Application of 2D Nmr Spectroscopy to the Structural Establishment of the Major Hydrolysis Product of Aescin

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APPLICATION OF 2D NMR SPECTROSCOPY TO THE STRUCTURAL ESTABLISHMENT OF THE MAJOR HYDROLYSIS PRODUCT OF AESCIN[1,2]

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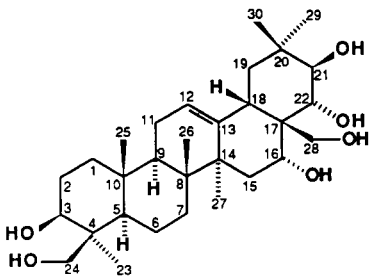
ABSTRACT.—The structure of the major product, protoaescigenin [**1**], obtained by acid hydrolysis of aescin from *Aesculus indica* was established as olean-12-ene-3β,16α,21β,22α,24,28-hexaol. Unambiguous ^{1}H- and ^{13}C-nmr spectral assignments were made on the basis of selected 2D nmr experiments (^{1}H-^{1}H and ^{1}H-^{13}C COSY) combined with standard 1D nmr experiments.

Aescin, an anti-inflammatory principle of *Aesculus indica* Hook. (Hippocastanaceae) (1), was considered to be a mixture of acylated and nonacylated glycosides derived from pentahydroxy and hexahydroxy oleanane triterpenoids as the aglycone residue and a trisaccharide unit as the sugar moiety (2). Earlier we carried out chemical studies on aescin (a mixture of four constituents) isolated from the MeOH extract of seeds of *A. indica*, which resulted in the isolation of two new triterpenoid glycosides: aesculuside A (3) and aesculuside B (4). Aescin, on acid hydrolysis, led to the formation of several products. Chromatographic purification of the hydrolysate afforded one major constituent, **1**. We report its structural establishment and ^{1}H- and ^{13}C-nmr assignments.



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RESULTS AND DISCUSSION

The 1D nmr spectra of **1** showed the number of hydrogens (except five hydroxyls) and carbons corresponding to the molecular formula $C_{30}H_{50}O_6$. In conjunction with DEPT-edited spectral data (5), the normal ^{13}C nmr spectrum (Table 1) confirmed the presence of six methyls, nine methylenes, eight methines, and seven quaternary carbons. The ratio of carbons to hydrogens in the molecule indicated five degrees of cyclization and/or unsaturation. Since there are two olefinic signals (δ 144.14 and 123.05) in the ^{13}C-nmr spectrum, the combined data strongly suggested it to be a pentacyclic triterpene having a trisubstituted olefinic bond. The oleanane skeleton appeared likely on the basis of the previously isolated constituents of aescin (2), and it was in conformity with the appearance of the olefinic methine at δ 123.05 and the olefinic quaternary resonance at δ 144.14 which are characteristic of olean-12-enes (6). Because

[1]Part 35 in the series "Studies on Medicinal Plants." For part 34 see G.C. Uniyal, P.K. Agrawal, O.P. Sati and R.S. Thakur, *Phytochemistry*, **30**, 1336 (1991). CIMAP publication no. 936.

[2]Part 28 in the series "Carbon-13 nmr Spectral Investigations." For part 27 see P.K. Agrawal, R.S. Thakur, A.W. Frahm, and M. Schneider, *J. Chem. Res.*, submitted (1991).

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TABLE 1. ^{1}H- and ^{13}C-nmr Spectral Assignments of Protoaeseigenin [**1**].

Carbon	δ ^{13}C	Multiplicity[a]	^{1}H[b]	Multiplicity and coupling constants[c]
C-13	144.14	C	—	—
C-12	123.05	CH	5.30	br s
C-3	80.08	CH	3.55	dd, J = 4.50, 10.5
C-21	78.61	CH	4.67	d, J = 10.5
C-22	77.08	CH	4.52	d, J = 10.5
C-28	66.16	CH_2	3.90, 3.59	each d, J = 10.5
C-16	67.79	CH	4.91, 5.73 (OH)	br s
C-24	64.53	CH_2	4.38, 3.57	each d, J = 10.5
C-5	56.26	CH	0.88	
C-19	48.11	CH_2	2.94, 1.34	dd
C-17	47.20	C	—	
C-9	47.09	CH	1.70	
C-4	43.01	C	—	
C-14	41.83	C	—	
C-18	41.01	CH	2.69	br dd
C-8	39.90	C	—	
C-1	38.75	CH_2	1.49, 0.93	
C-10	36.83	C	—	
C-20	36.27	C	—	
C-15	34.10	CH_2	1.98, 1.55	
C-7	33.37	CH_2	1.50, 1.19	
C-29	30.40	CH_3	1.22	s
C-2	28.22	CH_2	1.80, 1.91	
C-27	27.19	CH_3	1.73	s
C-11	23.92	CH_2	1.82, 1.75	
C-23	23.34	CH_3	1.43	s
C-30	19.25	CH_3	1.27	s
C-6	18.85	CH_2	1.55	
C-26	16.62	CH_3	0.80	s
C-25	16.01	CH_3	0.81	s

[a]Based on the DEPT analysis.
[b]Based on ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY.
[c] Coupling constant values given in Hz.

there were four hydroxylated methine resonances (δ 80.08, 78.61, 77.08, and 67.79) and two hydroxylated methylene resonances (δ 68.15 and 64.53) in its ^{13}C-nmr spectrum, **1** was considered to be a hexahydroxylated olean-12-ene.

A preliminary search of the literature revealed that there are only two known triterpenoids, protoaescigenin (7) and gmnemagenin (8), which contained the anticipated functional groups. Significant differences, particularly for ring-A resonances with gmnemagenin, were observed when the ^{13}C shielding data was compared. However, a close resemblance was noted for the calculated values for the aglycone of aesculuside B after deletion of glycosidation effects (9). Thus, **1** could be tentatively identified as olean-12-ene-3β,16α,21β,22α,24,28-hexaol (protoaescigenin).

The ^{1}H-nmr assignments were based upon the analysis of the ^{1}H-^{1}H COSY spectrum. The H-12 olefinic methine proton resonated at δ 5.30 as a broad singlet exhibiting cross peaks with the H-11 methylene protons at δ 1.82 and 1.75, which were in turn correlated with the H-9 methine at δ 1.70. The three protons on H-16 and H-15 were identified at δ 4.91 (H-16), and 1.98 and 1.55 (H-15) whereas the signal at δ 5.73 was assigned to OH-16. The H-28 and H-24 methylene protons were assigned

to the resonances at δ 3.90 and 3.59, and δ 4.38 and 3.57, respectively, related by the geminal coupling constant. The doublet (J = 10.5 Hz) resonances at δ 4.67 and 4.52 were ascribed to diaxial protons of the H-21 and H-22 positions, revealing diequatorial orientation of the hydroxyl groups at these positions. The H-3 proton was identified at δ 3.55 with two apparent ^3J couplings with H-2 at δ 1.91 and 1.80 indicating an axial orientation. The H-2 protons were further correlated with resonances at δ 1.49 and 0.93; hence these could be considered for the methylene at the 1 position. The non-equivalent protons of H-19 were observed at δ 2.94 and 1.34, exhibiting couplings with H-18 at δ 2.69. The most shielded methine resonance at δ 0.88 was assigned to H-5, which exhibited cross peak at δ 1.55 corresponding to the H-6 methylene protons, which were further correlated with the H-7 methylene protons at δ 1.50 and 1.19. The assignments for the methyl resonances at δ 0.81 and 0.80 to H-25 and H-26 were straightforward as cross peaks were observed with methylene protons of the 1 and 7 positions, respectively. Analogously, the methyl resonance at δ 1.73 was assigned to H-27 as it displayed cross peaks with methylene protons of the 15 position. The detailed analysis of the ^{1}H-^{1}H COSY led to the complete assignments of the ^{1}H-nmr resonances as presented in Table 1.

Based upon the ^{1}H-nmr assignments, the ^{13}C-nmr chemical shifts for the protonated carbon resonances could readily be correlated by the one-bond ^{1}H-^{13}C COSY spectrum. The assignment of the resonance at δ 144.14 to C-13 was straightforward, whereas assignments of other nonprotonated carbon resonances were made on the basis of comparison with those for related triterpenoids (6) and by consideration of the empirical rules. This led to the confirmation of the ^{13}C-nmr assignments proposed earlier (7).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—The ^{1}H and ^{13}C nmr were obtained with a Gemini-300 (300 MHz) nmr spectrometer (Varian associates) in pyridine-d_5 solutions with TMS as internal standard. Chemical shift values are reported in ppm downfield from TMS. The one-bond ^{1}H-^{13}C shift correlation experiment was carried out with the standard Varian HETCOR pulse sequence on the Gemini-300 spectrometer. The spectral width was 6334.9 Hz in the F1 dimension, with 12750 Hz in the F2 dimension with 2K data points. The ^{1}H-^{1}H COSY spectrum was recorded on a Varian-600 MHz nmr spectrometer. The spectral width was 6334.9 Hz in both dimensions with 2K data points.

PLANT MATERIAL AND EXTRACTION.—The collection data for plant material and the isolation procedure of aescin were detailed elsewhere (3,4). Aescin (3 g) was treated with 2 N HCl-dioxane (1:1) for 3 h under reflux, and the hydrolysate was partitioned between EtOAc and H_2O. The EtOAc extract was concentrated and chromatographed on Si gel and on elution with $CHCl_3$-MeOH (7:3) afforded compound **1** (350 mg), mp 308° (7).

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