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Studies on the Constituents of the Medicinal Plants. XXI.[1] Constituents of the Leaves of *Clethra barbinervis* Sieb. et Zucc. (2)[2] and the ^{13}C-Nuclear Magnetic Resonance Spectra of 19α-Hydroxyurs-12-en-28-oic Acid Type of Triterpenoids

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A new tetrahydroxy triterpene acid, named clethric acid (I), in addition to barbinervic- and rotundic-acids, has been isolated from the leaves of *Clethra barbinervis* Sieb. et Zucc and clethric acid was elucidated as 3α,19α,23,24-tetrahydroxyurs-12-en-28-oic acid by the chemical and spectral evidences.

The ^{13}C-nuclear magnetic resonance spectra of the methylates and acetyl methylates of clethric acid, pomolic acid, rotundic acid and barbinervic acid were studied.

Keywords——*Clethra barbinervis* Sieb. et Zucc.; Clethraceae; 19α-hydroxyurs-12-en-28-oic acid-type triterpenoid; clethric acid; ^{1}H-NMR; ^{13}C-NMR

It was reported that taraxerol and ceryl cerotinate were isolated from the barks[4] of *Clethra barbinervis* and ursolic acid from the fruits[4] and barbinervic acid and rotundic acid from the leaves.[2] This paper deals with the isolation and the structural elucidation of a new triterpene acid named clethric acid from the leaves. The methanolic extract of the leaves was extracted with petroleum ether, benzene, ethyl acetate and acetone, successively. The ethyl acetate-soluble and acetone-soluble fractions afforded clethric acid, $C_{30}H_{48}O_6$ (I), colorless needles of mp 284—287°, $[\alpha]_D^{17} = +50°$ (c=0.69, MeOH), Liebermann–Burchard test: red-violet. The acid (I) shows the infrared (IR) absorption bands (cm^{-1}) at 3500—3200 (OH), 1710 (COOH), 1650—1620 (trisubstituted double bond), 1065 (secondary OH), 1015 (primary OH), 920 (tertiary OH). The acid (I) afforded, by the methylation with diazomethane, monomethyl ester $C_{31}H_{50}O_6 \cdot 1/2\ H_2O$ (II), colorless needles of mp 206°, which afforded triacetyl methylate $C_{37}H_{56}O_9$ (III), colorless needles of mp 205—207° by the acetylation with acetic anhydride and pyridine. Acetyl methylate (III) shows the IR bands at 3600—3200 (OH), 1740, 1735, 1710 (acetyl and methyl ester groups), 1640—1630, 845 (trisubstituted double bond) and 935 (tertiary OH). Methylate (II) afforded two types of monoacetonides, $C_{34}H_{54}O_6$ (IV) of mp 145° (dec.) and $C_{34}H_{54}O_6$ (V) of mp 145° (dec.). The mass spectrum (MS) of I (M^+ =m/e 504) shows species at m/e 240 ($C_{14}H_{24}O_3$, A) and at m/e 264 ($C_{16}H_{24}O_3$, B), which would result from the M^+ ion by the retro-Diels–Alder (RDA) fragmentation, characteristic for Δ^{12}-amyrine type triterpenoid. The MS of I shows also species at m/e 222 (A$-H_2O$), m/e 204 (A$-2H_2O$), m/e 186 (A$-3H_2O$), m/e 246 (B$-H_2O$), m/e 201 (B$-H_2O$—COOH), m/e 218 B—HCOOH), m/e 200 (B—HCOOH$-H_2O$). The MS of III (M^+=644) shows species at m/e 366 ($C_{20}H_{30}O_6$, A′) and m/e 278 ($C_{17}H_{26}O_3$, B′) by the RDA fragmentation and also species at m/e 306 (A′—AcOH), m/e 246.163 ($C_{16}H_{22}O_2$, A′—2AcOH), m/e 186.136 ($C_{14}H_{18}$, A′—3AcOH) and at m/e 260.182 ($C_{17}H_{24}O_2$, B′$-H_2O$), m/e 245.153 ($C_{16}H_{21}O_2$, B′$-H_2O-CH_3$), m/e 219.172 ($C_{15}H_{23}O$, B′—$COOCH_3$), m/e 218.165 ($C_{15}H_{22}O$, B′—$HCOOCH_3$), m/e 203.147 ($C_{14}H_{19}O$, B′—$HCOOCH_3-CH_3$), m/e 201.162 ($C_{15}H_{21}$, B′$-H_2O-COOCH_3$), m/e 200.152 (C_{15}-

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3) Location: *Takaramachi, Kanazawa, 920, Japan.*
4) Y. Tanabe, T. Oda, and K. Takahashi, *Yakugaku Zasshi*, **86**, 441 (1966).

H_{20}, B′—$HCOOCH_3$—H_2O), and *m/e* 146.110 ($C_{11}H_{14}$, RDA fragment ion from species *m/e* 218.165). These chemical and spectral evidence suggest that clethric acid (I) has three OH groups on the ring A and/or B, which are easily acetylated and capable to form two types of acetonides IV and V, and a OH group on the ring E or D, which is sterically hindered, and a trisubstituted double bond at the C_{12}-carbon, and a carboxylic acid group on the ring D or E, although studies on the dehydration from species A should further be performed.

The ^1H nuclear magnetic resonance (NMR) spectra (δ-value) of II and III (Table I) could be interpreted as follows:

Table I. The ^1H-NMR Data (δ-value in $CDCl_3$, 100 MHz, J in Hz)

	$=C_{12}$–H	C_{18}–H	C_3–H	C_4–CH_2–O–	$COOCH_3$
II	5.35	2.58	4.24	3.92, q, 2H	3.59
	m, 1H	s, 1H	m, 1H	J=11, δ_{AB}=27	
			$W_{h/2}$=5	3.70, q, 2H	s, 3H
				J=15, δ_{AB}=29	
III	5.35	2.60	5.02	4.22, dull, 2H	3.60
	m, 1H	s, 1H	m, 1H		
			$W_{h/2}$=5	4.11, q, 2H	s, 3H
				J=11, δ_{AB}=7	
IV	5.34	2.60	4.06	3.84, q, 2H	3.60
	m, 1H	s, 1H	m, 1H	J=11, δ_{AB}=29	
			$W_{h/2}$=7	3.67, q, 2H	s, 3H
				J=12, δ_{AB}=22	
V	5.36	2.60	4.22	3.77, dull, 2H	3.60
	m, 1H	s, 1H	m, 1H	3.67, q, 2H	
			$W_{h/2}$=6	J=17, δ_{AB}=22	s, 3H

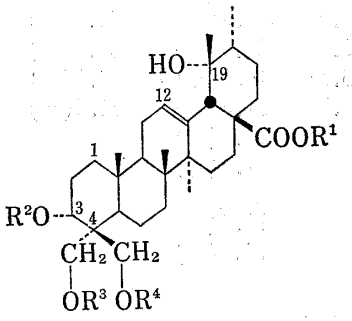
	CH_3					OAc	–O–CMe_2–O–
	C_{25}	C_{26}	C_{27}	C_{29}	C_{30}		
II	0.82	0.65	1.21	1.27	0.94		
	s, 3H	s, 3H	s, 3H	s, 3H	d, 3H		
					J=6		
III	0.97	0.69	1.21	1.31	0.94	1.99	
	s, 3H	s, 3H	s, 3H	s, 3H	d, 3H	s, 3H	
					J=6	2.06	
						s, 6H	
IV	0.92	0.69	1.22	1.31	0.95		1.40
	s, 3H	s, 3H	s, 3H	s, 3H	d, 3H		s, 3H
					J=6		1.43
							s, 3H
V	0.82	0.70	1.21	1.26	0.93		1.34
	s, 3H	s, 3H	s, 3H	s, 3H	d, 3H		s, 3H
					J=6		1.38
							s, 3H

Abbreviation: s, singlet; d, doublet; q, quartet; m, multiplet.

The δ-value (5.35) of the signal of II and that (5.35) of the signal of III, respectively assignable to the signal of the olefinic proton and that (2.58, s) of the signal of II and that (2.60, s) of the signal of III, respectively assignable to the C_{18}-proton are nearly equal to those of the corresponding signals of methyl esters and acetyl methyl esters of barbinervic acid,[2] rotundic acid,[2] and diacetyl tormentic acid,[5] respectively. But II does not exhibit the NMR signal assignable to the proton at the C_{19}-carbon, while siaresinolic acid methyl ester[6] and spinosic

5) K. Takahashi, M. Ogura, and Y. Tanabe, *Chem. Pharm. Bull.* (Tokyo), **20**, 2106 (1972).
6) R.T. Aplin, W.H. Hui, C.T. Ho, and C.W. Yee, *J. Chem. Soc.* (*C*), **1971**, 1067.

acid A methyl ester[6] exhibit respectively the signal of the proton at the C_{19}-carbon at 3.32 and 3.35. These spectral evidences indicate that clethric acid (I) could be assumed to be a trihydroxy derivative of 19α-hydroxyurs-12-en-28-oic acid type of triterpenoid. Methylate (II) shows the NMR methyl signals at 0.65 (s), 1.21 (s), 1.27 (s) and 0.94 (d, J=6 Hz). These δ-values are respectively nearly equal to those of the methyl signals of the C_{26}, C_{27}, C_{29} and C_{30} carbons of methyl barbinervate, suggesting the absence of the shielding effects of a secondary OH group on the C_{26} and C_{27} methyl groups of II and so the absence of an axial secondary OH group at the C_6 or the C_7 carbons of II. Acetyl methylate (III) shows five methyl signals at 0.97 (s), 0.69 (s), 1.21 (s), 1.31 (s) and 0.94 (d, J=6 Hz), whose values are respectively nearly equal to those of the methyl signals of the C_{25}, C_{26}, C_{27}, C_{29} and C_{30} of acetyl methyl barbinervate, but III does not show a methyl signal which corresponds to the C_{23}-methyl signal (0.93) of acetyl methyl barbinervate, suggesting that III has an equatorial CH_2OAc group in addition to an axial CH_2OAc at the C_4 carbon. Acetyl methylate (III) shows, as expected, two methylene signals of the CH_2OAc groups at 4.22 (2H, m) and 4.11 (2H, m), while methylate (II) shows these signals at 3.92 and 3.70, respectively, by the expected upfield shift. Acetyl methylate (III) shows also a methine signal of a HC-OAc group at 5.02 (m, $W_{h/2}$=5 Hz) as acetyl methyl barbinervate (4.94). A greatly reduced half band width ($W_{h/2}$=5 Hz) of this signal due to the methine group linked to the acetoxy group could be attributed to a combination of two factors: first its equatorial nature and second the presence of only two vicinal protons.[7] Hence, this acetoxy group must be linked to the C_3-carbon as axial. The NMR evidence and the formation of two types of acetonides IV and V suggest also that two hydroxymethyl groups of I, mentioned above, must be present at the C_4-carbon. The acetonides IV and V show the MS species at *m/e* 278, 260, 218, which are observed on the MS of III as the fragment ions from the ring D and E by the RDA fragmentation, indicating that acetonides formation occurred at the ring A. Acetonides IV and V show respectively the NMR signal of the methine proton at the C_3- carbon at 4.06 (m, $W_{h/2}$=7 Hz) and at 4.22 (m, $W_{h/2}$=6 Hz). The reduced half widths of these methine protons indicate that these protons are respectively equatorial as that of II and the conformation of each ring A of IV and V remains unchanged as a chair form and is not of boat form. The δ-value (4.22) of the methine proton at the C_3-carbon of V is nearly equal to that (4.24) of the methine proton at the C_3-carbon of II, suggesting that V could be considered to be C_{23}, C_{24}-acetonide and IV, whose methine proton resonates at rather high field (4.06), could be considered to be $C_{3\alpha}$, C_{23}-acetonide as shown in Chart 1. These chemical and spectral evidences indicate that clethric acid (I) could be elucidat-



I: R^1=R^2=R^3=R^4=H
II: R^1=Me, R^2=R^3=R^4=H
III: R^1=Me, R^2=R^3=R^4=Ac
IV: R^1=Me, R^2, R^3=$C(CH_3)_2$, R^4=H
V: R^1=Me, R^2=H, R^3, R^4=$C(CH_3)_2$

Chart 1

ed as 3α, 19α, 23, 24-tetrahydroxyurs-12-en-28-oic acid as shown in Chart 1, which is the first example of the naturally occurring urs-12-ene type of triterpenoid having geminal hydroxymethyl groups at the C_4-carbon in the ring A. The ^{13}C-NMR spectra of the methylates and

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acetyl methylates of clethric acid (I), pomolic acid,[8] rotundic acid[2] and barbinervic acid,[2] all of which belong to the 19α-hydroxyurs-12-en-28-oic acid type of triterpenoid were studied and all signals of each spectrum could be assigned to their appropriate carbons as expected for the proposal structures, respectively, as shown in Table II, taking the ^{13}C–NMR spectra of hederagenin[9] and ursolic acid[10,11] into consideration.

Table II. The ^{13}C–NMR Data (25.15 MHz, $CDCl_3$)

C	Pomolic acid		Rotundic acid		Barbinervic acid		Clethric acid	
	Methylate	Acetyl-methylate	Methylate	Acetyl-methylate	Methylate	Acetyl-methylate	II	III
1	38.7	38.1(t)	38.1(t)	37.9(t)	33.1	33.4(t)	32.7(t)	33.2(t)
2	27.4	23.6(t)	26.8(t)	23.0(t)	25.2	22.5(t)	24.6(t)	22.4(t)
3	79.0	80.9(d)	76.9(d)	74.6(d)	70.6	73.4(d)	67.0(d)	70.3(d)
4	38.5	37.6(s)	41.8(s)	40.6(s)	42.7	40.2(s)	45.3(s)	43.0(s)
5	55.2	55.2(d)	49.8(d)	47.9(d)	49.5	50.7(d)	46.4(d)	47.9(d)
6	18.4	18.3(t)	18.6(t)	18.1(t)	18.7	18.4(t)	18.4(t)	19.4(t)
7	32.8	32.7(t)	32.5(t)	32.5(t)	32.9	32.9(t)	32.7(t)	32.8(t)
8	39.9	39.9(s)	39.9(s)	40.0(s)	40.0	40.2(s)	39.8(s)	39.9(s)
9	47.2	47.1(d)	47.2(d)	47.3(d)	47.1	47.2(d)	46.9(d)	47.3(d)
10	36.9	36.9(s)	36.9(s)	36.8(s)	36.8	36.8(s)	36.6(s)	36.7(s)
11	23.7	23.6(t)	23.7(t)	23.7(t)	23.7	23.7(t)	23.8(t)	23.7(t)
12	129.1	129.0(d)	129.1(d)	129.0(d)	129.1	129.0(d)	128.9(d)	128.7(d)
13	138.0	138.0(s)	138.0(s)	138.2(s)	137.9	138.1(s)	138.0(s)	138.0(s)
14	41.1	41.1(s)	41.1(s)	41.2(s)	41.1	41.2(s)	41.2(s)	41.1(s)
15	28.1	28.2(t)	28.2(t)	28.3(t)	28.2	28.3(t)	28.2(t)	28.2(t)
16	25.5[a]	25.5(t)[a]	25.5(t)[a]	25.5(t)[a]	25.5[a]	25.5(t)[a]	25.5(t)[a]	25.5(t)[a]
17	47.9	47.9(s)	47.9(s)	47.9(s)	47.9	47.9(s)	47.9(s)	47.9(s)
18	53.2	53.3(d)	53.2(d)	53.3(d)	53.2	53.3(d)	53.3(d)	53.3(d)
19	73.1	73.0(s)	73.1(s)	73.2(s)	73.1	73.2(s)	73.1(s)	73.1(s)
20	41.1	41.1(d)	41.1(d)	41.2(d)	41.1	41.2(d)	41.1(d)	41.1(d)
21	26.0[a]	26.0(t)[a]	26.0(t)[a]	26.1(t)[a]	26.0[a]	26.1(t)[a]	26.0(t)[a]	26.0(t)[a]
22	37.4	37.4(t)	37.4(t)	37.4(t)	37.4	37.4(t)	37.5(t)	37.4(t)
23	28.1	28.2(q)	72.1(t)	65.5(t)	21.6	22.1(q)	70.3(t)	64.3(t)
24	15.2[b]	16.7(q)[b]	11.3(q)	13.1(q)	66.5	66.7(t)	66.0(t)	67.2(t)
25	15.5[b]	15.3(q)	15.6(q)	15.8(q)	15.6	15.5(q)	14.9(q)	15.2(q)
26	16.6[c]	16.7(q)[b]	16.6(q)[b]	16.7(q)[b]	16.5[b]	16.5(q)[b]	16.6(q)[b]	16.5(q)[b]
27	24.5	24.5(q)	24.5(q)	24.4(q)	24.6	24.6(q)	24.6(q)	24.4(q)
28	178.3	178.2(s)	178.3(s)	178.3(s)	178.2	178.3(s)	178.5(s)	178.2(s)
29	27.2	27.4(q)	27.4(q)	27.5(q)	27.4	27.5(q)	27.4(q)	27.4(q)
30	16.1[c]	16.1(q)[b]	16.1(q)[b]	16.1(q)[b]	16.1[b]	16.1(q)[b]	16.1(q)[b]	16.1(q)[b]
$COO\underline{C}H_3$	51.5	51.5(q)	51.5(q)	51.5(q)	51.5	51.5(q)	51.6(q)	51.5(q)
$OCO\underline{C}H_3$		21.3(q)		21.2(q)		20.9(q)		21.2(q)
				20.9(q)		21.3(q)		20.9(q)
								20.9(q)
$O\underline{C}OCH_3$		170.8(s)		170.9(s)		171.1(s)		170.5(s)
				170.6(s)		170.4(s)		170.5(s)
								169.9(s)

a), b), c): Values in any vertical column may be reversed although those given here preferred.
The letter in parentheses designates the multiplicity of signal with off-resonance decoupling.
Abbreviation: s, singlet; d, doublet; t, triplet; q, quartet.

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Experimental

The following instruments were used for the physical data. Melting point: Yanagimoto Micro-Melting apparatus (a hot plate type); IR (cm^{-1}): Nippon Bunko IR-G spectrometer in KBr; NMR (δ-value in ppm): JNM-PS-100 high resolution instrument at 100 MHz (^1H) and at 25.15 MHz (^{13}C) in $CDCl_3$ with $(CH_3)_4Si$ as an internal reference; MS: JMS-OISG mass spectrometer (direct inlet, 75 eV); Optical rotation: Nippon Bunko automatic polarimeter DIP-SL at 589 nm. The thin-layer chromatogram (TLC) was obtained on a glass plate coated with Kieselgel G (Stahl). The column chromatography was carried out using Kieselgel 60 (Stahl).

Isolation of I——Dried leaves (4.8 kg) of *Clethra barbinervis* were extracted with methanol and then the extract was concentrated *in vacuo* to afford a powder (500 g), which was adsorbed on a celite (100 g) and the celite was extracted with petr-ether, benzene, ethyl acetate and acetone, successively. The ethyl acetate–soluble and acetone–soluble fractions (49 g) were chromatographed on a column of silica gel (1 kg) with methanol–chloroform (1: 2). The fraction (840 mg) of *Rf* 0.34 (TLC, MeOH–$CHCl_3$=1: 10) was chromatographed on a column of silica gel (170 g) with acetone–$CHCl_3$ (1: 2) to afford colorless needles (304 mg) of mp 288° from methanol–benzene, which was proved to be identical with rotundic acid[2] by the mixed fusion and IR. The fraction (430 mg) of *Rf* 0.27 (TLC, MeOH–$CHCl_3$=1: 10) was chromatographed on a column of silica gel (170 g) with acetone–chloroform (2: 3) to afford colorless needles (427 mg) of mp 297—299° from methanol, which was proved to be identical with barbinervic acid[2] by the mixed fusion, IR and TLC. The fraction (900 mg) of *Rf* 0.25 (TLC, CH_3OH–$CHCl_3$=1: 10) was chromatographed on a column of silica gel (180 g) with acetone–chloroform (2: 3) to afford colorless needles (I) (130 mg) of mp 284—287° from methanol. *Anal.* Calcd. for $C_{30}H_{48}O_6 \cdot H_2O$: C, 68.93; H, 9.64. Found: C, 69.08; H, 9.86.

Methylation of I——I (260 mg) in methanol was methylated with diazomethane and the crude methylate was chromatographed on a column of silica gel (52 g) with acetone–chloroform (1: 2) to afford colorless crystalline powder (II) (59 mg) of mp 206° from petr-ether. *Anal.* Calcd. for $C_{31}H_{50}O_6 \cdot 1/2H_2O$: C, 70.55; H, 9.74. Found: C, 70.61; H, 9.52. MS *m/e*: 518 (M^+), 240 (A), 278 (B), 222 (A$-H_2O$), 204 (A$-2H_2O$), 186 (A$-3H_2O$), 260 (B$-H_2O$), 201 (B$-H_2O-COOCH_3$), 218 (B$-HCOOCH_3$), 200 (B$-HCOOCH_3-H_2O$).

Acetylation of II——II (164 mg) was acetylated with acetic anhydride (4.5 ml) and pyridine (2 ml) for 24 hr at room temperature and the crude acetyl methylate (176 mg) was chromatographed on a column of silica gel (35 g) with acetone–chloroform (1: 25) to afford colorless needles (III) of mp 205—207° from *n*-hexane. *Anal.* Calcd. for $C_{37}H_{56}O_9$: C, 68.91; H, 9.75. Found: C, 69.07; H, 8.84.

Acetonides of II——A mixture of II (150 mg), *p*-toluenesulfonic acid (15 mg) in dry acetone (20 ml) was refluxed for 1 hr. The reaction mixture was poured into 4% K_2CO_3 solution and extracted with $CHCl_3$, which was washed with water and evaporated, after being dried over Na_2SO_4. The residue was chromatographed over silica gel with benzene–ether (5: 1). The fraction of *Rf* 0.26 (TLC, benzene–ether=5: 1) was crystallized from petr-ether and methanol to afford acetonide IV, crystalline powder of mp 145° (dec.). *Anal.* Calcd. for $C_{34}H_{54}O_6$: C, 73.08; H, 9.74. Found: C, 72.45; H, 9.97. The fraction of *Rf* 0.19 (TLC, benzene–ether=5: 1) was crystallized from petr-ether and ether to afford acetonide V, crystalline powder of mp 145° (dec.). *Anal.* Calcd. for $C_{34}H_{54}O_6$: C, 73.08; H, 9.74. Found: C, 73.03; H, 9.98.

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