JOURNAL OF NATURAL PRODUCTS

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J. Nat. Prod., **1991**, 54 (2), 615-618• DOI:
10.1021/np50074a047 • Publication Date (Web): 01 July 2004

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Journal of Natural Products
Vol. 54, No. 2, pp. 615-618, Mar-Apr 1991

TWO NEW TRITERPENE GLUCOSIDES FROM *PALIURUS RAMOSISSIMUS*

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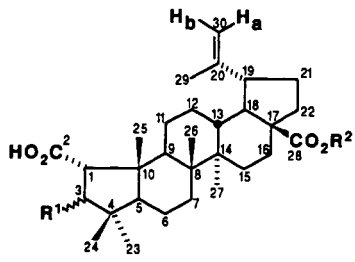
ABSTRACT.—Two new triterpene glucosides, ceanothic acid 28β-glucosyl ester [**2**] and isoceanothic acid 28β-glucosyl ester [**3**], together with ceanothic acid [**1**] were isolated from the stem bark of *Paliurus ramosissimus*. Their structures were determined by spectral analyses including nOe and 2D nmr.

It has been reported that several plants in the family Rhamnaceae contain ceanothic acid (1–3), a triterpene possessing two carboxylic functions. However, glycosides of ceanothic acid have not been reported. This paper describes the separation and structural elucidation of two glucosides, one of ceanothic acid and one of its isomer, isoceanothic acid, from the roots and stem bark of *Paliurus ramosissimus* Poir. (Rhamnaceae), a folk medicine used as a pain reliever in Taiwan.

Compound **1**, isolated during the separation of cyclopeptide alkaloids, showed identical spectral data with ceanothic acid (4). To facilitate the structural determination of the related compounds **2** and **3** the chemical shifts of all the methyl protons and some protons spatially close to the methyls of **1** were assigned by a series of nOe difference studies (Table 1). These studies confirmed also the trans relationship between H-1_β and H-3_α (1–3) by observation of the enhancement of H-25 and H-23 upon irradiation of the H-1 singlet (δ 3.18) and the H-3 singlet (δ 4.79), respectively.



1 R^1=β-OH, R^2=H
2 R^1=β-OH, R^2=β-glc
3 R^1=α-OH, R^2=β-glc

Further separation of the EtOH-insoluble fraction as described in the Experimental section led to the isolation of two glycosides, compounds **2** and **3**.

The fab super-accurate-measurement (fab-sam) mass spectrum of compound **2** shows $[M+Na]^+$ at *m/z* 671.3738, which affords the formula $C_{36}H_{56}O_{10}$ + Na (calcd 671.3771). The ^{1}H-nmr spectrum, excluding the sugar region, is almost identical to that of **1** (Table 1). The proton signals of the sugar are highly resolved in a high field ^{1}H-nmr spectrum and are further clarified by double resonance experiments. The chemical shifts and coupling patterns of H-1′ (δ 6.44, d, J=8.0 Hz) and C-1′ (δ 95.5, d) (Tables 1 and 2) indicate the sugar to be a β-glucose residue that is ester-linked (5). These data suggest **2** to be a ceanothic acid glucosyl ester.

That the position of the ester-linked glucose is at either C-2 or C-28 can be deduced from comparing the ^{13}C-nmr spectra of **2** and **1** (Table 2). Based on the proton assignments from the nOe results, the chemical shifts of the proton-attached carbons were assigned directly by a hetero-COSY experiment. The data of Table 2 indicate that **1** and **2** possess identical chemical shifts for C-1, C-3, and C-10 (δ 67.1, 84.9, and 49.7, respectively) whereas small differences for C-16, C-17, and C-22 ($\Delta\delta_{2\text{-}1}$ −0.6, +0.3, and −0.6, respectively) were observed. This comparison suggests that the glucose is ester-linked to C-28. Further direct evidence for this conclu-

TABLE 1. ^{1}H-nmr Data of Compounds **1**, **2**, and **3** Assigned from nOe's and COSY Experiments in C_5D_5N (δ in ppm, *J* in Hz).

Proton	Compound		
	1	**2**	**3**
H-1	3.18 s	3.18 s	3.34 d (7.1)
H-3	4.79 s	4.79 s	4.57 d (7.1)
H-13	2.76 dt (2.5, 11.3)	2.74 dt (2.5, 11.3)	2.72 dt (2.5, 11.3)
H-18	1.67 t (11.3)	1.67 t (11.3)	1.67 t (11.3)
H-19	3.49 m	3.49 m	3.40 m
H-23	1.42 s	1.42 s	1.42 s
H-24	1.27 s	1.27 s	1.04 s
H-25	1.37 s	1.37 s	0.92 s
H-26	1.14 s	1.14 s	1.18 s
H-27	1.03 s	1.03 s	1.05 s
H-29	1.60 s	1.60 s	1.60 s
H_a-30	4.85 br s	4.85 br s	4.85 br s
H_b-30	4.64 br s	4.64 br s	4.64 br s
glc			
H-1′		6.44 d (8.0)	6.45 d (8.0)
H-2′		4.18 t (8.5)	4.18 t (8.5)
H-3′		4.29 t (8.5)	4.29 t (8.5)
H-4′		4.37 dd (8.5, 9.1)	4.37 dd (8.5, 9.1)
H-5′		4.04 ddd (2.6, 3.7, 9.1)	4.04 ddd (2.6, 3.7, 9.1)
H-6′		4.45 dd (2.6, 12.1)	4.45 dd (2.6, 12.1)
		4.39 dd (3.7, 12.1)	4.39 dd (3.7, 12.1)

sion was obtained from a hetero-long range-COSY experiment (Table 2). In this 2D nmr spectrum the carbon signal at δ 177.9, coupled with H-1 (δ 3.18) and H-3 (δ 4.79) via two bonds and three bonds, respectively, is designated as C-2 while the carbon signal at δ 175.0, coupled with H-18 (δ 1.67, t) via three bonds, is designated as C-28. Because the signal of C-28 is shifted upfield by 3.7 ppm compared with the C-28 signal for ceanothic acid (from δ 178.7 to δ 175.0), the *O*-glucosidic linked carboxyl carbon must be C-28. From the above evidence, compound **2** is determined to be ceanothic acid 28β-glucosyl ester.

Compound **3**, mp 270–272°, has the same molecular formula (fab-sam: $C_{36}H_{56}O_{10}+Na$ at *m/z* 671.3679, calcd 671.3771) and a ^{1}H-nmr spectrum similar to that of compound **2**. The major difference is that the nmr signals of H-1 and H-3 appear as an AX coupling pattern ($J=7.1$ Hz) in **3**, whereas they appear as two singlets in **2** (Table 1). Thus, **3** might be an epimer of **2** at either C-1 or C-3. The ^{13}C-nmr spectrum of **3** differs only in the resonance of the ring A, which supports this suggestion (Table 2). Further information on the substituents at C-1 and C-3 was obtained by nOe studies. The H-25 signal (δ 0.92, s) was enhanced upon irradiation of either H-1 (δ 3.34) or H-3 (δ 4.57). These data indicate that H-1 and H-3 are in a cis relationship, i.e., the 1-carboxyl and 3-hydroxy functions are α-oriented. Compound **3**, therefore, is a C-3 epimer of **2** and is named as isoceanothic acid 28β-glucosyl ester.

The ^{13}C-nmr assignments of these three compounds were determined mainly on the basis of nOe's, COSY, and hetero-COSY. A proton-proton shift correlation (COSY) of **3** was performed to assign the protons that were not observed in nOe experiments. With the proton chemical shifts assigned, the proton-bearing carbons were designated directly by a hetero-COSY

TABLE 2. ^{13}C-nmr Data of Compounds **1**, **2**, and **3** in C_5D_5N (δ in ppm, m).

Carbon	Compound			Hetero-long range-COSY data of **2**	
	1	**2**	**3**	δ_C	δ_H
C-1	67.2 d	67.1 d	62.6 d	67.1	1.37 (H-25)
C-2	177.9 s	177.9 s	175.6 s	177.9	3.18 (H-1), 4.79 (H-3)
C-3	85.0 d	84.9 d	81.9 d	84.9	1.42 (H-23), 1.27 (H-24)
C-4	43.9 s	43.9 s	41.7 s	43.9	1.42 (H-23), 1.27 (H-24)
C-5	57.2 d	57.2 d	56.3 d	57.2	1.42 (H-23), 1.27 (H-24)
C-6	19.2 t	19.2 t	19.4 t		
C-7	34.9 t	34.7 t	34.7 t		
C-8	43.7 s	43.6 s	43.6 s	43.7	1.14 (H-26), 1.03 (H-27)
C-9	45.2 d	45.1 d	45.1 d	45.1	1.37 (H-25), 1.14 (H-26)
C-10	49.7 s	49.7 s	47.0 s	49.7	3.18 (H-1), 1.37 (H-25)
C-11	24.4 t	24.3 t	24.1 t		
C-12	26.4 t	26.3 t	26.2 t		
C-13	39.3 d	39.0 d	38.9 d		
C-14	42.3 s	42.4 s	42.3 s	42.4	1.14 (H-26), 1.03 (H-27)
C-15	30.7 t	30.5 t	30.4 t		
C-16	33.1 t	32.5 t	32.5 t		
C-17	56.8 s	57.2 s	57.1 s		
C-18	50.1 d	50.1 d	50.1 d		
C-19	47.7 d	47.4 d	47.5 d		
C-20	151.3 s	151.3 s	150.9 s	151.3	1.60 (H-29)
C-21	31.5 t	31.5 t	31.1 t		
C-22	37.6 t	37.0 t	37.0 t		
C-23	31.6 q	31.6 q	26.5 q		
C-24	20.1 q	20.2 q	25.2 q		
C-25	19.0 q	18.9 q	19.4 q		
C-26	17.1 q	17.1 q	17.2 q		
C-27	15.2 q	15.2 q	15.2 q		
C-28	178.7 s	175.0 s	175.0 s	175.0	1.67 (H-18)
C-29	19.7 q	19.7 q	19.6 q		
C-30	109.6 t	109.8 t	109.8 t	109.8	1.60 (H-29)
C-1′		95.5 d	95.5 d		
C-2′		74.4 d	74.4 d		
C-3′		78.9 d	79.0 d		
C-4′		71.5 d	71.5 d		
C-5′		79.3 d	79.3 d		
C-6′		62.5 t	62.5 t		

experiment. The methyl-attached quaternary carbons, however, were assigned by a hetero-long range-COSY experiment (Table 2). For example, the signal at C-4 in **2** is assigned at δ 43.9 via its two couplings to H-23 (δ 1.42) and H-24 (δ 1.27). Also, the long range hetero-COSY distinguished the carboxyls at C-2 and C-28 as described above. By means of these methods, most carbon signals of **2** were assigned (Table 2). The ambiguous carbon signals (C-11, C-12, C-15, and C-21) are designated partly by correlation with literature data for realted compounds (6). For a comparison with the assignments of **2**, the ^{13}C-nmr data of **1** and **3** are listed in Table 2.

EXPERIMENTAL

PLANT MATERIAL AND INSTRUMENTATION.—Stems and roots of *P. ramosissimus* were collected from the mid-west seashore of Taiwan in July 1988. A sample was deposited in the herbarium of the School of Pharmacy, National Taiwan University. Nmr spectra were measured on a Bruker WM-250 instrument using C_5D_5N (reference peaks 8.71 ppm for ^{1}H nmr and 149.9 ppm for ^{13}C nmr) or CD_3OD (reference peaks 3.30 ppm

for ^{1}H nmr and 49.0 ppm for ^{13}C nmr). The 2D nmr spectra were recorded by using Bruker's standard pulse programs. In the hetero-COSY and the hetero-long range-COSY experiment, a 1-sec delay was allowed between each scan and the coupling constant was optimized for $J = 125$ Hz and 10 Hz, respectively. The homo-COSY correlation maps consisted of 512 × 1 K data points per spectrum, each composed of 32 transients. The hetero-COSY correlation maps consisted of 512 × 1 K data points per spectrum, each composed of 256 transients. Ir spectra were recorded on a Perkin-Elmer 983 Infrared spectrophotometer. Uv spectra were measured on a Perkin-Elmer 320 spectrophotometer. Mass spectra were taken on a Ribermag 10-10 instrument or VG 70 EQ instrument using fab-sam technique.

EXTRACTION AND ISOLATION.—The dried ground powder of the bark (35 kg) was macerated with 95% EtOH (100 liters × 3). The EtOH solution was condensed under reduced pressure to about 3 liters. The precipitate (about 350 mg) was filtered. H_2O (3 liters) was added to the filtrate to yield H_2O-soluble and H_2O-insoluble fractions. The H_2O-insoluble fraction (about 200 g) was washed with hexane, and the insoluble residue (120 g) was further separated by a Si gel column (1 kg, 70–230 mesh) eluted with 5% (fractions 1 and 2), 10% and 20% (fraction 3) MeOH in $CHCl_3$. Tlc analysis indicated that fraction 1 contained alkaloids (cyclopeptides). Fraction 2 after recrystallization from MeOH yielded 10.5 g of ceanothic acid. The precipitate from the EtOH extract (370 mg) was separated repeatedly by Si gel columns (15 g, 230–400 mesh) eluted with MeOH-$CHCl_3$-H_2O (10:90:0.5) to give two glucosides **2** (150 mg) and **3** (40 mg).

CEANOTHIC ACID [**1**].—Mp 333–335°; $[\alpha]^{24}D$ +38° ($c = 0.8$, MeOH); ir (KBr) ν max (cm^{-1}) 2500–3500 (m, COOH, OH), 1690 (C=O), 1640 and 890 (C=CH_2); nOe's H-1 to H-25 (5%), H-3 to H-23 (4%), H-13 to H-19 (5%), H-13 to H-26 (9%), H-19 to H-13 (4%), H-19 to H_a-30 (6%), H-19 to H_b-30 (1%), H-23 to H-3 (12%), H-23 to H-24 (9%), H-25 to H-1 (14%), H-25 to H-24 (8%), H-25 to H-26 (11%), H-26 to H-25 (9%), H-26 to H-13 (10%), H-27 to H-16 (9%), H-27 to H-18 (9%), H-29 to H_b-30 (7%); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

CEANOTHIC ACID 28β-GLUCOSYL ESTER [**2**].—Mp 260–262°; $[\alpha]^{24}D$ +120° ($c = 0.5$, MeOH), ir (KBr, cm^{-1}) ν max 3420 (br OH), 2940 (s), 2860 (m), 1710 (br s, C=O), 1640 and 885 (C=CH_2), 1070 (br s, C-O); fab-sam *m/z* $[M + Na]^+$ 671.3738 ($C_{36}H_{56}O_{10}$ + Na, calcd 671.3771); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

ISOCEANOTHIC ACID 28β-GLUCOSYL ESTER [**3**].—Mp 270–272°; $[\alpha]^{24}D$ +26° ($c = 0.7$, MeOH); ir (KBr, cm^{-1}) ν max 3420 (br s, OH) 2940 (s), 2870 (m), 1712 (br s, C=O) 1640 and 885 (C=CH_2), 1070 (br s, C-O); fab-sam *m/z* $[M + Na]^+$ 671.3679 ($C_{36}H_{56}O_{10}$ + Na, calcd 671.3771); nOe's H-1 to H-25 (4%), H-3 to H-24 (7%), H-3 to H-25 (4%), H-23 to H-5 (7%), H-23 to H-24 (4%), H-24 to H-23 (5%), H-24 to H-3 (5%), H-24 to H-25 (1%), H-25 to H-1 (7%), H-25 to H-24 (5%), H-25 to H-26 (6%), H-26 to H-25 (4%), H-26 to H-11 (10%), H-26 to H-13 (9%); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

ACKNOWLEDGMENTS

We are grateful to NSC, Republic of China, for support of this research under Grant NSC 78-0420-B002-129.

LITERATURE CITED

1. P. De Mayo and A.N. Starratt, *Can. J. Chem.*, **40**, 788 (1962).
2. R.A. Eade, G. Kornis, and J.J.H. Simes, *Aust. J. Chem.*, **17**, 141 (1964).
3. G.B. Branch, D.V. Burgess, P.J. Dunstan, L.Y. Foo, G.H. Freen, J.P.G. Mack, E. Ritchie, and W.C. Taylor, *Aust. J. Chem.*, **25**, 2209 (1972).
4. J.N. Roitman and L. Jurd, *Phytochemistry*, **17**, 491 (1978).
5. K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai, and O. Tanaka, *Tetrahedron Lett.*, 1005 (1976).
6. G.V. Baddeley, J.J.H. Simes, and T.-H. Ai, *Aust. J. Chem.*, **33**, 2071 (1980).

Received 1 August 1990