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

Takashi Yoshida, Kazufumi Yazaki, M. Usman Memon,[+]
Izumi Maruyama, Kenji Kurokawa, and Takuo Okuda[*]
Faculty of Pharmaceutical Sciences, Okayama University,
Tsushima, Okayama 700, Japan

Abstract — Previously proposed structure (**1**) for alnusiin, a hydrolyzable tannin isolated from Alnus sieboldiana, has been revised to **2**, based on the ^1H-^{13}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**).[1] However, the orientation of the alnusinoyl 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 ^1H-^{13}C long-range shift correlation spectroscopy, leading to the gross structure (**2**) accompanied by the structural revision of the alnusinoyl 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}C nmr spectrum of tetradeca-O-methyl-α-alnusiin (**3**),[1] which was prepared by methylation of alnusiin, exhibited five ester carbonyl carbon resonances (δ163.0, 167.1, 167.3, 168.1 and 168.6), among which a signal in higher field (δ163.0) was readily assigned to a lactonic carbonyl carbon (ring-B, C-7''). In the ^1H-^{13}C *long-range shift correlation spectrum* (J_{CH}=7 Hz) of **3**, this carbon resonance was correlated with an aromatic one-proton singlet at δ7.19 through the three-bond

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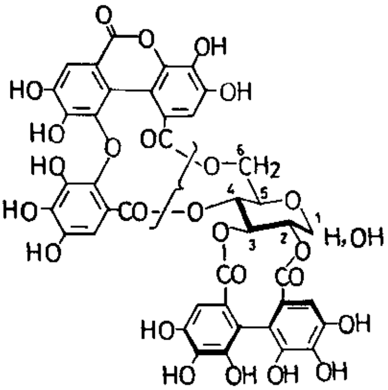
[+] Present address, University of Sind, Jamshoro, Pakistan



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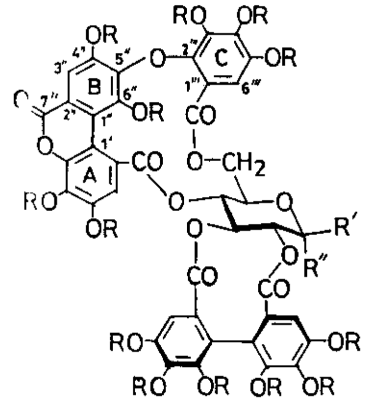
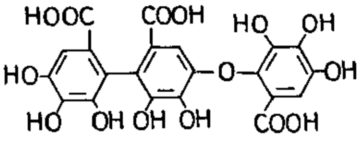


2 R=H, R′, R″=H,OH

3 R=Me, R′=H, R″=OMe



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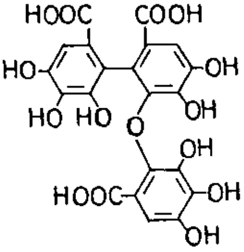

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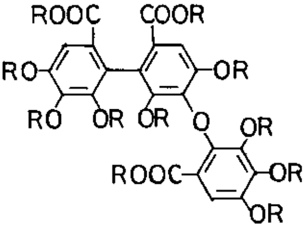
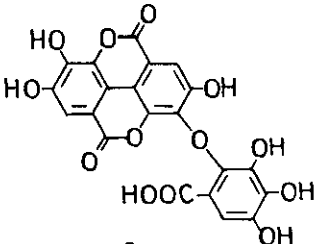


6 R=H

7 R=Me



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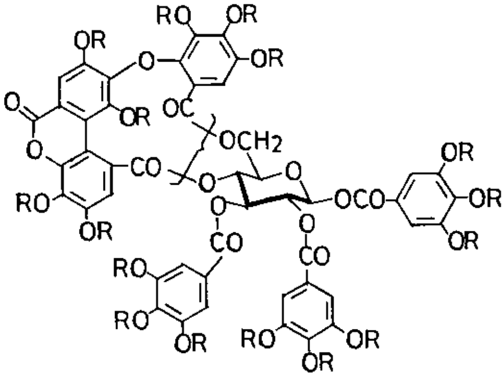
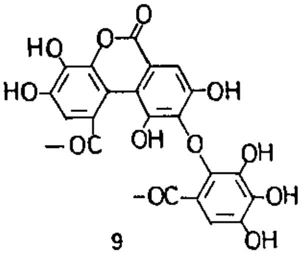


10 R=H

11 R=Me



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coupling, leading the assignment of the latter to H-3''. On the other hand, three ^{13}C resonances (δ150.1, 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 δ150.11 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**),[2] based on the spectral comparison of the permethylated derivatives of these two acids,[1] 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 H_2SO_4, with those of tergallic acid dilactone derived from tergallagin isolated from Terminalia catappa[3] provided a support for the structure (**6**). The ^1H nmr spectral data [δ 7.37, 7.34, 7.18 (1H each, s), 3.92 3.38 (11 x OMe)] of trimethyl octa-O-methylalnusinate (**7**)[1] which was obtained by methanolysis of **3** followed by further methylation, also coincided with the reported data[3] 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 ^1H-^{13}C long-range nmr spectrum of **3**, a signal at δ144.1 gave a cross peak with an aromatic proton at δ 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 δ167.3 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**), $[\alpha]_D$ +4.7° (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 ^1H nmr spectrum (500 MHz, acetone-d_6) exhibited three two-proton singlets (δ 7.11, 7.00 and 6.97) attributable to galloyl groups and three one-proton singlets (δ 7.99, 6.88 and 6.52) assignable to an alnusinic acid monolactone moiety, along with the signals of $^4C_1$ glucopyranose [δ 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 Hz, H-6')]. The ^{13}C nmr spectrum (126 MHz, acetone-d_6) indicated the glucose carbon signals at δ 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 δ 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 $(CH_3)_2SO_4$ and K_2CO_3 in acetone followed by further methylation with CH_2N_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 δ 162.7 in the ^{13}C nmr spectrum, which is analogous to that in **2**, and also from the ion peaks at m/z 1111 $[M+Na]^+$ and 1127 $[M+K]^+$ in the FAB-MS spectrum which support the molecular formula, $C_{48}H_{32}O_{30}$. 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 $^4C_1$ glucopyranose.[4] 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 β by the large coupling constant (J=8.5 Hz) of H-1 signal. Consequently, the structure of bicornin was concluded as **10**.

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