*, 1986 and Romo de Vivar *et al.*, 1990). These triterpenes were obtained in abundant quantities, during the isolation of antifungal agents from the resin of the guayule hybrid *P. argentatum* × *P. tomentosa* (Maatooq *et al.*, 1996). Several bioactive tetracyclic triterpenes are structurally related to these compounds (Williams *et al.*, 1992; Shi *et al.*, 1992). Microorganisms and chemical reactions can extend types of compounds by transferring abundant prototypes into new ones. Microorganisms are mimic of plant metabolism and thus produce rare compounds from abundant ones or biologically active and/or less toxic metabolites. In a previous communication, microbial metabolic products of argentatin A and incanilin were isolated (Maatooq and Hoffmann, 2002). The biotransformation and the chemical conversion of argentatin B, isolation and structural elucidation of the produced compound are described herein.

Results and Discussion

For the biotransformation of argentatin B, **1**, 25 microbes were used for the screening purpose Scale up the reactions with the three microorganisms, *Nocardia corallina* var. *taoka* ATCC 31338, *Mycobacterium species* NRRL B3683 and *Septomyxa affinis* ATCC 6737 gave metabolite **2** as a common product, while *Septomyxa affinis* ATCC 6737 produces two more metabolites (**3** and **4**).

Metabolites **2** and **3** were characterized as isoargentatin D and argentatin D, respectively (Rodriguez-Hahn *et al.*, 1970; Komoroski *et al.*, 1986 and Romo de Vivar *et al.*, 1990) (Table I and Fig. 1).

The ^{13}C-NMR spectrum of metabolite **4** displayed two new olefinic carbon atoms signals at δ 154.2 and 127.3, which were correlated to the proton doublets at 5.94 and 6.77 (J = 11 Hz each), respectively. This concluded that **4** should have a new double bond represented by two olefinic methine groups. The location of this double bond was concluded to be at position 1, since no significant changes in the chemical shifts values at rings C and D. However, position 3 carbonyl resonance is shielded and was found to be at δ 205.1 (ca. 11.4 ppm), which implies the likely dehydrogenation of positions-1 and 2 to give the α, β-unsaturated carbonyl group. This is supported by the ap-

Table I. ^{13}C-NMR spectral properties of compounds **1**–**8***.

C#	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	33.4	31.9	28.5	154.2	33.4	33.4	33.4	33.4
2	37.3	30.3	27.3	127.3	37.4	37.4	37.4	37.4
3	216.2	78.7	76.9	203.1	216.5	216.5	216.5	216.4
4	50.1	40.4	39.4	46.3	50.2	50.2	50.2	50.2
5	48.5	47.1	40.9	46.8	48.5	48.4	48.4	48.3
6	21.2	21.0	20.8	21.9	21.3	21.4	21.4	21.3
7	26.0	25.7	25.8	26.1	25.9	26.0	26.0	25.9
8	47.4	47.4	47.4	46.4	47.9	47.5	47.3	47.4
9	20.7	19.5	19.5	25.2	20.9	20.8	20.8	20.7
10	25.9	25.8	26.4	30.2	26.4	26.3	26.3	26.2
11	26.2	26.1	25.8	27.9	26.0	26.1	26.2	26.1
12	32.7	32.7	32.7	32.6	32.6	33.2	32.7	32.4
13	45.9	45.8	45.9	44.9	46.7	46.1	46.2	46.5
14	45.8	45.7	45.8	44.3	46.6	45.2	45.4	45.7
15	44.9	44.8	44.7	43.7	40.9	44.9	44.7	44.1
16	74.9	74.8	74.8	75.1	45.2	80.5	70.4	81.0
17	57.5	57.3	57.2	57.5	57.3	60.8	58.0	56.3
18	18.9	18.7	18.6	18.0	17.7	19.4	18.3	19.1
19	29.8	30.0	29.8	29.8	29.8	29.8	29.8	29.8
20	29.0	28.9	28.8	29.8	29.5	32.5	30.5	29.2
21	20.8	20.9	21.1	19.6	20.1	23.0	22.8	20.2
22	35.5	35.4	35.4	35.9	36.6	36.6	33.8	31.3
23	23.4	23.4	23.4	23.9	29.3	33.8	32.5	29.9
24	82.7	82.4	82.4	83.1	216.8	109.6	101.2	174.2
25	73.3	73.2	73.2	73.7	72.1	–	–	–
26^{+}	23.9	23.8	237	24.0	18.3	–	–	–
27^{+}	25.6	25.6	25.5	24.4	18.4	–	–	–
28	19.5	14.0	20.9	20.0	20.8	20.8	20.8	20.8
29	22.3	25.7	25.7	21.5	22.1	22.1	22.1	22.1
30	20.7	19.4	19.4	19.2	18.9	19.6	18.9	19.7
OCH_3	–	–	–	–	–	55.4	54.5	–

* 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.

pearance of a strong absorption bands at 1610 (double bond) and 1670 cm^{-1} in the IR spectrum and a UV absorption of 265.0 nm (Silverstein *et al.*, 1991). The EIMS gave the parent ion peak at *m/z* 454 analyzed for $C_{30}H_{46}O_3$ which is consistent with the proposed structure for **4** as 16, 24-epoxycycloart-1-en-25-ol (1, 2-didehydroargentatin B).

Compound **5** was obtained after reflux with hydrochloric acid. The EIMS gave *m/z* 456 analyzed for $C_{30}H_{48}O_3$. The ^{1}H-NMR demonstrated the absence of both carbinol methine proton signals of positions 16 and 24 in the range of δ 3.0–5.0. The ^{13}C-NMR spectrum showed the presence of only one carbinol methine proton signal at δ 72.1 assigned to position 25 and confirmed the loss of the epoxide linkage between positions 16 and 24 and the absence of any other hydroxylations. Two carbonyl signals were observed at δ 216.5 and 216.8, one of them has to be assigned to position 3, while the new one at δ 216.8 has to be assigned to position 16 or 24. The location of this carbonyl was excluded from position 16 and was confirmed to be at position 24 based on the appearance of *m/z* 369 $[M\text{-}C_4H_7O_2]^{+}$ and the strong peak at *m/z* 313 $[M\text{-}C_8H_{15}O_2]^{+}$. Thus, HCl reaction demonstrated an opening of the side chain epoxide ring followed by oxidation to produce 25-hydroxycycloartan-3, 24-dione.

Oxidation of argentatin B with cerium sulfate/H_2SO_4/aq. MeOH, produced an isomeric mixture. The analysis of the NMR spectral data of this mixture indicated that this reagent induced scission of 24–25 bond, followed by methoxylation of position-24 to give an isomeric mixture of 24-methoxy-25–27-trinorargentatin B. The EIMS gave *m/z* 428 $[M]^{+}$ analyzed for $C_{28}H_{44}O_3$. The two isomers were partially separated chromatographically, where **6** was obtained in a pure form and the remaining mixture was oxidized with pyridinium chlorochromate. It was noticed that **7** is completely oxidized to the corresponding lactone, **8**, 24-oxo-25–27-trinorargentatin B, leaving behind the remains of the unreacted **6**.

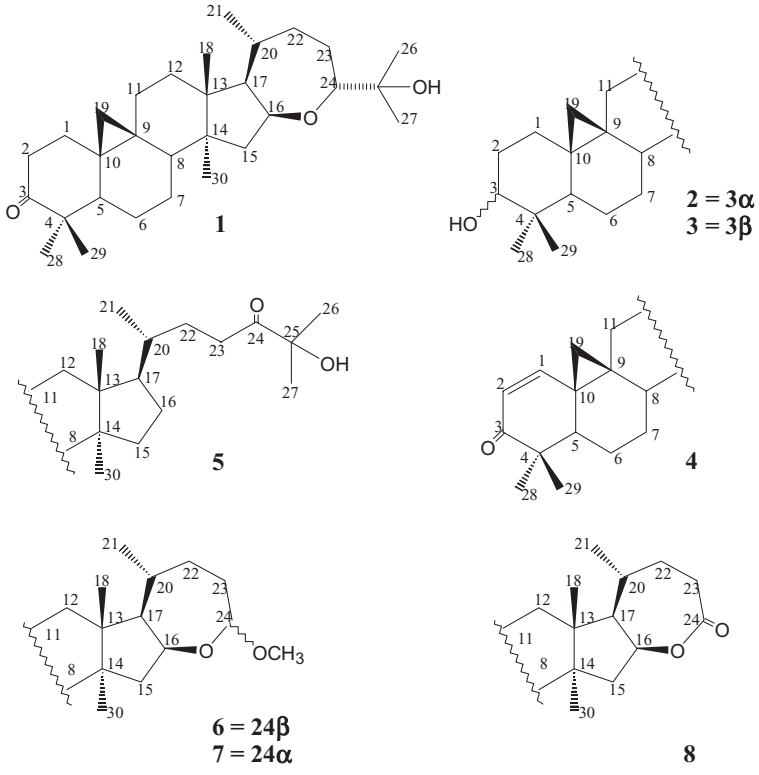

Fig. 1. The structure of argentatin B and its transformation products.

The ^{1}H-NMR spectral data of **6** displayed five signals at δ 0.92 (3H, s, H-18), 0.95 (3H, d, *J* = 7.0 Hz, H-21), 1.05 (3H, s, H-30), 1.11 (3H,s, H-28) and 1.19 (3H, s, H-29). This indicated the likely loss of the two skeletal methyl groups at positions-26 and 27. HETCOR showed that the proton multiplet at δ 4.09–4.20 (2H) was correlated to two methine (DEPT) carbon signals at δ 80.5 and 109.6 which were assigned to positions-16 and 24, respectively. The δ 109.6 chemical shift value is consistent for a deshielded position-24 as a result of another oxygenation. The proton singlet at δ 3.38 (3H) correlated to the carbon signal at δ 55.4 (HETCOR) was assigned to the methoxyl group at position-24. The relative stereochemistry of this methoxyl group at position-24 was determined to be β-oriented which inferred from the observed relatively high deshielding of the ^{13}C-NMR chemical shift of position-24 (δ 109.6) compared to that of **7** (δ 101.2). The overlapping of the proton signals of positions-16 and 24 made it difficult to add further confirmations. The ^{13}C-NMR data of **6** (Table I) were significantly affected with this transformation compared to that of the substrate (Komoroski *et al.*, 1986). This is evidenced only at rings-D and E, while rings A, B and C are not affected. The EIMS of **6** gave *m/z* 428 $[M]^+$, 413 $[M\text{-}CH_3]^+$ and 397 $[M\text{-}OCH_3]^+$, which are consistent with the proposed structure for **6**. The ^{1}H and ^{13}C-data for **7** was obtained by subtracting those of **6** from those of the isomeric mixture. The ^{1}H-NMR spectral data of **7** showed few differences from those of **6**. Positions-16 and 24 protons signals appeared at δ 4.57–4.68 as multiplet and were correlated (HETCOR) to the carbon signals at δ 70.4 and 101.2, respectively. The protons singlet at δ 3.31 integrated for three protons and was correlated to the methyl carbon signal (DEPT) at δ 54.5 which was assigned to position-24 methoxyl group. The relative stereochemistry of this methoxyl group was assigned to be α-oriented based on the relative shielding of position-24 carbon signal (δ 101.2), compared to that of **6** (δ 109.6). The overlapping of positions-16 and 24 anomeric proton signal made it impossible to get more confirmations.

The ^{1}H-NMR spectrum of **8** indicated the absence of the methoxyl group, since the proton signal at δ 3.31 was vanished. A downfield shifted single proton multiplet observed at δ 4.92, which was correlated to the carbon signal at δ 81.0, was assigned to position-16, while the anomeric position-24 carbon and proton signals were absent. A new downfield carbon signal at δ 174.2, in ^{13}C-NMR spectrum, was assigned to position-24-oxo group forming a lactone ring. The location of the lactone ring was also confirmed by its observed deshielding effect on H-16 (ca. δ 0.3) and C-16 (ca. δ 10.0). The IR spectrum showed a strong absorption band at 1680 cm^{-1} (lactone ring). The EIMS of **8** gave *m/z* 412 as a parent ion peak analyzed for $C_{27}H_{40}O_3$, which is consistent with the proposed structure for the oxidation product to be 24-oxo-25–27-trinorargentatin B.

Experimental

Instrumentation

Melting points are uncorrected. IR was conducted on Beckman Acculab I IR spectrometer. UV data were obtained from Beckman Model 26 Spectrophotometer. Optical rotations were measured on Autopole III Automatic Polarimeter (Rudolph Scientific, Fairfield, New Jersey). ^{1}H-NMR and ^{13}C-NMR were measured on a Bruker WM 250 NMR Spectrometer, at 250 MHz and 62.5 MHz, respectively, using $CDCl_3$ as a solvent and TMS as the internal standard. The chemical shifts (δ) are expressed in ppm. DEPT and HETCOR were measured on a Bruker WM 300 NMR Spectrometer. CIMS (CH_4) and EIMS (70 eV) were conducted on a Hewlett Packard 5988A Spectrometer, equipped with a Hewlett Packard RTE-6/VM data system.

Substrate material

Argentatin B was isolated from *Parthenium argentatum* × *P. tomentosa* and was characterized by ^{1}H-, ^{13}C-NMR and mass spectrometry (Rodriguez-Hahn *et al.*, 1970; Komoroski *et al.*, 1986 and Romo de Vivar *et al.*, 1990).

Fermentation methods

Microbial transformation studies were carried out by incubating the cultures, by shaking at 250 rpm, at 25°. Fermentation was carried out according to the standard two-stages fermentation protocol (Betts *et al.*, 1974). Preliminary screening experiments were carried out in 125 ml stainless steel capped DeLong 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 was adjusted to 7.0 using 6 N HCl before autoclaving for 20 m at 121° and 15 psi. After inoculation with *Nocardia corallina* var. *taoka* ATCC 31338, or *Mycobacterium species* B3683 NRRL or *Septomyxa affinis* ATCC 6737, stage I cultures were incubated at 27° and 250 rpm for 72 h before being used to inoculate stage II culture flasks. Usually, 10% inoculum volumes are recommended. For screening scale experiments 10 mg of the triterpene in 0.2 ml of DMF-EtOAc, 1:1 v/v, mixture was added to 24-h-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 × *g* for 1 min in a desk-top centrifuge. EtOAc extract of all samples were spotted on Si gel GF_{254} TLC plates, and developed in a suitable solvent system. All the chromatograms were visualized after spraying with 0.01% vanillin/H_2SO_4, followed by heating for 5–10 s with a heat gun.

Preparative scale conversion of argentatin B with Nocardia corallina (Reaction-A)

Five 2-liter stage II cultures received 2.0 g of argentatin B in 10 ml of DMF-EtOAc, 1:1 (1 mg substrate per ml of culture medium). After incubation for 10 days under the usual condition, the cultures were combined and exhaustively extracted with 3 × 1.5 liter of 10% MeOH/EtOAc. The ethyl acetate extract was concentrated and dried to yield 2.49 g of a dark brown residue.

Preparative scale conversion of argentatin B with Mycobacterium species (Reaction-B)

Seven 2-liter stage II cultures received 2.8 g of argentatin B in 14 ml of DMF-EtOAc, 1:1 (1 mg substrate per ml of culture medium). After incuba-

tion for 18 days under the usual condition, the cultures were combined and exhaustively extracted with 3 × 2 liter of 10% MeOH/EtOAc. The ethyl acetate extract was concentrated and dried to yield 3.9 g of a brown residue.

Preparative scale conversion of argentatin B with Septomyxa affinis (Reaction-C)

Ten 2-liter stage II cultures received 4.0 g of argentatin B in 20 ml of DMF-EtOAc, 1:1 (1 mg substrate per ml of culture medium). After incubation for 13 days at 250 rpm and 27°, the cultures were combined and exhaustively extracted with 3 × 3 liter of 10% MeOH/EtOAc. The extract was concentrated and dried to yield 5.2 g residue.

Isolation and purification of reactions A and B metabolites

The TLC indicated the presence of one major spot more polar than the substrate, in both reactions, at R_f = 0.50 (Si gel GF_{254}, hexane-EtOAc; 75:25). The crude extract of reactions A (2.49 g) and B (3.9 g) were, separately flash chromatographed, 200 g silica gel, 63–200 μ, 2.5 × 45 cm. The elution was adopted using EtOAc/hexane 500 ml each of 5%, 10%, 15%, 20%, 25%, 30% and 100%. Frs 100–200 ml each were collected and TLC investigated. Similar frs were pooled together. Frs eluted with 10–15% in both reactions gave 1.51 g and 1.72 g of recovered substrate, respectively. Frs eluted with 20% EtOAc/hexane gave **2** as needles (109 mg from reaction A and 256 mg from reaction B), respectively, after prep TLC on 1 mm-thick silica gel GF_{254} plates and using 30% EtOAc/hexane as a solvent.

Isolation and purification of reaction C metabolites

The TLC displayed two new reddish-brown spots at R_f = 0.52 and 0.50, respectively (Si gel GF_{254}, hexane-EtOAc; 75:25). The crude reaction mixture (5.2 g) was subjected to flash chromatography, silica gel, 400 g, 63–200 μ, 3.5 × 45 cm. The elution profile was EtOAc/hexane, 1000 ml each of 5%,10%, 15%, 20%, 25%, and 50%. Twenty four frs were collected, 200–300 ml each, and TLC investigated. Similar frs were pooled together. Frs eluted with 10–15% EtOAc/hexane gave 2.22 gm of recovered substrate. Frs eluted with 15–20% EtOAc/hexane gave 2.01 g residue which displaying 3 spots one of them is the substrate. This 2.01 g was subjected to MPLC, 140 g silica gel 15–25 μ, 2.5 × 45 cm. The elution was adopted using EtOAc/hexane 500 ml each of 5%, 10%, 15%, 20%, 25% and 50%. Frs 4–5 eluted with 10% EtOAc/hexane gave 59 mg of the substrate. Frs eluted with 15% EtOAc/hexane gave three different groups. Frs 8–10 afforded 96 mg of **2** as needles. Fr 7 gave 170 mg of **3** as needles. Fr 6 gave 38 mg of **4** which further purified on 1 mm-thick prep TLC silica gel GF_{254} plates, using 30% EtOAc/hexane as a solvent system. This gave 26 mg of **4** as yellowish gum. Compound **4** is strongly quenching under Uv light. Compounds **2**, **3** and **4** possess R_f = 0.5, 0.52 and .054 (25% EtOAc/hexane), respectively.

Sodium hydroxide reaction

Argentatin B, 0.50 g was refluxed with 50% methanolic NaOH, 50 ml, overnight. The reaction mixture was diluted with H_2O (200 ml) and extracted with EtOAc (3 × 150 ml) to give 0.42 g residue. The reaction product was subjected to c.c., 140 g silica gel 63–200 μ, 1.5 × 45 cm. The elution was adopted using EtOAc/hexane, 250 ml each of 5%, 10%, 15%, 20%, 25% and 50%. Frs eluted with 15–20% displayed 2 spots (R_f = 0.5 and 0.52 in 25% EtOAc/hexane). After prep TLC, on 1 mm-thick silica gel GF_{254} plates, it gave 93 mg and 52 mg of **2** and **3**, respectively.

Hydrochloric acid reaction

Argentatin B, 1.0 g, was dissolved in 50 ml MeOH and 30 ml conc. HCl was added. The mixture was refluxed overnight, then diluted with 300 ml H_2O and extracted with EtOAc, 3 × 300 ml, to give 0.82 g residue. The reaction product was subjected to c.c., 150 g silica gel 63–200 μ, 2.5 × 45 cm. The elution was adopted using 2.0 l 5% EtOAc/hexane, 1.0 l 10%, 1.0 l 15% and 1.0 l 20% v/v. Frs eluted with 10–15% gave 240 mg of recovered substrate. Frs eluted with 15–20% gave 503 mg of **5** as needles which was purified on 1 mm-thick prep TLC silica gel GF_{254} plates, using 30% EtOAc/hexane as a solvent (R_f = 0.53).

Cerium sulfate reaction

Argentatin B, 0.5 g, was dissolved in MeOH, 40 ml, then 2.0 g cerium sulfate was added, followed by 10 ml 1:1 H_2SO_4/H_2O. The reaction mixture was refluxed for four h where 100% conversion was observed. The reaction mixture was diluted with H_2O, 200 ml, and extracted with EtOAc, 3 × 300 ml, to give 0.44 g residue. The reaction product, 0.44 g, was subjected to c.c., 150 g silica gel 63–200 μ, 2.5 × 35 cm. The elution was achieved using 2000 ml 5% EtOAc/hexane and 1000 ml 10% EtOAc/hexane. Twelve frs were obtained. Frs 9–11 eluted with 10% EtOAc/hexane afforded 168 mg needles (R_f = 0.71, 30% EtOAc/hexane). By NMR this product proved to be an isomeric mixture of equal proportions of **6** and **7**. Partial chromatographic separation was obtained by c.c, 140 gm 63–200, 1.5 × 45 cm. The eluting solvent was 500 ml 0.5% *iso*-Pr-OH/hexane, 3000 ml of 1%, 500 ml 2%, 500 ml 5%, 250 ml 10% and 300 ml Me_2CO. Thirty frs were obtained (100–200 ml each). Frs 7–10 eluted with 1% *iso*-Pr-OH/hexane gave 62 mg of pure **6** as needles (R_f = 0.31, 2% *iso*-Pr-OH/hexane). Frs 4–6 eluted with 1% *iso*-Pr-OH/hexane gave 95 mg unresolved mixture. This isomeric mixture was dissolved in 5 ml CH_2Cl_2 and 200 mg of pyridinium chlorochromate was added. The reaction mixture was left at room temperature for four h where equilibrium was obtained. Water was added (50 ml) and the reaction mixture was extracted with EtOAc, 3 × 50 ml. The residue left after solvent evaporation was subjected to successive prep TLC on 1 mm-thick silica gel GF_{254} plates using 4% *iso*-Pr-OH/hexane then 20% Me_2CO/hexane as a solvent systems. This gave 20 mg of **6** as needles and 23 mg of **8** as needles (R_f = 0.32, 20% Me_2CO/hexane).

*Compound **2**, isoargentatin D, (–) 16, 24-epoxycycloartan-3α, 25-diol*

Needles, mp 148–149°, $\alpha[D]^{25}$, – 31.4° (CH_2Cl_2; c. 1.5). UV λ_{max} nm; 218.0. EIMS, 70 eV, *m/z* (rel. int.); 458 $[M]^+$ (8), 457 $[M\text{-}H]^+$ (20), 443 $[M\text{-}CH_3]^+$ (22), 441 (60), 440 (9), 424 (40), 423 (100), 400 (30), 381 (39), 341 (10), 260 (22), 233 (18), 203 (30), 202 (30), 161 (22) and 127 (52). ^{1}H-NMR (250 MHz, $CDCl_3$, (δ) ppm, *J* = Hz); 4.59 (1H, m, H-16), 3.58 (1H, dd, 5, 5, H-24), 3.28 (1H, dd, 3, 9, βH-3), 1.15 (3H, s, H-28), 1.09 (6H, s, H-26 and H-27), 0.97 (3H, s, H-29), 0.93 (3H, d, 7, H-21), 0.88 (3H,s, H-30), 0.82 (3H, s, H-18), 0.59 (1H, d, 5, H-19) and 0.34 (1H, d, 5, H-19′).

*Compound **3**, argentatin D, (–) 16, 24-epoxycycloartan-3β, 25-diol*

Needles, mp 225°. $\alpha[D]^{25}$, – 13.8° (CH_2Cl_2; c. 0.5). UV λ_{max} nm; 221.0. EIMS, 70 eV, *m/z* (rel. int.); 458 $[M]^+$ (5), 457 $[M\text{-}H]^+$ (12), 443 $[M\text{-}CH_3]^+$ (8), 441 (50), 439 (40), 424 (40), 423 (100), 399 (41), 382 (30), 381 (44), 313 (10), 233 (18), 201 (30), 175 (22), 149 (20) and 127 (42). ^{1}H-NMR (250 MHz, $CDCl_3$, (δ) ppm, *J* = Hz); 4.55 (1H, m, H-16), 3.56 (1H, dd, 5, 5, H-24), 3.44 (1H, br t, αH-3), 1.11 (3H, s, H-28), 1.07 (3H, s, H-26), 1.06 (3H, s, H-27), 0.92 (3H, s, H-29), 0.87 (3H, d, 7, H-21), 0.85 (3H,s, H-30), 0.83 (3H, s, H-18), 0.51 (1H, d, 5, H-19) and 0.31 (1H, d, 5, H-19′).

*Compound **4**, (–) 16, 24-epoxycycloartan-1-en-25-ol-3-one*

Yellow gum, $\alpha[D]^{25}$, – 45° (CH_2Cl_2; c. 1.0). IR υ_{max}^{cm-1}; 3410, 2940, 2910, 2860, 1670, 1610, 1460, 1340, 1270, 1160 , 1100, 1060 and 730. UV λ_{max} nm; 265.0. EIMS, 70 eV, *m/z* (rel. int.); 454 $[M]^+$ (2), 439 $[M\text{-}CH_3]^+$ (2), 436 $[M\text{-}H_2O]^+$, (40), 421 $[M\text{-}H_2O\text{-}CH_3]^+$, (2), 395 $[M\text{-}C_3H_7O]^+$, (2), 396 (3), 381 (4), 337 (2), 297 (5), 233 (6), 203 (11), 159 (23), 137 (25), 109 (29), 93 (33), 59 (100) and 42 (77). ^{1}H-NMR (250 MHz, $CDCl_3$, (δ) ppm, *J* = Hz); 5.94 (1H, d, 11, H-1), 6.77 (1H, d, 11, H-2), 4.59 (1H, m, H-16), 3.60 (1H, dd, 5, 5, H-24), 1.12 (3H, s, H-28), 1.11 (6H, s, H-26 and H-27), 1.10 (3H, s, H-29), 0.95 (3H,s, H-30), 0.92 (3H, d, 7, H-21), 0.88 (3H, s, H-18), 0.91 (1H, d, 5, H-19) and 0.74 (1H, d, 5, H-19′).

*Compound **5**, (–) 25-hydroxycycloartan-3, 24-dione*

Needles, mp 136–138°, $\alpha[D]^{25}$, – 4.2° (CH_2Cl_2; c. 1.5). IR υ_{max}^{cm-1}; 3390, 2930, 2850, 1710, 1340, 1100, 1010 and 740. UV λ_{max} nm; 228.0. EIMS, 70 eV, *m/z* (rel. int.); 456 $[M]^+$ (2), 441 $[M\text{-}CH_3]^+$ (4), 438 $[M\text{-}H_2O]^+$, (5), 423 (8), 369 $[M\text{-}C_4H_7O_2]^+$, (4), 313 $[M\text{-}C_8H_{15}O_2]$ (13), 311 (14), 285 (3), 271 (3), 245 (2), 219 (40), 173 (8), 133 (18), 105 (17),

71 (42), 54 (40), 43 (100) and 42 (33). ^{1}H-NMR (250 MHz, $CDCl_3$, (δ) ppm, *J* = Hz); 1.17 (3H, s, H-28), 1.12 (3H, s, H-29), 1.11 (3H, d, 7, H-21), 1.10 (3H, s, H-26), 1.09 (3H, s, H-27), 1.05 (3H,s, H-30), 0.91 (3H, s, H-18), 0.82 (1H, d, 5, H-19) and 0.57 (1H, d, 5, H-19′).

*Compound **6**, (+) 24-β methoxy-25–27-trinorargentatin B*

Needles, mp 162–164°, $\alpha[D]^{25}$, + 6.1° (CH_2Cl_2; c. 2.5). IR υ_{max}^{cm-1}; 3090, 2930, 2850, 1710, 1340, 1100, 1010 and 740. UV λ_{max} nm; 226.0. EIMS, 70 eV, *m/z* (rel. int.); 428 $[M]^+$ (2), 413 $[M\text{-}CH_3]^+$ (2), 397 $[M\text{-}CH_3O]^+$ (3), 381 (3), 363 (2), 311 (6), 290 (12), 258 (4), 219 (10), 159 (32), 133 (40), 121 (55), 107 (62), 71 (100), 55 (90) and 43 (75). CIMS (CH_4), *m/z* (rel. int.); 429 $[M+1]^+$ (7), 428 $[M]^+$ (18), 427 (14), 411 (20), 397 $[M\text{-}CH_3O]^+$ (100), 379 (85), 311 (11), 269 (7), 231 (12), 219 (14), 193 (27), 177 (26), 175 (32), 163 (12), 219 (14), 193 (27), 177 (26), 175 (35), 163 (12), 133 (10), and 85 (17). ^{1}H-NMR (250 MHz, $CDCl_3$, (δ) ppm, *J* = Hz); 4.09–4.20 (2H, m, H-16 and H-24), 3.38 (3H, s, H-24 methoxy), 1.19 (3H, s, H-28), 1.11 (3H, s, H-29), 1.05 (3H,s, H-30), 0.95 (3H, d, 7, H-21), 0.92 (3H, s, H-18), 0.83 (1H, d, 5, H-19) and 0.59 (1H, d, 5, H-19′).

*Compound **7**, 24-α methoxy-25–27-trinorargentatin B*

^{1}H-NMR (250 MHz, $CDCl_3$, (δ) ppm, *J* = Hz); 4.57–4.68 (2H, m, H-16 and H-24), 3.31 (3H, s, H-24 methoxy), 1.19 (3H, s, H-28), 1.11 (3H, s, H-29), 1.05 (3H,s, H-30), 0.95 (3H, d, 7, H-21), 0.92 (3H, s, H-18), 0.83 (1H, d, 5, H-19) and 0.59 (1H, d, 5, H-19′).

*Compound **8**, (–) 24-oxo-25–27-trinorargentatin B*

Needles, mp 191–192°, $\alpha[D]^{25}$, – 35.8° (CH_2Cl_2; c. 1.0). IR υ_{max}^{cm-1}; 2910, 2860, 1730, 1680, 1350, 1090, 1030 and 790. UV λ_{max} nm; 232.0. EIMS, 70 eV, *m/z* (rel. int.); 412 $[M]^+$ (10), 397 $[M\text{-}CH_3]^+$ (10), 311 (11), 275 (11), 274 (32), 259 (20), 219 (10), 193 (20), 173 (28), 133 (40), 91 (45), 67 (43), 55 (100), 43 (60) and 42 (80). ^{1}H-NMR (250 MHz, $CDCl_3$, (δ) ppm, *J* = Hz); 4.92 (H, m, H-16), 1.18 (3H, s, H-28), 1.14 (3H, s, H-29), 1.06 (3H,s, H-30), 1.00 (3H, d, 7, H-21), 0.95 (3H, s, H-18), 0.85 (1H, d, 5, H-19) and 0.61 (1H, d, 5, H-19′).

Dedication

This work is dedicated to the spirit of my late colleague Prof. Dr. Joseph J. Hoffmann, Southwest Center for Natural Products Research and Commercialization, Office of Arid Lands Studies, College of Agriculture, University of Arizona, USA.

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.

Komoroski R. A., Gregg E. C., Shockcer J. P., and Geckle J. M. (1986), Identification of guayule triterpenes by two-dimentional and multiple 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**, 519–523.

Maatooq G. T., and Hoffmann J. J. (2002), Microbial transformation of a mixture of argentatin A and Incanilin. Z. Naturforsch. **57 c**, 498–495.

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

Romo de Vivar A., Martinez-Vazquez M., Matsubara C., Prez-Sanchez and Joseph-Nathan P. (1990), Triterpenes in *Parthenium argentatum*, structures of argentatins C and D. Phytochemistry, **29**, 915–918.

Shi Q., Chen K., Fujiboka T. I., Kashiwada Y., Chang J., Kozoka M., Estes J. R., McPhail A. T., McPhail D. R., and Lee K. (1992), Antitumor agents, 135. Structure and stereochemistry of polacandrin, A new cytotoxic triterpene from *Polanisia dodecandra*. J. Nat. Prod. **55**, 1488–1497.

Silverstein R. M., Bassler G. C., and Morrill T. C. (1991), Spectrometric Identification of Organic Compounds, 5th ed. John Wiley & Sons, New York, pp. 115 & 303.

Williams D. E., Sinclair A. R. E., and Andersen R. J. (1992), Triterpene constituents of the dwarf birch, *Betula glandulosa*. Phytochemistry **31**, 2321–2324.