The Structures of Four Novel C_{31}-Secodammarane-type Triterpenoid Saponins from the Female Flowers of *Alnus serrulatoides*

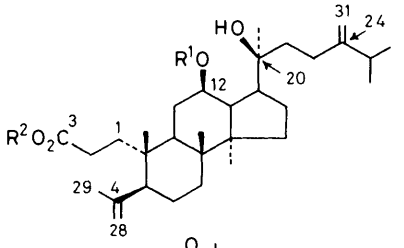
By **Tadashi Aoki, Shinji Ohta, Satoshi Aratani, Toshifumi Hirata,** and **Takayuki Suga,*** Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Naka-ku, Hiroshima 730, Japan

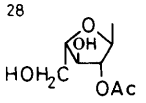
Four novel C_{31}-secodammarane-type triterpenoid saponins (1)—(4), in addition to the recently structurally elucidated alnustic acid (9), were isolated from the female flowers of *Alnus serrulatoides*. On the basis of their physico-chemical data, these saponins were characterized as the 12-*O*-(2′-*O*-acetyl)-α-L-arabinofuranoside, the 12-*O*-α-L-arabinofuranoside, the 12-*O*-β-D-xylopyranoside, and the 12-*O*-β-D-glucopyranoside of alnustic acid, respectively.

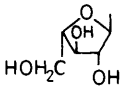
IN our previous studies we isolated four new triterpenoids, alnuserol (5),[1] alnuseric acid (6),[2] alnuselide (7),[2] and alnuserrudiolone (8)[3] from the male flowers of *Alnus serrulatoides* Call., and alnustic acid (9)[4] from the male flowers of *A. sieboldiana* Matsum. These triterpenoids all possess a C_{31}-dammarane- or secodammarane-type skeleton. In a continuation of our studies, we investigated the chemical constituents of the female flowers of *A. serrulatoides*. Four novel C_{31}-secodammarane-type triterpenoid saponins, in addition to alnustic acid (9) (whose structure has been recently elucidated[4]), were isolated, and these saponins all were found to be monoglycosides of alnustic acid. In this paper we report the evidence which led to the establishment of their structures.

RESULTS AND DISCUSSION

The saponins (1)—(4), in addition to alnustic acid (9), were extracted from the female flowers by the methods described in the Experimental section. Pure samples of these compounds were obtained by centrifugal chromatography and/or continuous preparative thin-layer chromatography (p.l.c.). The saponins are numbered in the order of increasing polarity on their respective thin-layer chromatogram. On the basis of their physico-chemical data (*vide infra*), these saponins were characterized as the 12-*O*-(2′-*O*-acetyl)-α-L-arabinofuranoside (1), the 12-*O*-α-L-arabinofuranoside (2), the 12-*O*-β-D-xylopyranoside (3), and the 12-*O*-β-D-glucopyranoside (4) of alnustic acid, respectively.



(1) R^1 =  R^2 = H

(2) R^1 =  R^2 = H

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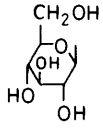
(4) R^1 =  R^2 = H

(9) R^1 = R^2 = H

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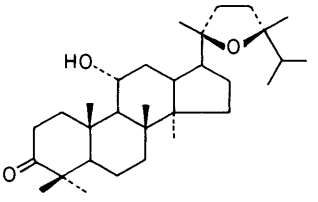

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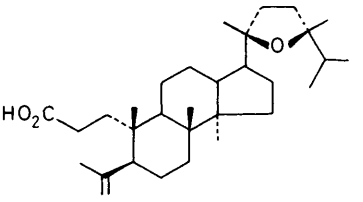

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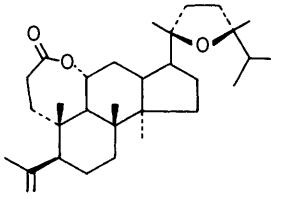

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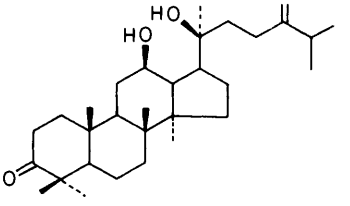

(8)

Saponin (2).—This compound gave the peaks due to the $[(M - H) + 2\ Na]^+$, $[M + Na]^+$, and $[(M - H) + 3\ Na]^{++}$ ions at *m/z* 665, 643, and 344, respectively, in the field-desorption mass spectrum (f.d.–m.s.). The appearance of the doubly charged cluster ion at *m/z* 344, in addition to the fragment ions at *m/z* 665 and 643, indicated that the saponin had a molecular weight of 620 and also that it contained a carboxy-group.[5] The presence of the carboxy-group was supported by the i.r. and ^1H n.m.r. spectra, which also indicated the presence of both a hydroxy-group and a terminal methylene moiety. The chemical ionisation mass spectrum (c.i.–m.s.) of compound (2) exhibited peaks due to the fragment ions $[(M - H_2O) - C_5H_9O_4]^+$ and $[C_5H_9O_4]^+$ at *m/z* 469 and 133, respectively. The appearance of these fragment ions indicated that compound (2) is composed of an aglycone moiety having the elementary composition $C_{31}H_{52}O_4$ and a pentose moiety. The saponin (2) on methylation with CH_2N_2 afforded its methyl ester, whilst on exhaustive methylation using Hakomori's method [6] it gave a tetra-*O*-methylated product. Acetylation of compound (2) with acetic anhydride gave a tri-*O*-acetate which, on methylation (CH_2N_2), was converted into a tri-*O*-acetate methyl ester. All this indicated that compound (2) possesses one carboxy-group and (at least) three hydroxy-groups. However, the i.r. spectra of the tetra-*O*-methylated product and the tri-*O*-acetate methyl ester showed the presence of another hydroxy-group. This indicated that the saponin (2) had one more (tertiary) hydroxy-group.

The ^{13}C n.m.r. chemical shifts (Table 1) of the methyl ester of the saponin (2) were similar to those of methyl alnustate (10) and α-L-arabinofuranose,[7-9] with the exception of those for C-11, C-12, and C-13. The ^{13}C n.m.r. signals due to C-11 and C-13 of saponin (2) methyl ester were shielded by 4.0 and 1.8 p.p.m., respectively, while that due to C-12 was shifted downfield by 5.3 p.p.m., with respect to those of the corresponding carbon atoms of the ester (10) (Table 1). On application of the glycosidation shift rule [10-12] to the displacement of these three signals, it was found that C-1′ of L-arabinofuranose is bonded to the C-12 hydroxy-group of alnustic acid (9) by an α-glycosidic linkage. This was supported by the observation of the coupling constant (J 2 Hz) for the anomeric proton of the tetra-*O*-methylated product [13] as well as by application of Klyne's [14,15] and Hudson's [16] rules to the $[M]_D$ value of L-arabinofuranose as shown in Table 2. The $[M]_D$ value for the carbohydrate moiety was evaluated using the molecular rotation values listed in Table 3. The α-glycosidic linkage of L-arabinofuranose in compound (2) agrees with the general rule that, for natural glycosides, the glycosidic linkages of the sugars in the D- and L-series are β and α, respectively.[17-19] Consequently, the structure of the saponin (2) is proposed as (12*R*,20*S*)-12-*O*-α-L-arabinofuranosyl-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid.

Saponin (1).—The f.d.–m.s. of this saponin exhibited the $[M + H]^+$ ion peak at *m/z* 663, indicating that the molecular weight of compound (1) is larger than that of compound (2) by 42 a.m.u. The i.r. and ^1H n.m.r. spectra of compound (1) showed the presence of an acetoxy-group, in addition to a carboxy- and a hydroxy-group and a terminal methylene moiety. The saponin (1), on saponification followed by methylation with CH_2N_2, gave a deacetyl methyl ester whose spectral data were identical with those of the methyl ester of saponin (2). From these observations, the structure of saponin (1) was presumed to be that of compound (2) but with a hydroxy-group replaced by an acetoxy-group.

TABLE 1

^{13}C N.m.r. chemical shifts (δ_C) for compound (10) and the methyl esters of compounds (1)—(4), for solutions in C_5D_5N.

		Methyl ester of compound				
		(2)				
Carbon	Compound (10)	*a*	*b*	(1)	(3)	(4)
1	24.9	24.6	24.7	24.6	24.7	24.5
2	28.8 [c]	28.5	28.5 [c]	28.4	28.7 [c]	28.3
3	174.1	174.0	173.9	173.8	174.1	174.0
4	147.6	147.3	147.2	147.1	147.3	147.2
5	40.8	40.6	40.7	40.7	40.5	40.1
6	28.7 [c]	28.5	28.5 [c]	28.4	28.7 [c]	28.3
7	33.7	33.3	33.4	33.4	33.5	33.4
8	39.6	39.5	39.5	39.5	39.6	39.5
9	50.6	50.3	50.4	50.5	50.4	50.2
10	39.6	39.5	39.5	39.5	39.6	39.5
11	32.5	28.5	28.0 [c]	28.4	28.2 [c]	28.3
12	70.4	75.7	75.7	76.0	76.4	76.7
13	48.5	46.7	46.6	46.5	46.8	46.6
14	52.2	52.7	52.7	52.8	52.8	52.5
15	31.4	31.8	31.8	31.7	31.6	31.1
16	27.0	27.1	27.1	27.1	27.2	26.5
17	54.7	53.4	53.4	53.4	53.5	53.7
18	15.6	15.4	15.3	15.4	15.4	15.4
19	20.3	20.0	20.0	20.1	20.2	20.0
20	72.7	72.9	72.9	73.0	72.6	72.8
21	27.0	27.1	27.1	26.8	27.2	26.7
22	34.4 [d]	34.6	34.8 [d]	34.3	34.3 [d]	34.4 [d]
23	34.9 [d]	34.6	34.3 [d]	34.3	35.1 [d]	34.7 [d]
24	157.1	157.2	157.2	157.0	157.3	157.0
25	34.4	34.6	34.3	34.3	34.3	34.1
26	22.2	22.0	22.0	22.0	22.0	21.9
27	22.2	22.0	22.0	22.0	22.0	21.9
28	114.0	113.9	114.0	113.9	114.0	113.9
29	23.4	23.2	23.2	23.2	23.3	23.1
30	17.0	17.3	17.4	17.4	17.4	17.1
31	106.5	106.2	106.2	106.3	106.2	106.3
OMe	51.4	51.5	51.5	51.4	51.4	51.5
C(:O)Me				169.6		
C(:O)*Me*				20.5		
1′		105.4	105.4	103.5	100.5	99.6
2′		83.5	83.4	85.9 [e]	74.7	74.5
Sugar 3′		77.9	77.8	76.5	78.6	77.9 [f]
moiety 4′		85.3	85.2	85.5 [e]	70.5	70.4
5′		62.2	62.2	61.6	67.2	77.7 [f]
6′						62.0

[a] Prepared by methylation of compound (2). [b] Prepared by deacetylation of compound (1), followed by methylation. [c-f] Values in any vertical column may be interchanged although those given here are preferred.

The location of the acetoxy-group was established by f.d.–m.s. and ^{13}C n.m.r. spectral measurements of compound (1) as follows. The presence of the $[(M + H) - CH_3CO\cdot\text{arabinofuranosyl}]^+$ and $[CH_3CO\cdot\text{arabinofuranosyl}]^+$ ion peaks at *m/z* 489 and 175, respectively, in the f.d.–m.s. indicated that the acetoxy-group was situated on

the arabinofuranosyl moiety. This was supported by the appearance, of the $[CH_3CO\cdot arabinofuranosyl\cdot(SiMe_3)_2]^+$ ion peak at *m/z* 319 on converting the saponin (1) into the bis(trimethylsilyl) derivative. In addition it was observed that the ^{13}C n.m.r. signal due to C-2′ of the methyl ester of compound (1) (prepared by methylation with CH_2N_2) shifted downfield by 2.4 p.p.m., whilst those of C-1′ and C-3′ were shielded by 1.9 and 1.4 p.p.m., respectively, in comparison with those due to the corresponding carbon atoms of the methyl ester of compound (2) (Table 1). The other carbon atoms of the methyl esters of compounds (1) and (2) showed similar chemical shifts. Application of the acetylation shift rule [21-24] to the displacements of the above three signals showed that the acetoxy-group is located at C-2′ of the α-arabinofuranose moiety. Consequently, the saponin (1) was defined as (12*R*,20*S*)-12-*O*-(2′-*O*-acetyl-α-L-arabinofuranosyl)-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid.

TABLE 2

Observed molecular rotation values, $[M]_D$(obs.), of the saponin (2) and the methyl esters of compounds (3) and (4), calculated $[M]_D$(calc.) values for the sugar moieties in the saponins, and the assigned configuration at C-1′ of the sugars

Compound	$[M]_D$(obs.) (°)	$[M]_D$(calc.) (°) (sugar) [a]	Assigned configuration at C-1′
Saponin (2)	−44.6		
Arabinofuranose moiety		−180.3	α
Methyl ester of compound (3)	+29.5		
Xylopyranose moiety		−184.9	β
Methyl ester of compound (4)	+10.0		
Glucopyranose moiety		−204.4	β

[a] The $[M]_D$ value for the sugar moiety in the saponin (2) was evaluated from that of alnustic acid (9) [4] (Table 3), and those for the sugar moieties in the methyl esters from that of methyl alnustate (10) [4] (Table 3).

TABLE 3

Observed $[M]_D$ values of alnustic acid (9), methyl alnustate (10), and some methyl glycosides

Compound	$[M]_D$ (°)	Reference
Alnustic acid (9)	+135.7	4
Methyl alnustate (10)	+214.4	4
Methyl α-L-arabinofuranoside	−226	*a*
Methyl β-L-arabinofuranoside	+208	*a*
Methyl β-D-xylopyranoside	−107	20
Methyl α-D-xylopyranoside	+249	20
Methyl β-D-glucopyranoside	−62	16
Methyl α-D-glucopyranoside	+305	16

[a] S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.*, 1974, **22**, 421.

Saponin (3).—This saponin, on methylation with CH_2N_2, gave a methyl ester. The f.d.–m.s. of this methyl ester exhibited peaks at *m/z* 635 and 133, which were assigned to an $[M + H]^+$ ion and a fragment ion originating from the pentose moiety, respectively. On the basis of the ^{13}C n.m.r. spectrum (Table 1) and the molecular rotation (Table 2) of its methyl ester, it was apparent that compound (3) is the glycoside of alnustic acid (9) and β-D-xylopyranose.[20] Application of the glycosidation shift rule [10-12] to the chemical shifts for the C-11, C-12, and C-13 carbon atoms of the methyl ester of compound (3) indicated that the C-1′ carbon atom of the D-xylopyranose bonds to the C-12 hydroxy-group of alnustic acid (9) *via* a β-glycosidic linkage. Thus, the identity of the saponin (3) was established as (12*R*,20*S*)-12-*O*-β-D-xylopyranosyl-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid.

Saponin (4).—Methylation of the saponin (4) with CH_2N_2 gave a methyl ester whose f.d.–m.s. exhibited peaks due to the ions $[M + H]^+$ and $[M + Na]^+$ at *m/z* 665 and 687, respectively. The structural elucidation of the methyl ester was achieved by considering the ^{13}C n.m.r. chemical shifts (Table 1), the molecular rotation (Table 2), and the coupling constant (J 7 Hz) [25] of the anomeric proton in the same manner as for the other compounds. From these data we propose that the saponin (4) is (12*R*,20*S*)-12-*O*-β-D-glucopyranosyl-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid.

EXPERIMENTAL

The n.m.r. spectra were measured, with Me_4Si as internal standard, at 60 and 90 MHz (^1H-) and 22.6 MHz (^{13}C n.m.r.). E.i.–m.s. (electron-impact mass spectra) were recorded on a Hitachi RMU-6L mass spectrometer at 70 eV. The c.i.–m.s. of saponin (2) was obtained on a Shimazu GCMS-6020 mass spectrometer using isobutane at 100 eV. The f.d.–m.s. of saponin (1) was taken on a JEOL JMS-D 300 mass spectrometer equipped with a silicone emitter; the emitter current was 5—25 mA. The f.d.–m.s. of saponins (2)—(4) were obtained on a Hitachi M-80 mass spectrometer equipped with a carbon (graphite) emitter; the emitter current was 0—20 mA. Analytical t.l.c. and preparative t.l.c. (p.l.c.) were carried out on Merck 60 GF_{254} silica-gel plates (adsorbent thickness 0.25 and 0.75 mm, respectively). Compounds were visualized as coloured spots by spraying with HNO_3–H_2SO_4 (1 : 19 v/v) and then by heating on a hot plate or by spraying with 0.3% *p*-Bromocresol Green solution in H_2O–MeOH (1 : 4) adjusted to pH 8.0.

Extraction and Isolation.—Female flowers (444 g) of *Alnus serrulatoides* Call., which grows naturally on river banks in the suburbs of Hiroshima City, were collected in April (about 1 month after the flowering of the male flowers). The flowers, after they had been minced mechanically, were extracted with acetone at room temperature for 2 months. Removal of the solvent from the extract gave a brown, viscous oil (76.0 g). A portion (20.0 g) of this oil was subjected to centrifugal chromatography on silica gel with gradient elution using $CHCl_3$–MeOH as eluant (with MeOH increasing from 1—100%) and separated into 3 fractions: (i) a mixture of the saponin (1) and alnustic acid (9) (1.42 g), (ii) the saponin (2) (0.60 g), and (iii) a mixture of the saponins (3) and (4) (0.32 g). These fractions gave spots with R_F 0.45, 0.27, and 0.16, respectively, on analytical t.l.c. with $CHCl_3$–MeOH (17 : 3 v/v) as developer. Fraction (i) was then subjected to continuous p.l.c. with EtOH–

benzene (1 : 19 v/v) as eluant for 10 h, and separated into two bands (R_F 0.36 and 0.14) which, on extraction with $CHCl_3$, gave alnustic acid (9) (0.04 g) and the *saponin* (1) (1.0 g), respectively. Fraction (iii), after methylation with CH_2N_2, was separated into two bands (R_F 0.33 and 0.24) on analytical t.l.c. with acetone–hexane (1 : 1 v/v) as developer; the bands, on extraction with $CHCl_3$, gave the *methyl ester* of the *saponin* (3) (0.10 g) and the *methyl ester* of the *saponin* (4) (0.20 g), respectively.

Saponin (2).—This was obtained as an amorphous solid; $[\alpha]_D^{25}$ −7.2° (*c* 0.70, $CHCl_3$); $\nu_{max.}$ (Nujol) 3 350 (OH), 3 080, 1 640, and 890 (C=CH_2), and 1 690 cm^{-1} (CO_2H); δ_H ($CDCl_3$) 0.88, 0.96, 1.01, 1.04, 1.08, and 1.13 (6 × Me), 1.73 (3 H, s, C=CMe), 4.68, 4.74, and 4.86 (br) (4 H, 2 × C=CH_2), 5.05br (1 H, CO_2H), and 5.27 (1 H, s, anomeric H); δ_C ($CDCl_3$) 182.2 (s, CO_2H), 157.1 (s, C-24), 147.4 (s, C-4), 113.8 (t, C-28), 106.0 (t, C-31), 104.1 (d, anomeric C), 74.0 (s, C-20), 26.4 (q, Me), 23.4 (q, Me), 22.1 (q, 2 × Me), 20.4 (q, Me), 17.7 (q, Me), and 15.5 p.p.m. (q, Me); f.d.–m.s. *m/z* (rel. intensity) 665 {$[(M - H) + 2Na]^+$, 47}, 643 ($[M + Na]^+$, 100), and 344 {$[(M - H) + 3Na]^{++}$, 33}; c.i.–m.s. *m/z* 469 {$[(M - H_2O) - C_5H_9O_4]^+$} and 133 ($C_5H_9O_4$).

Methylation of compound (2) with CH_2N_2 yielded the *methyl ester* as an oil; $\nu_{max.}$ (neat) 3 360 (OH), 3 080, 1 630, and 890 (C=CH_2), and 1 735 cm^{-1} (CO_2Me); δ_H ($CDCl_3$) 0.89, 0.94, 1.02, 1.05, 1.09, and 1.15 (6 × Me), 1.74 (3 H, s, C=CMe), 3.69 (3 H, s, CO_2Me), 4.67, 4.74, and 4.87 (br) (4 H, 2 × C=CH_2), and 5.22 (1 H, s, anomeric H).

Acetylation of compound (2) (35 mg) with a mixture of acetic anhydride (2 ml) and pyridine (2 ml) under the usual conditions gave the 2′,3′,5′-*tri*-O-*acetate* (10 mg) as an oil; $\nu_{max.}$ (neat) 3 470 and 3 370 (OH), 3 080, 1 640, and 890 (C=CH_2), and 1 740 cm^{-1} (C=O); δ_H ($CDCl_3$) 0.89, 0.94, 1.01, 1.04, 1.09, and 1.12 (6 × Me), 1.73 (3 H, s, C=CMe), 2.11 (9 H, s, 3 × C(:O)Me), 4.67, 4.74, and 4.86 (br) (4 H, 2 × C=CH_2), and 5.26br (1 H, s, anomeric H). Methylation of the triacetate with CH_2N_2 gave the 2′, 3′, 5′-*tri*-O-*acetate methyl ester* as an oil; $\nu_{max.}$ (neat) 3 480 (OH), 3 080, 1 640, and 890 (C=CH_2), and 1 745 cm^{-1} (C=O); $\nu_{max.}$ (0.0002M in CCl_4) 3 620 (free OH) and 3 470 cm^{-1} (intramolecularly hydrogen-bonded OH).

Exhaustive Methylation of the Saponin (2).—Following the method developed by Hakomori,[6] a solution of compound (2) (56 mg) in dimethyl sulphoxide (DMSO) (10 ml) was added to a mixture of sodium hydride (300 mg) and DMSO (10 ml) which had been prepared by stirring for 1 h at room temperature under nitrogen. The solution was stirred for 1 h under N_2 and an excess of methyl iodide (0.7 ml) was added with stirring during 4 h. The mixture was then poured into ice-cold water (300 ml) and extracted with ethyl acetate. The extracts were washed with water (3 × 300 ml) and evaporated to dryness to afford a *tetra*-O-*methylated* product (the 2′,3′,5′-*tri*-O-*methyl ether methyl ester*) (15 mg) as an oil; $\nu_{max.}$ (neat) 3 430 (OH), 3 080, 1 635, and 890 (C=CH_2), and 1 735 cm^{-1} (CO_2Me); δ_H ($CDCl_3$) 0.83—1.12 (6 × Me), 1.72 (3 H, s, C=CMe), 3.35, 3.37, and 3.45 (3 × OMe), 3.73 (3 H, s, CO_2Me), 4.57 (1 H, s, OH, disappeared in D_2O), 4.69, 4.73, 4.85 (br) (4 H, 2 × C=CH_2), and 5.36 (1 H, d, *J* 2 Hz, anomeric H); e.i.–m.s. *m/z* (rel. intensity) 175 (43), 143 (46), 123 (39), 115 (44), and 101 (100).

Saponin (1).—Work-up of fraction (i) gave the *saponin* (1) as an oil; $[\alpha]_D^{25}$ −13.5° (*c* 0.74, $CHCl_3$); $\nu_{max.}$ (neat) 3 370 (OH), 3 080, 1 640, and 890 (C=CH_2), 1 730 (C=O); δ_H ($CDCl_3$) 0.86, 0.96, 1.01, 1.04, 1.09, and 1.13 (6 × Me), 1.72 (3 H, s, C=CMe), 2.12 (3 H, s, C(:O)Me), 4.68, 4.74, and 4.86 (br) (5 H, 2 × C=CH_2 and C*H*OAc), 5.37 (1 H, s, anomeric H), and 5.80br (1 H, CO_2H); δ_C ($CDCl_3$) 177.5 (s, CO_2H), 170.4 (s, *C*(:O)Me), 156.7 (s, C-24), 146.9 (s, C-4), 113.9 (t, C-28), 106.2 (t, C-31), 101.6 (d, anomeric C), 74.1 (s, C-20), 26.4 (q, Me), 23.4 (q, Me), 22.1 (q, 2 × Me), 20.7 (q, CO*Me*), 20.0 (q, Me), 17.4 (q, Me), and 15.5 p.p.m. (q, Me); f.d.–m.s. *m/z* (rel. intensity) 663 ($[M + H]^+$, 55), 489 {$[(M + H) - 174]^+$, 30}, 473 {$[(M + H) - 174 - 16]^+$, 15}, and 175 (100).

Trimethylsilylation of compound (1) with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) gave the expected *trimethylsilyl derivative;* f.d.–m.s. *m/z* (rel. intensity) 879 ($[M + H]^+$, 30), 319 {$[C_7H_{11}O_5(SiMe_3)_2]^+$, 80}, and 73 ($Me_3Si^+$, 100); e.i.–m.s. *m/z* (rel. intensity) 319 (100), 247.100 2 (58, $C_{10}H_{19}O_5Si$ requires *m/z* 247.100 1), 187.078 1 (32, $C_8H_{15}O_3Si$ requires *m/z*, 187.0789), and 147.0800 (81, $C_6H_{15}O_2Si$ requires *m/z*, 147.0840).

Methylation of compound (1) with CH_2N_2 gave the *methyl ester* as an oil; $\nu_{max.}$ (neat) 3 380 (OH), 3 080, 1 640, and 890 (C=CH_2), and 1 735 cm^{-1} (C=O); δ_H ($CDCl_3$) 0.88, 0.93, 1.01, 1.05, 1.09, and 1.14 (6 × Me), 1.73 (3 H, s, C=CMe), 2.10 (3 H, s, C(:O)Me), 3.67 (3 H, s, CO_2Me), 4.67, 4.74, and 4.87 (br) (5 H, 2 × C=CH_2 and C*H*OAc), and 5.24br (1 H, s, anomeric H).

Saponification of the Saponin (1).—A solution of compound (1) (91 mg) in 5% NaOH–MeOH (10 ml) was refluxed for 2 h. The mixture was diluted with water (20 ml) and was extracted with chloroform to give the deacetyl derivative (26 mg) as an amorphous solid; $\nu_{max.}$ (Nujol) 3 350 (OH), 3 080, 1 640, and 890 (C=CH_2), and 1 700 cm^{-1} (CO_2H); δ_H ($CDCl_3$) 0.87, 0.97, 1.00, 1.05, 1.11, and 1.14 (6 × Me), 1.73 (3 H, s, C=CMe), 4.68, 4.74, and 4.87 (br) (4 H, 2 × C=CH_2), 5.21br (1 H, s, anomeric H), and 5.98br (1 H, CO_2H) [*cf.* the spectra of compound (2)]. Methylation of the deacetyl derivative with CH_2N_2 gave its methyl ester whose spectral data were identical with those of the methyl ester of compound (2).

Methylation of the Saponin (3).—Methylation of compound (3) with CH_2N_2 gave its methyl ester as an oil; $[\alpha]_D^{25}$ +4.65° (*c* 0.86, $CHCl_3$); $\nu_{max.}$ (neat) 3 380 (OH), 3 080, 1 635, and 890 (C=CH_2), and 1 735 cm^{-1} (C=O); δ_H ($CDCl_3$) 0.89, 0.91, 1.02, 1.05, 1.09, and 1.14 (6 × Me), 1.74 (3 H, s, C=CMe), 3.68 (3 H, s, CO_2Me), 4.68, 4.74, and 4.86 (br) (4 H, 2 × C=CH_2), and 5.14br (1 H, s, anomeric H); f.d.–m.s. *m/z* (rel. intensity) 635 ($[M + H]^+$, 100) and 133 ($C_5H_9O_4$, 90).

Methylation of the Saponin (4).—Methylation of compound (4) with CH_2N_2 gave its methyl ester as an oil; $[\alpha]_D^{25}$ +1.50° (*c* 0.66, $CHCl_3$); $\nu_{max.}$ (neat) 3 370 (OH), 3 080, 1 640, and 890 (C=CH_2), and 1 735 cm^{-1} (C=O); δ_H ($CDCl_3$) 0.89, 0.90, 1.02, 1.05, 1.09, and 1.15 (6 × Me), 1.74 (3 H, s, C=CMe), 3.66 (3 H, s, CO_2Me), 4.68, 4.74, and 4.87 (br) (4 H, 2 × C=CH_2), and 5.31br (1 H, s, anomeric H); δ_H (C_5D_5N) 1.70 (3 H, s, C=CMe), 3.70 (3 H, s, CO_2Me), 4.76, 4.83, and 4.90 (br) (4 H, 2 × C=CH_2), and 5.20 (1 H, d, *J* 7 Hz, anomeric H);[25] f.d.–m.s. *m/z* (rel. intensity) 687 ($[M + Na]^+$, 32) and 665 ($[M + H]^+$, 100).

Identification of Alnustic Acid (9).—The band with R_F 0.36 obtained from fraction (i) by continuous p.l.c. with EtOH–benzene (1 : 19 v/v) as eluant gave, on extraction with chloroform, the acid (9) as an amorphous solid which was methylated (CH_2N_2) to give its methyl ester (10); m.p. 154—156 °C (from $CHCl_3$—MeOH); $[\alpha]_D^{25}$ +25.7° (*c* 0.39, $CHCl_3$). The spectral data (i.r., ^1H n.m.r., and ^{13}C n.m.r.) and t.l.c. of compounds (9) and (10) were identical with

those of authentic samples of alnustic acid and its methyl ester,[4] respectively.

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REFERENCES

[1] T. Hirata and T. Suga, *J. Chem. Soc., Perkin Trans. 2*, 1978, 347.
[2] T. Suga and T. Hirata, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1153.
[3] T. Hirata, T. Aoki, and T. Suga, *Bull. Chem. Soc. Jpn.*, 1981, **54**, 3059.
[4] T. Suga, T. Aoki, T. Hirata, K. Aoki, and Y. Asakawa, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1698.
[5] H.-R. Schulten, T. Komori, T. Nohara, R. Higuchi, and T. Kawasaki, *Tetrahedron*, 1978, **34**, 1003.
[6] S. Hokamori, *J. Biochem.* (*Tokyo*), 1964, **55**, 205.
[7] P. A. J. Gorin and M. Mazurek, *Carbohydr. Res.*, 1976, **48**, 171.
[8] P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 1975, **53**, 1212.
[9] R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch, and A. S. Perlin, *Can. J. Chem.*, 1975, **53**, 1424.
[10] R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, 1977, 175.
[11] K. Tori, S. Seo, Y. Yoshimura, H. Arita, and Y. Tomita, *Tetrahedron Lett.*, 1977, 179.
[12] S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, 1978, **100**, 3331.
[13] B. Capon and D. Thacker, *Proc. Chem. Soc.* (*London*), 1964, 369.
[14] W. Klyne, *Biochem. J.*, 1950, **47**, xli.
[15] W. Klyne, ' Determination of Organic Structures by Physical Methods,' Academic Press, New York, 1955, vol. 1, pp. 73—130.
[16] C. S. Hudson, *J. Am. Chem. Soc.*, 1909, **31**, 66; C. S. Hudson, *ibid.*, 1916, **38**, 1566.
[17] H. Hariharon and S. Rangaswami, *Phytochemistry*, 1970, **9**, 409.
[18] R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.*, 1972, **20**, 2143.
[19] Y. Kumekawa, H. Itokawa, and M. Fujita, *Chem. Pharm. Bull.*, 1974, **22**, 2294.
[20] A. Tada, M. Kobayashi, and J. Shoji, *Chem. Pharm. Bull.*, 1973, **21**, 308.
[21] H. Ishii, S. Seo, and K. Tori, *Tetrahedron Lett.*, 1977, 1227.
[22] Y. Terui, K. Tori, and N. Tsuji, *Tetrahedron Lett.*, 1976, 621.
[23] K. Yamasaki and O. Tanaka, *Tetrahedron Lett.*, 1977, 1231.
[24] O. Tanaka and S. Yahara, *Phytochemistry*, 1978, **17**, 1353.
[25] S. Yahara, R. Kasai, and O. Tanaka, *Chem. Pharm. Bull.*, 1977, **25**, 2041.