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NEW KAURANE DITERPENOIDS FROM *ASTER TONGOLENSIS*

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ABSTRACT.—The MeOH-Et_2O (1:1) extract of the aerial parts of *Aster tongolensis* gave one known (**1**) and three novel (**2–4**) kaurane diterpenoids. Structures were established by spectral analyses (ir, ms, ^1H and ^{13}C nmr) and chemical transformation. The taxonomic significance of kaurane diterpenes is discussed in brief.

In a previous paper (1), we reported the phytochemical investigation of *Aster poliothamnus*, which is used frequently in traditional Chinese medicine to treat fever, influenza, etc. However, very little is known about the chemical constituents of *Aster tongolensis* Franch., a desirable substitute for *A. poliothamnus* especially in Tibet. Continuing our characterization of terpenoids from the Compositae (2–4), the above observation provided impetus for chemical study of the title species to reveal whether these two plants are chemically related. The results are discussed in this article.

RESULTS AND DISCUSSION

Cc of the MeOH-Et_2O (1:1) extract of the aerial parts of *A. tongolensis* afforded fractions showing red/purple spots by tlc after spraying with H_2SO_4 followed by heating. Further purification of these fractions by filtration over activated charcoal, repeated cc and preparative tlc led to isolation of eight constituents. Four of these were identified as β-sitosterol, α-amyrin, β-amyrin, and shionone by direct comparison with the literature data using ir, ms, and ^1H and/or ^{13}C nmr (5–8). Structures of the four isolated diterpenoids were established by spectrometric interpretation and chemical transformation as 16β,17-dihydroxykauran-18-oic acid [**1**], 16β-hydroxy-17-acetoxykauran-18-oic acid [**2**], 16β,17-dihydroxykauran-18-oic acid 18-*O*-β-D-glucopyranoside [**3**], and 16β-hydroxy-17-acetoxykauran-18-oic acid 18-*O*-β-D-glucopyranoside [**4**].

A molecular formula of $C_{20}H_{32}O_4$ was inferred for compound **1** by eims, which displayed a weak molecular ion at *m/z* 336, and from its elemental analysis. The ^{13}C-nmr spectrum of **1** and DEPT experiments showed 20 resonance lines consisting of two methyl, ten methylene (an oxygen-bearing C at δ 69.5), three methine, and five quaternary carbons (an oxygenated C at δ 79.7). As no carbon-carbon unsaturated bond was indicated by ^{13}C nmr, the five degrees of unsaturation of **1** were assigned to a tetracyclic system with a carboxylic acid. The ^1H-nmr spectrum (Table 1) displayed two methyl singlets at δ 1.19 and 0.98, and a pair of doublets (J=11.3 Hz) at δ 3.43 and 3.32, indicating that it was most likely a kaurane diterpene acid possessing a tertiary hydroxy group at C-16 and a hydroxymethyl function at C-17 (9–12). Comparison between the ^{13}C-nmr spectral data of compound **1** and those of 16α,17-dihydroxykauran-18-oic acid (13) revealed that the chemical shifts of C-1 to C-12, C-14 to C-16, and C-18 to C-20 were nearly identical ($\Delta\delta \leq 0.5$ ppm, Table 2). This ^{13}C-nmr comparison supported the proposed ring system with a carboxylic acid function on C-18 and reinforced the above proposed C-16,-17 diol unit. Nevertheless, the most striking

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TABLE 1. ^{1}H-nmr Spectral Data of Compounds **1–4**.[a]

Proton	Compound 1	Compound 2	Compound 3	Compound 4	Multiplicity
H-17	3.43	4.06	3.45	4.08	d
H-17′	3.32	3.88	3.31	3.90	d
H-19	1.19	1.23	1.18	1.21	s
H-20	0.98	0.95	0.94	0.95	s
OAc	—	2.11	—	2.09	s
H-1′	—	—	6.18	6.20	d
H-2–H-6′ (6H)	—	—	3.50–4.30	3.55–4.40	m

[a]J (Hz): $J_{17,17'}=11.3$, $J_{1',2'}=8$.

difference in the δ shift values of C-13 and C-17 could be explained by assuming that compound **1** was 16β,17-dihydroxykauran-18-oic acid, previously isolated from *Helianthus petiolaris* (Compositae) (12). For further confirmation, **1** was treated with CH_2N_2 to yield its methyl ester, whose ^{1}H-nmr spectral data were consistent with those in the literature (12).

With the structure of diterpene acid **1** in hand, the structures of the related novel diterpene acids **2–4** were readily elucidated. Compound **2**, isolated as a white gum, was less polar than acid **1**. The molecular formula $C_{22}H_{34}O_5$ of **2** was indicated by its

TABLE 2. ^{13}C-nmr Spectral Data of Compounds **1–4**.

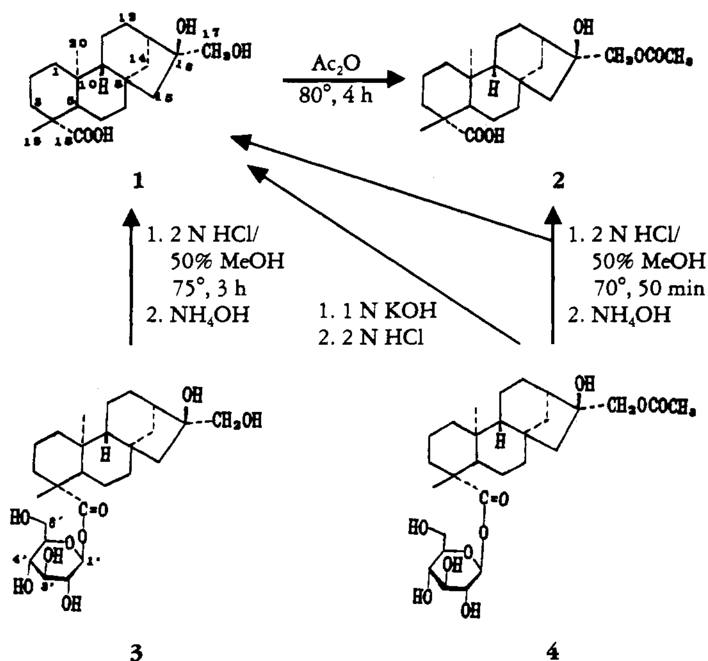
Carbon	1	2	3	4	DEPT
C-1	41.3[a]	41.2[a]	41.4[a]	41.2[a]	CH_2
C-2	18.9[b]	19.0[b]	19.1[b]	19.2[b]	CH_2
C-3	38.0	38.0	38.1	38.1	CH_2
C-4	43.5[c]	43.7[c]	43.4[c]	43.5[c]	C
C-5	56.9[d]	56.8[d]	56.4[d]	56.7[d]	CH
C-6	21.7[b]	21.6[b]	21.5[b]	21.6[b]	CH_2
C-7	40.8[a]	40.7[a]	40.6[a]	40.8[a]	CH_2
C-8	43.4[c]	43.5[c]	43.7[c]	43.7[c]	C
C-9	56.3[d]	56.4[d]	57.0[d]	57.1[d]	CH
C-10	39.4	39.3	39.5	39.6	C
C-11	19.0[b]	19.1[b]	19.2[b]	19.3[b]	CH_2
C-12	26.8	26.5	27.0	26.9	CH_2
C-13	40.1	41.1	41.2	41.3	CH
C-14	37.5	37.8	37.9	37.8	CH_2
C-15	52.4	52.8	53.0	52.9	CH_2
C-16	79.7	79.5	79.8	79.6	C
C-17	69.5	70.9	70.3	70.6	CH_2
C-18	179.3	179.5	176.1	176.4	C
C-19	29.0	28.7	28.5	28.8	Me
C-20	15.1	15.3	15.2	15.2	Me
Ac	—	170.8	—	170.9	C
Ac	—	20.4	—	20.1	Me
C-1′	—	—	95.5	95.6	CH
C-2′	—	—	73.6	73.7	CH
C-3′	—	—	79.1	79.0	CH
C-4′	—	—	70.9	70.5	CH
C-5′	—	—	78.9	78.8	CH
C-6′	—	—	61.7	62.0	CH_2

[a–d]Interchangeable assignments within the same column.

elemental analysis. The ir absorption bands were characteristic for carboxyl and acetoxy groups. The eims of **2** was similar to that of **1** and exhibited the highest discernable ion at *m/z* 336, produced presumably by loss of ketene from the molecular ion. Comparison of the ^{1}H- and ^{13}C-nmr spectra of **2** with those of **1** indicated that **2** was an acetate of **1** (Tables 1 and 2). Furthermore, the presence of a C-17 acetoxy group was shown by the carbon resonance lines at δ 20.4 (Me) and 170.8 (carbonyl), and proton signals at δ 2.11 (3H, s, OAc), 4.06 (1H, d, J=11.3 Hz, H-17), and 3.88 (1H, d, J=11.3 Hz, H-17′) moved downfield by ca. 0.6 ppm from those of **1**. Compound **2** (16β-hydroxy-17-acetoxykauran-18-oic acid) was confirmed by conversion of **1** to its acetate.

Compound **3**, a white amorphous powder, was more polar than **1** and **2**. Its fabms gave intense quasi-molecular ions at *m/z* 521 [M+Na]$^{+}$ and 505 [M+Li]$^{+}$. Its molecular formula $C_{26}H_{42}O_9$ was deduced by elemental analysis. The ir spectrum of **3** indicated hydroxy and ester groups. Its ^{1}H-nmr spectrum exhibited, in addition to two methyl singlets and a pair of hydroxymethyl doublets, a downfield anomeric proton doublet (J=8 Hz) and a heavily overlapped six-proton multiplet in the δ 3.50–4.30 region (Table 1). These data indicated that compound **3** was a kaurane-type diterpene carboxylic acid glycoside. The proposed structure was confirmed by the DEPT experiments and the ^{13}C-nmr spectrum in which an oxygenated methylene signal at δ 61.7, an anomeric carbon resonating at δ 95.5, and four methinoxy resonances at δ 79.1, 78.9, 73.6, and 70.9 could be readily assigned to a β-D-glucopyranosyl group (13,14) connecting through an ester linkage to the aglycone, which gave in total 20 carbon signals closely similar to those of **1** (Δδ≤0.6 ppm except for C-18 chemical shift, Table 2). On the other hand, hydrolysis of **3** with 2 N HCl/50% MeOH liberated compound **1** and β-D-glucopyranoside (Scheme 1). Thus, compound **3** was 16β,17-dihydroxykauran-18-oic acid 18-*O*-β-D-glucopyranoside.

A molecular formula of $C_{28}H_{44}O_{10}$ for **4** was disclosed by elemental analysis consistent with quasi-molecular ions at *m/z* 563 [M+Na]$^{+}$ and 547 [M+Li]$^{+}$ as



SCHEME 1

determined by fabms. The ir spectrum of **4** showed a pair of strong ester carbonyl bonds at 1744 and 1728 cm^{-1} and a broadened hydroxyl absorption band centered at 3400 cm^{-1}. Its ^1H-nmr spectrum differed from that of **2** by the presence of an anomeric proton doublet (J=8 Hz) at δ 6.20 coexistent with an overlapping six-proton multiplet in the range of δ 3.55–4.40. Moreover, the ^1H-nmr spectrum of **4** differed from that of **3** by the presence of an acetate singlet at δ 2.09 together with the methylenoxy doublets at δ 4.08 and 3.90, both shifted ca. 0.6 ppm downfield relative to those of **3** (Table 1). Furthermore, except for resonance lines at δ 170.9 (carbonyl) and 20.1 (Me), most carbon signals in the ^{13}C-nmr spectrum were close to those of **3**. The above findings led to the conclusion that compound **4** was 16β-hydroxy-17-acetoxykauran-18-oic acid 18-*O*-β-D-glucopyranoside. For further confirmation of the proposed structure, compound **4** was hydrolyzed with 2 N HCl/50% MeOH. Products were identified as compounds **1**, **2**, and β-D-glucopyranoside as well as unreacted starting material (Scheme 1). Treatment of compound **4** with 1 N KOH for 1 h at room temperature gave a transparent yellowish solution which afforded, upon neutralization with 2 N HCl, a white precipitate identical with compound **1**.

The present chemical investigation of *A. tongolensis* confirms that some constituents are identical with those of *A. poliothamnus* whereas the glycosides they contain are quite different (1). To our surprise, *A. tongolensis* was a rich source of kaurane-type diterpene acid derivatives, which are relatively rare in *Aster* species (15). However, triterpenoids and steroids are more commonly found as constituents of the *Aster* genus (1, 16–18) than are mono-, sesqui- and diterpenes (15, 18–22).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a 5DX-FT IR spectrometer; mass spectra were taken on a ZAB-HS mass spectrometer. Except for DEPT experiments carried out at 126 MHz on a Bruker AM-500 spectrometer, ^1H-nmr measurements were performed at 80 MHz and ^{13}C at 19.8 MHz on a Bruker AC-80 instrument [chemical shifts reported in δ (ppm) units downfield from the internal standard TMS (δ=0) with the samples dissolved in $CDCl_3$ containing a few drops of DMSO-d_6]. Si gel (200–300 mesh) for cc and Si GF_{254} (10–40 μ) for tlc were obtained from Qingdao Marine Chemical Factory, People's Republic of China. Tlc spots (bands) were visualized by the uv lamp and/or spraying with 22% H_2SO_4 followed by heating.

PLANT MATERIAL.—The aerial parts of *A. tongolensis* were collected in July 1992 in Zhang County, Gansu Province, and were identified by Prof. X. Pan (Lanzhou Medical College). A specimen is preserved at the Department of Pharmacy, Lanzhou Medical College, Lanzhou 730000, People's Republic of China.

EXTRACTION AND ISOLATION.—The powdered, air-dried plant material (800 g) was extracted twice for a two-day period with Et_2O-MeOH (1:1) by cold percolation. The residue (ca. 50 g) obtained after evaporation of solvent from the combined extracts was refluxed with 350 ml MeOH until the residue was completely dissolved. The solution was cooled to room temperature and kept at −5° for 30 h. The yellowish waxy precipitate that formed was filtered, and the filtrate was concentrated to a black gum (24 g). The gum was subjected to Si gel (800 g) cc using petroleum ether-Et_2O (9:1↦1:10) followed by Et_2O and Et_2O-MeOH (100:3↦7:8). Cc eluates (300 ml each) were, according to their tlc patterns, combined into five fractions: F-1 (3.4 g), F-2 (4.2 g), F-3 (4.1 g), F-4 (8.7 g), and F-5 (1.3 g). F-1 dissolved in 30 ml of EtOAc afforded a yellowish amorphous powder. Preparative tlc of the powder using petroleum ether-Et_2O (5:1) (developed twice) gave shionone (R_f 0.51), β-sitosterol (R_f 0.27), and β-amyrin (R_f 0.30). F-2 was chromatographed (100-ml fractions) through a Si gel column, eluted with solvents of increasing polarity from cyclohexane through EtOAc to provide α-amyrin [R_f 0.33, cyclohexane-EtOAc (6:1), developed twice) and β-sitosterol. F-3 was separated by Si gel cc utilizing a CH_2Cl_2/MeOH gradient into a mixture of no interest and a fraction containing compounds **1** and **2**. Acids **1** (R_f 0.61) and **2** (R_f 0.53) were separated by preparative tlc with CH_2Cl_2-MeOH-HCOOH (20:1:0.1) (developed twice). F-4 dissolved in 50 ml of Me_2CO-MeOH (1:1) was stirred with 5 g of activated charcoal at 60° for 45 min and filtered over a short Si gel (10 g) column to afford a yellowish transparent filtrate. The residue obtained from removal of the solvent of the filtrate was chromatographed by Si gel cc, with solvents of gradually increasing polarity, from CH_2Cl_2 through MeOH. Two fractions were collected (F-4-1 and F-4-2). Preparative tlc of F-4-1 with

CH_2Cl_2-MeOH-HCOOH (20:3:0.2) supplied compound **3** (153 mg, R_f 0.13). F-4-2 was rechromatographed through a Si gel column with a CH_2Cl_2/MeOH gradient containing 0.2% of HCOOH, which yielded compound **4** [276 mg, R_f 0.21, CH_2Cl_2-MeOH-HCOOH (20:3:0.2), developed twice]. Reaction of F-5 with spray reagents showed that it contained mainly flavonoid glycosides and saccharides.

16β-Hydroxy-17-acetoxykauran-18-oic acid [**2**].—A colorless gum: found C 69.74, H 9.01 ($C_{22}H_{34}O_5$ requires C 69.81, H 9.05); ir (KBr) 3400–2500 (broad), 1725, 1705, 1245, 1018 cm^{-1}; eims *m/z* $[M-ketene]^+$ 336 (0.4), $[336-HOAc]^+$ 318 (1.1), 305 (100), 287 (40), 259 (61); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

SYNTHESIS OF COMPOUND **2** FROM COMPOUND **1**.—Compound 1 (50 g) was refluxed for 4 h at 80° with Ac_2O. From the reaction mixture, compound **2** (gum, 42 mg) was isolated by preparative tlc with CH_2Cl_2-MeOH-HCOOH (20:1:0.1). The synthetic diterpene acid was identified by co-tlc, ir, eims, and ^1H nmr.

*16β,17-Dihydroxykauran-18-oic acid 18-*O*-β-*D*-glucopyranoside* [**3**].—An amorphous powder: found C 62.19, H 8.45 ($C_{26}H_{42}O_9$ requires C 62.26, H 8.50); ir (KBr) 3404, 1727, 1376, 1247 cm^{-1}; fabms *m/z* $[M+Na]^+$ 521 (79), $[M+Li]^+$ 505 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2 respectively.

ACID HYDROLYSIS OF COMPOUND **3**.—Compound **3** (50 mg) was stirred at 75° for 3 h with 2 N HCl/ 50% MeOH. The resulting mixture was adjusted to pH 7 with dilute NH_4OH and partitioned, after evaporation of MeOH under vacuum, with EtOAc. The organic phase was concentrated, and the residue was purified using preparative tlc with CH_2Cl_2-MeOH-HCOOH (20:1:0.1). A product (gum, 25 mg) was isolated and identified as compound **1** based on co-tlc, ir, and ^1H-nmr comparison. The aqueous layer was concentrated under vacuum to 5 ml, in which β-D-glucopyranoside was detected by pc employing C_5H_5N–C_6H_6–*n*-BuOH–H_2O (3:5:1:3) co-developed with authentic monosaccharide standards.

*16β-Hydroxy-17-acetoxykauran-18-oic acid 18-*O*-β-*D*-glucopyranoside* [**4**].—A white gum: found C 62.16, H 7.79 ($C_{28}H_{44}O_{10}$ requires C 62.20, H 7.83); ir (KBr) 3358, 1744, 1728, 1465, 1450, 1240 cm^{-1}; fabms *m/z* $[M+Na]^+$ 563 (67), $[Na+Li]^+$ 547 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

ACID HYDROLYSIS OF COMPOUND **4**.—Compound **4** (50 mg) was stirred at 70° for 50 min with 2 N HCl/ 50% MeOH. The reaction mixture was worked up and separated following the procedure described above for the acidic hydrolysis of compound **3**. This procedure resulted in the isolation of compound **1** (15 mg), compound **2** (8 mg), and β-D-glucopyranoside.

ALKALINE HYDROLYSIS OF COMPOUND **4**.—Compound **4** (50 mg) was stirred with 2 N KOH for 1 h at room temperature to form a transparent solution which, upon neutralization with 3 N HCl, gave a white precipitate identical with compound **1**, by co-tlc, ir, and ^1H nmr. The mother liquor was concentrated, and β-D-glucopyranoside was detected by direct pc comparison with monosaccharide samples using C_5H_5N–C_6H_6–*n*-BuOH–H_2O (3:5:1:3).

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