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STRUCTURE OF ALNUSELIDE, THE FIRST REPORTED NATURALLY OCCURRING C_{31}-SECODAMMARANE-TYPE TRITERPENE LACTONE FROM *ALNUS SERRULATOIDES*

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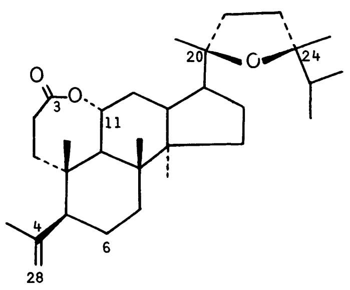
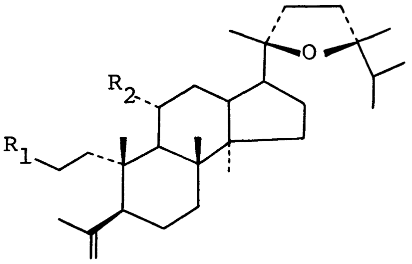
A novel C_{31}-3,4-secodammarane-type triterpene lactone, alnuselide, was isolated from the male flowers of *Alnus serrulatoides* Call. (Betulaceae). The structure of alnuselide was elucidated to be (11*R*,20*S*,24*R*)-20,24-epoxy-24-methyl-3,4-secodammar-4(28)-en-3,11-carbolactone (I) by a combination of the chemical and spectroscopic methods and the synthesis.

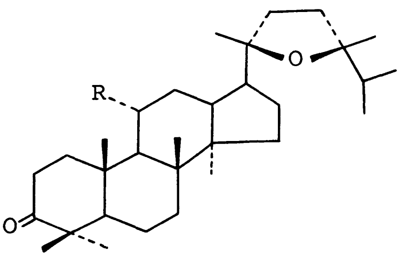
In connection with the biochemical and physiological studies of pollination,[1)] we investigated chemical constituents of the male flowers of *Alnus serrulatoides* Call. and reported the isolation and the structure elucidation of three new C_{31}-dammarane-type triterpenes, alnuserrudiolone,[2)] alnuserol,[3)] and alnuseric acid.[4)] We further have isolated a novel C_{31}-secodammarane-type triterpene lactone, named



I



II: R_1=COOH, R_2=OH
III: R_1=COOMe, R_2=OH
IV: R_1=COOMe, R_2=H
VII: R_1=CN, R_2=OH



V: R=OH
VI: R=OAc

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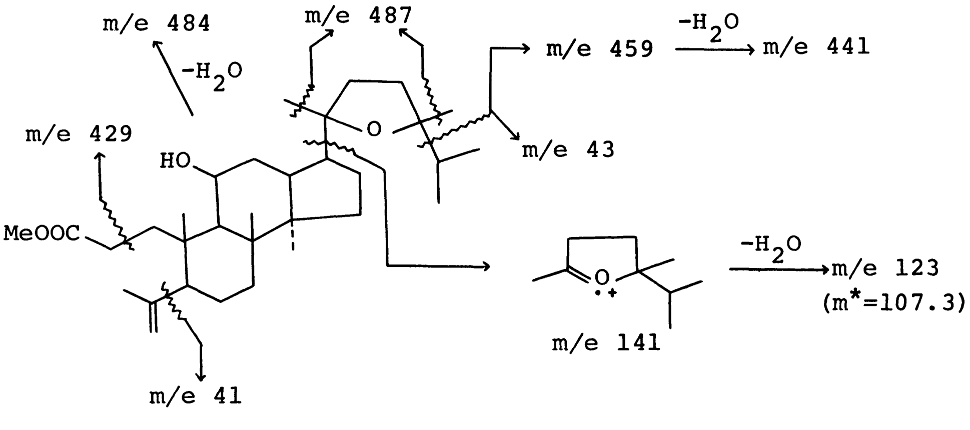
alnuselide (I), from the male flowers. In naturally occurring dammarane-type triterpenes, the newly-isolated C_{31}-3,4-secodammaren-3,11-carbolactone-type triterpene is quite unique. We here wish to report evidence which led to the establishment of its structure.

The *n*-hexane soluble fraction of the male flowers on chromatographic separation with a centrifugal liquid chromatograph (SiO_2) and then a preparative TLC (SiO_2) gave alnuselide (I) as colorless needles [mp 139~140°C; $[\alpha]_D^{25}$ +107° (*c* 0.33, MeOH); m/e 470 (M^+), 141, 123, 43, and 41; $C_{31}H_{50}O_3$ (requires: C 79.10, H 10.71. found: C 79.01, H 10.93%)]. The IR spectrum showed the absorption bands due to an end-methylene group [ν_{max} (Nujol) 3080, 1635, and 890 cm^{-1}]. The ^1H-NMR and MS spectra demonstrated the presence of an isopropenyl group [δ_{ppm} ($CDCl_3$) 1.79 (bs, 3H), 4.73 (bs, 1H), and 4.89 (bs, 1H); m/e 41]. The ^{13}C-NMR spectrum indicated the presence of a tertiary carbon attached to an oxygen atom [δ_{ppm} ($CDCl_3$) 76.5 (d)] and two quaternary carbons bearing an oxygen atom (probably an ether oxygen) [85.4 (s) and 84.9 (s)] in addition to an ester [176.2 (s)], an end-methylene [146.7 (s) and 114.1 (t)], and eight methyl [24.9 (q), 23.7 (q), 22.9 (q), 18.8 (q), 18.7 (q), 17.5 (q), 17.0 (q), and 15.6 (q)] carbons.

Appearance of the absorption band at 1740 cm^{-1} in CCl_4 or 1735 cm^{-1} in Nujol in the IR spectrum of alnuselide (I) suggested that the alnuselide has the partial structure of a six- or a seven-membered lactone. This was confirmed by formation of a hydroxy acid II from I on hydrolysis with KOH/MeOH and reproduction of the alnuselide (I) from II on acidification with HCl/MeOH. Methylation of II with



SCHEME 1. The mass spectral fragmentation pattern of hydroxy methyl ester III.

CH_2N_2 gave a hydroxy methyl ester III [$C_{32}H_{54}O_4$; m/e 502 (M^+); ν_{max} (Nujol) 1731 cm^{-1} (ester); ^1H-NMR ($CDCl_3$) δ_{ppm} 3.67 (s, 3H, OMe)], which exhibited the ^{13}C signal at δ 71.2 ppm (d) due to the carbon occupied with a secondary hydroxyl group. The MS spectrum of III (Scheme 1) showed the fragmentation pattern quite similar to that of alnuseric acid methyl ester (IV) reported recently by the authors.[4)] The similarity in the mass fragmentation patterns of III and IV suggested that III possesses the structure of a secodammarane-type triterpene having the side chain with a tetrahydrofuran ring[4,5)] similarly to IV. The molecular formula of III revealed that it comprises four rings, and is in a seco-form. Accordingly, the hydroxy methyl ester (III) may be a C-6 or a C-11 hydroxylated derivative of alnuseric acid methyl ester (IV), because alnuselide (I) involves a six- or a seven-membered lactone comprised of a secondary hydroxyl group as described above. On the basis of the fact that the carbon signals due to the C- and the D-rings and the tetrahydrofuran ring of III in the ^{13}C-NMR spectrum were identical with the signals due to these rings of alnuserol (V), it was clarified that the secondary hydroxyl group is located at C-11 of III. The ^{13}C-NMR spectrum of III exhibited the following signals due to the carbons assignable to the structural formula III; δ_{ppm} ($CDCl_3$) 175.8 (C-3), 147.4 (C-4), 113.7 (C-28), 71.2 (C-11), 85.3 (2×ether-ring carbon; C-20 and C-24), 51.4 (COO$\underline{Me}$), and 24.6, 23.1, 22.9, 20.4, 18.7, 17.5, 16.4 and 16.0 (8×Me). The structure III is now assigned to the hydroxy methyl ester, and the structure I is proposed for the novel triterpene lactone, alnuselide. The proposed structure (I) possesses all the features necessary to explain its spectral data.

The structure (I) proposed for alnuselide was confirmed by means of the synthesis involving cleavage of the A-ring of alnuserol (V),[3)] followed by lactonization of the resulting hydroxy acid. Following the method established previously for cleaving the 3,4-position in the A-ring of β-amyrenone,[6)] the oxime derivative of alnuseryl acetate (VI) prepared from alnuserol (V) in the usual manner was treated with *p*-toluenesulfonyl chloride in dry pyridine to yield an abnormal Beckmann rearrangement product (VII) with the IR absorption bands (Nujol) at 2245 (-C≡N), 1730 (OAc), and 3076, 1639, and 890 cm^{-1} ($>C=CH_2$), and then the product (VII) was hydrolyzed with 5% KOH/MeOH to give a hydroxy acid. The hydroxy acid on dissolving in ether saturated with 5% hydrochloric acid and then standing at room temperature suffered lactonization to yield (11*R*,20*S*,24*R*)-20,24-epoxy-24-

methyl-3,4-secodammar-4(28)-en-3,11-carbolactone [mp 139~140°C; m/e 470 (M^+), 141 (base), 123, 43, and 41; ν_{max} (Nujol) 1735 (lactone), 3080, 1635, and 890 cm^{-1} ($\rangle C=CH_2$); ^{1}H-NMR ($CDCl_3$) δ_{ppm} 1.80 (bs, 3H), 4.73 (bs, 1H), and 4.89 (bs, 1H)]. Identity of naturally occurring alnuselide with this authentic sample was established by comparison of mixed mp, TLC, IR, ^{1}H-NMR, and MS.

Thus, the structure of alnuselide (I) has been elucidated to be (11*R*,20*S*,24*R*)-20,24-epoxy-24-methyl-3,4-secodammar-4(28)-en-3,11-carbolactone. To our knowledge, alnuselide (I) is the first reported naturally occurring C_{31}-3,4-secodammarane-type triterpene lactone.

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