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Studies on Constituents of Medicinal Plants. XIII.[1] Constituents of the Pericarps of the Capsules of *Euscaphis japonica* Pax. (1)

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From the pericarps of the capsules of *Euscaphis japonica* Pax., oleanolic, pomolic, tormentic acids and a new triterpene acid, named euscaphic acid were isolated and the structure of euscaphic acid was elucidated to be 2α,3α,19α-trihydroxyurs-12-en-28-oic acid by chemical and spectral evidences.

Very little is known about the constituents of *Euscaphis japonica*. This paper deals with the structural elucidation of a new triterpene acid, to be named euscaphic acid hereafter. The ether extract of the pericarps of the capsules afforded faint yellow powder, whose thin layer chromatogram showed six spots, designated A, B, C, D, E and F from the top downward. From the fraction of the spot A, colorless needles $C_{30}H_{48}O_3$, mp>300° were obtained, which gave monoacetate $C_{32}H_{50}O_4$, mp 265—266° and monoacetyl methyl ester $C_{33}H_{52}O_4$, mp 220°. The monoacetate was proved to be identical with monoacetyl oleanolic acid by the mixed fusion and infrared spectra (IR). From the fraction of the spot B, colorless glossy crystalline powder $C_{30}H_{48}O_4$, mp>300° was obtained, which gave monoacetate $C_{32}H_{50}O_5$, mp 282° and monoacetyl methyl ester $C_{33}H_{52}O_5$, mp 249—250° and methyl ester $C_{31}H_{50}O_4$, mp 126°. The monoacetyl methyl ester was proved to be identical with methyl monoacetyl pomolate[3] by the mixed fusion and IR. From the fraction of the spot E, colorless crystalline powder $C_{30}H_{48}O_5 \cdot 1/2H_2O$, mp 264—266° was obtained, which gave diacetate $C_{34}H_{52}O_7$, mp 194°. The diacetate was proved to be identical with diacetyl tormentic acid[4] by the mixed fusion and IR. From the fraction of the spot D, colorless crystalline powder $C_{30}H_{48}O_5 \cdot 1/2H_2O$, mp 270°, $[\alpha]^{23°}=12°$ (c=1.00, MeOH), euscaphic acid (I) was obtained, which gave diacetate (II) $C_{34}H_{52}O_7$, mp 188° with acetic anhydride and pyridine and methyl ester (III) $C_{31}H_{50}O_5$, mp 125—130° (decomp.) with diazomethane. I gave the Liebermann-Burchard reaction (red) and showed IR bands (cm^{-1}) at 3400 (OH), 1690 (COOH), 1650—1630, 820, 805 (trisubstituted double bond), 1030, 1000 (secondary OH), 930 (tertiary OH) and its mass spectrum (MS) showed M^+-peak at 488. Generally, pentacyclic triterpenes with Δ^{12}-unsaturation readily undergo the retro-Diels-Alder cleavage of the ring C under electron impact. Diacetate (II) showed MS peaks (m/e, relative intensity), which closely resemble that[4b] of diacetyl tormentic acid, at 572 (12, M^+), 526 (48, M^+-HCOOH), 454 (24, RDA ion of 526), and 264 (30, A) and 308 (2, B), 307 (7) by the RDA fragmentation and also 246 (35, A-H_2O), 201 (30, 246-COOH), 200 (22, 246-HCOOH) and 248 (5, B-CH_3COOH), 188 (39, B-2CH_3COOH). I, after being oxidated with CrO_3 in pyridine, gave the positive 1,2-diketone reaction (red) with *o*-phenylenediamine and HCl, indicating that I has 1,2-diol group which is easily acetylated and a

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3) C.H. Brieskorn and H. Wunderer, *Chem. Ber.*, **100**, 1252 (1967). The sample was furnished through the courtesy of Prof. Dr. C.H. Brieskorn.
4) *a*) H.T. Cheung and D.G. Williamson, *Tetrahedron*, **25**, 119 (1969); *b*) K. Takahashi, M. Ogura, and Y. Tanabe, *Chem. Pharm. Bull.* (Tokyo), **17**, 2223 (1969); *c*) P. Potier, B.C. Das, Anne-Marie Bui, and M.M. Jonot, *Bull. Soc. Chim. Fr.*, **11**, 3458 (1966).

TABLE I. NMR Signals of II and III (δ-values, J in Hz, 100 Mc)

	C_{12}-H	C_{18}-H	C_2-H	C_3-H	$COOCH_3$	OAc	C-CH_3						
							C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{29}	C_{30}
II	5.31[a] m,1H	2.53 s,1H	5.15[a] m,1H $W_{h/2}=14$	4.93 d,1H $J=3$		1.95 s,3H 2.05 s,3H	0.87 s,3H	0.98 s,3H	1.03 s,3H	0.73 s,3H	1.19 s,3H	1.30 s,3H	0.98 s,3H
III	5.32 m,1H	2.58 s,1H	3.96 m,1H $W_{h/2}=20$	3.39 d,1H $J=3$	3.56 s,3H		1.00 s,3H	0.85 s,3H	0.94 s,3H	0.66 s,3H	1.19 s,3H	1.25 s,3H	0.94 s,3H

a) The signal of the C_2-H and that of the C_{12} overlapped each other.
abbreviation: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

sterically hindered OH group. These chemical and spectral data suggest that euscaphic acid (I) might be an isomer of tormentic acid and so the nuclear magnetic resonance spectra (NMR) (δ-value, ppm) of II and III were analysed and all the signals of II and III could be assigned to their appropriate groups as shown in Table I.

Analyses of the NMR of II and III

Angular Methyl Groups——It is well known that for the triterpenoid skeleton, modifications in the substituent pattern are accompanied by systematic changes in the NMR chemical shifts of the neighbouring angular methyl group and such changes are, to the first approximation, additive. The pattern of the NMR signals of the angular methyl groups of II and III

TABLE II

		C_{23}	C_{24}	C_{25}	C_{26}	C_{27}	C_{29}	C_{30}
	tormentic acid diacetate[a]	0.89	0.89	1.04	0.71	1.17	1.23	0.95
Effect	2α-OAc[b]	0.04	0.04	0.13	−0.01	−0.01	−0.02	−0.02
	2β-OAc[b]	0.03[c]	0.18	0.26	0.03	0	0	0
	3α-OAc[c]	−0.02	0.07	0.01	0	0	0	0
	3β-OAc[b]	0	0.05	0.02	0	0	0.01	0.01
Calcd.	2β,3β-diacetoxy-19α-hydroxy-urs-12-en-28-oic acid	0.88	1.03	1.17	0.75	1.18	1.25	0.97
	2β,3α-diacetoxy-	0.86	1.05	1.16	0.75	1.18	1.24	0.96
	2α,3α-diacetoxy-	0.87	0.91	1.03	0.71	1.17	1.22	0.94
	II	0.87	0.98	1.03	0.73	1.19	1.30	0.98
	methyl tormentate[b]	1.03	0.83	0.98	0.69	1.21	1.25	0.94[d]
Effect	2α-OH[b]	0.03	0.03	0.03	−0.01	−0.01	−0.02	−0.02
	2β-OH[c]	0.03	0.22	0.28	0.02	0	0.01	0.01
	3α-OH[c]	0.10	0.03	0.01	0	0	0	0
	3β-OH[b]	0.12	−0.03	0.01	0.02	0	0	0
Calcd.	2β,3β-dihydroxy-19α-hydroxy-urs-12-en-28-oic acid Me ester	1.03	1.02	1.23	0.72	1.22	1.28	0.97
	2β,3α-dihydroxy-	1.01	1.08	1.23	0.70	1.22	1.28	0.97
	2α,3α-dihydroxy-	1.01	0.89	0.98	0.67	1.21	1.25	0.94
	III	1.00	0.85	0.94	0.66	1.19	1.25	0.94

a) See, ref. 4*b*. Some of assignments of angular methyl signals were revised.
b) Shielding effect on the angular methyl groups of urs-12-ene. See, ref. 4*a*.
c) Shielding effect on the angular methyl groups of olean-12-ene. See, ref. 5.
d) The data was calculated from the data of tormentic acid diacetate. For example, the chemical shift of the signal of the C_{23}-methyl of 2β,3β-diacetoxy 19α-hydroxyurs-12-en-28-oic acid was calculated as follows: 0.89−0.04+0.03=0.88

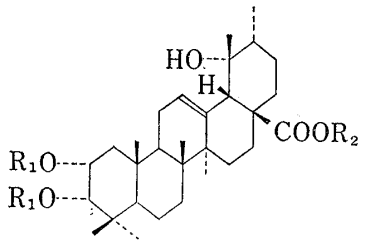
suggests that I is α-amyrin type of triterpenoid as tormentic acid. With the chemical shifts of the angular methyl groups of tormentic acid diacetate[4] (2α,3β-diacetoxy-19α-hydroxyurs-12-en-28-oic acid) as references, 2β,3β-diacetoxy, 2β,3α-diacetoxy and 2α,3α-diacetoxy derivatives of 2,3-dihydroxy-19α-hydroxyurs-12-en-28-oic acid were expected to exhibit the angular methyl signals at δ-values, respectively as shown in Table II. II and III exhibit the NMR signals of the angular methyl groups at δ-values as shown in Table II, which nearly equal the δ-values of the calculated angular methyl signals of diacetate and methyl ester of 2α,3α,19α-trihydroxyurs-12-en-28-oic acid, respectively. The 1,3-diaxial effect which is expected in the case of 2β,3α,19α- or 2β,3β,19α-trihydroxyurs-12-en-28-oic acid methyl ester, namely the deshielding effect of the C_2-OH group (β, ax.) on the C_{24}-methyl (β, ax.) and C_{25}-methyl (β, ax.) groups, was not observed in the case of III, also indicating that III has the C_2-OH (α, equ.) group, as shown in Table II.

C_{12}-Proton——II exhibited the NMR signal at 5.31 (m) and III at 5.32 (m), which closely resemble the signal of the C_{12}-proton of diacetyl tormentic acid at 5.27 (m), that of monoacetyl pomolic acid at 5.24 (m) and that of methyl 2α,3α-dihydroxyolean-12-en-28-oate[5] at 5.3 (t, J=4 Hz), but they do not resemble that of methyl acetylastilbate[6] at 5.61, indicating that both II and III have a doble bound at C_{12}-C_{13} and a COOH group at C_{17}, respectively.

C_{18}-Proton——II showed the NMR signal at 2.53 (s) and III at 2.58 (s), which closely resemble the signal of the C_{18}-proton of diacetyl tormentic acid at 2.51 (s, β) and that monoacetyl pomolic acid at 2.48 (s, β), respectively, but they do not resemble those of methyl oleanolate at 2.75 (q) and of methyl ursolate at 2.2 (d), probably indicating that II and III have a β-proton at C_{18} and also that II and III are α-amyrin type of triterpenoid with a methyl group and a OH group at C_{19} as tormentic and pomolic acids.

C_2-Proton——II showed the NMR signal at 5.15 (m, $W_{h/2}$=14 Hz), which resembles the signal of the C_2-H (β, ax.) of methyl 2α,3α-diacetoxyolean-12-en-28-oate[5] at 5.2 (m, $W_{h/2}$=18 Hz), but does not resemble the signal of the C_2-H (α, equ.) of methyl 2β,3β-diacetoxyolean-12-en-28-oate[5] at 5.3 (m, $W_{h/2}$=8 Hz). III showed the NMR signal at 3.96 (m, $W_{h/2}$=20 Hz), which resembles the signal of the C_2-H (β, ax.) of methyl 2α,3α-dihydroxyolean-12-en-28-oate[5] at 3.9 (m, $W_{h/2}$=21 Hz), but does not resemble that (α, equ.) of methyl 2β,3β-dihydroxyolean-12-en-28-oate[5] at 4.05 (m, $W_{h/2}$=8 Hz). These data also suggest that the C_2-protons of II and III are in β (ax.) conformation, respectively. In this case, the difference between the shielding effect of OAc group and that of OH group is calculated at 1.19 ppm (5.15—3.96).

C_3-Proton——II showed the NMR signal at 4.93 (d, J=3 Hz), which resembles the signal of the C_3-H (β, equ.) of methyl 2α,3α-diacetoxyolean-12-en-28-oate[5] at 4.95 (d, J=3 Hz), but does not resemble that (d, J=4 Hz) of methyl 2β,3β-diacetoxyolean-12-en-28-oate[5] at 4.6. III showed the NMR signal at 3.39 (d, J=3 Hz), which resembles the signal of the C_3-H (β, equ) of methyl 2α,3α-hydroxyolean-12-en-28-oate[5] at 3.35 (d, J=3 Hz), but does not resemble that (d, J=4 Hz) of methyl 2β,3β-dihydroxyolean-12-en-28-oate[5] at 3.15. In this case, the difference between the shielding effect of OAc group and that of OH group is calculated at 1.54 ppm (4.93—3.39). These spectral data suggest that the C_3-protons of II and III are in β (equ.) conformation, respectively. These spectral and chemical evidences indicate that euscaphic acid (I) might be 2α,3α,19α-trihydroxyurs-12-en-28-oic acid, as shown in Chart 1.



I : R_1=R_2=H
II : R_1=Ac, R_2=H
III : R_1=H, R_2=CH_3

Chart 1

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Experimental

The IR were taken in KBr pellet with Nippon Bunko DS-402G spectrometer, the NMR in $CDCl_3$ with a Varian NMR instrument at 100 Mc with $(CH_3)_4Si$ as internal reference, the MS with JMS-01SG mass spectrometer and the optical rotation with Nippon Bunko automatic polarimeter DIP-SL at 589 nm. The *Rf*-values were obtained with Kieselgel nach Stahl as adsorbent and $CHCl_3$: MeOH=40: 1 as developer, not otherwise stated.

Isolation——The pericarps of capsules (300 g) were extracted with ether for 80 hr in a soxhlet and the ether solution was evaporated *in vacuo* to give faint yellow powder (2—3 g), three grams of which were adsorpted on Kieselgel (10 g) in methanol and it was chromatographed over Kieselgel (500 g) with $CHCl_3$–MeOH (40: 1).

Oleanolic Acid——The fractions (20 g each) from No 13 to 15 gave colorless needles (100 mg) of mp>300° from MeOH. *Rf*: 0.45. *Anal.* Calcd. for $C_{30}H_{48}O_3$: C, 78.90; H, 10.59. Found: C, 78.42; H, 10.56. It gave monoacetate of mp 265—266° and monoacetyl methyl ester of mp 220°, the former of which was proved to be identical with acetyl oleanolic acid by the mixed fusion and IR.

Pomolic Acid——The fractions from No 16 to 35 gave colorless crystalline powder (200 mg) of mp> 300° from MeOH, which gave the positive Liebermann-Burchard reaction (red to red-violet). *Rf*: 0.40. IR: 3400 (OH), 1695 (COOH), 1640, 825, 805 (trisubstituted double bond), 1030, 995 (secondary OH), 925 (tertiary OH). *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 76.34; H, 10.28. M^+: 472. It gave monoacetate of mp 282° with acetic anhydride and pyridine. *Anal.* Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.47; H, 9.79. It gave methylester of mp 126° (colorless needles) with diazomethane. *Anal.* Calcd. for $C_{31}H_{50}O_4$: C, 76.50; H, 10.36. Found: C, 76.21, H, 10.48. The monoacetate gave monoacetyl methyl ester of mp 249—250° (colorless prisms) from MeOH. $[\alpha]^{20}=40°$ ($c=1.5$, $CHCl_3$). IR: 3450—3550 (OH), 1720 (C=O), 1640, 825, 805 (trisubstituted double bond), 930 (tertiary OH). *Anal.* Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.86. Found: C, 75.22; H, 9.84. The acetyl methyl ester was proved to be identical with an authentic sample of methyl monoacetyl pomolate[3] by the mixed fusion and IR.

Euscaphic Acid——The fractions from No 141 to 170 gave colorless crystalline powder (I) of mp 270—271° from MeOH (250 mg). Liebermann-Burchard reaction: red. *Anal.* Calcd. for $C_{30}H_{48}O_5 \cdot 1/2H_2O$: C, 72.40; H, 9.92. Found: C, 72.15; H, 9.91. $M^+=488$. *Rf*: 0.21 (brown with conc. H_2SO_4). In the same condition, tormentic acid showed a spot at $Rf=0.15$ (violet brown with conc. H_2SO_4).

Acetylation of I—— I (160 mg) in pyridine (1 ml) was added to acetic anhydride (2 ml) and the mixture was allowed to stand for 24 hr and then poured into ice water to give colorless needles (II) of mp 188° from benzene–hexane. (68 mg). IR: 3750—3100 (OH), 1745 (acetate), 1697 (COOH), 802 (trisubstituted double bond). *Anal.* Calcd. for $C_{34}H_{52}O_7$: C, 71.29; H, 9.15. Found: C, 71.04; H, 9.09. *Rf*: 0.42. In the same condition, diacetyltormentic acid showed a spot at $Rf=0.48$.

1,2-Diketone Reaction——To a solution of CrO_3 (12 mg) in pyridine (1 ml), I (12 mg) was added and the mixture was agitated for 1 hr at room temperature and then was poured into ice water-HCl. The aqueous layer was extracted with $CHCl_3$ and then the $CHCl_3$ solution was evaporated *in vacuo* to give a product, which gave red coloration with *o*-phenylenediamine and conc. HCl.

Methylation of I——I in methanol was methylated with diazomethane to give a syrupy substance, which was purified by column chromatography with Kieselgl and $CHCl_3$–MeOH (80: 1). The eluate of $Rf=0.28$ ($CHCl_3$–MeOH=40: 1) was crystallized from ether–*n*-hexane to give colorless crystalline powder of mp 125—130° (decomp.) IR: 3450 (OH), 1720 (ester), 1640, 810 (trisubstituted double bond). *Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 73.68; H, 9.91.

Tormentic Acid——The fractions from No 171 to 200 gave tormentic acid of mp 264—266° from methanol–water. Yield: 50 mg. Liebermann-Burchard reaction: red to violet-red. IR: 3300 (OH), 1695 (COOH), 1640, 825, 805 (trisubstituted double bond), 1030, 995 (secondary OH). *Anal.* Calcd. for $C_{30}H_{48}O_5 \cdot 1/2H_2O$: C, 72.40; H, 9.92. Found: C, 72.20; H, 9.66.

Acetylation of Tormentic Acid——Tormentic acid (50 mg) was acetylated with acetic anhydride (4 ml) and pyridine (2 ml) to give diacetate of mp 194° from benzene. It was undepressed with authentic sample of 2,3-diacetyl tormentic acid. *Anal.* Calcd. for $C_{34}H_{52}O_7$: C, 71.29; H, 9.15. Found: C, 71.06; H, 9.12.

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