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TRITERPENES FROM *ILEX ROTUNDA* FRUITS

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Key Word Index—*Ilex rotunda;* Aquifoliaceae; pentacyclic triterpene; rotungenic acid; rotundioic acid; ulsolic acid; rotundic acid, pceduncloside.

Abstract-Two new triterpenes have been isolated from the fruits of *Ilex rotunda* along with three known triterpenoids ulsolic acid, rotundic acid and peduncloside. Their structures were characterized as 3β,19α,24-trihydroxyurs-12-ene-28-oic acid and 3β,19α-dihydroxyurs-12-ene-23,28-dioic acid by spectral and chemical means.

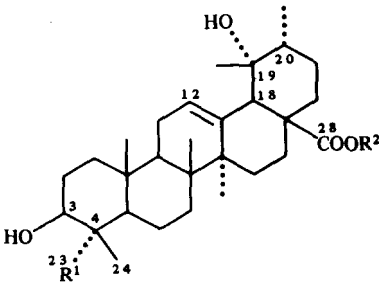
INTRODUCTION

There are some reports on the triterpenoids from *Ilex* rotunda. Rotundic acid (2) has been isolated from the seeds [1] and peduncloside (3) from the leaves [2]. *Ilex rotunda* yields red fruits in winter, from which we isolated two new ursene-type triterpenes rotungenic acid (4) and rotundioic acid (5) along with three known triterpenoids ulsolic acid (**1**), rotundic acid (2) and its ester glucoside peduncloside (3). This paper describes the isolation and identification of these triterpenoids.

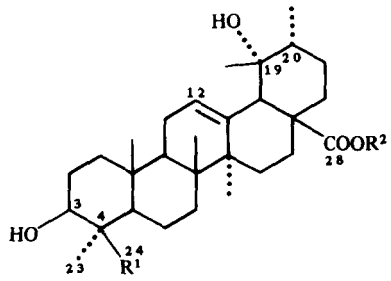
RESULTS AND DISCUSSION

The methanol extract of the fresh fruits afforded a mixture of triterpenoids which were separated by extensive chromatography to give ulsolic acid (**1**), rotundic acid (**2**), peduncloside (28-β-glucopyranosyl rotundate) (**3**), and two new triterpenes 4 and 5.

Rotungenic acid (**4**), $C_{30}H_{48}O_5$, mp 295-298" (dec), exhibited the following spectral data. SIMS: m/z 489 [M+ 1]$^+$; $[\alpha]_D + 50°$ (MeOH); UV 209 nm (ε 4000). Its IR spectrum showed the presence of hydroxyl (3600-3200 cm-') and carboxyl (1690 cm^{-1}) groups and double bond (1640 cm^{-1}). The ^{13}C NMR spectrum revealed 30 carbon signals (Me-x6, $-CH_2-$ x 9, >CH- x 4, >C< x 5, $-CH_2-O$ x 1, >CH–O x 1, >C–O x 1, > C=CH- x 1, -CO-O x 1). Unequivocal information for the ring system and substitution mode in 4 was obtained from its EI mass spectrum. There were two characteristic peaks at *m/z* **264** (rel. int. 24%) and **223 (15%)** denoting the retro-Diels-Alder cleavage fragments commonly found in the spectra of olean-12-ene or urs-12-ene derivatives possessing two hydroxyl groups in rings A/B and hydroxyl and carboxyl groups in rings D/E [1, 33, and three peaks at *m/z* **246 (44%), 219 (20%)** and **201 (56%)** indicated further successive losses of water and CO_2H



	R¹	R²	
2	CH_2OH	H	
3	CH_2OH	glu	
5	COOH	H	
8	CH_2OH	Me	
10	COOMe	Me	
13	COOH	H	3β-OAc
14	COOMe	Me	3β-OAc
16	CHO	H	
18	CH_2OH	28-CH_2OH	



	R¹	R²	
4	CH_2OH	H	
6	CH_2OH	H	3α-OH
7	COOH	H	
9	CH_2OH	MC	
11	CH_2OH	Me	3α-OH
12	CH_2OAc	H	3β-OAc
15	CHO	H	
17	Me	H	

from the *m/z* 246 peak. Furthermore, there was a prominent peak at *m/z* 146 (74%) apparently due to the secondary retro-Diels-Alder cleavage of the m/z 218 fragment ion. These fragmentations resemble very closely those of rotundic acid (2). The ^{13}C NMR spectrum also indicated that it was closely related in structure to compound 2 except for the substitution mode of A ring (Table 1). The signal at δ23.7 was assigned to the 4α-methyl group because it was observed at about δ10 higher field than the 4β-methyl in most ursene and oleane triterpenes possessing a 3β-hydroxy group [4–7]. Therefore, the hydroxymethyl group giving a signal at δ64.6 must be present in the 4β-position.

A further proof on the substitution was obtained from the ^{1}H NMR spectrum of methyl rotungenate (9) (Table 2). The H-3α signal (6 3.44, *dd*) showed the upfield shift of SO.16 compared to that of methyl rotundate (**8**), attributed to the loss of a shielding effect by the equatrial 4α-CH_2OH group. On the other hand, a W-type long range coupling was observed between the H-3α signal and one (6 3.35) of the 4β-hydroxymethyl signals. These data indicated that compound 4 could be 19α,24-dihydroxyursolic acid, an epimer of barbinervic acid 6 [8], and the structure was also elucidated chemically as follows.

Rotungenic acid (4) was oxidized with chromic anhydride in pyridine to give an 24-aldehyde (**15**), followed by Wolff-Kishner reduction to give 3β,19α-dihydroxyurs-12-ene-28-oic acid (**17**), mp > 300° [9–11]. Therefore, the structure of rotungenic acid was established to be 3β,19α,24-trihydroxyurs-12-ene-28-oic acid (4).

Table 1. ^{13}C NMR spectral data (δ) for compounds **2**, **4**, **5** and **7**

c	2	4	5	7*
1	38.9	38.8	38.5	39.8
2	21.1	28.5	26.9	29.1
3	73.7	80.3	73.3	78.3
4	42.9	43.2	52.2	49.2
5	48.8	56.5	49.6	56.9
6	18.9	19.3	19.5	20.9
7	33.4	34.0	34.5	33.9
8	40.4	40.4	39.8	40.2
9	47.9	47.9	45.9	47.2
10	37.3	37.2	36.8	37.9
11	24. I	24.3	24.1	24.5
12	128.1	127.9	125.6	128.1.
13	140.0	140.0	137.6	139.9
14	42.2	42.1	40.0	42.2
15	29.4	29.1	31.1	29.1
16	26.5	26.5	25.4	26.5
17	48.3	48.4	45.7	48.3
18	54.7	54.7	52.3	54.7
19	72.7	72.8	70.4	72.7
20	42.4	42.4	39.8	42.2
21	27.0	27.0	24.8	27.0
22	38.5	38.6	36.1	38.4
23	68.2	23.7	178.2	24.2
24	13.1	64.6	9.8	180.6
25	17.3	17.2	13.8	13.9
26	16.8	16.8	14.4	17.1
27	24.9	24.7	24.6	24.5
28	180.7	180.8	178.3	180.6
29	27.2	27.2	26.9	27.0
30	16.0	16.1	14.8	16.8

*Cited from ref. [10].
Measured in C_5D_5N.

Rotundioic acid (**5**), mp 295–298°, $[\alpha]_D$ + 50° (MeOH), revealed a molecular ion at *m/z* 502 $[M]^+$ corresponding to $C_{30}H_{46}O_6$ and retro-Diels-Alder fragmentation ions at *m/z* 264 and 238 from the cleavage of the C-ring of urs-12-ene derivative. The *m/z* 238 ion suggested the presence of one carboxyl group in rings A/B. This fragmentation and the IR and UV spectra (see Experimental) showed the presence of similar groups in 5 to those in 3 and 4 except for one additional carboxyl group instead of the hydroxymethyl group. Its ^{13}C NMR spectrum revealed thirty carbon signals including characteristic signals due to two carboxyl groups (6 178.2 and 178.3), a trisubstituted double bond (6 125.6 and 137.6) and two alcoholic carbons (6 70.4 and 73.3). It afforded a dimethyl acetate (14) which showed the presence of a 3β-acetoxyl group (6 5.17: 1H, *dd*, *J* = 11.5 and 4.5 Hz; H-3α) in the 'HNMR spectrum. A signal at δ2.60(1H, *br s*; H-l 8) suggested the presence of a 19-*O*-substituted urs-12-ene skeleton [12].

These facts indicated that compound **5** was related to 2 and 3 except for the exchanging of $-CH_2OH$ group to $-COOH$. In the ^{13}C NMR spectrum of5, the C-4 signal at δ 52.3 was shifted to downfield by *ca* 9 ppm from those of 2 or 3, and the chemical shift was very similar to that (653.0) of an oleane triterpene gypsogenic acid [13] possessing an equatorial carboxyl group at C-23. On the other hand, the comparison of the spectrum with that of ilexgenin A (7) [14] having a C-24 axial carboxyl group revealed significant differences in the chemical shifts of the A and B ring carbons (Table 1). Therefore, one of the carboxyl groups in 5 should be restricted to C-23 and the structure 5 was also established as follows.

Oxidation of the C_{23}-aldehyde 16 derived from 2 with silver oxide gave rotundioic acid (**5**), while reduction of 5 and 8 with lithium aluminium hydride afforded the same tetraol 18. The spectral and chemical evidence elucidated the structure of 5 as 3β,19α-dihydroxyurs-12-ene-23,28-dioic acid.

EXPERIMENTAL

Mps: uncorr. Concentrations were performed under red. pres. at bath temps not exceeding 50°. ^{1}H NMR spectra were obtained at 360 MHz and ^{13}C NMR spectra at 25.2 MHz. All the compounds were finally purified by HPLC on a C_{18} semiprep column using H_2O–MeOH solvent system.

Plant material. The ripe fruits of the plant were collected at Kagoshima University in March 1980.

Extraction and isolation. The ripe fruits (2 kg) were extracted with MeOH (3 × 10 1). After concn to 500 ml, H_2O (500 ml) was added and the mixture extracted with Et_2O and EtOAc to give 15 and 1.2 g of extracts, respectively. The Et_2O extract (5 g) was fractionated by CC using a MeOH–CH_2Cl_2 solvent system to give six fractions, I (1% MeOH–CH_2Cl_2): 350 mg, II (2% MeOH–CH_2Cl_2): 2.5 g. III (3% MeOH–CH_2Cl_2): 600 mg. IV (5% MeOH–CH_2Cl_2): 600 mg, V (7% MeOH–CH_2Cl_2): 750 mg and VI (10% MeOH–CH_2Cl_2): 100 mg. Rechromatography of the fraction I afforded ursolic acid (1) (35 mg). After rechromatography, the fraction II (125 mg) was separated by repeated HPLC using 3040% H_2O/MeOH as the solvent to give 2 (80 mg), 4 (8 mg) and 5 (4.5 mg). HPLC separation of the fraction IV (60 mg) with 40–55% H_2O–MeOH afforded **3** (15 mg)

Table 2. ^{1}H NMR spectral data for compounds **8–11** (CDCl$_3$, 360 MHz)

	8			9			10			11*		
H	δ	Mult	J(Hz)	δ	Mult	J(Hz)	δ	Mult	J(Hz)	δ	Mult	J(Hz)
3	3.60	*dd*	10, 6	3.44	*br dd*	11.5, 4.5	4.00	*dd*	11, 4.5	3.83	*t*	3
12	5.35	*brt*	3.5	5.35	*brt*	3.5	5.35	*br t*	3.5	5.31	*m*	
18	2.59	brs		2.60	*brs*		2.60	*brs*		2.58	*s*	
23	3.39	*d*	10.5	1.26	*s*					0.89	*s*	
	3.68	*d*	10.5									
24	0.95	*s*		3.35	*brd*	11	1.15	*s*		3.49	*d*	11
				4.20	*d*	11				3.69	*d*	11
25	0.95	*s*		0.86	*s*		0.94	*s*		1.08	*s*	
26	0.68	*s*		0.66	*s*		0.68	*s*		0.66	*s*	
27	1.25	*s*		1.25	*s*		1.27	*s*		1.21	s	
29	1.21	*s*		1.25	*s*		1.22	*s*		1.25	*s*	
30	0.94	*d*	6	0.97	*d*	7	0.95	*d*	6.5	0.94	*d*	6
OMe	3.60	*s*		3.60	*s*		3.61	s		3.57	*s*	
							3.73	*s*				

*Cited from ref. [8].

and an unknown compound (1.5 mg). The EtOAc fraction (100 mg) also gave **3** (21 mg).

Rotungenic acid (4). Columns from H_2O–MeOH; mp 295-298" (d); $[\alpha]_D$ + 16" (MeOH); SIMS m/z: 489 [M + 1]$^+$; EIMS m/z (rel. int.): 488 $[M]^+$ (4), 470 [M $-H_2O]^+$ (9), 452 [M $-2H_2O]^+$ (9), 442 [M-HCOOH]+ (16), 264 (24), 246 [264 $-H_2O]^+$ (44), 223 (15), 219 (20), 218 (20), 205 [223–$H_2O]^+$ (30), 201 [264–COOH]$^+$ (56), 175 (55), 146 (74). IR ν_{max}^{nujol} cm^{-1}: 3600-2600, 1690, 1640; UV λ_{max}^{MeOH} nm *(E): 209 (4000).*

Rotundioic acid (5). Prisms from MeOH; mp 295-298" (d); $[\alpha]_D$ + 50° (MeOH); SIMS m/z: 503 [M + 1]$^+$; EIMS m/z (rel. int.): 502 [M]' (4), 484 [M $-H_2O]^+$ (26), 456 [M $-$HCOOH]$^+$ (28), 264 (6), 246 (48), 238 (21), 219 (8), 218 (8), 201 (68), 175 (63), 146 (94); IR ν_{max}^{nujol} cm^{-1}: 3600–3200, 1690, 1635; UV λ_{max}^{MeOH} nm (E): 210 (4500); ^{1}H NMR (pyridine-d,): δ 1.00, 1.09, 1.42, 1.60, 1.66 (each 3H, *s*), 1.12 (3H, *d*, *J* = 7 Hz), 3.02 (1H, *s*), 3.04 (1H, *m*), 4.61 (1H, *dd*, *J*= 10 and 6 Hz), 4.71 (1H, s, -OH), 5.59 (1H, *br t*).

Ursolic acid(l), Mp 290°; EIMS ***m/z: 456*** $[M]^+$, ***438,410, 248*** (base peak), ***207, 203, 189.***

Rotundic acid (2). Mp 272–274° (d); $[\alpha]_D$ + 24" (MeOH); EIMS m/z: 488 $[M]^+$, 470, 452, 442, 264, 246, 223, 201, 175, 146; IR ν_{max}^{nujol} cm^{-1}: 3500–3200, 1690, 1640.

Peduncloside (3) Mp 213-214"; $[\alpha]_D$ + 22° (MeOH); SIMS m/z: 673 $[M+Na]^+$, 489 $[651-C_6H_{11}O_5]^+$, 471 $[489-H_2O]^+$, 425 $[453-CO]^+$, 407 $[425-H_2O]^+$; IR ν_{max}^{nujol} cm^{-1}: 3500-3200, 1720.

Methyl ***rotungenate (9).*** Rotungenic acid (4) was treated with CH_2N_2 to give the methyl ester 10, mp 208-211" (d); $[\alpha]_D$ + 5.5" (MeOH); EIMS ***m/z: 502*** $[M]^+$; IR ν_{max}^{nujol} cm^{-1}: ***3500, 1725.***

Diacetylrotungenic acid (12). Compound 4 was acetylated with Ac_2O in pyridine to give the diacetate (**12**), mp 164-167"; SIMS m/z: 573 [M + 1]$^+$; IR ν_{max}^{nujol} cm^{-1}: 3500, 1735, 1690.

Dimethyl rotundioate (10). Rotundioic acid (5) was treated with CH_2N_2 to give the dimethyl ester 13; EIMS m/z: 530 $[M]^+$.

Acetylrotundioic acid (13). Compound **5** was acetylated with Ac_2O in pyridine to give the acetate 13, mp > 300"; SIMS m/z: 545 [M + 1]$^+$; IR ν_{max}^{nujol} cm^{-1}: 3500, 1735, 1710, 1690.

Dimethyl acetylrotundioate (14). Compound 13 was methylated with CH_2N_2 to give the dimethyl ester 14, mp 210-212"; EIMS m/z: 572 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 0.67, 0.97, 1.19, 1.22, 1.26, 1.98 (each 3H, *s*), 0.95 (3H, *d*, *J* = 7 Hz), 2.60 (1H, *br s*), 3.60, 3.67 (each 3H, s), 5.17 (1H, *dd*, *J* = 11.5 and 4.5 Hz), 5.35 (1H, *br t*, *J* = 3.5 Hz).

Methyl ***rotundate (8).*** Mp 257-259" (d); $[\alpha]_D$ + 43° (CHCl$_3$); EIMS m/z: 502 $[M]^+$.

Aldehyde 15. Compound 4 was oxidized with chromic anhydride in pyridine at room temp. to give 15, mp 200–202°; EIMS ***m/z:*** 486 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ 0.76, 0.84, 1.20 (each 3H, s), 0.94 (3H, *d*, *J* = 6.5 Hz), 1.27 (6H, *s*), 2.53 (1H, *s*), 3.19 (1H, *m*), 5.34 (1H, *br t*), 9.76 (1H, s).

Aldehyde 16. Mp 205.5-207.5"; EIMS ***m/z:*** 486 $[M]^+$; ^{1}H NMR (pyridine-d,): 69.61 (1H, *s*, –CHO).

Wolff–Kishner reduction of 15. Compound 15 was treated with hydrazine hydrate (64%) and KOH in triethyleneglycol at 195–200° for 6 hr. The product gave 17 (15%), mp > 300°; EIMS ***m/z:*** 472 $[M]^+$; ^{1}H NMR (CDCl, + MeOH-d_4): δ 0.76, 0.78, 0.91, 0.99, 1.21, 1.26 (each 3H, s), 0.95 (3H, *d*, *J* = 6.6 Hz), 2.50 (1H, s), 3.22 (1H, *dd*, *J* = 10 and 5.5 Hz), 5.36 (1H, *br t*), which was also obtained from 16. Methyl ester; mp 123-125".

Tetraol 18. Rotundioic acid (5) was treated with $LiAlH_4$ in THF to give a tetraol **18**, mp 159-161"; SIMS m/z: 475 [M + 1]$^+$; ^{1}H NMR (CDCl,): δ 0.91, 0.97, 0.99, 1.18, 1.30 (each 3H, *s*), 0.95 (3H, *d*, *J* = 6 Hz), 3.19 (1H, *d*, *J*= 10.5 Hz), 3.46 (2H, *t*, *J*= 10 Hz), 3.67 (1H, *dd*, *J* = 9 and 7 Hz), 3.74 (1H, *d*, *J* = 10.5 Hz), 5.26 (1H, *br t*), which was also obtained from 8. Triacetate; 'HNMR (CDCl,): δ 0.85, 0.95, 0.98, 1.00, 1.17, 1.29, 2.03, 2.05, 2.07 (each 3H, *s*), 2.36 (1H, *dt*, *J*= 15 and 18.5 Hz), 3.64, 4.00 (each 3H, *d*, *J* = 11 Hz), 3.72, 3.89 (each, 1H, *d*, *J* = 11.5 Hz), 4.80 (1H, *dd*, *J* = 5 and 11 Hz), 5.26 (1H, *br t*).

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