Journal of Natural Products
Vol. 58, No. 2, pp. 288-290, February 1995

A NEW EUDESMENOIC ACID FROM *ARTEMISIA PHAEOLEPIS*

R.X. TAN,* W.Z. WANG,

Institute of Biotechnology, Department of Biological Sciences and Technology, Nanjing University, Nanjing 210093, People's Republic of China

L. YANG,

National Laboratory of Applied Organic Chemistry at Lanzhou University, Lanzhou 730000, People's Republic of China

and J.H. WEI

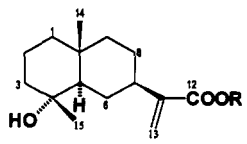
State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing 210093, People's Republic of China

ABSTRACT.—A new eudesmenoic acid **2**, along with a number of known compounds, was isolated from the aerial parts of *Artemisia phaeolepis*, with the structure of **2** determined by spectroscopic methods.

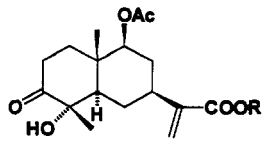
In Tibet, a folk medicine based on *Artemisia phaeolepis* Krasch. (Asteraceae) endemic to the area has been used for generations to cure colds and icterus hepatitis (C. Guo, personal communication, Hezuo Pharmaceutical Factory, Hezuo, People's Republic of China). However, the nature of its chemical constituents is not known. In a continuation of our investigation of *Artemisia* species (1,2), we have collected and examined this medicinally useful plant.

Through a variety of chromatographic techniques, the aerial parts of the title species afforded α- and β-amyrin, sitosterol, daucosterol, costic acid, ilicic acid [**1**], eudesma-4,11(13)-dien-12-oic acid, 9β-acetoxyeudesma-4,11(13)-dien-12-oic acid, 2β-hydroxyguaia-3,10(14),11(13)-trien-12,6α-olide, and a minor new acid [**2**].

Compound **1** was isolated as a methyl ester **1a**. The identity of **1a** with ilicic acid methyl ester was established by direct comparison of the $[\alpha]^{20}D$, ir, eims, ^1H- and ^{13}C-nmr data (see Experimental) with those in the literature (3,4). Compound **2** was also obtained as a methyl ester [**2a**]. The structure of **2a** was elucidated from its spectral data, with the eudesman-12-oic acid carbon framework based on the characteristic signals at δ 1.04 s (H-14), 1.39 s (H-15), 6.14 br s (H-13), 5.56 br s (H-13′), and 3.77 s (COOMe) (3,5). All ^1H-nmr signals were assigned by spin decoupling. Comparison of the ^1H-nmr spectrum of **2a** with those of its analogues reported earlier (3,5) indicated the presence of a 9β-acetoxy group. Furthermore, the ir absorption band at 3470 cm^{-1} and the singlet of H-15 at δ 1.39 revealed the presence of hydroxylation on C-4. These structural inferences were reinforced by analysis of the ^{13}C-nmr spectrum of **2a** in which resonance lines due to C-2 and C-4 were shifted remarkably downfield compared with analogous signals of ilicic acid methyl ester **1a** (Table 1). This observation, along with the carbonyl singlet at δ



1 R=H
1a R=CH_3



2 R=H
2a R=CH_3

TABLE 1. ^{13}C-Nmr Data of Compounds **1a** and **2a** (125 MHz, $CDCl_3$, TMS, δ values).

Carbon	**1a**[a]	**2a**	DEPT
C-1	40.9	36.9	CH_2
C-2	20.0	32.0[b]	CH_2
C-3	43.4	213.7	C
C-4	72.0	82.6	C
C-5	55.0	54.5	CH
C-6	27.3	24.4	CH_2
C-7	40.4	32.9	CH
C-8	26.4	33.4[b]	CH_2
C-9	44.4	77.2	CH
C-10	34.5	47.8	C
C-11	145.7	143.1	C
C-12	167.7	167.1	C
C-13	122.3	123.5	CH_2
C-14	18.6	12.4	CH_3
C-15	22.5	24.8	CH_3
OMe	51.6	51.9	CH_3
Ac	—	170.9	C
Ac	—	21.2	CH_3

[a]Cited from Daniewski *et al.* (3).
[b]Interchangeable assignments.

213.7, could only be explained by the presence of a 3-ketone functionality in the molecule of **2a**. Regarding the stereochemistry, the pronounced nOe effects of H-14 with H-15 (5.3%), of H-5 with H-7 (2.3%), and of H-7 with H-9 (2.7%) established the formulated configuration in **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker AMX-500 nmr spectrometer at 500 and 125 MHz, respectively. DEPT experiments were carried out to determine multiplicities of carbon signals using the standard 45°, 90°, and 135° pulse sequences. Other apparatus used in this study was as described previously (2).

PLANT MATERIAL.—The aerial parts of *A. phaeolepis* were collected in September 1991 in Luqiu County, Gansu Province, People's Republic of China, and were identified by Prof. G.L. Zhang (Lanzhou University). A voucher specimen has been preserved at the Department of Biology, Lanzhou University, Lanzhou 730000, People's Republic of China.

EXTRACTION AND ISOLATION.—The pulverized, air-dried aerial parts (400 g) of *A. phaeolepis* were extracted twice for 48 h with petroleum ether (60–90°)-Et_2O-MeOH (1:1:1) by cold percolation. The solid material (ca. 20 g) afforded after removal of solvent from the combined extracts was refluxed with ca. 150 ml MeOH until the solid was entirely dissolved. The solution was cooled to room temperature and then kept at −5° for 24 h. The tarry substance that formed was filtered and the filtrate was subsequently concentrated to a black residue (13.5 g). This residue was fractionated by Si gel (400 g) cc eluting successively with petroleum ether-Et_2O (10:1→1:50), Et_2O, and Et_2O-MeOH (100:5→1:1). Evaporation of solvent from the cc fractions (200 ml each) combined according to tlc monitoring gave five major portions: F-1 (1.4 g), F-2 (1.7 g), F-3 (2.1 g), F-4 (2.6 g), and F-5 (3.1 g). β-Sitosterol (16 mg) was crystallized from F-1 using 20 ml EtOAc. Prep. tlc of the mother liquor utilizing petroleum ether-Et_2O (5:1) (developed twice) gave β-amyrin (30 mg, R_f 0.30), as well as additional β-sitosterol (16 mg, R_f 0.44), and a band which upon further prep. tlc using petroleum ether-CH_2Cl_2-Et_2O (5:5:1) (developed 7 times) yielded α-amyrin (9 mg, R_f 0.27). Treatment of F-2 with CH_2N_2, followed by cc over Si gel (50 g) eluted with petroleum ether-Me_2CO (9:1→1:3), gave costic acid Me ester (6 mg), eudesm-4,11(13)-dien-12-oic acid Me ester (9 mg), and a mixture which afforded 9β-acetoxyeudesm-4,11(13)-dien-12-oic acid Me ester (8 mg, R_f 0.23) by prep. tlc with petroleum ether-CH_2Cl_2-Et_2O (5:5:1) (developed 6 times). F-3, dissolved in Me_2CO, was filtered through an activated charcoal column. Evaporation of Me_2CO from the filtrate gave a yellow gum (1.45 g) which afforded pure 2β-hydroxyguaia-3,10(14),11(13)-trien-12,6α-olide (6 mg) and three fractions (F-3a, F-3b, and F-3c) by means of cc using petroleum ether-Et_2O (50:2→1:5). Treatment of F-3a with CH_2N_2 followed by prep. tlc utilizing CH_2Cl_2-MeOH (25:1) (developed 3 times) furnished **1a** in pure form.

Ilicic Acid Methyl Ester [**1a**].—Me ester **1a** (9 mg, R_f 0.36) exhibited: $[\alpha]^{20}D$ −41.0° (c=1.021, MeOH); ir ν max (CCl_4) 3535, 3245, 1713, 1620 cm^{-1}; ^{1}H nmr ($CDCl_3$, TMS, 500 MHz) δ 6.10 (1H, br s, H-13), 5.52 (1H, br s, H-13′), 3.77 (3H, s, OMe), 1.06 (3H, s, H-15), 0.88 (3H, s, H-14); ^{13}C-nmr data, see Table 1; eims *m/z* $[M]^+$ 266, $[M^+-18]$ 248, 232, 206, 191, 149, 121, 97, 55.

3-Keto-9β-acetoxyilicic acid methyl ester [**2a**].—By the same procedure described for F-3a, F-3b gave **2a** (8 mg, R_f 0.36): gum; $[\alpha]^{20}D$ −17.5° (c=0.2391, MeOH); ir ν max (CCl_4) 3470, 1715, 1706, 1628, 1236, 1145, 1090 cm^{-1}; ^{1}H nmr δ 6.14 (1H, br s, H-13), 5.56 (1H, br s, H-13′), 4.70 (1H, dd, $J_{9,8\alpha}$=5.1 Hz, $J_{9,8\beta}$=11.3 Hz, H-7), 2.08 (3H, s, OAc), 1.86 (1H, ddd, $J_{8\alpha,8\beta}$=12.5 Hz, $J_{8\alpha,9}$=5.1 Hz, $J_{8\alpha,7}$=3.7 Hz, H-8α), 1.79 (1H, dd, $J_{5,6\alpha}$=4.2 Hz, $J_{5,6\beta}$=12.5 Hz, H-5), 1.73–1.50 (3H, m, H-1β, H-2β, H-6α), 1.53 (1H, ddd, $J_{8\beta,8\alpha}$=12.5 Hz, $J_{8\beta,7}$=12.5 Hz, $J_{8\beta,9}$=11.3 Hz, H-8β), 1.47 (1H, ddd, $J_{6\beta,5}$=$J_{6\beta,6\alpha}$=$J_{6\beta,7}$=12.5 Hz,

H-6β), 1.39 (3H, s, H-15), 1.27 (1H, ddd, $J_{1\alpha,1\beta}$=13 Hz, $J_{1\alpha,2\beta}$=12.5 Hz, $J_{1\alpha,2\alpha}$=4.5 Hz, H-1α), 1.04 (3H, s, H-14); ^{13}C-nmr data, see Table 1; eims *m/z* [M]$^+$ 338 (11), [M$^+$−HOAc] 278 (45), 260 (18), 171 (13), 91 (50), 55 (100).

Fractions F-3c and F-4 were combined and separated by cc over Si gel with CH_2Cl_2-MeOH (30:1→1:1) to yield daucosterol (35 mg) and a tarry solid. Further cc of this solid and F-5 afforded no pure compound.

ACKNOWLEDGMENTS

This study was financed by the State Education Commission of China. We are very grateful to C. Guo (Hezuo Pharmaceutical Factory, Hezuo 747000, People's Republic of China) for collecting the plant material.

LITERATURE CITED

1. R.X. Tan and Z.J. Jia, *Phytochemistry*, **31**, 2534 (1992).
2. R.X. Tan, Z.J. Jia, and Y. Zhao, *Planta Med.*, **58**, 459 (1992).
3. W.M. Daniewski, W. Kroszczyński, E. Bloszyk, B. Drozdz, J. Nawrot, U. Rychlewska, M. Budĕšínský, and M.M. Holub, *Coll. Czech. Chem. Commun.*, **51**, 1710 (1986).
4. W. Herz, H. Chikamatsu, and L.R. Tether, *J. Org. Chem.*, **31**, 1632 (1966).
5. J.F. Sanz and J.A. Marco, *Liebigs Ann. Chem.*, 541 (1990).

Received 27 July 1994