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TWO TRITERPENES FROM *PALIURUS RAMOSISSIMUS*

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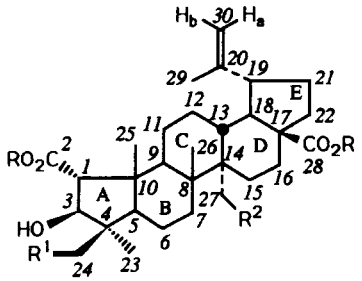
ABSTRACT.—Two additional triterpenes, 24-hydroxyceanothic acid and 27-hydroxyceanothic acid, were isolated as dimethyl esters **2** and **4** from the roots of *Paliurus ramosissimus*. Their structures were determined by correlation with ceanothic acid [**5**] and spectral analysis including nOe and COLOC. Compound **4** is a new triterpene, while **2** is identical to granulosic acid dimethyl ester.

A recent report described the characterization of two new triterpene glucosides (1), ceanothic acid 28β-glucosyl ester and isoceanothic acid 28β-glucosyl ester, from the stem barks of *Paliurus ramosissimus* Poir. (Rhamnaceae), a Taiwan folk medicine used in the treatment of toothache and abdomen ache. Here we report the separation and structure elucidation of two additional ceanothic acid analogues, 24-hydroxyceanothic acid and 27-hydroxyceanothic acid, isolated from the roots, as their dimethyl esters.

The EtOH extract of the powdered roots of *P. ramosissimus* was fractionated into *n*-C_6H_{14}-soluble, $CHCl_3$-soluble, EtOAc-soluble, *n*-BuOH-soluble and H_2O-soluble fractions. Partitioning the $CHCl_3$-soluble fraction between 1% NaOH and $CHCl_3$ allowed separation of acidic compounds (aqueous layer) and neutral compounds ($CHCl_3$ layer). The acidic components, obtained as a precipitate while acidifying the aqueous layer, were then separated by repeated Si gel cc which resulted in the isolation of ceanothic acid, betulic acid, and a mixture of ceanothic acid [**5**] and two components **1** and **3**. We attempted to separate **1** and **3**, but only a very small amount of pure **1** was obtained. To facilitate the separation, the mixtures of **1**, **3**, and **5** were methylated with CH_2N_2, and the structures of **1** and **3** were characterized as their corresponding dimethyl esters, **2** and **4**.

Ceanothic acid dimethyl ester [**6**], mp 224–226°, $[M]^+$ at *m/z* 514 ($C_{32}H_{50}O_5$), shows two *O*-methyl signals at δ 3.65. Other than this difference, the proton signals of ceanothic acid [**5**] and **6** were almost superimposable (Table 1). The structure of **6** was confirmed by its physical data, identical with those of ceanothic acid *O,O*-dimethyl ester prepared from **5** by reaction with CH_2N_2. The ^{13}C-nmr spectrum of **6** was assigned by correlation with that of ceanothic acid (1) and is shown in Table 2.



1 R=H, R^1=OH, R^2=H
2 R=Me, R^1=OH, R^2=H
3 R=R^1=H, R^2=OH
4 R=Me, R^1=H, R^2=OH
5 R=R^1=R^2=H
6 R=Me, R^1=R^2=H

TABLE 1. ^{1}H-nmr Data of Compounds **2**, **4**, and **6** in $CDCl_3$ (δ in ppm, *J* in Hz).

Proton	Compound		
	6	**2**	**4**
H-1	2.57 s	2.65 s	2.59 s
H-3	4.14 s	4.14 s	4.25 s
H-19	2.95 m	2.94 m	2.95 m
H-23	1.09 s	1.29 s	1.09 s
H-24	0.89 s	4.18 d (10.7, H_X) 3.26 d (10.7, H_A)	0.90 s
H-25	1.04 s	1.14 s	1.07 s
H-26	0.90 s	0.87 s	0.91 s
H-27	0.89 s	0.87 s	4.11 d (12.4) 3.76 d (12.4)
H-29	1.64 s	1.64 s	1.64 s
H_a-30	4.70 br s	4.70 br s	4.70 br s
H_b-30	4.57 br s	4.57 br s	4.57 br s

TABLE 2. ^{13}C-nmr Data of Compounds **2**, **4**, **5** and **6** (δ in ppm).[a]

Carbon	Compound				COLOC data of **4**	
	5	**6**	**2**	**4**	δ_C	δ_H
C-1	67.2 d	65.6 d	64.9 d	65.7 d	65.7	1.07 (H-25)
C-2	177.9 s	175.1 s	174.8 s	175.2 s	175.2	2.59 (H-1), 4.14 (H-3), 3.65 (2-OMe)
C-3	85.0 d	84.9 d	85.4 d	85.2 d		
C-4	43.9 s	43.3 s	47.9 s	43.4 s	43.4	0.90 (H-24), 1.09 (H-23)
C-5	57.2 d	56.7 d	56.9 d	57.0 d	57.0	1.07 (H-25), 1.09 (H-23)
C-6	19.2 t	18.5 t	17.8 t	18.5 t		
C-7	34.9 t	34.1 t	34.5 t	35.3 t	35.3	0.91 (H-26)
C-8	43.7 s	42.9 s	42.9 s	42.5 s	42.5	0.91 (H-26)
C-9	45.2 d	44.6 d	44.9 d	46.1 d		
C-10	49.7 s	49.5 s	49.9 s	49.7 s	49.7	1.07 (H-25)
C-11	24.4 t	23.6 t	23.7 t	23.8 t		
C-12	26.4 t	25.5 t	25.5 t	25.4 t		
C-13	39.3 d	38.7 d	38.7 d	39.5 d		
C-14	42.3 s	41.7 s	41.6 s	46.8 s	46.8	0.91 (H-26)
C-15	30.7 t	29.9 t	29.9 t	24.0 t		
C-16	33.1 t	32.3 t	32.3 t	33.3 t		
C-17	56.8 s	56.6 s	56.6 s	56.5 s		
C-18	50.1 d	49.6 d	49.6 d	50.0 d		
C-19	47.7 d	47.0 d	47.0 d	46.9 d	46.9	4.57 (H-30)
C-20	152.4 s	150.3 s	150.3 s	150.3 s	150.3	1.64 (H-29)
C-21	31.5 t	30.8 t	30.7 t	30.8 t		
C-22	37.6 t	36.9 t	36.9 t	36.8 t		
C-23	31.6 q	30.8 q	24.5 q	30.9 q	30.9	0.90 (H-24)
C-24	20.1 q	19.1 q	66.6 t	19.1 q	19.1	1.09 (H-23)
C-25	19.0 q	19.0 q	19.0 q	19.0 q		
C-26	17.1 q	18.5 q	18.4 q	17.0 q		
C-27	15.2 q	16.5 q	16.5 q	61.5 t		
C-28	178.7 s	176.5 s	176.7 s	176.7 s		
C-29	19.7 q	19.4 q	19.4 q	19.6 q		
C-30	109.6 t	109.4 t	109.5 t	109.6 t	109.6	1.64 (H-29)
2 × OCH_3		51.1 q	51.1 q	51.2 q		

[a]Compounds **2**, **4**, and **6** were measured in $CDCl_3$ while **5** was measured in C_5D_5N.

Compound **2**, mp 237.5–238.5°, showed the molecular ion at *m/z* 530.3605, corresponding to the formula $C_{32}H_{50}O_6$ (calcd 530.3607) with one more oxygen atom than that of **6**. The ^{1}H-nmr spectrum of **2** is similar to that of **6** and only a few differences are observed (Table 1). Compound **2** shows five methyl signals, one fewer than **6**, and an additional AX coupling system at δ 4.18 and 3.26 (J = 10.7 Hz). These differences are also reflected in the ^{13}C-nmr spectra, in which a methyl signal of **6** is replaced by a hydroxylated methylene signal (δ 66.7) suggesting that **2** is a hydroxyceanothic acid.

The ^{13}C-nmr data of **2** and **6** are very similar, and the carbon with the largest chemical shift difference is assigned to C-4 (Δδ **2**–**6** 4.6 ppm). Consequently, the hydroxylated position must be either C-23 or C-24. This substitution will cause a β effect on C-4, which accounts for the downfield shift (2). The location of this hydroxymethylene was confirmed by nOe studies (Figure 1).

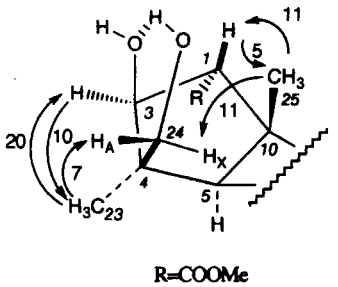


FIGURE 1. NOe's (%) and conformation of **2** in ring A.

The similarity of the ^{1}H-nmr spectra of **2** and **6** allows the assignment of H-1 and H-3 of **2** to δ 2.65 and 4.25, respectively. Irradiation of H-1 (s, δ 2.65) enhanced H-25 (s, δ 1.14). Upon irradiation of H-3 (s, δ 4.25), a methyl singlet at δ 1.29 was enhanced. This study indicated the presence of a methyl group (C-23) at C-4α and suggested that the hydroxyl group was at C-24. The AX methylene protons at δ 3.26 (A) and 4.18 (X) (J_{AX} = 10.8) were enhanced by irradiation of H-23 (δ 1.29) and H-25 (δ 1.14), respectively. These data also indicate a rigid orientation of the methylene protons. This rigid conformation probably results from an intramolecular H-bond between 3-OH and 24-OH. This postulation is supported by a broad absorption at 3400 cm^{-1} in the ir spectrum (3). Without this interaction, the O-H appears as a sharper peak, at 3500 cm^{-1}, in **4** and **6**.

These data taken together, and its identity to an authentic sample (^{1}H nmr, tlc and mp) of granulosic acid dimethyl ester (4), established **2** as 24-hydroxyceanothic acid dimethyl ester.

Compound **4**, mp 253.5–255.0°, showed the molecular ion at *m/z* 530.3640, corresponding to the formula $C_{32}H_{50}O_6$ (calcd 530.3607). The ^{1}H-nmr spectrum of **4** is very similar to that of **6** except for the absence of a methyl group and the presence of an additional AB quartet at δ 4.11 and 3.76 (J = 12.4 Hz). These data suggest **4** to be another hydroxyceanothic acid, an isomer of **2**. NOe studies (Figure 1) indicate four methyl signals at δ 1.09 (H-23), 0.90 (H-24), 1.07 (H-25), and 0.91 (H-26). The methyl singlet appearing at relatively low field (δ 1.64) was assigned to H-29 by correlation with that of **2** or **6**. These data indicate C-27 or C-28 as the hydroxylated position.

The COLOC spectrum of **4** located the signals of C-7 and C-14 at δ 35.3 (t) and 46.8 (s), respectively, based on their three-bond couplings to H-26 (δ 0.91). This 2D

spectrum also located C-1 (δ 65.7), C-2 (δ 175.2), C-4 (δ 43.4), C-5 (δ 57.0), C-10 (δ 49.7), C-19 (δ 46.9), C-20 (δ 150.3), C-23 (δ 30.9), C-24 (δ 19.1), and C-30 (δ 109.6) via the couplings with the protons of two- and three-bond distance as shown in Table 2. Using these assignments as markers and comparing them with the ^{13}C-nmr data of **6** enabled complete assignment of the ^{13}C-nmr of **4** (Table 2). These data indicate that carbon signals of ring E in **4** are almost identical to the corresponding signals in **6**, while some of the carbon signals of rings B, C, and D show differences among them [$\Delta\delta_{4-6}$ = −1.2 (C-7), −0.3 (C-8), 1.5 (C-9), 0.8 (C-13), 5.1 (C-14), −5.9 (C-15), 1.0 (C-16), ring E: $\Delta\delta_{4-6}$ = −0.1 (C-17), −0.1 (C-19), 0.0 (C-21), −0.1 (C-22), 0.2 (C-28)]. These correlations require that both compounds possess the same substituent at C-17 (-COOMe) but a different substituent at C-14. Thus, C-27 is a hydroxymethylene. The hydroxyl group causes a β and a γ effect on C-14 and C-15, respectively, accounting for the large shifts indicated above (2). Consequently, **4** is 27-hydroxyceanothic acid dimethyl ester.

24-Hydroxylated or 27-hydroxylated triterpenes have been isolated from several plants including soyasapogenol A (24-hydroxylated) from *Glycine max* Merill (5), and senegenin II (27-hydroxylated) from *Polygala* sp. (6). Most of these triterpenes are the oleanane type. Our study reveals that the ceanothane type also possesses such modifications. Other analogues are expected, and the isolation of these relatively minor and polar natural products is still in progress.

EXPERIMENTAL

PLANT MATERIAL AND INSTRUMENTATION.—Stems and roots of *P. ramosissimus* were collected from the mid-west seashore of Taiwan in July 1988. A voucher specimen was deposited in the herbarium of the School of Pharmacy, National Taiwan University. Melting points were measured on a Fisher-Johns melting point apparatus and not corrected. Optical rotations were measured on a Jasco DIP-181 digital polarimeter. Ir spectra were recorded on a Perkin-Elmer 1760-X Infrared FT spectrometer. Eims were recorded on a Finnigan Mat 4500 series gcms and on a JEOL JMS-HX 110 mass spectrometer. The ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a Bruker AM-300 spectrometer. They were measured in $CDCl_3$ or C_5D_5N using each solvent peak as internal standard. In the COLOC experiment, a 1-sec delay was allowed between each scan, and the coupling constant was optimized for J = 8 Hz. The 2D nmr map consisted of 512 × 1K data points, each composed of 320 transients.

EXTRACTON AND ISOLATION.—Dried ground powders of the roots (16.6 kg) were macerated with 95% EtOH (42 liters × 3) at 40°. The EtOH solution was condensed under reduced pressure to about 722 g of EtOH extract. The extract was then triturated with *n*-C_6H_{14} (3 liters × 3, 26 g extract), $CHCl_3$ (2 liters × 3, 277 g extract) and H_2O (1 liter × 2, 300 g extract). Part of the $CHCl_3$-soluble fraction (120 g) was triturated with 2% citric acid to separate alkaloids. The residue was dissolved in $CHCl_3$ (1 liter) and partitioned with 1% NaOH (500 ml × 2). The $CHCl_3$ layer (9.64 g), containing neutral and very nonpolar alkaloids, was set aside, and the aqueous layer was acidified with 1% aqueous HCl to pH 3 to precipitate the acidic components (88 g). Part of the precipitate (40 g) was separated by a Si gel column (400 g, 70–230 mesh) eluted with MeOH/$CHCl_3$ from 5% to 30% stepwise to give ceanothic acid [**5**] (8.90 g), a mixture of **1** and **5** (3.10 g, fraction A), a mixture of **1** and **3** (3.27 g, fraction B) and other more polar substances. Repeated Si gel cc of fraction B yielded **1** (1.05 g) and mixture of **1** and **3** (300 mg) (fraction C).

Fraction A (0.82 g) dissolved in MeOH (20 ml) was treated with ethereal CH_2N_2 freshly prepared from 2.14 g of Diazald and kept at 4° overnight. After removing solvent, the residue (1.04 g) was separated on a Si gel column (42 g, 230–400 mesh) eluted with $CHCl_3$ and 0.5% to 2% MeOH stepwise to give 117 mg of **2** and 410 mg of **6**.

CH_2N_2 freshly prepared from 0.53 g of Diazald was added to fraction C (153 mg) dissolved in MeOH (10 ml) and the resultant solution was kept at 4° overnight. After removing solvent, the residue (145 mg) was separated on a Si gel column (7 g, 230–400 mesh) eluted with $CHCl_3$ and 0.5% to 2% MeOH stepwise to give 17 mg of **2** and 20 mg of **4**.

Ceanothic acid [**5**].—Mp 333–335° from MeOH; $[\alpha]^{24}D$ +38° (c = 0.8, MeOH), ir (KBr, cm^{-1}) ν max 2500–3500 (m, COOH, OH), 1690 (C=O), 1640 and 890 (C=CH_2); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Ceanothic acid dimethyl ester [**6**].—Mp 224.0–226.0° from MeOH; $[\alpha]^{24}D$ +41.4° (c = 1.0, $CHCl_3$);

ir (KBr, cm^{-1}) ν max 3540 (br m, OH), 2960 (s), 1720 (s, C=O), 1645 and 890 ($C=CH_2$), 1180 (br s), 1050 (br s); eims *m/z* (rel. int. %) $[M]^+$ 514 (calcd for $C32H_{50}O_5$) (3), $[M-OMe]^+$ 483 (3), 465 (3), 263 (22), 204 (30), 189 (20), 175 (50), 173 (38), 147 (32), 133 (32), 121 (70), 103 (80), 69 (100); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

24-Hydroxyceanothic acid dimethyl ester [**2**].—Mp 237.5–238.5° from MeOH; $[\alpha]^{24}D$ +51.5° (*c* = 1.0, $CHCl_3$); ir (KBr, cm^{-1}) ν max 3400 (br m, OH) 2960 (s), 1725 (s, C=O), 1645 and 890 ($C=CH_2$), 1200 (s), 1180 (br s), 1050 (br s); hrms *m/z* $[M]^+$ 530.3605 (calcd for $C_{32}H_{50}O_6$, 530.3607); eims *m/z* (rel. int. %) 498 (8), 273 (45), 262 (69), 219 (95), 203 (75), 189 (100), 187 (48), 175 (63), 173 (40), 147 (10), 133 (38), 121 (30), 119 (65), 107 (58), 105 (60); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

27-Hydroxyceanothic acid dimethyl ester [**4**].—Mp 253.5–255° from MeOH; $[\alpha]^{24}D$ +24.0° (*c* = 1.0, $CHCl_3$); ir (KBr, cm^{-1}) ν max 3500 (br s, OH), 2960 (s), 1710 (s, C=O), 1645 and 890 ($C=CH_2$), 1200 (s), 1180 (br s), 1050 (br s); hrms $[M]^+$ *m/z* 530.3640 (calcd for $C_{32}H_{50}O_6$, 530.3607), $[M-OMe]^+$ 499.3402 (calcd $C_{31}H_{47}O_5$, 499.3423); eims *m/z* (rel. int. %) 500 (25), $[M-OMe]^+$ 499 (100), 485 (6), 439 (42), 421 (20), 233 (9), 201 (11), 189 (12), 187 (22), 175 (17), 173 (16), 147 (8), 133 (11), 121 (18), 119 (17), 107 (17), 105 (18); nOe data H-1 to H-25 (4%), H-3 to H-23 (6%), H-23 to H-3 (20%), H-23 to H-24 (4%), H-25 to H-24 (16%), H-25 to H-26 (16%), H-26 to H-25 (12%), H-26 to H-13 (17%); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

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