ALNUSIIN, A NOVEL ELLAGITANNIN FROM ALNUS SIEBOLDIANA FRUITS

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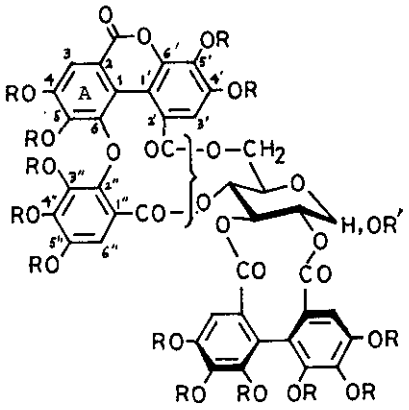
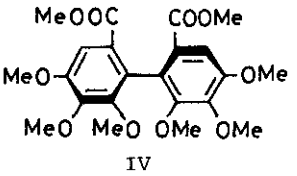
Abstract — A new ellagitannin, named alnusiin has been isolated from the fruits of Alnus sieboldiana, and the structure (I) was elucidated.

Fruits of Alnus sieboldiana (Betulaceae) are known to be rich in tannins, and have been used for dyeing in Japan. We have isolated a new ellagitannin from the fruits, and named it alnusiin.

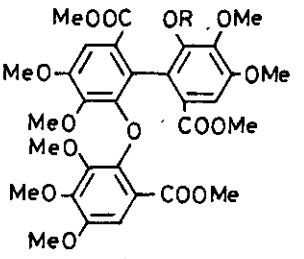
The crushed fresh fruits were homogenized in an aqueous acetone and the concentrated solution was extracted with ethyl acetate. The extract was then fractionated by droplet counter-current chromatography followed by Sephadex LH-20 column chromatography to give alnusiin (I), $C_{41}H_{26}O_{26}\cdot 4H_2O$, $[\alpha]_D$ -32° (c=1.4, acetone), as an off-white amorphous powder.

The ^1H nmr spectrum (acetone-d_6) of I exhibited six singlets due to the five aromatic protons at δ 7.01 (0.4H), 6.99 (0.6H), 6.96, 6.41 (1H each), 6.59 (0.4H) and 6.64 (1.6H), and unresolved sugar protons at δ 5.0~5.6 and 3.9. The signal pattern of the aromatic protons, and absence of the anomeric proton signal in the region of δ 6.0~6.5, imply that the anomeric hydroxyl group in the sugar moiety of I is free. The formation of an anomer mixture was shown by the signals of α- and β-anomeric carbons at δ 92.6 and 96.2, and by the double signals for the other carbons in the ^{13}C nmr spectrum (acetone-d_6) of I. The double signals of the peak area ratio ca. 3:2 for α- and β-anomer, were also observed in the ^1H nmr spectrum of trideca-O-methylalnusiin (II), $C_{54}H_{52}O_{26}$, $[\alpha]_D$ -34° (c=1.0, $CHCl_3$), which was prepared by methylation of I with diazomethane. Acetylation of II gave a monoacetate (III) [$C_{56}H_{54}O_{27}$; M^+ 1158; ^1H nmr ($CDCl_3$) δ 6.44 (d, J=4 Hz, anomeric β-H), 5.90 (d, J=9 Hz, anomeric α-H)]. Upon methanolysis with sodium methoxide in methanol, II yielded D-glucose, (S)-dimethyl hexamethoxydiphenoate (IV) {$[\alpha]_D$





IV



V : R=H

VI : R=Me

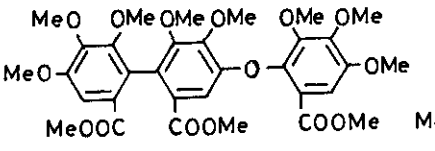
I : R=R'=H

II : R=Me, R'=H

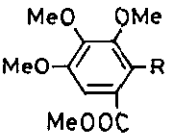
III: R=Me, R'=Ac

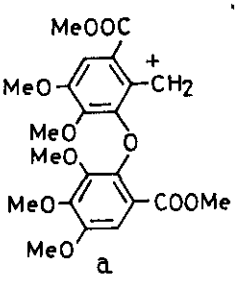
XI : R=R'=Me (β-anomer)

XII: R=R'=Me (α-anomer)

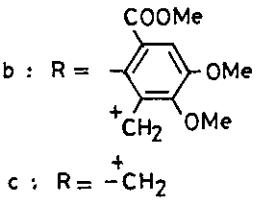


VII





a



b : R =

c :

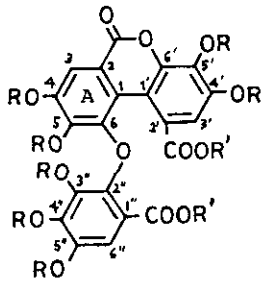
-24° (c=0.56, $CHCl_3$); M^+ 450}, and decamethyl derivative (V) {$C_{31}H_{34}O_{15}$; $[\alpha]_D$ 0° (c=1.0, $CHCl_3$); m/e 646 (M^+) and 614 (M^+-MeOH, 100%); ^1H nmr ($CDCl_3$) δ 7.03, 7.23 and 7.38 (1H each, s) and 3.60~3.95 (10 x OMe)}. Methylation of V with diazomethane gave an undecamethyl derivative (VI) [$C_{32}H_{36}O_{15}$; ^1H nmr ($CDCl_3$) δ 7.20, 7.36 and 7.41 (1H each, s) and 3.40~3.95 (11 x OMe); m/e 660 (M^+), 449, 433 and 239 (fragment ions a, b and c, respectively, arising upon cleavage of biphenyl and ether linkages with rearrangement of CH_2[1])]. This derivative (VI) was shown to be an analogue of trimethyl octa-O-methylvaloneate (VII)[2] by close similarity in the mass spectra, and differences in the ^1H nmr spectra, and hence the free phenolic tricarboxylic acid was named alnusinic acid.

The formation of V, not VI, upon methanolysis of II, along with the presence of a carbonyl signal at δ 164.10, which resonates at higher field than the other four ester carbonyls in the ^{13}C nmr spectrum of I, suggests that alnusinic acid is bonded to glucose forming a monolactone in I. This assumption was supported by the prominent peaks at m/e 586 and 568 in the mass spectrum of II, which are

attributable to the fragments $[VIII]^{\bullet+}$ and its anhydride, respectively.

Based on the above observations, the structure of alnusinic acid monolactone (AAML) is represented by either IXa or Xa, between which the former is supported by the ^{13}C nmr analysis as follows.

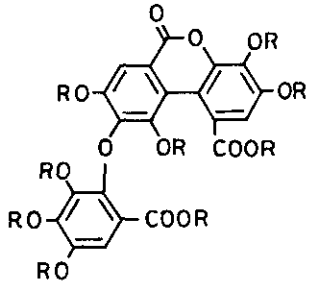
Methylation of I with dimethyl sulfate and potassium carbonate in acetone, followed by separation using preparative TLC, gave tetradeca-O-methylalnusiin (β-anomer)(XI), $C_{55}H_{54}O_{26}$, M^+ 1130, and its α-anomer (XII) in the ratio of ca. 3:2. Among the ^{13}C nmr signals of XI, most of the peaks corresponding to a hexamethoxydiphenoyl group in the permethylated β-anomer of pedunculagin[3] [2,3-4,6-di-(hexahydroxydiphenoyl)-glucose], and of the signals attributable to C_1,~C_6, and $C_{1''}$~$C_{6''}$ in the AAML moiety by the comparison with those of XIII[4] and XIV[5], were subtracted. The residual peaks assignable to the carbons in ring A (C_1, δ 116.4; C_5, δ 145.0 or 144.4; C_4 and C_6, two peaks among those at δ 149.5~153.9) were found more compatible with the ^{13}C nmr chemical shifts predicted applying the substituent additivity rule[6] for IXb, than with those for Xb (Table 1).



VIII: R=Me, R'=H

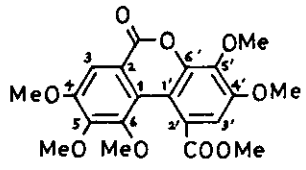
IXa: R=R'=H

IXb: R=R'=Me

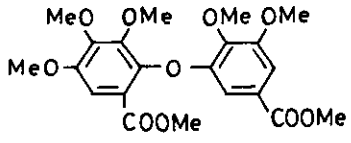


Xa: R=H

Xb: R=Me



XIII



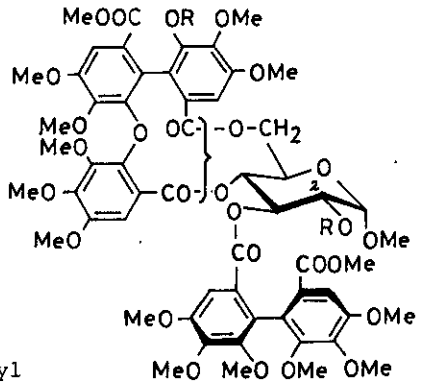
XIV

Table 1. Chemical shifts predicted for ring A carbons of IXb and Xb

	C_1	C_2	C_3	C_4	C_5	C_6
IXb	115.5	119.3	111.8	152.1	144.9	151.6
Xb	110.0	121.5	109.7	156.6	137.8	158.7

The linkages of hexahydroxydiphenoyl (HHDP) and AAML groups to glucose moiety in I were determined as follows. The coupling constants observed for the sugar protons ($J_{1,2}$=4 Hz, $J_{2,3}$=$J_{3,4}$=$J_{4,5}$=9 Hz, $J_{5,6}$=7 Hz) in the ^1H nmr spectrum of XII

indicate that the glucose exists in the pyranose form having the Cl conformation, which permits the HHDP linkages only at 2,3- or 4,6-position. Mild methanolysis of XII gave a partial methanolyzate (XV), $C_{57}H_{62}O_{28}$, whose ^1H nmr spectrum showed retention of five aromatic singlets, and absence of the proton signal due to O-acylated C_2 of glucose. Presence of the free hydroxyl group at C_2 in XV was established by production of methyl 2-O-methyl-α-D-glucoside upon methanolysis of the permethylated derivative (XVI), $C_{59}H_{66}O_{28}$, M^+ 1222,



XV : R = H
XVI : R = Me

which was obtained by methylation of XV with diazomethane and boron trifluoride-ether. The mass spectrum of XV exhibited the base peak at m/e 436, attributable to hexamethoxydiphenic acid monomethyl ester, which was verified by its replacement by m/e 439 peak upon methanolysis with deuterated methanol containing sodium. These results indicate that the methanolysis of one of the ester linkages of HHDP in XII occurred at O-2 of glucose. Therefore, the HHDP group in XII is bonded to O-2 and O-3, and the AAML group is hence bonded to O-4 and O-6 of glucose.

Consequently, the structure of alnusiin is represented by I.

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References and Notes

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