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A C_{31}-SECODAMMARANE-TYPE TRITERPENIC ACID, 12-DEOXY ALNUSTIC ACID, FROM THE FEMALE FLOWERS OF *ALNUS PENDULA*

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Key Word Index—*Alnus pendula*; Betulaceae; female flowers; 12-deoxy alnustic acid; alnustic acid; (20*S*)-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid.

Abstract—A new C_{31}-secodammarane-type triterpenic acid, 12-deoxy alnustic acid, was isolated from the female flowers of *Alnus pendula*. Its structure was elucidated to be (20*S*)-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid by a combination of chemical and spectroscopic methods.

INTRODUCTION

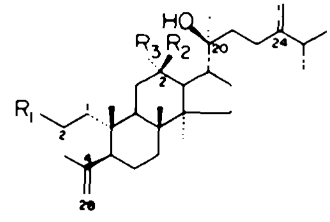
In our previous studies on the chemical constituents of the male flowers of *Alnus pendula* Matsum. (Japanese name: Himeyashabushi), we have characterized fatty acids and 16 aromatic compounds [1] as well as alnustic acid (3) and six 12-*O*-monoglycosides of the alnustic acid [2]. In the process of the investigation on the chemical constituents of the female flowers, a new C_{31}-secodammarane-type triterpenic acid was found in addition to alnuseric acid (1) [3] and alnustic acid (3) [2, 4]. We now wish to report the evidence which led to the establishment of the structure of the new triterpenic acid isolated from the female flowers.

RESULTS AND DISCUSSION

The female flowers were collected just before flowering in March. An ether-soluble fraction of the acetone extract of the female flowers exhibited 16 spots on a silica gel plate with ethyl acetate–hexane (2:3) as a solvent; a major spot (A) was observed between those of alnuseric acid (1) [3] and alnustic acid (3) [2, 4]. The ether-soluble fraction was then subjected to preparative TLC to isolate a constituent corresponding to the spot (A), which gave a new C_{31}-secodammarane-type triterpenic acid (2). These compounds (1–3) are numbered in the order of increasing polarity on TLC.

The mass spectrum of this acid (2) exhibited a molecular ion peak at m/z 472, indicating that the molecular weight of compound 2 is smaller than that of alnustic acid (3) by 16 amu. The IR and ^{1}H NMR spectra of compound 2 and its methyl ester (4) revealed the presence of carboxyl and hydroxyl groups and a terminal methylene moiety. Comparisons of the ^{13}C NMR chemical shifts of compounds 2 and 4 with those of alnustic acid (3) and its methyl ester (5) [4] indicated that compound 2 possesses a C_{31}-secodammarane-type skeleton with the carboxyl group on C-3 (δ_C 179.3), the tertiary hydroxyl group on C-20 (δ_C 75.7) and two terminal methylenes (δ_C 147.2 and 113.6 due to C-4 and C-28 and δ_C 156.2 and 106.3 due to C-24 and C-31, respectively), as shown in Table 1. On the basis of the co-occurrence of alnustic acid (3), possessing the *S* configuration at C-20 in the female flowers, the configuration at C-20 of compound 2 was suggested to be *S*. These observations suggested that compound 2 was a 12-deoxy derivative of alnustic acid (3).

Finally, the proposed structure (2) was established by relating its 3-ol derivative with a 12-deoxy, 3-ol derivative derived from alnustic acid (3). The methyl ester (5) of alnustic acid (3) was converted to the 12-deoxy, 3-ol derivative (6) on tosylation with *p*-TsCl followed by reduction with lithium aluminium hydride. On the other



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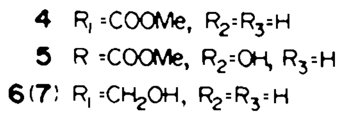

2 R_1 =COOH, R_2=R_3=H
3 R_1 =COOH, R_2=OH, R_3=H
4 R_1 =COOMe, R_2=R_3=H
5 R =COOMe, R_2=OH, R_3=H
6(7) R_1 =CH_2OH, R_2=R_3=H

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Table 1. ^{13}C NMR chemical shifts for compounds **2**, **4**, **6** and **7** (δ_c, in $CDCl_3$)

Carbon	**2**	**4**	**6**	**7**
1	25.0	24.7	24.7	24.7
2	28.4	28.4	26.3	26.2
3	179.3	177.4	63.5	63.5
4	147.2	147.2	147.9	148.0
5	41.1	41.0	41.0	41.0
6	28.4	28.4	28.4	28.4
7	34.4	34.4	35.7	35.7
8	40.0	39.9	40.0	40.0
9	50.8	50.7	50.9	50.9
10	40.0	39.9	39.0	38.9
11	22.0	22.0	22.0	22.0
12	24.7	24.7	24.7	24.7
13	42.4	42.4	42.4	42.5
14	50.8	50.7	50.9	50.9
15	31.3	31.3	31.3	31.3
16	27.5	27.4	27.6	27.5
17	49.5	49.7	49.9	49.8
18	15.3	15.3	15.3	15.3
19	20.0	20.1	20.6	20.5
20	75.7	74.9	75.2	75.3
21	25.0	25.3	25.3	25.2
22	39.1	39.1	39.6	39.6
23	34.0	33.9	34.0	34.0
24	156.2	156.3	156.3	156.3
25	34.0	33.9	34.0	34.0
26	22.0	22.0	22.0	22.0
27	22.0	22.0	22.0	22.0
28	113.6	113.5	113.1	113.0
29	23.3	23.3	23.1	23.1
30	16.4	16.4	16.5	16.4
31	106.3	106.2	106.4	106.2
OMe		51.4		

hand, reduction of the methyl ester (**4**) of the compound (**2**) with lithium aluminium hydride gave its 3-ol derivative (**7**). The physical and IR, ^{1}H NMR, ^{13}C NMR (Table 1) and mass spectral data of this derivative (**7**) were identical with those of compound **6**. Thus, the structure of compound **2** has been established to be (20*S*)-20-hydroxy-24-methylene-3,4-secodammar-4(28)-en-3-oic acid. Although an ether-soluble fraction of the male flowers also gave the spot corresponding to this acid (**2**) on a silica gel plate, the content was too low to be isolated.

EXPERIMENTAL

The same analytical methods as those described in refs [4] and [5] were used.

Extraction and isolation. Female flowers (365 g) of *A. pendula*, naturally grown on a hill in the suburbs of Hiroshima City, were collected just before flowering in March. The flowers, after they had been minced mechanically, were extracted with Me_2CO at room temp. for 2 months. The Me_2CO soln, after concn *in vacuo*, was extracted with Et_2O. Removal of the solvent from the extract gave a brown, viscous oil (18.9 g). A portion (8.9 g) of this oil was subjected to prep. TLC with EtOAc–hexane (2:3) to give the acid **2** (889 mg) as an oily substance.

12-Deoxy alnustic acid (**2**). $[\alpha]_D^{25}$ +36.7° ($CHCl_3$; *c* 1.14); IR ν_{max}^{film} cm^{-1}: 3400 and 1705 (COOH), 3080, 1640 and 890 ($C{=}CH_2$); ^{1}H NMR ($CDCl_3$): δ0.86–1.17 (18H, Me × 6), 1.73 (3H, *s*, $>C{=}C(Me)-$), 4.68, 4.75 and 4.85 (4H, *br*, $>C{=}CH_2 \times 2$), 6.11 (1H, *br*, $-COOH$); ^{13}C NMR ($CDCl_3$): see Table 1; EIMS *m/z* (rel. int.): 472 $[M]^+$ (1), 454 $[M-H_2O]^+$ (6), 141 (32), 123 (100). Methylation of compound **2** with CH_2N_2 yielded a methyl ester (**4**) as an oil; $[\alpha]_D^{25}$ +42.0° ($CHCl_3$; *c* 0.17); IR ν_{max}^{film} cm^{-1}: 3490 (OH), 3080, 1638 and 890 ($C{=}CH_2$), 1738 (COOMe); ^{1}H NMR ($CDCl_3$): δ0.85–1.16 (18H, Me × 6), 1.74 (3H, *s*, $>C{=}C(Me)-$), 3.66 (3H, *s*, $-COOMe$), 4.68, 4.74 and 4.84 (4H, *br*, $>C{=}CH_2 \times 2$); ^{13}C NMR ($CDCl_3$): see Table 1; EIMS *m/z* (rel. int.): 486 $[M]^+$ (3), 468 $[M-H_2O]^+$ (8), 141 (59), 123 (100).

Conversion of methyl alnustate (**5**) *to 12-deoxy, 3-ol derivative* (**6**). Methylation of alnustic acid (**3**) with CH_2N_2 gave a methyl ester (**5**). *p*-Toluenesulphonyl chloride (50 mg) was added to a soln of the methyl alnustate (**5**, 80 mg) in dry pyridine (2 ml) at 0°. The mixture was stirred for 1 hr at 0° and then allowed to stand for 3 days at 4°. The reaction mixture was poured into ice-cold 5% HCl and then extracted with Et_2O. The Et_2O soln was washed with 5% $NaHCO_3$ and satd NaCl solns, successively, dried over Na_2SO_4 and concd *in vacuo* to give tosylate as an oily substance, which was used for the next preparation without further purification. $LiAlH_4$ (200 mg) was added to a soln of the tosylate in dry THF (20 ml) under ice-cooling. This suspension was refluxed for 5 hr. After decomposition of an excess of $LiAlH_4$ with a soln of MeOH in THF and then 5% HCl, the mixture was extracted with $CHCl_3$. The $CHCl_3$ soln separated was washed with satd NaCl soln and then evaporated to dryness *in vacuo* to give an oily substance. This oily substance was subjected to flash chromatography [6] with a 15:85 mixture of EtOAc–hexane to afford a 12-deoxy,3-ol derivative (**6**, 24 mg): $[\alpha]_D^{25}$ +48.7° ($CHCl_3$; *c* 0.48); IR ν_{max}^{film} cm^{-1}: 3370 (OH), 3080, 1640 and 893 ($C{=}CH_2$); ^{1}H NMR ($CDCl_3$): δ0.82–1.16 (18H, Me × 6), 1.73 (3H, *s*, $>C{=}C(Me)-$), 3.58 (2H, *t*, $J = 6$ Hz, $-CH_2OH$), 4.60–4.90 (4H, *m*, $>C{=}CH_2 \times 2$); EIMS *m/z* (rel. int.): 440 $[M-H_2O]^+$ (11), 425 (8), 359 (39), 123 (100); CIMS (isobutane) *m/z* (rel. int.): 457 $[M-H]^+$ (4), 441 $[M-H_2O+H]^+$ (100).

Conversion of methyl 12-deoxy alnustate (**4**) *to its 3-ol derivative* (**7**). $LiAlH_4$ (100 mg) was added to a soln of methyl 12-deoxy alnustate (**4**, 66 mg) in dry THF (20 ml). The suspension was stirred for 5 hr at room temp. and then worked up by use of the same manner as described above to give a 3-ol derivative (**7**, 45 mg): $[\alpha]_D^{25}$ +48.1° ($CHCl_3$; *c* 1.54); IR ν_{max}^{film} cm^{-1}: 3370 (OH), 3080, 1641 and 893 ($C{=}CH_2$); ^{1}H NMR ($CDCl_3$): δ0.82–1.15 (18H, Me × 6), 1.72 (3H, *s*, $>C{=}C(Me)-$), 3.56 (2H, *t*, $J = 6$ Hz, $-CH_2OH$), 4.60–4.87 (4H, *m*, $>C{=}CH_2 \times 2$); EIMS *m/z* (rel. int.): 440 $[M-H_2O]^+$ (19), 425 (8), 359 (39), 123 (100); CIMS (isobutane) *m/z* (rel. int.): 457 $[M-H]^+$ (4), 441 $[M-H_2O+H]^+$ (100). The physical and spectral data of compound **7** were identical with those of the 12-deoxy,3-ol derivative (**6**) prepared from alnustic acid (**3**).

Identification of alnuseric acid (**1**) *and alnustic acid* (**3**). The behaviour on TLC of these acids (**1** and **3**) and their corresponding methyl esters were identical with those of known samples [3, 4].

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