

ALNUSIIN, A NOVEL ELLAGITANNIN FROM ALNUS SIEBOLDIANA FRUITS

Takashi Yoshida, M. Usman Memon and Takuo Okuda*

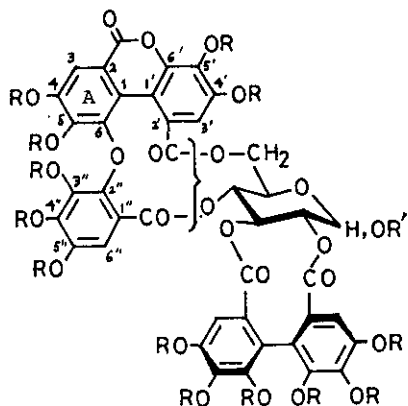
Faculty of Pharmaceutical Sciences, Okayama University, Tsushima,
Okayama, Japan

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), $C_{41}H_{26}O_{26} \cdot 4H_2O$, $[\alpha]_D -32^\circ$ ($c=1.4$, 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), $C_{54}H_{52}O_{26}$, $[\alpha]_D -34^\circ$ ($c=1.0$, $CHCl_3$), 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) $\{[\alpha]_D$



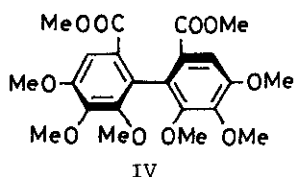
I : R=R'=H

II : R=Me, R'=H

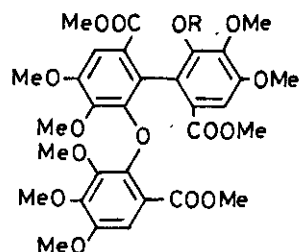
III : R=Me, R'=Ac

XI : R=R'=Me (β -anomer)

XII : R=R'=Me (α -anomer)

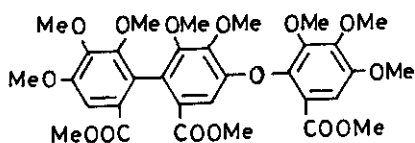


IV

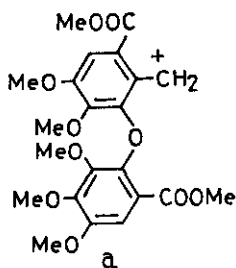
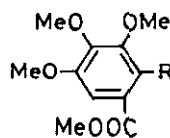


V : R=H

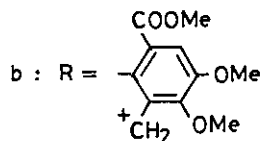
VI : R=Me



VII



a



c : R = $-\text{CH}_2$

-24° ($c=0.56$, CHCl_3); M^+ 450}, and decamethyl derivative (V) [$\text{C}_{31}\text{H}_{34}\text{O}_{15}$; $[\alpha]_D^{20}$ ($c=1.0$, CHCl_3); m/e 646 (M^+) and 614 ($M^+ - \text{MeOH}$, 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) [$\text{C}_{32}\text{H}_{36}\text{O}_{15}$; ^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^+)]. This derivative (VI) was shown to be an analogue of trimethyl octa-O-methylvalonate (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]⁺ 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 ¹³C nmr analysis as follows.

Methylation of I with dimethyl sulfate and potassium carbonate in acetone, followed by separation using preparative TLC, gave tetradeca-O-methylalnutiin (β -anomer) (XI), C₅₅H₅₄O₂₆, M⁺ 1130, and its α -anomer (XII) in the ratio of ca. 3:2. Among the ¹³C nmr signals of XI, most of the peaks corresponding to a hexamethoxydiphenoyl group in the permethylated β -anomer of pedunculagin³ [2,3-4,6-di-(hexahydroxydiphenoyl)-glucose], and of the signals attributable to C₁, -C₆, and C₁"-C₆" in the AAML moiety by the comparison with those of XIII⁴ and XIV⁵, were subtracted. The residual peaks assignable to the carbons in ring A (C₁, δ 116.4; C₅, δ 145.0 or 144.4; C₄ and C₆, two peaks among those at δ 149.5~153.9) were found more compatible with the ¹³C nmr chemical shifts predicted applying the substituent additivity rule⁶ for IXb, than with those for Xb (Table 1).

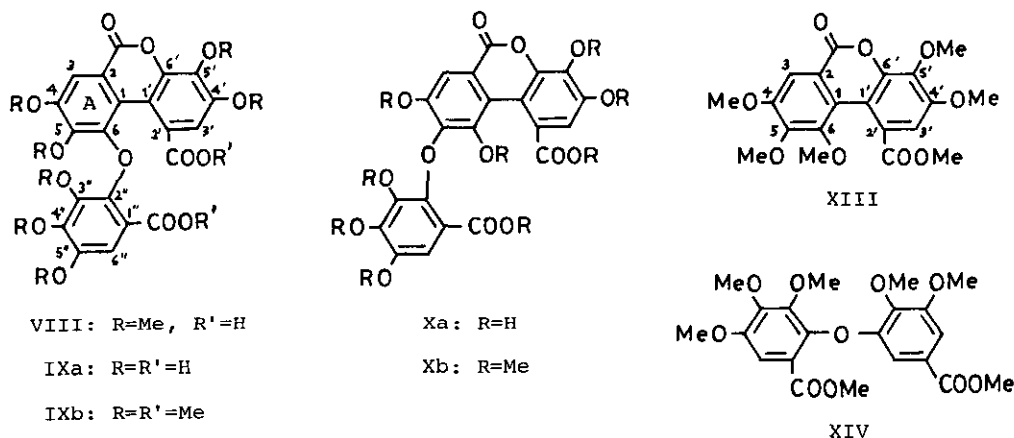
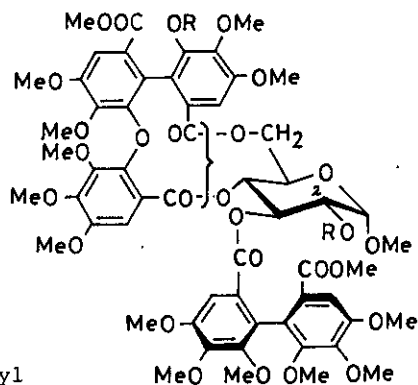


Table 1. Chemical shifts predicted for ring A carbons of IXb and Xb

	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 C1 conformation, which permits the HHDP linkages only at 2,3- or 4,6-position. Mild methanolysis of XII gave a partial methanol-
 yzate (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 replace-
 ment 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.

Acknowledgements — The authors thank Dr. K. Kawazu, Department of Agricultural
 Chemistry, Okayama University, for measuring high resolution mass spectrum. One
 of us (M. U. M) is grateful to the Japanese Government (Monbusho) for the scholar-
 ship.

References and Notes

1. C. W. A. Sachs and W. Mayer, Tetrahedron, 1969, 25, 73.
2. T. Okuda and K. Seno, Tetrahedron Letters, 1978, 139; J. Chem. Soc. Japan, 1981, 671.
3. O. T. Schmidt, L. Würtele, and A. Harréus, Ann., 1965, 690, 150.
4. Synthesized by a route essentially analogous to Schmidt's method: O. T. Schmidt, E. Komarek, and H. Rentél, Ann., 1957, 602, 50. The assignment of the ^{13}C resonances of this compound was made by comparison with those of IV, and by applying the chemical shift rule⁶.
5. T. Ozawa and Y. Takino, Agric. Biol. Chem., 1979, 43, 1173. The resonances of the carbons not reported in this reference were assigned by the chemical shift rule⁶.
6. J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic, New York, 1972, p. 197. G. Miyajima, Y. Sasaki, and M. Suzuki, Chem. Pharm. Bull., 1971, 19, 2301.

Received, 9th March, 1981