

**BICORNIN, A NEW HYDROLYZABLE TANNIN FROM TRAPA BICORNIS,  
AND REVISED STRUCTURE OF ALNUSIIN**

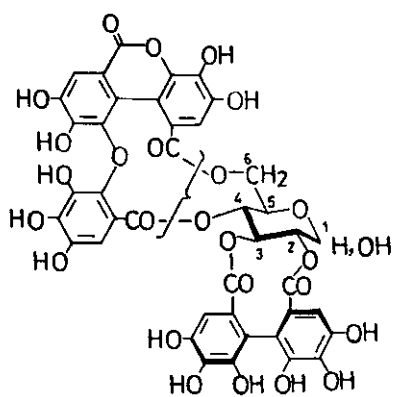
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**Abstract** — Previously proposed structure (1) for alnusiin, a hydrolyzable tannin isolated from Alnus sieboldiana, has been revised to 2, based on the  $^1\text{H}$ - $^{13}\text{C}$  long-range shift correlation spectroscopy. Bicornin (10), a new tannin was isolated from Trapa bicornis and its structure related to alnusiin was determined.

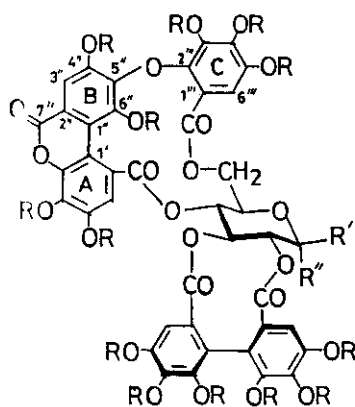
We previously isolated a new hydrolyzable tannin, alnusiin, from the fruits of Alnus sieboldiana (Betulaceae) and proposed its structure (1).<sup>1</sup> However, the orientation of the alnusiinoyl group, a new component unit at O-4 and O-6 in 1, was remained unsolved. Full characterization of alnusiin has now been carried out with the aid of the  $^1\text{H}$ - $^{13}\text{C}$  long-range shift correlation spectroscopy, leading to the gross structure (2) accompanied by the structural revision of the alnusiinoyl group in the molecule. We have also isolated a new tannin, named bicornin (10), from Trapa bicornis L. f. (Trapaceae), and elucidated its structure which is related to alnusiin.

The  $^{13}\text{C}$  nmr spectrum of tetradeca-O-methyl- $\alpha$ -alnusiin (3),<sup>1</sup> which was prepared by methylation of alnusiin, exhibited five ester carbonyl carbon resonances ( $\delta$ 163.0, 167.1, 167.3, 168.1 and 168.6), among which a signal in higher field ( $\delta$ 163.0) was readily assigned to a lactonic carbonyl carbon (ring-B, C-7''). In the  $^1\text{H}$ - $^{13}\text{C}$  long-range shift correlation spectrum ( $J_{\text{CH}}=7$  Hz) of 3, this carbon resonance was correlated with an aromatic one-proton singlet at  $\delta$ 7.19 through the three-bond

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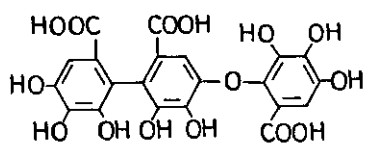


1

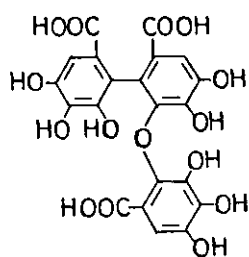


2 R=H, R', R''=H, OH

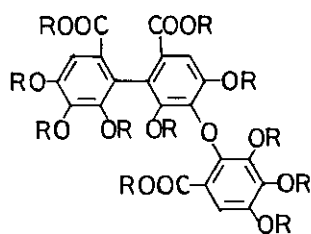
3 R=Me, R'=H, R''=OMe



4

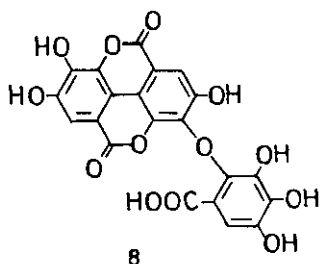


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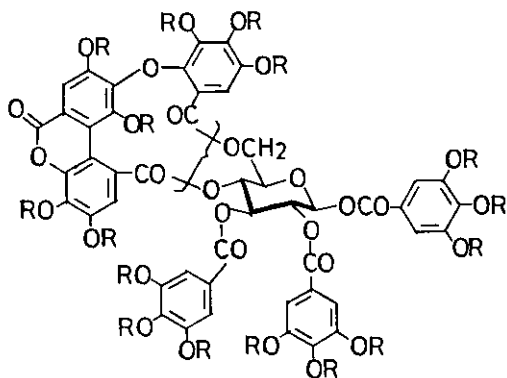


6 R=H

7 R=Me

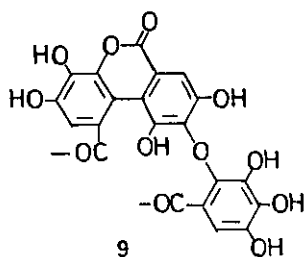


8



10 R=H

11 R=Me



9

coupling, leading the assignment of the latter to H-3''. On the other hand, three  $^{13}\text{C}$  resonances ( $\delta$ 150.1, 145.3 and 144.1) in the region of oxygen-bearing carbons, gave no cross peak with the methoxyl protons. Among them, the signal at  $\delta$ 150.11 was definitely correlated with the H-3'' signal through the two- or three-bond coupling, indicating the presence of an aroyl ether linkage at C-4'' or C-5'' of ring B. As alnusinic acid has already been proved to be an isomer of valoneic acid (4),<sup>2</sup> based on the spectral comparison of the permethylated derivatives of these two acids,<sup>1</sup> the location of this ether linkage should be at C-5''. The previously proposed structure (5) for alnusinic acid was then revised to 6. Identity of the data of the dilactone (8) obtained by hydrolysis of alnusiin with 1N  $\text{H}_2\text{SO}_4$ , with those of tergallic acid dilactone derived from tergalagin isolated from Terminalia catappa<sup>3</sup> provided a support for the structure (6). The  $^1\text{H}$  nmr spectral data [ $\delta$  7.37, 7.34, 7.18 (1H each, s), 3.92-3.38 (11 x OMe)] of trimethyl octa-O-methylalnusinate (7)<sup>1</sup> which was obtained by methanolysis of 3 followed by further methylation, also coincided with the reported data<sup>3</sup> for the permethylated derivative of tergallic acid. Consequently, the monolactone form of alnusinic acid in alnusiin was concluded to be represented by 9.

The orientation of the alnusinoyl (tergalloyl) group at O-4 and O-6 in alnusiin was established as follows. Among the three oxygen bearing carbon signals mentioned above, which gave no cross peak with the methoxyl protons in the  $^1\text{H}$ - $^{13}\text{C}$  long-range nmr spectrum of 3, a signal at  $\delta$ 144.1 gave a cross peak with an aromatic proton at  $\delta$  7.18, and this proton was assigned to H-6''' of ring C in 3. This proton signal was found to be correlated through the three-bond long-range coupling with an ester carbonyl carbon signal at  $\delta$ 167.3 which also exhibited the cross peak with one of the C-6 protons of the glucose residue. Based on these data, the structure of alnusiin was established as 2.

Bicornin (10),  $[\alpha]_{\text{D}} +4.7^\circ$  (MeOH), was isolated as an off-white amorphous powder from the aqueous acetone extract of the fruits of Trapa bicornis by column chromatography on Toyopearl HW-40 developing with water-ethanol-acetone, followed by preparative HPLC. The  $^1\text{H}$  nmr spectrum (500 MHz, acetone- $d_6$ ) exhibited three two-proton singlets ( $\delta$  7.11, 7.00 and 6.97) attributable to galloyl groups and three one-proton singlets ( $\delta$  7.99, 6.88 and 6.52) assignable to an alnusinic acid monolactone moiety, along with the signals of  $^4\text{C}_1$  glucopyranose [ $\delta$  6.21 (d, J=8.5 Hz, H-1), 5.61 (dd, J=8.5, 10 Hz, H-2), 5.85 (t, J=10 Hz, H-3), 5.29 (t, J=10 Hz, H-4), 4.59 (ddd, J=1, 7, 10 Hz, H-5), 5.32 (dd, J=7, 13 Hz, H-6), 3.95 (dd,

$J=1, 13 \text{ Hz, H-6'}$ ]. The  $^{13}\text{C}$  nmr spectrum (126 MHz, acetone- $d_6$ ) indicated the glucose carbon signals at  $\delta$  93.6 (C-1), 71.8 (C-2), 73.1 (C-3), 70.8 (C-4), 72.8 (C-5), 63.9 (C-6), together with ester and lactone carbonyl carbon signals at  $\delta$  167.7, 167.0, 166.3, 165.4, 164.9, 162.7. These structural units were confirmed by methanolysis of hexadeca-O-methylbicornin (11) which was prepared by methylation of 10 with  $(\text{CH}_3)_2\text{SO}_4$  and  $\text{K}_2\text{CO}_3$  in acetone followed by further methylation with  $\text{CH}_2\text{N}_2$ , to afford methyl tri-O-methylgallate and trimethyl octa-O-methylalnusinate (7) in addition to glucose. The existence of alnusinoyl group as the monolactone form in 10 was evident from the lactone carbonyl carbon resonance at  $\delta$  162.7 in the  $^{13}\text{C}$  nmr spectrum, which is analogous to that in 2, and also from the ion peaks at  $m/z$  1111  $[\text{M}+\text{Na}]^+$  and 1127  $[\text{M}+\text{K}]^+$  in the FAB-MS spectrum which support the molecular formula,  $\text{C}_{48}\text{H}_{32}\text{O}_{30}$ . The location of this group in 10 was indicated to be at O-4 and O-6 by the marked difference ( $\Delta\delta$  1.37 ppm) between the chemical shifts of each proton of the C-6 methylene in the glucose residue, which is characteristic of ellagitannins having a bridged ester group at O-4 and O-6 on the  $^4\text{C}_1$  glucopyranose.<sup>4</sup> Three galloyl groups are therefore determined to be bonded to O-1, O-2 and O-3. The configuration of the anomeric center in 10 was determined to be  $\beta$  by the large coupling constant ( $J=8.5 \text{ Hz}$ ) of H-1 signal. Consequently, the structure of bicornin was concluded as 10.

#### ACKNOWLEDGMENTS

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