The Structure of Alnuserrutriol, a New C_{31} Dammarane-type Triterpenoid from the Male Flowers of *Alnus serrulatoides*

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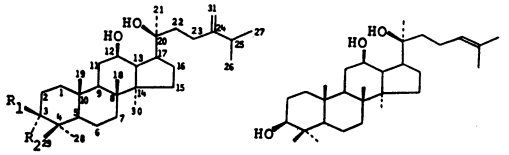
Synopsis. The structure of a new C_{31} dammarane-type triterpenoid, alnuserrutriol, isolated from the male flowers of *Alnus serrulatoides* CALL. (Betulaceae) has been elucidated to be (3*S*,12*R*, 20*S*)-3,12,20-trihydroxy-24-methylene-dammarane by a combination of chemical and spectroscopic methods.

In our previous studies, we have elucidated the structures of novel C_{31} dammarane-type triterpenoids and glycosides, such as alnuserol,[1] alnuseric acid,[2] alnuselide,[2] and alnuserrudiolone[3] isolated from the male flowers of *Alnus serrulatoides* CALL. (Japanese name: Kawara-hannoki) and monoglycosides of alnustic acid[4] isolated from the female flowers of the plant. In a continuation of these studies, a new C_{31} dammarane-type triterpenoid, named alnuserrutriol (I), has now been isolated from the male flowers of the plant. We here wish to describe evidence leading to the establishment of the structure of this new triterpenoid.

Results and Discussion

The male flowers of *Alnus serrulatoides* CALL. grown naturally on a river side were collected just before the flowering and immersed in acetone. An ether-soluble fraction of the acetone extract exhibited 15 spots on a silica-gel TLC plate with hexane–EtOAc (7: 3 v/v) as a solvent; a small spot (A) was observed between those of alnuserrudiolone (II)[3] and a residue at the origin. The ether-soluble fraction was then subjected to chromatography using silica gel to isolate a constituent corresponding to the spot (A), which gave a new triterpenoid, alnuserrutriol (I).



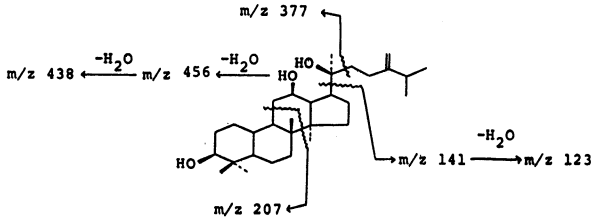
I: R_1=OH, R_2=H III
II: R_1, R_2=O

The molecular formula $C_{31}H_{54}O_3$ of I was determined by elemental analysis and appearance of a molecular ion peak at m/z 474 in the mass spectrum. The IR band at 3350 cm^{-1} and the ^1H NMR signal at δ 3.58 indicated the presence of a hydroxyl group. The existence of a terminal methylene was proved by appearance of the IR bands at 3080, 1635, and 885 cm^{-1} and the ^1H NMR signal at δ 4.78. Comparison of the ^{13}C NMR chemical shifts of I with those of alnuserrudiolone (II)[3] and protopanaxadiol (III)[5] indicated that I possesses a C_{31} dammarane-type skeleton with two secondary hydroxyl groups on C-3 (δ_c 78.9) and C-12 (δ_c 71.1), a tertiary hydroxyl group on C-20 (δ_c 74.1), and a terminal methylene (δ_c 157.0 and 106.5 due to C-24 and C-31, respectively), as shown in Table 1. The configurations of C-3, C-12, and C-20 were suggested to be *S*, *R*, and *S*, respectively, by comparing the ^{13}C NMR chemical shifts with those of (3*S*, 12*R*, 20*S*)-

TABLE 1. ^{13}C CHEMICAL SHIFTS (δ_c) OF I—III IN $CDCl_3$

Carbon No.	I	II	III[a]
1	38.8	39.7	39.0
2	27.4	33.5	27.4
3	78.9	217.8	78.8
4	39.1	47.3	39.0
5	56.0	55.3	56.0
6	18.4	19.7	18.3
7	34.9	33.9	34.8
8	39.8	39.6	39.8
9	50.3	49.4	50.2
10	37.2	36.8	37.1
11	31.5	31.7	31.2
12	71.1	70.5	70.8
13	47.8	47.7	47.7
14	51.7	51.5	51.6
15	31.1	31.0	31.1
16	26.5	26.7	26.6
17	53.8	53.6	53.6
18	16.2[b]	15.9[b]	16.2[b]
19	15.7[b]	15.3[b]	15.7[b]
20	74.1	73.6	74.0
21	27.0	26.7	26.8
22	33.9[c]	34.0	34.8
23	33.5[c]	34.0	22.4
24	157.0	156.5	125.2
25	28.4	28.2	131.4
26	22.0	21.9	25.8
27	22.0	21.9	17.8
28	28.1	26.4	28.1
29	15.5[b]	21.0	15.5[b]
30	17.0	16.8	16.9
31	106.5	106.2	—

a) Referred to the chemical shifts in the Ref. 5. b) and c) The values in any vertical column may be reversed although those given here are preferred.



Scheme 1. The MS spectral fragmentation pattern of alnuserrutriol (I).

protopanaxadiol (III)[5] and its related compounds.[5,6] The structure I was thus proposed for alnuserrutriol and possessed all the feature necessary to explain its MS spectrum (Scheme 1). The MS spectral fragmentation pattern was completely supported by the high-resolution MS.

Finally, the proposed structure (I) was confirmed by relating it with a triol which was obtained by the reduction of alnuserrudiolone (II) with $LiAlH_4$. The configuration of C-3 of this triol should be *S*, because it is well known that the $LiAlH_4$ reduction of dammarane-type triterpenoids with the carbonyl group at the 3-position gives exclusively the 3β-hydroxyl derivatives.[7,8] The physical and spectral data of naturally occurring alnuserrutriol (I) were perfectly identical with those of the prepared triol. Thus, the structure of alnuserrutriol (I) has been established to be (3*S*, 12*R*,-20*S*)-3,12,20-trihydroxy-24-methylenedammarane.

Experimental

The ^1H NMR spectra were taken on a varian T-60 spectrometer using TMS as an internal standard. The ^{13}C NMR spectra were obtained on a JEOL JNM FX-60 (15.1 MHz) and a Hitachi R-42 FT NMR (22.6 HMz) spectrometers ($\delta_{TMS}=0$). The EI-MS were obtained on Hitachi RMS-4 and RMU-7L mass spectrometers at 70 eV.

Extraction and Isolation. The male flowers (10.3 kg) of *Alnus serrulatoides* CALL. grown naturally on a river side in the suburbs of Hiroshima city were collected just before the flowering in December. The flowers, after minced mechanically, were immersed in acetone (54 dm^3) at room temp for 2 months. Removal of the solvent from the acetone soln gave a viscous sirup, which was extracted with ether (500 cm^3×5) to give a viscous oil (66.0 g). A part (7.0 g) of the viscous oil was subjected to centrifugal chromatography using silica gel (160 g) and a hexane–EtOAc mixture with EtOAc increasing 0 to 100% as a solvent, and then to preparative TLC on a silica-gel plate (Merck; Type 60, GF_{254}, 0.75 mm thick) with hexane–EtOAc (7 : 3 v/v) to give alnuserrutriol (I) (75 mg; R_f 0.05).

Alnuserrutriol (I). Mp 200—202 °C; $[\alpha]_D^{25}$ +89.1° (*c* 0.15, MeOH); IR (Nujol) ν_{max} 3350 (OH), 3080, 1635, and 885 cm^{-1} (C=CH$_2$); ^1H NMR ($CDCl_3$) δ=0.78—1.12 (Me×8), 3.58 (2H, br, >C<u>H</u>–OH×2), 4.78 (2H, br, >C=CH$_2$); ^{13}C NMR ($CDCl_3$) (see Table 1); MS *m/z* (rel intensity) 474 (0.5, M$^+$), 456 (11), 438 (23), 377 (12), 341 (37), 207 (43), 189 (26), 141 (69), 123 (71), and 108 (100). High-resolution MS *m/z* 438.3941 ($C_{31}H_{50}O$ (M−2H$_2$O): 438.3859), 377.3010 ($C_{24}H_{41}O_3$: 377.3053), 207.1750 ($C_{14}H_{23}O$: 207.1748), 141.1290 ($C_9H_{17}O$: 141.1279), and 123.1138 (C_9H_{15}: 123.1172).

Found: C, 78.17; H, 11.37%. Calcd for $C_{31}H_{54}O_3$: C, 78.42; H, 11.47%.

Preparation of I from Alnuserrudiolone (II). A soln of alnuserrudiolone (II) (100 mg), which was isolated from *A. serrulatoides*,[3] in dry ether (2 cm^3) was dropped into a suspension of $LiAlH_4$ (10 mg) in dry ether (2 cm^3), followed by stirring for 20 min at 0 °C. The reaction mixture, after acidification, was extracted with ether to give a triol (97 mg): mp 200—203 °C; $[\alpha]_D^{25}$ +87.3° (*c* 0.30, MeOH); IR (Nujol) ν_{max} 3350 (OH), 3080, 1640, and 885 cm^{-1} (>C=CH$_2$); ^1H NMR ($CDCl_3$) δ=3.58 (2H, br, >C<u>H</u>–OH×2), 4.76 (2H, br, >C=CH$_2$); ^{13}C NMR ($CDCl_3$) δ_c=157.2 (s, C-24), 106.3 (t, C-31), 78.8 (d, C-3), 74.0 (s, C-20), 71.1 (d, C-12), 28.0, 27.1, 21.9, 17.0, 16.0, 15.6, 15.3 (q, Me×8); MS *m/z* (rel intensity) 474 (0.5, M$^+$), 456 (12), 438 (21), 377 (9), 341 (35), 207 (37), 189 (30), 141 (60), 123 (65), and 108 (100). The physical and spectral data of the triol were identical with those of the naturally occurring alnuserrutriol (I).

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