

Structure Determination of a New Lupane-type Triterpene, Tiarellic Acid, Isolated from *Tiarella polyphylla*

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(Received January 8, 2002)

A new 27-carboxylic lupane-type triterpene, tiarellic acid (1), was isolated from *Tiarella polyphylla* together with corosolic acid (2) and tormentic acid (3). Tiarellic acid was characterized as 3,23-dihydroxy-20(29)-lupen-27-oic acid and its NMR data were unambiguously assigned using 2-D NMR techniques.

Key words: *Tiarella polyphylla*, Saxifragaceae, 3,23-Dihydroxy-20(29)-lupen-27-oic acid, Tiarellic acid, Corosolic acid, Tormentic acid

INTRODUCTION

The plant *Tiarella polyphylla* D. Don (Saxifragaceae) is single species belonging to this genus in Korea, and it inhabits Ullung island (Lee, 1996). The Korean names of this plant are 'Heul-teu-ki-pul' or 'Cheun-sik-yak-pul', which mean healing of asthma. The whole plant of *T. polyphylla* has been traditionally used for the treatment of asthma, however, its chemical constituents have not been reported (Hotta *et al.*, 1989). Earlier and more recently, we reported the isolation of oleanolic saponins from this plant and their anticomplement activities (Park *et al.*, 1999).

The present report deals with the isolation and structure elucidation of a 27-carboxylic lupane-type triterpene, as a new component tiarellic acid (1), as well as two known ursane-type triterpenes of corosolic acid (2) and tormentic acid (3) (Yang *et al.*, 1992), which were characterized by comparisons of their spectral data with those of references.

MATERIALS AND METHODS

General procedure

Melting point was measured using an Electrothermal

9100 and was uncorrected. Optical rotation was recorded on a Jasco DIP-370 digital polarimeter. IR spectrum was recorded on a Magna 550 as KBr disc. EIMS spectra were taken from a Hewlett-Packard 5889A. ¹H-NMR and ¹³C-NMR spectra were obtained from a Bruker DRX-600 or a Varian Unity 300 spectrometers.

Plant material

The whole plants of *Tiarella polyphylla* were collected in September 1997, Ullung island, Korea. A voucher specimen (NDC-209) is deposited in our laboratory.

Extraction and isolation

Dried and chopped whole plants (1 kg) were extracted with MeOH (4 L × 5). The extract was concentrated *in vacuo* to yield a dark green residue (120 g), which was suspended in water and extracted with EtOAc. The EtOAc layer was concentrated *in vacuo*, and the extract (40 g) was subjected to column chromatography (CC) on silica gel eluting with hexane followed gradiently with the mixed eluting solvent of hexane-EtOAc and finally with CHCl₃-MeOH (1:1) mixture. Eight fractions were obtained from the EtOAc-soluble fraction. Column chromatography of Fr.VI on silica gel (CHCl₃-MeOH = 9:1) and reverse phase C-18 (MeOH-H₂O, 2:1), followed by recrystallization (in MeOH) afforded tiarellic acid (1), corosolic acid (2), and tormentic acid (3).

Tiarellic acid (1). Needles (MeOH), mp 254-256°C; $[\alpha]_D^{23} +94^\circ$ (pyridine, c 0.14); IR ν_{\max}^{KBr} (cm⁻¹): 3491 (OH),

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1689 (CO), 1645 (C=C), 1450, 1388, 1262, 1222, 1044; HRMS m/z 472.3552 (M^+ , Calcd for $C_{30}H_{48}O_4$: 472.3553); EIMS (rel. int.) m/z : 472 [M] $^+$ (61), 454 [$M-H_2O$] $^+$ (34), 436 [$M-2H_2O$] $^+$ (62), 424 (42), 396 (26), 205 (75), 187 (71), 175 (87), 173 (100); 1H - and ^{13}C -NMR (pyridine- d_5): see Table I.

Corosolic acid (2) White amorphous powder, ^{13}C -NMR (75 MHz, pyridine- d_5): δ 16.8 (C-24), 16.9 (C-29), 17.5 (C-25), 17.7 (C-26), 18.9 (C-6), 23.7 (C-16), 23.8 (C-27), 23.8 (C-30), 23.9 (C-11), 28.3 (C-15), 29.3 (C-23), 30.0 (C-21), 33.2 (C-7), 34.2 (C-22), 38.5 (C-10), 39.5 (C-19), 40.1 (C-14), 40.4 (C-8), 42.0 (C-20), 42.2 (C-4), 46.6 (C-17), 48.1 (C-9), 48.4 (C-1), 55.9 (C-18), 55.9 (C-5), 68.6 (C-2), 83.8 (C-3), 122.5 (C-12), 144.9 (C-13), 180.2 (C-28); 1H -NMR (300 MHz, pyridine- d_5): δ 0.98 (3H, d, J = 6.6 Hz, H-29), 0.99 (3H, s, H-25), 1.00 (3H, d, J = 6.2 Hz, H-30), 1.01 (3H, s, H-24), 1.09 (3H, s, H-26), 1.22 (3H, s, H-27), 1.29 (3H, s, H-23), 3.36 (1H, d, J = 9.4 Hz, H-3), 4.06 (1H, ddd, J = 4.4, 9.4, 11.1 Hz, H-2), 5.44 (1H, br.s, H-12).

Tormentic acid (3) White amorphous powder, ^{13}C -NMR (150 MHz, pyridine- d_5): δ 16.7 (C-30), 16.8 (C-25), 17.2 (C-26), 17.6 (C-24), 18.9 (C-6), 24.1 (C-11), 24.7 (C-27), 26.3 (C-16), 26.9 (C-21), 27.1 (C-29), 29.2 (C-15), 29.3 (C-23), 33.5 (C-7), 38.5 (C-10), 38.5 (C-22), 39.8 (C-4), 40.4 (C-8), 42.1 (C-14), 42.3 (C-20), 47.7 (C-1), 47.8 (C-9), 48.2 (C-17), 54.5 (C-18), 55.9 (C-5), 68.6 (C-2), 72.7 (C-19), 83.8 (C-3), 127.9 (C-12), 139.9 (C-13), 180.7 (C-28); 1H -NMR (600 MHz, pyridine- d_5): δ 0.98 (3H, s, H-25), 1.05 (3H, s, H-24), 1.08 (3H, s, H-26), 1.10 (3H, d, J = 6.4 Hz, H-30), 1.24 (3H, s, H-23), 1.41 (3H, s, H-29), 1.68 (3H, s, H-27), 2.21 (1H, dd, J = 3.6, 12.5 Hz, H-1), 2.30 (1H, ddd, J = 4.1, 13.0, 13.8 Hz, H-15), 3.02 (1H, s, H-18), 3.10 (1H, ddd, J = 3.7, 13.0, 13.0 Hz, H-16), 3.36 (1H, d, J = 9.2 Hz, H-3), 4.07 (1H, ddd, J = 3.6, 9.2, 11.2 Hz, H-2), 5.55 (1H, br.s, H-12).

RESULTS AND DISCUSSION

An EtOAc-soluble fraction of the MeOH extract of *T. polyphylla* was chromatographed on columns of silica gel and reverse phase C-18, followed by solvent recrystallization to give three triterpenes (1-3) (Fig. 1).

Compound 1, white needles, was revealed to have the molecular formula $C_{30}H_{48}O_4$ ($[M]^+$ at m/z 472.3552, Calcd 472.3553) by high resolution electron impact mass spectrum. Two fragment ion peaks at m/z 454 [$M-H_2O$] $^+$ and 436 [$M-2H_2O$] $^+$ indicated this compound contained two hydroxyl groups. The IR spectrum exhibited absorption bands at 3491 cm^{-1} (OH), 1689 cm^{-1} (C=O, carboxylic acid), and 1645 cm^{-1} (C=C). The ^{13}C -NMR and DEPT spectra

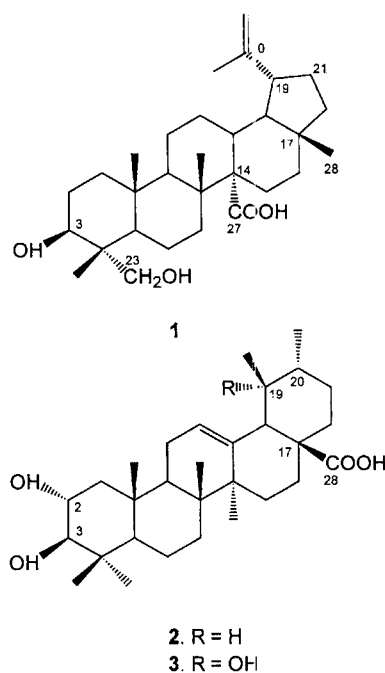


Fig. 1. Structures of compounds (1-3) isolated from *Tiarella polyphylla*

revealed 30 carbon signals; five methyls, twelve methylenes, six methines, and seven quaternary carbons (Table I). All of the methyl signals appeared as singlet in 1H -NMR spectrum, and four methyl signals were observed at the upper field region (δ 0.9-1.2), but one methyl signal at considerably downfield region (δ 1.86). This result indicated the signal at δ 1.86 can be attributed to a vinylic methyl, and this fact was further confirmed by HMBC. This methyl signal crossed with the signals at δ 150.90 (C-20) and 110.17 (C-29, CH_2) in HMBC, which indicated compound 1 had a terminal olefin group (Fig. 2). In HMQC spectrum, the signal at δ 73.60 (C-3) coupled with δ 4.02 (H-3), and the signal at δ 68.20 (C-23) coupled with δ 4.07 and 3.57 (H-23), respectively, indicated that this compound possessed one primary and one secondary hydroxyl groups. From these results, compound 1 indicated to be a

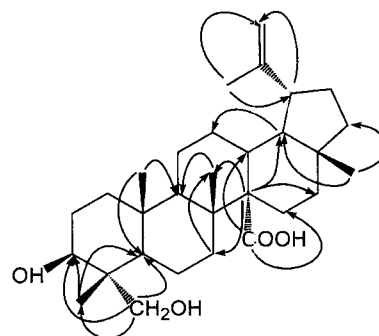


Fig. 2. Long-range correlations observed in the HMBC spectrum of compound 1

Table I. ^1H - (600 MHz) and ^{13}C - (150 MHz) NMR spectral data of compound 1 (pyridine- d_5)

Position	δ_{C}	δ_{H} (J, Hz)	NOE	HMBC (C-H)
1	39.2	α 1.05, <i>ddd</i> (2.9, 12.5, 13.0) β 1.71, <i>m</i>	3α , 5α , 9α 11α	5, 25
2	27.9	α 1.83, <i>m</i> β 1.91, <i>m</i>	25	1, 3
3	73.6	4.02, <i>dd</i> (4.7, 11.6)	1α , 5α	1, 2, 5, 23, 24
4	42.9		--	3, 5, 23, 24
5	49.2	α 1.51, <i>dd</i> (1.5, 12.0)	1α , 3α , 7α , 9α , 23	1, 6, 9, 23, 24, 25
6	18.7	α 1.65, <i>dddd</i> (1.5, 1.5, 3.0, 13.0) β 1.48, <i>dddd</i> (1.7, 12.0, 12.5, 13.0)	23 25, 26	5, 7
7	38.2	α 2.06, <i>ddd</i> (3.0, 12.5, 13.0) β 1.87, <i>ddd</i> (1.5, 1.7, 13.0)	5α 15α	26
8	40.8		--	7, 9, 26
9	51.6	α 2.02, <i>dd</i> (1.7, 12.7)	1α , 5α , 12α	5, 11, 25, 26
10	37.7		--	1, 5, 9, 25
11	21.3	α 1.64, <i>m</i> β 1.32, <i>dddd</i> (4.4, 12.7, 13.0, 13.1)	1β 25, 26	9, 12
12	26.7	α 2.60, <i>m</i> β 1.87, <i>m</i>	9α --	9, 11, 13
13	39.6	β 1.88, <i>m</i>	15β , 19β , 26, 28	12, 15, 18
14	60.4		--	7, 9, 13, 15, 16, 18, 26
15	25.8	α 2.28, <i>ddd</i> (3.0, 3.0, 13.1) β 1.67, <i>m</i>	7β 13β , 26, 28	16, 26
16	38.3	α 1.78, <i>m</i> β 1.70, <i>m</i>	22α 28	15, 28
17	43.0		--	16, 18, 22, 28
18	51.4	α 1.81, <i>m</i>	22α , 29	12, 13, 19, 21, 22
19	48.1	β 2.60, <i>m</i>	21β , 28, 29	18, 21, 22, 29, 30
20	150.9		--	18, 19, 21, 29, 30
21	30.1	α 1.36, <i>m</i> β 1.97, <i>m</i>	-- 19β	19, 22
22	40.4	α 1.16, <i>ddd</i> (10.5, 10.5, 10.5) β 1.40, <i>dd</i> (10.2, 10.5)	16α , 18α 28	21
23	68.2	3.57, <i>d</i> (10.4) 4.07, <i>d</i> (10.4)	5α , 6α , 24	3, 5, 24
24	13.0	1.04, <i>s</i>	23	3, 5, 23
25	17.4	1.01, <i>s</i>	$2b$, 6β , 11β , 26	5, 9
26	17.5	1.21, <i>s</i>	11β , 13β , 15β , 25	9
27	178.3	--	--	13, 15
28	18.8	0.90, <i>s</i>	13β , 15β , 16β , 19β , 21β , 22β	18, 22
29	110.2	4.76, <i>s</i> 4.96, <i>s</i>	19, 30 18, 19	19, 30
30	19.4	1.86, <i>s</i>	29	19, 29

lupane-type triterpene. Furthermore, a primary hydroxyl at C-23 and a secondary hydroxyl at C-3 were verified by HMBC and HMQC experiments. The signal at δ 4.02 (H-3) was splitted into doublet of doublets ($J = 4.7$ and 11.6 Hz). The smaller one ($J = 4.7$ Hz) was due to coupling with equatorial H-2 and the larger one ($J = 11.6$ Hz) was due to coupling with axial H-2 proton. Thus, H-3 must be in axial position and the hydroxyl group must be β -posi-

tion. The hydroxy-methyl proton signals (H-23) showed cross peaks with 5α and 6α proton signals in NOESY spectrum, and thus this hydroxymethyl must be in α -orientation (Fig. 3).

The carboxyl group is commonly located at C-28 in lupan-type triterpenes (Mahato *et al.*, 1988). However, the carboxyl carbon signal of this compound showed cross peaks with H-13 and H-15 in HMBC (Fig. 2). The methyl

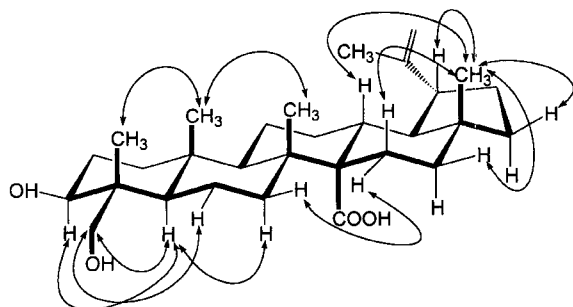


Fig. 3. NOE correlations observed in NOESY spectrum of compound 1

signal of C-28 (δ 18.84) showed cross peaks with H-18 and H-22 in HMBC. Furthermore, this methyl (H-28, δ 0.90) showed cross peaks with 13β , 15β , 16β , 19β , 21β , and 22β protons in NOESY (Fig. 3). These results confirmed that the carboxyl group should be located at C-27, and the structure of tiarellic acid (**1**) was established as 3,23-dihydroxy-20(29)-lupen-27-oic acid. 27-Carboxyl triterpenes are rare in nature. Two 20(29)-lupen-27-oic acids (Misra *et al.*, 1983) and three 20(29)-lupen-27,28-dioic acids (Nair *et al.*, 1993) were reported as natural compounds.

Compound **2** showed the molecular ion at m/z 472, which agrees with the molecular formula $C_{30}H_{48}O_4$. The 1H -NMR spectrum of **2** showed the presence of five singlet methyls, two doublet methyls, two carbinol methine protons assignable to H-2 (δ 4.06) and H-3 (δ 3.36), and one vinylic proton (δ 5.44) on C-12. These results indicated that **2** was an ursane-type triterpene. Splitting pattern ($J=9.4$ Hz) of the H-3 in 1H -NMR spectrum indicated the axial interaction with H-2. Each of the coupling constant of H-2 could

explained the axial-axial ($J=11.1$ Hz) and axial-equatorial ($J=4.4$ Hz) coupling with corresponding geminal protons at C-1. Furthermore, ^{13}C NMR data showed a good agreement with reported data of corosolic acid (Yang *et al.*, 1992). Therefore, the structure of **2** was established as (2 α ,3 β)-2,3-dihydroxy-12-ursen-28-oic acid.

Compound **3** was also an ursane-type triterpene. NMR spectra of **3** were similar to those of **2** except for an additional hydroxyl group located at C-19 (δ 72.7). The structure of **3** was determined to be (2 α ,3 β ,19 α)-2,3,19-trihydroxy-12-ursen-28-oic acid (tormentenic acid). Since the previously reported NMR data of tormentenic acid (Yang *et al.*, 1992) do not agree with our assignment, we report the revised assignments of those aided by HMQC and HMBC NMR data.

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