ISOLATION OF 11-HYDROXYLATED KAURANIC ACIDS FROM *ADENOSTEMMA LAVENIA*

PING C. CHENG[1], CHARLES D. HUFFORD[2] and NORMAN J. DOORENBOS[3]

Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Mississippi 38677

ABSTRACT.—Four 11-hydroxylated kauranic acids were characterized from *Adenostemma lavenia* (L.) O. Kuntze, namely: *ent*-11α-hydroxy-15α-acetoxykaur-16-en-19-oic acid (**1**), *ent*-11α, 15α-dihydroxykaur-16-en-19-oic acid (**2**), (16R)-*ent*-11α-hydroxy-15-oxokauran-19-oic acid (**3**), and *ent*-11α-hydroxy-15-oxokaur-16-en-19-oic acid (**4**).

Adenostemma lavenia (L.) O. Kuntze, a perennial herb of the Compositae family, is widely distributed in the temperate to tropical parts of Asia and the Pacific Islands. In Taiwan, according to folklore, the whole plant is used to treat lung congestion, pneumonia, edema and inflammation (1). In a phytochemical survey of some plants of North Borneo, Arthur (2) indicated positive tests for bitterness, alkaloids, and essential oils in the leaves of *A. laevenia*.

A sample of *A. lavenia* was extracted with hexane and then ethanol. The concentrated ethanolic extract was partitioned between chloroform and water. From the chloroform extract and the interfacial solids, seven acidic substances were separated by chromatography. Characterization of four of these as 11-hydroxylated kauranic acids **1–4** is reported in this paper. The other three will be discussed in a separate paper.

The spectral data of **1** suggested it was a carboxylic acid with a hydroxyl, an acetyl, a terminal methylene, and two tertiary methyl groups. The molecular formula $C_{22}H_{32}O_5$ obtained from the high resolution mass spectrum, elemental analysis, and previous chemotaxonomic studies of the diterpenoids of Compositae suggested that **1** could be an *ent*-kaurenic acid derivative (3, 4).

The presence of a carboxylic acid was confirmed by the conversion of **1** into its methyl ester **9**. The hydroxyl group was oxidized by Jones reagent (5) to the corresponding ketone **12**, thus demonstrating that it was a secondary alcohol. Hydrolysis of **1** with 5% ethanolic KOH gave a diol identical with the natural **2**. Both **1** and **2** yielded the same diacetate **5** upon acetylation. Catalytic isomerization (6, 7) of **2** gave a product identical with the natural **3**. The formation of **3** from **2** suggested that the 15-hydroxyl group in **2** was *ent* α and axial (7, 8). The fact that **2** contained an allylic secondary hydroxyl function was also confirmed by oxidation with active manganese dioxide (9). The resulting ketone **4** was found to be identical with the natural **4**. The ketone **4** showed uv λmax 235 nm (ε5,426) and cd $[\theta]_{345}$ −1,764 in accordance with what would be expected for a cyclopentenone structure.

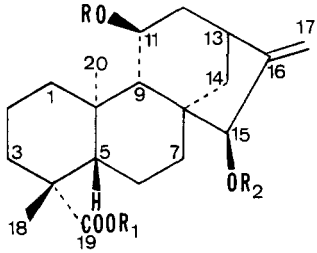
While this work was in progress, Tanaka and his co-workers (10, 11) reported the application of ^{13}C nmr to elucidate the structures of the diterpenoid glycosides of *Stevia*. Two of the aglycones of these glycosides had properties similar to those of **2** and **4**. An authentic sample of one of these aglycones was obtained and was

[1]Present address: Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi 38677.

[2]To whom inquiries should be addressed.

[3]Present address: College of Science, Southern Illinois University at Carbondale, Carbondale, Illinois 62901.

found to be identical (ir, tlc, mp, and mmp) with **2**. Since the interrelationships of **1**, **2**, **3**, and **4** have been clearly established and since **2** has been compared with an authentic sample, the structures for **1**, **3**, and **4** are also established. The diterpene acids **2**, **3**, and **4** have been reported from *Eupatorium album* (12) and more recently from *Adenostemma caffrum* (13). Compounds **3** and **4** have also been reported from the fern *Pteris disper* (14). The properties as reported in these references for **2**, **3**, and **4** agreed with those reported here.



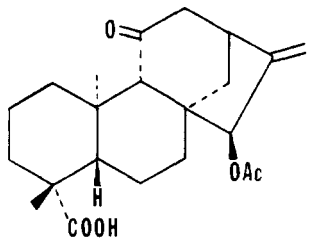
1 R=R1=H, R2=Ac

2 R=R1=R2=H

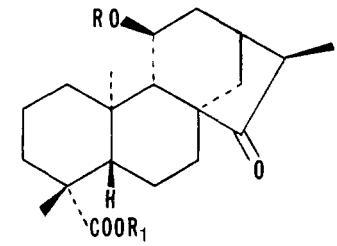
5 R=R2=Ac, R1=H

8 R=H, R1=CH3, R2=Ac

9 R=R2=H, R1=CH3



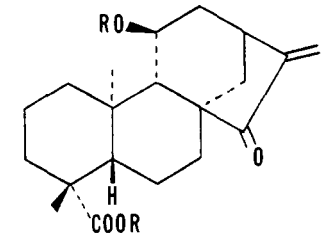
12



3 R=R1=H

6 R=Ac, R1=H

10 R=H, R1=CH3



4 R=R1=H

7 R=Ac, R1=H

11 R=H, R1=CH3

EXPERIMENTAL

PLANT MATERIAL.—The plant material was collected in Chiayi, Taiwan, during August, 1973. A voucher specimen identified by Mr. Muh-Tsuen Kao (curator, Herbarium of National Taiwan University) was placed in the Herbarium, Department of Pharmacognosy, School of Pharmacy, University of Mississippi. The plant material was air dried and milled to a coarse powder.

EXTRACTION AND FRACTIONATION.—The whole plant (6.0 kg) was extracted by percolation first with hexane and then with 95% ethanol. Concentration of the ethanolic extract gave a semisolid (250 g). A portion of this (100 g) was partitioned between chloroform and water (2 liters each) to obtain the chloroform-soluble fraction (45 g), water solubles (freeze-dried, 24 g), and an interfacial solid (5 g).

A 12 g sample of the chloroform-soluble fraction was mixed with 20 g silica gel (Grade II, 70–230 mesh) and chromatographed over silica gel (Grade I, 70–230 mesh) (600 g). The column was eluted with hexane-benzene-ethyl ether (1:1:1) [fractions 1–133 (20 ml each)] followed by benzene-chloroform (1:1) [fractions 134–151 (20 ml each)].

ISOLATION AND IDENTIFICATION OF ENT-11α-HYDROXY-15α-ACETOXYKAUR-16-EN-19-OIC ACID (**1**).—Fractions 73–80 were combined and concentrated to dryness and the solid crystallized

from benzene to give colorless crystals of **1** (205 mg), mp 244–5°; $[\alpha]^{25}D-111°$ (c 0.90, chloroform); uv: λmax 204 nm (ε4,546); ir: νmax (chloroform) 3600, 1745, 1700, 1655, and 1240 cm^{-1}; pmr ($CDCl_3$): δ0.88 (s, H-20), 1.26 (s, H-18), 2.20 (s, acetyl), 2.70 (m, H-13), 3.80 (m, H-11), 4.90 (t, H-15, $J=2.5$ Hz), 5.10 (t, H-17, $J=2.5$ Hz), and 5.23 (t, H-17, $J=2.5$ Hz); ms: *m/e* 376 (M^+, 4%), 358 (12%), 334 (32%), 316 (100%).

Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57. Found: C, 70.07; H, 8.60.

ISOLATION AND IDENTIFICATION OF ENT-11α, 15α-DIHYDROXYKAUR-16-EN-19-OIC ACID (**2**).—Recrystallization of the solid obtained from fractions 82–86 from ethanol and benzene (20:1) gave colorless crystals of **2** (175 mg), mp 229–230°; $[\alpha]^{25}D-73.5°$ (c 1.47, chloroform); uv: λmax 208 nm (ε4,555); ir: νmax (KBr) 3280, 1700, 1650, and 1450 cm^{-1}; pmr (deuterated pyridine): δ1.15 (s, H-20), 1.35 (s, H-18), 2.60 (m, 2H), 4.1–4.2 (m, H-11, H-15), 5.16 (m, H-17), 5.40 (m, H-17), and 6.0–7.0 (m, 3H, exchangeable); ms: *m/e* 334 (M^-, 6%), 316 (74%), 301 (18%), 298 (12%), 270 (24%), 148 (44%), and 83 (100%).

Anal. Calcd for $C_{20}H_{30}O_4$: C, 71.81; H, 9.04. Found: C, 71.72; H, 9.02.

A comparison of the ir, tlc, mp, and mmp of **2** with an authentic sample (10, 11) showed that they were identical.

ISOLATION AND IDENTIFICATION OF (16R)-ENT-11α-HYDROXY-15-OXOKAURAN-19-OIC ACID (**3**).—Fractions 134–136 were combined and concentrated to dryness and the solid crystallized from ethanol and benzene (20:1) to give colorless needles of **3** (35 mg), mp 236–7°, $[\alpha]^{25}D-95°$ (c 1.21, methanol); cd $[\theta]_{309}=-1870$; ir: νmax (KBr) 3440, 3270, 1725, 1704, and 1160 cm^{-1}; pmr (deuterated pyridine): δ1.13 (s, H-20), 1.26 (s, H-18), 1.56 (d, H-17, $J=6$ Hz), 4.10 (d, H-11, $J=6$ Hz), 7.5–8.5 (m, 2H, exchangeable); ms: *m/e* 334 (M^-, 2%), 319 (63%), 316 (18%), 288 (43%), 273 (40%), 215 (40%), and 74 (100%).

Anal. Calcd for $C_{20}H_{30}O_4$. Found: C, 71.69; H, 9.02.

ISOLATION AND IDENTIFICATION OF ENT-11α-HYDROXY-15-OXOKAUR-16-EN-19-OIC ACID (**4**).—Recrystallization of the solid from fractions 139–151 from ethanol and benzene (20:1) gave colorless crystals of **4** (104 mg), mp 272° (d.); $[\alpha]^{25}D-132°$ (c 1.0, methanol); uv: λmax 235 nm (ε5,425.8); cd: $[\theta]_{345}-1764$, $[\theta]_{242}+3041$, $[\theta]_{213}-13308$; ir: νmax (chloroform) 3260, 2525, 1735, 1700, 1650, 1265, and 940 cm^{-1}; pmr ($CDCl_3$): δ0.95 (s, H-20), 1.30 (s, H-18), 3.10 (m, H-13), 4.07 (m, H-11), 5.25 (s, H-17), and 5.85 (s, H-17); ms: *m/e* 332 (M^-, 3%), 317 (50%), 314 (21%), 299 (2%), 286 (32%), 164 (48%), and 78 (100%).

Anal. Calcd for $C_{20}H_{28}O_4$: C, 72.26; H, 8.49. Found: C, 71.50; H, 8.80.

HYDROLYSIS OF **1** TO **2**.—A 50 mg sample of **1** was hydrolyzed by refluxing (15 hr) in 5% ethanolic KOH (10 ml) solution. The solution, after neutralizing with HCl, was extracted with ether. The residue from the evaporated ether extract was crystallized from ethanol and benzene (20:1) to obtain colorless crystals (35 mg) mp 215–6°. After recrystallization from benzene (mp 228–9°), the product was found to be identical with **2** (mp, mmp, ir, and tlc).

CATALYTIC ISOMERIZATION OF **2** TO **3**.—A 50 mg sample (**2**) in 10 ml ethanol was stirred with 10% palladium-charcola in a hydrogen atmosphere (one atmosphere pressure, room temperature) for one hour. The catalyst was then filtered and the solvent removed. Recrystallization of the product from ethanol gave colorless needles (30 mg), mp 238–240°. The product was identical in ir, tlc, mp, and mmp with **3**.

MANGANESE DIOXIDE OXIDATION OF **2** TO **4**.—Freshly prepared MnO_2 (50 mg) was added to 50 mg of **2**, dissolved in 5 ml acetone (9). The mixture was stirred at room temperature overnight. After filtration and evaporation, the residue was chromatographed through a silica gel column (hexane-benzene-ether 1:1:1) to obtain 8 mg of white crystals, mp 270° (d.). The product was identical with **4** (ir, tlc, mp, and mmp).

PREPARATION OF ACETATES **5**, **6**, AND **7**.—The acetates were prepared by reacting the hydroxylated kauranic acids with acetic anhydride in pyridine. Both **1** and **2** (50 mg each) yielded the same acetate **5** (45 mg and 50 mg respectively), mp 236–7° (hexane), $[\alpha]^{25}D-104.6°$ (c 1.3, chloroform); ir: νmax (chloroform) 1730 and 1260 cm^{-1}; pmr ($CDCl_3$): δ0.90 (s, H-20), 1.25 (s, H-18), 1.92 (s, acetyl), 2.18 (s, acetyl), 2.68 (s, H-13), 4.75–5.06 (m, 3H), and 5.20 (t, H-15, $J=2.5$ Hz), ms: *m/e* 418 (M^-, 6%), 376 (10%), 358 (40%), 316 (100%), 298 (75%), and 283 (40%).

Anal. Calcd for $C_{24}H_{34}O_6$: C, 68.87; H, 8.20. Found: C, 68.73; H, 8.39.

Acetylation of **3** (50 mg) gave **6** (45 mg) mp 230–2° (ethanol); ir: νmax (KBr) 1740 and 1240 cm^{-1}; pmr ($CDCl_3$) δ0.98 (s, H-20), 1.27 (s, H-18), 1.16 (d, $J=6$ Hz, H-17), 1.95 (s, acetyl), and 5.10 (m, H-11); ms: *m/e* 376 (M^-, 1%), 345 (8%), 316 (24%), 310 (15%), 295 (100%), 243 (15%), 150 (32%), and 105 (36%).

Anal. Calcd for $C_{22}H_{32}O_5$: C, 70.19; H, 8.57. Found: C, 69.91; H, 8.54.

Acetylation of **4** (50 mg) gave **7** (47.5 mg) mp 239–240° (ethanol); $[\alpha]^{25}D-139.7°$ (c 1.45, chloroform). The ir, pmr, and ms spectra of **7** were similar to those reported (12).

Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.07. Found: C, 70.52; H, 7.98.

PREPARATION OF METHYL ESTERS **8**, **9**, **10**, AND **11**.—Methylation of 50 mg of **1** with diazomethane and recrystallization of the product from ether furnished pure **8** (51 mg) mp 117–8°, $[\alpha]^{24}D$ −97.7° (c 1.7, chloroform), ir: νmax (chloroform): 1730, 1220, 1170 and 1150 cm^{-1}; pmr ($CDCl_3$): 0.76 (s, H-20), 1.20 (s, H-18), 3.75 (s, methoxyl), 5.03 (m, H-15), 5.23 (m, H-17), 5.36 (m, H-17); ms: *m/e* 390 (M+, 5%), 372 (15%), 348 (35%), 339 (100%), 313 (45%), 312 (30%), 281 (50%), and 280 (30%).

Anal. Calcd for $C_{23}H_{24}O_5$: C, 70.72; H, 8.78. Found: C, 70.60; H, 8.76.

Methylation of **2** (50 mg) afforded **9** (45 mg) mp 160–2° (ethanol); reported mp 161–3° (11) and mp 125–7° (12). Methylation of **3** (50 mg) afforded **10** (47 mg) mp 202–3° (ethanol), reported mp 195–7° (12). Methylation of **4** (50 mg) gave 45 mg of methyl ester **11**, mp 153–4° (ethanol); ir: νmax 1730, 1240–1210, and 1165 cm^{-1}; pmr: δ0.92 (s, H-20), 1.25 (s, H-18), 2.67 (m, H-13), 3.73 (s, methoxyl), 4.10 (m, H-11), 4.68 (d, H-17, J=3 Hz), and 4.80 (t, H-17, J=3 Hz).

PREPARATION OF **12**.—An acetone solution of **1** (98 mg) was stirred in an ice bath and four drops of Jones reagent (5) were added. After one minute, 5 ml of methanol was added to destroy the excess reagent. The reaction mixture was extracted with ether. The solvent was evaporated to give 80 mg of white residue. The reaction product was purified through a silica gel column (hexane-benzene-ether 1:1:1) to yield the ketone **12**, 71 mg, mp 224–5° (ethanol); λmax 216 nm (ε=993), and 300 nm (ε=75); $[\theta]_{309}$+6,370; ir νmax (chloroform): 1210 and 1700 cm^{-1}; pmr ($CDCl_3$): δ0.98 (s, H-20), 1.27 (s, H-18), 2.15 (s, acetyl), 2.50 (s, H-9), 2.57 (d, J= 12 Hz, H-14a), 2.93 (s, H-13), 4.95 (m, H-15), 5.08 (d, J=3 Hz, H-17), 5.25 (t, J=3 Hz, H-17), and 11.5 (m, 1H, exchangeable); ms: *m/e* 374 (M^+, 6%), 331 (80%), 315 (32%), and 229 (100%).

Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.07. Found: C, 70.23; H, 8.50.

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