New C_{31} Secodammarane-type Triterpenoids, Alnuseric Acid and Alnuselide, in the Male Flowers of *Alnus serrulatoides*

Takayuki SUGA* and Toshifumi HIRATA

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Hiroshima 730

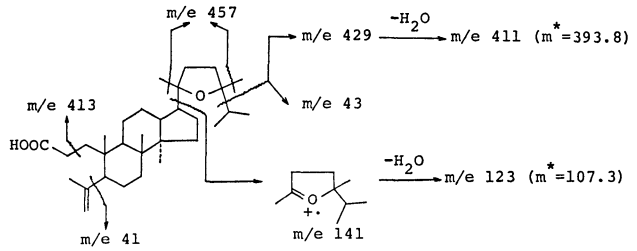
(Received September 4, 1978)

Two novel C_{31} 3,4-secodammarane-type triterpenoids, alnuseric acid and alnuselide, were isolated from the male flowers of *Alnus serrulatoides* Call. (Betulaceae); their structures were elucidated to be (20*S*,24*R*)-20,24-epoxy-24-methyl-3,4-secodammar-4(28)-en-3-oic acid and (11*R*,20*S*,24*R*)-20,24-epoxy-24-methyl-3,4-secodammar-4(28)-en-3,11α-olactone, respectively, by a combination of chemical and spectroscopic methods.

In connection with the chemical and biochemical studies on pollination of *Alnus* species,[1] we investigated chemical constituents of the male flowers of *Alnus serrulatoides* Call. (Japanese name: Kawara-hannoki) and reported the presence of novel C_{31} dammarane-type dihydroxy keto and 11α-hydroxylated triterpenoids.[2-4] A further investigation on constituents of the male flowers has led to the isolation of two novel triterpenic acid and triterpene lactone, named alnuseric acid and alnuselide, and preliminary accounts of the structural work have been presented in the previous papers, respectively.[5,6] We here wish to report *en bloc* detailed evidence which led to the establishment of the structures of these novel triterpenoids, which are featured in the C_{31} 3,4-secodammarane form.

Results and Discussion

The ether soluble fraction of the male flowers on separation by means of a centrifugal liquid chromatograph and then a preparative TLC gave alnuseric acid (I) and alnuselide (II), along with several dammarane-type triterpenoids, alnincanone,[2,7] alnuserol,[4] and alnuserrudiolone.[2]

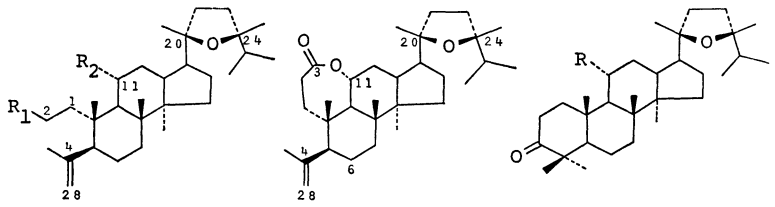


Scheme 1. The mass spectral fragmentation pattern of alnuseric acid (I).

Alnuseric acid (I), $C_{31}H_{52}O_3$, showed IR absorption bands of a terminal methylene and a carboxyl group. The CMR spectrum in $CDCl_3$ also indicated the presence of the terminal methylene, the carboxyl, the ether ring, and eight methyl carbons. The peaks at *m/e* 141 (base), 123, and 43 in the mass spectrum was similar to those in it of alnincanone (III), a naturally occurring dammarane-type triterpenoid with a tetrahydrofuran ring. Ryabinin *et al.*[8] has proved the occurrence of such ions from the tetrahydrofuran ring of alnincanone (III). This has also been established in the mass spectra of alnuserol (IV)[4] and various compounds with a tetrahydrofuran ring.[9,10] Therefore, the occurrence of the peaks in the mass spectrum of alnuseric acid (Scheme 1) indicates clearly that this acid includes a side chain comprised of a tetrahydrofuran ring similarly to III and IV.

The molecular formula indicated that alnuseric acid has four rings, in contrast to alnincanone (III) and alnuserol (IV). Accordingly, alnuseric acid surely contains no ring A located with a carbonyl or hydroxyl group at position 3, though such functional groups appear almost without exception in plant triterpenoids. These structural features of alnuseric acid may result from the cleavage of ring A of a 3-keto triterpenoid having a side chain comprised of a tetrahydrofuran ring as III and IV. To the alnuseric acid, we here propose a biogenetically acceptable structure (I),[11] which is probably formed biologically from alnincanone by ring-cleavage at the 3,4-position.

The structure (I) proposed for alnuseric acid was confirmed by its synthesis starting from alnincanone (III) following the method established for ring-cleavage at the 3,4-position in ring A of β-amyrenone.[12] Alnincanone (III) was transformed to the corresponding ketoxime, which then was treated with *p*-toluenesulfonyl



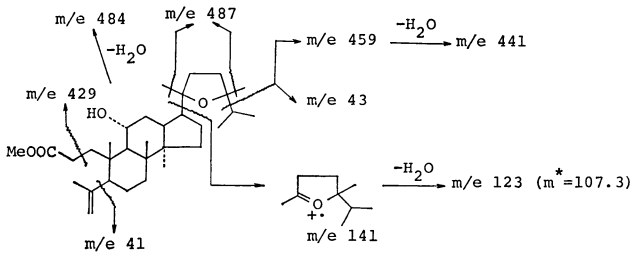
I: R_1=COOH, R_2=H
V: R_1=CN, R_2=H
VI: R_1=COOH, R_2=OH
VII: R_1=COOMe, R_2=OH
VIII: R_1=COOMe, R_2=H
X: R_1=CN, R_2=OAc

II

III: R=H
IV: R=OH
IX: R=OAc

chloride in pyridine to yield an abnormal Beckmann rearrangement product (V) with infrared bands due to a cyano group and a terminal methylene. Alkaline hydrolysis of this product (V) under milder conditions gave (20*S*,24*R*)-20,24-epoxy-24-methyl-3,4-secodammar-4(28)-en-3-oic acid. Identity of this authentic acid with the naturally occurring alnuseric acid was established by comparison of the mixed melting point and spectral data. The structure of alnuseric acid has been elucidated to be in **I**.

Alnuselide (II), $C_{31}H_{50}O_3$, showed IR absorption bands due to a terminal methylene. The CMR spectrum indicated the presence of an ester or a lactone carbonyl, a terminal methylene, and eight methyl carbons in addition to two quaternary carbons bearing an oxygen atom. Appearance of the IR absorption band at 1740 cm^{-1} in CCl_4 suggested the presence of a six- or a seven-membered lactone moiety. This was confirmed by the formation of a hydroxy acid VI from II on hydrolysis with KOH/MeOH and the reproduction of II from VI on acidification with HCl/MeOH. Methylation of VI with CH_2N_2 yielded a hydroxy methyl ester VII, which exhibited the CMR signal at δ 71.2 ppm (d) due to the carbon atom occupied with a secondary hydroxyl group. The mass spectrum of VII (Scheme 2) showed the



Scheme 2. The mass spectral fragmentation pattern of hydroxy methyl ester VII derived from alnuselide (II).

fragmentation pattern quite similar to that of alnuseric acid methyl ester (VIII). This suggested a secodammarane-type triterpenoid with the side chain comprised of a tetrahydrofuran ring. The seco-form was also indicated by the molecular formula revealing that VII comprises four rings. Accordingly, the hydroxy methyl ester (VII) may be a 6- or a 11-hydroxylated derivative of VIII, because alnuselide (II) is found to involve a six- or a seven-membered lactone comprised of a secondary hydroxyl group as described above. The agreement between the carbon signals due to the C, D, and tetrahydrofuran rings of VII and the signals due to these rings of alnuserol (IV)[4] demonstrated that the secondary hydroxyl group should be located on C-11 of VII. Structure VII is assigned to the hydroxy methyl ester, and structure II is proposed for alnuselide, a novel triterpenoid lactone.

The structure (II) was confirmed by means of its synthesis involving the cleavage of the A-ring of alnuserol (IV)[4] followed by the lactonization of the resulting hydroxy acid. The oxime derivative of alnuseryl acetate (IX) prepared from alnuserol (IV) in the usual manner was treated[12] with *p*-toluenesulfonyl chloride in pyridine to yield a product (X) resulting from the abnormal Beckmann rearrangement. The hydrolysis of the product (X) with KOH/MeOH gave a hydroxy acid. This hydroxy acid on dissolving in ether saturated with 5% hydrochloric acid and then standing at room temperature suffered lactonization to form (11*R*,20*S*,24*R*)-20,24-epoxy-24-methyl-3,4-secodammar-4(28)-en-3,11α-olactone. Identity of naturally occurring alnuselide with this authentic sample was established by comparisons of the mixed melting point, and spectral data. Thus, the structure of alnuselide has been elucidated to be II. Biogenetically, it is fascinating to note that biological cleavage of ring A in alnuserol (IV), similarly to the case of alnuseric acid (I), followed by lactonization between the 3-carboxyl and the 11-hydroxyl groups might result in the formation of alnuselide (II).

Experimental

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

Extraction and Isolation. The male flowers (10.3 kg) of *Alnus serrulatoides* Call. grown wildly on a river side in suburbs of Hiroshima city were collected just before the flowering in late February. After minced mechanically, the flowers were immersed in acetone at room temp for 2 months. Removal of the solvent from the acetone solution gave a viscous sirup, which was extracted with ether to give a viscous oil (66.7 g). A part (20.0 g) of the viscous oil was subjected to a centrifugal liquid chromatography (silica gel; 3 mm in thickness) with a hexane–EtOAc mixture with EtOAc increasing 0 to 30% and then to preparative TLC (silica gel; 0.75 mm in thickness) with hexane–EtOAc (7: 3 v/v) to give alnuseric acid (I) (144 mg) and alnuselide (II) (198 mg).

Alnuseric Acid (I). Mp 107—109 °C; $[\alpha]_D^{25}$+24.3° (*c* 0.21, MeOH); IR (KBr) ν_{max} 3300—2800, 1708 (COOH), 3077, 1638, and 888 cm^{-1} (>C=CH$_2$), 1084 (C–O–C); PMR ($CDCl_3$) δ 0.8—1.2 (7×Me), 1.75 (3H, broad s, C(4)-Me), 4.70 and 4.85 (2H, broad s, >C=CH$_2$); CMR ($CDCl_3$) δ 180.2 (s, C-3), 147.4 (s, C-4), 113.4 (t, C-28), 85.8 and 85.3 (s, C-20 and C-24), 25.1, 23.2, 22.9, 20.1, 18.8, 17.6, 16.3, 15.4 (q, 8×Me); MS *m/e* (rel. intensity) 472 (M^+, 1), 457 (3), 429 (20), 411 (8), 141 (100), 123 (61), 43 (63), 41 (52). Found: C, 79.06; H, 11.01%. Calcd for $C_{31}H_{52}O_3$: C, 78.76; H, 11.09%. Methylation of I with CH_2N_2 gave the methyl ester (VIII): IR (liquid film) ν_{max} 1735 (ester), 3078, 1637, and 890 cm^{-1} (>C=CH$_2$); PMR ($CDCl_3$) δ 0.8—1.2 (7×Me), 1.76 (3H, broad s, C(4)-Me), 3.36 (3H, s, OMe), 4.72 and 4.86 (2H, broad s, >C=CH$_2$); MS *m/e* (rel. intensity) 486 (M^+, 2), 471 (3), 141 (100), 123 (60), 43 (65), 41 (59).

Alnuselide (II). Mp 139—140 °C; $[\alpha]_D^{25}$+ 107° (*c* 0.33, MeOH); IR (KBr) ν_{max} 1735 (lactone), 3066, 1635, and 877 cm^{-1} (>C=CH$_2$); UV (MeOH) λ_{max} 220 nm (ε 34.9); PMR ($CDCl_3$) δ 0.8—1.2 (7×Me), 1.79 (3H, broad s, C(4)-Me), 4.73 and 4.88 (2H, broad s, >C=CH$_2$); CMR ($CDCl_3$) δ 176.2 (s, C-3), 146.7 (s, C-4), 114.1 (t, C-28), 85.4 and 84.9 (s, C-20 and C-24), 76.5 (d, C-11), 24.9, 23.7, 22.9, 18.8, 18.7, 17.5, 17.0, and 15.6 (s, 8×Me); MS *m/e* (rel. intensity) 470 (M^+, 2), 426 (60), 408 (18), 141 (100), 123 (62), 43 (60), 41 (67), 28 (83); ORD (*c* 0.33, MeOH) $[\Phi]_{400}$+2090°, $[\Phi]_{219}$ +12300°; CD (*c* 0.33, MeOH) $[\theta]_{219}$+203°. Found: C, 79.01; H, 10.93%. Calcd for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71%.

Preparation of Hydroxy Methyl Ester VII from Alnuselide. A solution of II (20 mg) in 5% NaOH/MeOH (5 ml) was reflux-

ed for 30 min. The reaction mixture, after acidification, was extracted with ether to give a crude product (18 mg), which was then purified by preparative TLC [silica gel; hexane–EtOAc (7:3 v/v)] to afford hydroxy acid VI (15 mg): IR (Nujol) ν_{max} 3350—2500 and 1710 cm^{-1} (OH and COOH). The hydroxy acid (VI) (4.0 mg) was treated for 12 h at room temp with ether (3 ml) saturated with 5% hydrochloric acid to reproduce the original lactone (II) (3.3 mg): mp and mixed mp 139—140 °C. Treatment of the hydroxy acid (VI) (10 mg) with CH_2N_2 gave hydroxy methyl ester VII (10 mg): IR (Nujol) ν_{max} 3350 (OH), 1731 (ester), 3080, 1635, and 883 cm^{-1} (>C=CH_2); MS *m/e* (rel. intensity) 502 (M^+, 0.5), 487 (2), 484 (4), 141 (100), 123 (71), 43 (45), 41 (47); PMR ($CDCl_3$) δ 1.77 (3H, broad s, C(4)–Me), 3.35 (3H, s, OMe), 4.71 and 4.87 (2H, broad s, >C=CH_2); CMR ($CDCl_3$) δ 175.8 (s, C-3), 147.4 (s, C-4), 113.7 (t, C-28), 85.3 (s, C-20 and C-24), 71.2 (d, C-11), 51.4 (q, OMe), 24.6, 23.1, 22.9, 20.4, 18.7, 17.5, 16.4, 16.0 (q, 8×Me). Found: C, 76.69; H, 10.70%. Calcd for $C_{32}H_{54}O_4$: C, 76.45; H, 10.83%.

Synthesis of I. *i) Cleavage of the A Ring of Alnincanone (III)*: According to the previously-described procedure,[12] a mixture of alnincanone oxime (30 mg; prepared from alnincanone (III)[2,7] and hydroxylamine hydrochloride in pyridine) and *p*-toluenesulfonyl chloride (30 mg) in dry pyridine (1.0 ml) was kept for 20 h at room temp. After addition of a few drops of water, the mixture was stirred for 30 min at room temp. The reaction mixture on acidification with 5% hydrochloric acid (10 ml) was extracted with ether to give a solid mass (27 mg), which was subjected to preparative TLC to give 3-cyano-20,24-epoxy-24-methyl-3,4-secodammar-4-(28)-ene (V) (8.2 mg): mp 101—103 °C; IR (Nujol) ν_{max} 2240 (C≡N), 3075, 1632, and 899 cm^{-1} (>C=CH_2); PMR ($CDCl_3$) δ 1.79 (3H, broad s, C(4)–Me) and 4.75 and 4.94 (2H, broad s, >C=CH_2). Found: C, 82.16; H, 11.20%. Calcd for $C_{31}H_{51}ON$: C, 82.06; H, 11.33%. This reaction, as has been described previously,[12] yielded an undesired product, aza-A-homodammarane derivative (12 mg): mp 195—197 °C; IR (Nujol) ν_{max} 1665 cm^{-1} (amide), which was not further investigated.

ii) Hydrolysis of V: A solution of V (5.0 mg) in 20% KOH/EtOH (2 ml) was refluxed for 6 h. The reaction mixture, after acidification was extracted with ether to give 20,24-epoxy-24-methyl-3,4-secodammar-4(28)-en-3-oic acid (I) (3.9 mg): mp 107—109 °C; IR (Nujol) 3300—2800 and 1710 (COOH), 3079, 1638, and 890 cm^{-1} (>C=CH_2); MS *m/e* (rel. intensity) 472 (M^+, 2), 141 (100), 123 (78), 43 (49), 41 (45); PMR ($CDCl_3$) δ 1.75 (3H, broad s, C(4)–Me), 4.70 and 4.85 (2H, broad s, >C=CH_2).

Synthesis of II. *i) Cleavage of the A Ring of Alnuserol (IV)*: By the same procedure as described above, the oxime derivative (228 mg) of alnuseryl acetate (IX)[4] was treated with *p*-toluenesulfonyl chloride (140 mg) in dry pyridine (10 ml) to give 11-acetoxy-3-cyano-20,24-epoxy-24-methyl-3,4-secodammar-4(28)-ene (X) (66 mg): mp 160—162 °C; IR (Nujol) ν_{max} 2245 (C≡N), 1730 (OAc), 3076, 1639, and 890 cm^{-1} (>C=CH_2); PMR ($CDCl_3$) δ 1.77 (3H, broad s, C(4)–Me), 2.07 (3H, s, OAc), 4.72 and 4.92 (2H, broad s, >C=CH_2). Found: C, 77.66; H, 10.51%. Calcd for $C_{33}H_{53}O_3N$: C, 77.45; H, 10.44%. In a similar manner as above, this reaction gave the undesired aza-A-homodammarane derivative (48 mg): mp 225—226 °C; IR (Nujol) ν_{max} 1666 cm^{-1} (amide).

ii) Hydrolysis of X: A solution of X (20 mg) in 20% KOH/MeOH (5 ml) was refluxed for 12 h. The reaction mixture on usual work-up gave 20,24-epoxy-11-hydroxy-24-methyl-3,4-secodammar-4(28)-en-3-oic acid (VI): IR (Nujol) 3350—2500 (OH and COOH), 1708 (COOH), 3080, 1640, and 890 cm^{-1} (>C=CH_2); PMR ($CDCl_3$) δ 1.73 (3H, broad s, C(4)–Me), 4.71 and 4.86 (2H, broad s, >C=CH_2).

iii) Lactonization of VI: Hydroxy acid VI (10 mg) dissolved in ether (5 ml) saturated with 5% hydrochloric acid was left stand for 12 h at room temp to give (11*R*, 20*S*, 24*R*)-20,24-epoxy-24-methyl-3,4-secodammar-4(28)-en-3,11α-olactone (II) (9.0 mg): mp 139—140 °C; IR (Nujol) ν_{max} 1735 (lactone), 3080, 1635, and 890 cm^{-1} (>C=CH_2); MS *m/e* (rel. intensity) 470 (M^+, 2), 141 (100), 123 (68), 43 (59), 41 (67); PMR ($CDCl_3$) δ 1.80 (3H, broad s, C(4)–Me), 4.73 and 4.89 (2H, broad s, >C=CH_2).

We thank Miss Reiko Ideo for having charge of a part of the experiments and JEOL Co., Ltd. for obtaining the ^{13}C-NMR spectra. The present work was partially supported by a Grant-in-Aid for Scientic Research from the Ministry of Education (Nos. 247027 and 234033 to T.S.) and Matsunaga Science Foundation in 1977 (to T.H.).

References

1) J. Heslop-Harrison, "Pollen: Development and Physiology," London Butherworths Ltd., London (1971).
2) T. Suga, T. Hirata, and N. Iwata, *Chem. Lett.*, **1974**, 971.
3) T. Hirata, K. Murai, R. Ideo, and T. Suga, The preprint of The 20th Symposium on the Chemistry of Natural Products, Sendai (1976), p. 273.
4) T. Hirata, K. Murai, T. Suga, and A. Christensen, *Chem. Lett.*, **1977**, 95; T. Hirata and T. Suga, *J. Chem. Soc., Perkin Trans. 2*, **1978**, 347.
5) T. Hirata, R. Ideo, and T. Suga, *Chem. Lett.*, **1977**, 283.
6) T. Hirata, R. Ideo, and T. Suga, *Chem. Lett.*, **1977**, 711.
7) R. Labriola and G. Ourisson, *Tetrahedron*, **29**, 2105 (1973).
8) A. A. Ryabinin, L. H. Matyukhina, I. A. Saltikova, F. Patil, and G. Ourisson, *Bull. Soc. Chim. Fr.*, **1968**, 1089.
9) Q. N. Porter and J. Baldas, "Mass Spectrometry of Heterocyclic Compounds," Wiley-Interscience Inc., New York, N.Y. (1971), p. 39.
10) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day Inc., San Francisco, Calif. (1967), p. 227.
11) A. A. Newman, "Chemistry of Terpenes and Terpenoids," Academic Press Inc., New York, N.Y. (1972), p. 214.
12) G. H. Whitham, *J. Chem. Soc.*, **1960**, 2016.