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AESCULUSIDE-B, A NEW TRITERPENE GLYCOSIDE FROM *AESCULUS INDICA*[1]

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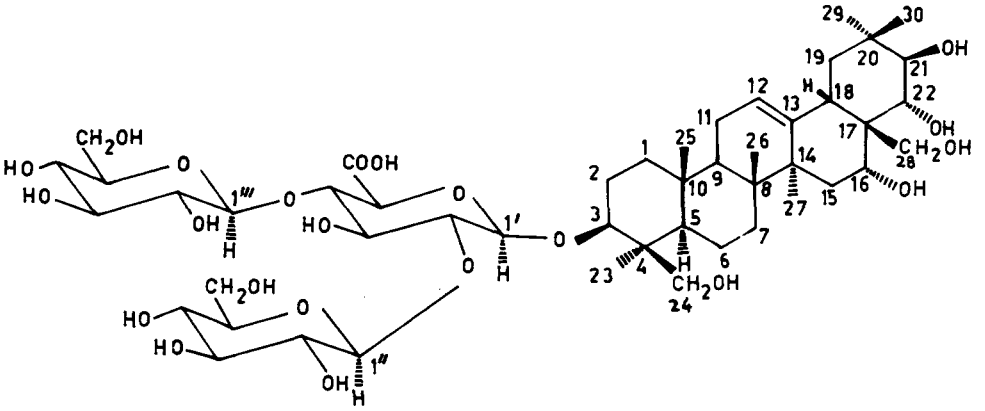
ABSTRACT.—A new triterpene glycoside, aesculuside-B, isolated from *Aesculus indica* was identified as 3β-*O*-[β-D-glucopyranosyl (1→2), β-D-glucopyranosyl (1→4)]-β-D-glucuronopyranosyl]-16α,21β,22α,24,28-pentahydroxy-olean-12-ene [**1**] on the basis of ^{13}C nmr and other spectral and chemical evidence.

Recently, we reported the characterization of a new saponin, aesculuside-A, from the MeOH extract (1) of *Aesculus indica* L. (Hippocastanaceae). We have now isolated another saponin, named aesculuside-B, from the same MeOH extract. The precipitate obtained on addition of Me_2CO to the MeOH extractives of *A. indica* (1) was fractionated over Si gel to afford aesculuside-B [**1**] as colorless crystals from a mixture of MeOH and H_2O. The ir spectrum exhibited broad absorption bands at 3400 and 1060 cm^{-1} for hydroxyls, indicating its glycosidic nature. On acid hydrolysis, as with aesculuside-A (1), glucose and glucuronic acid (pc) were identified as the sugar residues, whereas a complex mixture of sapogenins was detected on tlc (2-4). The complete structure of aesculuside-B was deduced on the basis of its ^{13}C-nmr spectral analysis.

The ^{13}C-nmr spectrum of **1** showed 48 carbon resonances inferring the presence of a trisaccharide moiety which was in conformity with the appearance of the three anomeric



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carbon signals at δ 103.37, 102.93, and 102.31. On the basis of analysis of the DEPT spectrum, the molecular formula could be surmized as $C_{48}H_{78}O_{22}$. The olefinic resonances at δ 143.09 and 121.78, corresponding to quaternary and methine behavior, suggested the presence of unsaturation at the 12-position in an oleanane skeleton. The presence of the three secondary hydroxyls, two primary hydroxyls, and one glycoxy substituent in the aglycone moiety was readily deduced by the signals at δ 66.56, 76.99, 76.48, 62.61, 65.58, and 89.95, respectively, and by comparison with the reported literature data for oleanane triterpenoids (5,6). This led to the establishment of the presence of free hydroxyl groups at the 16, 21, 22, 24, and 28 positions and a sugar moiety linked at C-3. The chemical shift for rings E and F very much resembled the reported

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^{13}C shielding data for protoaescigenin (7-9) but differed markedly with the ^{13}C chemical shifts of compounds having different orientations of hydroxyl groups in these positions (5-10). This led us to infer the existence of free hydroxyl groups at the 16α,21β,22α,24, and 28 positions. The 3β-hydroxyl group was glycosylated in view of glycosidation induced shifts (11,12).

This was also in conformity with the fact that the triterpenoid saponins so far characterized from *Aesculus* species were glycosides of protoaescigenin (2,3). Interestingly, the chemical shifts of the sugar carbon atoms quite remarkably resembled the observed values for the sugar moiety as in aesculuside A (1), thus establishing the substitution of two β-D-glucopyranosyl moieties at the 2- and 4-positions of β-D-glucuronopyranoic acid, which was the first sugar being directly bonded to C-3 of protoaescigenin. Hence, on the basis of foregoing evidence, aesculuside-B was elucidated as 3β-*O*-[{ β-D-glucopyranosyl (1↦2), β-D-glucopyranosyl (1↦4) } β-D-glucuronopyranosyl]-16α, 21β,22α,24,28-pentahydroxy-olean-12-ene [**1**].

Moreover, aesculuside-A (1), which is structurally 21-angeloyl aesculuside-B, on alkaline hydrolysis yielded aesculuside-B (co-tlc, ^1H nmr, and ^{13}C nmr). The presence of aesculuside-B was detected in the methanolic extract prior to alkali treatment; thus, it was not an artifact.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: ir, Perkin-Elmer 399B model, ^1H nmr and ^{13}C nmr, Brucker WM-400 spectrometer in DMSO-d_6 with TMS as internal standard. Melting points were recorded on a Toshniwal melting point apparatus and not corrected. The adsorbents for cc and tlc were from BDH.

PLANT MATERIAL AND EXTRACTION.—The seeds of *A. indica* were collected at Kulu, Himachal Pradesh, India, during the autumn 1983 and were identified at the Department of Botany of this Institute. A voucher specimen No. 1851 is deposited in the CIMAP Herbarium. The air dried, powdered seeds (1 kg) were defatted with hexane (5×2.5 liters) and then extracted with cold MeOH (5×2.5 liters). The MeOH extract was concentrated in vacuo to give a residue (400 g). A part (100 g) of the residue was dissolved in MeOH (500 ml) and Me_2CO (2 liters) was added to give a brown precipitate which was filtered and dried to an amorphous powder (20 g). Part of the precipitate (10 g) was dissolved in *n*-BuOH saturated with H_2O, treated with 1% aqueous NaOH solution, and extracted successively with *n*-BuOH saturated with H_2O. The combined *n*-BuOH extract was washed with H_2O saturated with *n*-BuOH. These washings were collected and dried in vacuo to give a white solid (3 g). This crude mixture was chromatographed on a column (3.5×110 cm) packed with Si gel (80-120 mesh) and eluted with $CHCl_3$-MeOH-H_2O (65:15:10) with increasing proportions of MeOH up to (65:40:10).

AESCULUSIDE-B [**1**].—The $CHCl_3$-MeOH-H_2O (65:28:10) eluates, on concentration, gave a solid (1 g) which on crystallization from a MeOH and H_2O mixture yielded the saponin **1** mp 255-260°, $[\alpha]D$ −1.6° (H_2O), Rf 0.31 on Si gel plates using $CHCl_3$-MeOH-H_2O (65:40:10); ir (KBr) 3400, 2930, 2857, 1690, 1410, 1380, 1160, 1073, 1035 cm^{-1}; ^1H nmr δ 0.75 (9H, 3×3H, S, 3Me), 0.79 (6H, 2×3H, S, 2Me), 1.29 (3H, S, Me), 5.11 (1H, brs, 12-H); ^{13}C nmr quartets at 15.31 (C-25), 16.32 (C-26), 18.68 (C-30), 21.97 (C-23), 26.63 (C-27), 29.90 (C-29), triplets at 18.00 (C-6), 23.18 (C-11), 24.47 (C-2), 32.59 (C-7), 33.14 (C-15), 38.15 (C-1), 47.16 (C-19), 60.33, 60.96 (C-6″, C-6‴), 62.21 (C-24), 65.58 (C-28), doublets at 39.89 (C-18), 46.02 (C-9), 55.44 (C-5), 66.56 (C-16), 68.82 (C-4‴), 69.92 (C-4″), 73.52 (C-2″), 73.97 (C-3′), 74.45 (C-2‴), 75.21 (C-3″), 75.72 (C-3‴), 76.26 (C-4‴), 76.48 (C-22), 76.62 (C-5″), 76.90 (C-5‴), 76.99 (C-21), 78.26 (C-4′), 82.07 (C-2′), 89.95 (C-3), 102.31 (C-1″), 103.37 (C-1′), 121.78 (C-12), singlets at 35.27 (C-20), 35.83 (C-10), 40.93 (C-14), 42.87 (C-4), 47.16 (C-17), 143.09 (C-13), 171.77 (C-6′). The signal for C-8 was obscured with solvent peaks.

ACID HYDROLYSIS OF AESCULUSIDE-B.—Compound **1** (30 mg) was treated with 2N HCl-dioxane (1:1) for 3 h under reflux and worked up as usual. The organic layer exhibited the presence of several spots in tlc, while the presence of D-glucose and D-glucuronic acid in the hydrolysate was confirmed by paper chromatography (BuOH-HOAc-H_2O, 4:1:5) and spraying with aniline hydrogen phthalate.

ALKALINE HYDROLYSIS OF AESCULUSIDE-A.—Aesculuside-A (1) (15 mg) was heated at reflux with

2% NaOH with a few drops of MeOH for 0.5 h, which on usual work-up gave a single spot of aesculuside-B [**1**] (co-tlc).

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