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Structure of Alnustic Acid, a New Secodammarane-type Triterpenic Acid from *Alnus sieboldiana*

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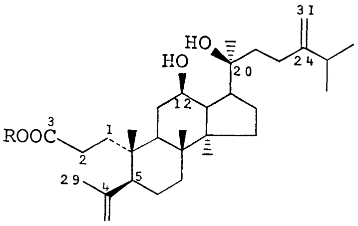
A novel C_{31} 3,4-secodammarane-type triterpenoid, alnustic acid, was isolated from the male flowers of *Alnus sieboldiana* Matsum. (Betulaceae). Its structure was elucidated to be (12*R*,20*S*)-12,20-dihydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid by a combination of chemical and spectroscopic methods.

The occurrence of only aromatic ring-containing compounds, such as phenyl propane derivatives, flavonoids, and stilbenes, in the male flowers of *Alnus sieboldiana* Matsum. (Betulaceae) was previously reported from our laboratory.[1–5] Ohmoto *et al.* also described the presence of a flavone glucoside in the pollens of this plant.[6] Our further investigation of the male flowers has newly led to the isolation of a novel C_{31} 3,4-secodammarane-type triterpenic acid, named alnustic acid, differing from the above-described group of compounds. We here wish to report evidence which led to the establishment of its structure.



1 R=H
2 R=CH_3

Table 1. ^{13}C-Chemical shifts of **1**, **2**, **5**, and **6** in $CDCl_3$

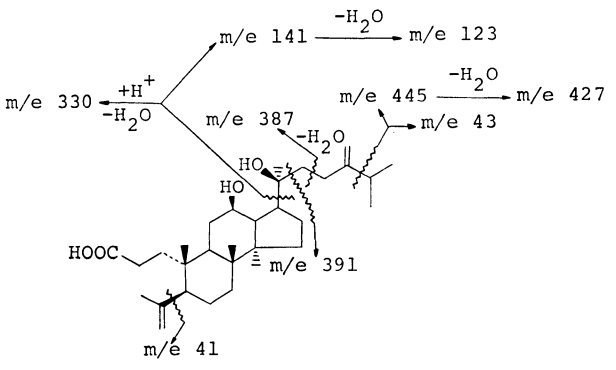
Carbon No.	Compds 1	2	5	6
1	24.8	24.7	24.7	24.9
2	33.6	30.4	34.0	29.7
3	177.8	174.5	180.2	175.8
4	147.3	147.1	147.4	147.4
5	40.6	40.6	41.0	39.9
6	28.4	28.4	28.4	29.5
7	33.6	33.5	34.3	34.7
8	39.5	39.4	40.1	40.5
9	50.7	50.7	50.4[a]	52.1
10	39.5	39.1	39.1	38.8
11	31.8	31.8	22.1	71.2
12	70.6	70.6	25.5	42.0
13	47.5	47.7	43.2	42.3
14	52.1	52.0	50.4	50.1
15	31.2	31.1	31.1	30.5
16	26.6	26.5	26.8	25.5
17	53.5	53.7	50.9[a]	49.9
18	15.5	15.5	15.4	16.0
19	20.0	20.1	20.1	20.4
20	74.2	73.6	85.3	85.3
21	26.6	26.7	25.1	24.6
22	34.0[a]	33.9[a]	37.3[b]	37.4[a]
23	34.5[a]	34.4[a]	36.1[b]	36.0[a]
24	156.7	156.9	85.8	85.3
25	28.6	29.0	38.0	38.0
26	22.0	22.0	18.8	18.7
27	22.0	22.0	17.6	17.5
28	113.6	113.6	113.4	113.7
29	23.2	23.2	23.2[c]	23.1[b]
30	16.9	16.7	16.3	16.4
31	106.3	106.2	22.9[c]	22.9[b]
OMe		51.6		51.4

a), b), and c): Values in any vertical column may be reversed although those given here are preferred.

Results and Discussion

The male flowers of *Alnus sieboldiana* Matsum. were collected just before the flowering and immersed in benzene. The benzene extract was subjected to chromatography on a silica gel column and then to preparative thin layer chromatography to give alnustic acid.

Alnustic acid (**1**), $C_{31}H_{52}O_4$, showed the IR absorption bands due to a carboxyl and a hydroxyl group. Methylation of **1** with CH_2N_2 gave a methyl ester (**2**), which upon Jones oxidation yielded a keto alcohol (**3**). Acetylation of **2** with acetic anhydride under mild conditions gave a methyl ester monoacetate (**4**), which was found to possess another unaltered hydroxyl group from the IR spectrum. This inidcates that **1** possesses both a secondary and a tertiary hydroxyl group. The ORD curve pattern, as well as the CD curve, of **3** resembled to those of the previously-described dammaran-12-one derivatives,[7–9] implying the presence of the secondary hydroxyl group located at position 12 in the acid (**1**) and the similarity in surroundings of the newly-formed carbonyl chromophore in the keto alcohol (**3**). The presence of both the carboxyl group located at position 3 and the secondary hydroxyl group occupying the *R* position on C-12 (δ_C 70.6 ppm) was demonstrated by comparing the CMR chemical shifts (Table 1) with those of the corresponding carbon atoms of alnuseric acid (**5**) and the methyl ester (**6**)[10,11] and protopanaxadiol.[12] Simultaneously, the presence of an isopropenyl group on C-5 was shown by the CMR spectra (δ_C 147.3 and 113.6 ppm), as well as by the IR bands, the PMR signals, and the MS fragment. These findings indicate that alnustic acid is a 3,4-secodammarane-type derivative. On the other hand, the mass spectral fragmentation pattern (Scheme 1) indicated the presence of an



Scheme 1. Mass spectral fragmentation pattern of alnustic acid (**1**).

acyclic side chain. The presence of a terminal methylene and a tertiary hydroxyl group located, respectively, on C-24 and C-20 of the side chain was established by a combination of the IR bands, the PMR signals, the MS peaks (Scheme 1), and the CMR signals (Table 1). In addition, the comparison of these CMR chemical shifts with those of alnuserrudiolone (**7**),[8,9] alnuserrutriol,[9] and protopanaxadiol[12] demonstrated that the chirality at C-20 (δ_C 74.2 ppm) is *S*. This was supported by occurrence of both the free and the intramolecularly hydrogen-bonded hydroxyl bands between the 12-carbonyl and the 20-hydroxyl groups in **3**.[13] We here wish to propose structure **1** for alnustic acid. This proposed structure (**1**) possesses all the features necessary to explain all the spectral data of the acid.

The structure (**1**) was confirmed by its synthesis starting from alnuserrudiolone (**7**)[8,9] following the method established for ring-opening at the 3,4-position in ring A of β-amyrenone.[14] Acetylation of **7** gave a monoacetate, which was then transformed to the corresponding ketoxime, followed by treating with *p*-toluenesulfonyl chloride to yield an abnormal Beckmann rearrangement product (**8**). Hydrolysis of the product (**8**) with alkali under mild conditions gave (12*R*,20*S*)-12,20-dihydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid. Identity of an authentic sample of this acid with naturally occuring alnustic acid was established by direct comparison of a mixed melting point, thin layer chromatogram, and the infrared, mass, and nuclear magnetic resonance spectra.

Thus, the structure of alnustic acid (**1**) has been elucidated to be (12*R*,20*S*)-12,20-dihydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid.

Experimental

The mass spectral analyses were performed on a Hitachi RMS-4 mass spectrometer at 70 eV. The PMR spectra were taken on a Varian T-60 spectrometer using TMS as an internal standard. The CMR spectra were obtained on a JEOL JNM FX-100 spectrometer operating at 15.1 MHz ($\delta_{TMS}=0$).

Extraction and Isolation. The male flowers (95.0 kg) of *Alnus sieboldina* Matsum. naturally grown on a hill in suburbs of Hiroshima city were collected just before the flowering in March. After minced mechanically, the flowers were immersed in benzene at room temp for 2 months. The benzene extract was chromatographed on a silica gel column (Merck; type 60) with a hexane-EtOAc mixture with EtOAc increasing 0 to 100%. The eluate with 50% EtOAc in hexane, after removal of the solvent, was subjected to repeated preparative TLC (silica gel, Merck GF_{254}; EtOAc–$CHCl_3$–hexane (5: 1: 1, v/v); R_f 0.47) to give alnustic acid (577 mg), which was recrystallized from a hexane–EtOAc mixture to yield colorless needles.

Alnustic Acid (1). Mp 195—196 °C; $[\alpha]_D^{25}$ +27.8° (*c* 0.52, $CHCl_3$); IR (Nujol) ν_{max}3400—2800 and 1710 (COOH), 1640 and 892 cm^{-1} ($>C=CH_2$); PMR ($CDCl_3$) δ 0.88—1.19 (Me×6), 1.75 (3H, s, $>C=C(CH_3)-$), 3.60 (1H, br, $>C\underline{H}-OH$), 4.71 and 4.87 (4H, br, $>C=CH_2\times2$), 7.36 (1H, s, -COOH); MS, *m/e* (rel intensity), 470 ($(M-H_2O)^+$, 8), 445 (4), 427 (6), 391 (13), 387 (7), 330 (21), 141 (42), 123 (68), 43 (100), and 41 (71).

Found: C, 75.88; H, 10.56%. Calcd for $C_{31}H_{52}O_4$: C, 76.18; H, 10.72%.

Methylation of 1. **1** (363 mg) was methylated with CH_2N_2, followed by purification with preparative TLC [silica gel; benzene–dioxane–acetic acid (90: 25: 4, v/v)], to give a methyl ester (**2**) (389 mg): mp 157—158 °C; $[\alpha]_D^{25}$ +42.7° (*c* 0.59, $CHCl_3$); IR (0.001 M, CCl_4) ν_{max} 3610 (free OH), 3435 (intramolecularly hydrogen-bonded OH), 1730 cm^{-1} (COOMe); PMR ($CDCl_3$) δ 0.98—1.18 (Me×6), 1.73 (3H, s, $>C=C(CH_3)-$), 3.60 (1H, br, $>C\underline{H}-OH$), 3.65 (3H, s, $-COOCH_3$), 4.73 and 4.86 (4H, br, $>C=CH_2\times2$).

Found: C, 76.20; H, 10.92%. Calcd for $C_{32}H_{54}O_4$: C, 76.44: H, 10.83%.

Acetylation of 2. A mixture of **2** (380 mg), dry pyridine (4 ml), and acetic anhydride (8 ml) was allowed to stand overnight at room temp and the product, obtained on treatment of the reaction mixture in the usual method, was subjected to preparative TLC [silica gel; hexane–EtOAc (7: 3, v/v)] to give a methyl ester monoacetate (**4**) (376 mg): IR (Liquid) ν_{max} 3530 (OH), 1738 (C=O), 1642 and 900 cm^{-1} ($>C=CH_2$); PMR ($CDCl_3$) δ 0.85—1.10 (Me×6), 1.71 (3H, s, $>C=C(CH_3)-$), 2.02 (3H, s, $-OCOCH_3$), 3.65 (3H, s, $-COOCH_3$), 4.67 (1H, br, $>CH-OAc$), 4.77 and 4.84 (4H, br, $>C=CH_2\times2$).

Oxidation of 2. A soln of **2** (58 mg) in dry pyridine (2 ml) was added to the CrO_3–pyridine complex prepared from 100 mg of CrO_3 and 4 ml of pyridine. The reaction mixture was stirred overnight to give a keto alcohol (**3**) (49 mg): IR (0.001 M, CCl_4) ν_{max} 3620 (free OH), 3450 (intramolecularly hydrogen-bonded OH), 1735 (COOMe), 1707 cm^{-1} (C=O); PMR ($CDCl_3$) δ 0.80—1.20 (Me×6), 1.72 (3H, s, $>C=C(CH_3)-$), 2.92 (2H, d, J=10 Hz, $>CH-C\underline{H}_2-CO-$), 3.65 (3H, s, -COOMe), 4.70 and 4.88 (4H, br, $>C=CH_2\times2$); ORD (*c* 0.75, dioxane) $[\Phi]_{600}$ +117°, $[\Phi]_{589}$ +117°, $[\Phi]_{309}$ −780°, $[\Phi]_{303}$ −552°, $[\Phi]_{300}$ −584°, $[\Phi]_{263}$ +1218°; CD (*c* 0.75, dioxane) $[\theta]_{312}$ 0, $[\theta]_{284}$ −244, $[\theta]_{250}$ 0.

Synthesis of 1. *i) Acetylation of 7:* A mixture of **7** (290 mg), dry pyridine (2 ml), and acetic anhydride (2.5 ml) was left overnight at room temp and, after treatment as usual method, the product obtained was subjected to preparative TLC [silica gel; hexane–EtOAc (7: 3, v/v)] to give a monoacetate (260 mg): IR (Nujol) ν_{max} 3550 (OH), 1730 (COOMe), 1710 (C=O); PMR ($CDCl_3$) δ 2.06 (3H, s, -COOMe).

ii) Cleavage of Ring A of 7: According to the previously-described procedure,[14] a mixture of 12β-acetoxy-alnuserrudiolone oxime (145 mg) and *p*-toluenesulfonyl chloride (100 mg) in dry pyridine (4 ml) was kept for 24 h at room temp. After addition of a few drops of water, the reaction mixture was further stirred for 30 min at room temp, acidified with 5% hydrochloric acid (15 ml), and extracted with ether to give a solid mass. This mass was subjected to preparative TLC to give (12*R*,20*S*)-12-*O*-acetoxy-3-cyano-20-hydroxy-24-methylene-3,4-secodammar-4(28)-ene (**8**) (16 mg): MS, *m*/*e* (rel intensity), 511 (M^+, 1), 414 (3), 311 (7), 141 (10), 123 (14), 43 (100), and 41 (72); IR (Nujol) ν_{max} 3550 (OH), 2250 (–C≡N), 1730 (OCOMe), 3077, 1638, and 888 cm^{-1} ($>$C=CH_2); PMR ($CDCl_3$) δ 2.05 (3H, s, –OCOMe), 4.73 and 4.90 (4H, br, $>$C=CH_2×2).

iii) Hydrolysis of 8: A soln of **8** (16 mg) in 20% KOH/MeOH (6 ml) was refluxed for 15 h. The reaction mixture, after acidification with 5% hydrochloric acid, was extracted with ether. The ether extract was subjected to preparative TLC [silica gel; EtOAc–benzene (7:3, v/v)] to give (12*R*,20*S*)-12,20-dihydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid (**1**) (14 mg): mp 195—196 °C; IR (Nujol) ν_{max} 3400—2800 and 1713 (COOH), 1638 and 890 cm^{-1} ($>$C=CH_2); PMR ($CDCl_3$) δ 0.88—1.18 (Me×6), 1.73 (3H, s, $>$C=C(CH_3)–), 3.58 (1H, br, $>$C$\underline{H}$–OH), 4.73 and 4.90 (4H, br, $>$C=CH_2×2), 5.90 (1H, br, –COOH); MS, *m*/*e* (rel intensity), 470 ($(M-H_2O)^+$, 5), 445 (4), 427 (4), 391 (6), 387 (4), 330 (6), 141 (20), 123 (26), 43 (100), and 41 (82).

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