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AN ACETYLATED SAPONIN FROM *SCHEFFLERA IMPRESSA*[1]

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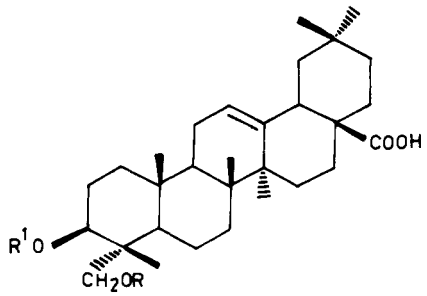
ABSTRACT.—A new acetylated saponin, hederagenin-3-*O*-β-D-6′-acetylglucopyranoside [**1**], has been isolated from the bark and stem of *Schefflera impressa*. Its structure has been deduced from spectroscopic data and by chemical correlation with compounds of established structure.

In a previous report (1) we described the isolation and identification of six compounds including two new saponins from the stem and bark of *Schefflera impressa* C.B. Clarke (Araliaceae). The present communication describes the isolation and structure elucidation of a new acetylated saponin, hederagenin-3-*O*-β-D-6′-acetylglucopyranoside [**1**], and three other known compounds from the stem and bark of *S. impressa*.

The stem and bark of *S. impressa* were extracted with MeOH, and the crude saponin fraction on chromatographic separation afforded saponin **1**. The eims ($[M]^+$ 472, base peak at *m/z* 248) and ^{13}C-nmr data (180.6, 122.5, and 145.2 ppm) indicate that **1** is an olean-12-ene derivative having a free carboxyl group at C-17 (2,3), which was supported by acid hydrolysis of **1** to afford hederagenin [**2**] (4) and D-glucose. The presence of a D-glucose unit in **1** was further confirmed by the eims of compound **4**, an acetate of **1**, which showed a characteristic fragment ion for tetraacetyl glucose [glucosyl $(Ac)_4]^+$ at *m/z* 331. The signals at 83.2 and 26.3 ppm assigned to C-3 and C-2 are 9.2 ppm downfield and 1.2 ppm upfield compared to the parent compound **2**, clearly indicating α and β effects due to glycosidation at C-3 (5). The signals at δ 4.95 (1H, d, $J = 7$ Hz) in the ^{1}H nmr and at 104.8 ppm in the ^{13}C nmr are characteristic for β configuration of the sugar at C-3 (6). This evidence led to the partial structure of **1** as hederagenin-3-*O*-β-D-glucopyranoside [**3**].



1 R=H, R^1=β-D-6′-Acetyl Glc
2 R=R^1=H
3 R=H, R^1=β-D-Glc
4 R=Ac, R^1=β-D-2′,3′,4′,6′-Tetraacetyl Glc

The presence of an acetyl group in **1** was shown by ir bands at 1730 and 1265 cm^{-1}, ^{1}H-nmr signal at δ 1.85, and ^{13}C-nmr signals at 170.8 and 19.5 ppm. The eims of **1** showed fragment peaks at *m/z* 472 [hederagenin]$^+$, 222 [(glc) Ac]$^+$, 180 [222 − 42]$^+$, and 162 [222 − HOAc]$^+$. This evidence indicated that **1** must be a monoacetate of hederagenin-3-*O*-β-D-glucopyranoside [**3**]. This was confirmed by alkaline hydrolysis of **1**, which yielded **3**. Compound **3** lacked acetate bands and was identified as HN-saponin D1 (7) by spectral data and its acidic hydrolysis product **2** and D-glucose. In partially acetylated glycosides the location of acyl linkages is determined by ^{13}C-nmr spectroscopy. On acetylation, a methine carbon signal is somewhat deshielded and the carbon signals of β positions are displaced upfield, while other carbon signals of the alcohol moiety remain almost unaffected (8). On going through ^{13}C nmr from **3** to **1**, the signal due to C-5 of the glucose moiety was displaced upfield by 2.4 ppm, whereas the

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C-6 signal was deshielded by 2.8 ppm. The other signals remained almost unshifted. These data confirmed that the acetyl group in **1** was present at C-6 of the glucose unit. Thus, **1** was identified as hederagenin-3-*O*-β-D-6′-acetylglucopyranoside.

Similarly, *n*-hexane and C_6H_6 extracts on cc followed by vacuum liquid chromatography and preparative tlc yielded β-sitosterol (9), triacontanoic acid (10), and tetracosanoic acid (11) identified on the basis of their mp, ir, ^{1}H nmr, ms, and co-tlc with authentic samples.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Toshniwal melting point apparatus and are uncorrected. Ir spectra were taken with a Perkin-Elmer 399B spectrometer. ^{1}H- (80 MHz), and ^{13}C- (20 MHz) nmr spectra were measured with a Varian FT80A spectrometer. TMS was used as the internal standard, and chemical shifts were given in δ (ppm) values. Mass spectra were determined on a JMX DX-300 spectrometer. Optical rotation was measured with a JASCO model DIP-181. Si gel 60–120 mesh (BDH) was used for cc, and Si gel plates (0.25 mm, Merck) were used for tlc. The spots on tlc were visualized by spraying with 10% H_2SO_4 followed by heating at 100° for few minutes. The following chromatographic solvent systems were used: (A) $CHCl_3$-MeOH-H_2O (13:7:2) (lower phase), (B) $CHCl_3$-MeOH (9:1), (C) $CHCl_3$-MeOH-HCOOH (100:8:1), (D) toluene-HCOOH-HCOOEt (5:1:4), (E) $CHCl_3$-MeOH-H_2O (25:11:2) (lower phase), and (F) *n*-BuOH–HOAc–H_2O (4:1:5).

PLANT MATERIAL.—The plant material used was collected from Darjeeling, India, and identified as *S. impressa* by Dr. S.P. Jain of the Botany Division. A voucher specimen (no. 1909) was deposited in the herbarium of this Institute.

ISOLATION PROCEDURES.—Powdered air-dried stems and bark of *S. impressa* (2.3 kg) were extracted with MeOH at room temperature for 10 days. The extract was concentrated in vacuo. The residue was dissolved in H_2O. The H_2O solution was extracted with *n*-hexane, C_6H_6, $CHCl_3$, and EtOAc, and finally with *n*-BuOH saturated with H_2O. The *n*-BuOH fraction concentrated under vacuum yielded a brown viscous mass (62.8 g), which was subjected to Si gel (725 g) cc and eluted with EtOAc with increasing amounts of MeOH. Fractions (250 ml each) were collected and monitored by tlc (solvents A, B, and E). The EtOAc-MeOH (47:3) eluate afforded a solid (0.3 g), which on repeated chromatography furnished saponin **1** (95 mg). The other eluates yielded saponins reported earlier (1).

SAPONIN **1**.—White powder (95 mg): mp 215–217°; $[\alpha]^{25}D$ +23°, ir ν max (KBr) 3400, 2920, 2880, 1730, 1695, 1630, 1460, 1390, 1265, 1130, 1080, 1040, 810 cm^{-1}; ^{1}H nmr (C_5D_5N) δ 0.75, 0.82, 1.06 (each s, tert, Me × 6), 1.85 (3H, s, -OAc), 4.95 (1H, d, J = 7 Hz, H-1 of glucose unit), 5.25 (1H, br s, H-12); ^{13}C nmr (C_5D_5N) 30.0 (C-1), 26.3 (C-2), 83.2 (C-3), 43.8 (C-4), 48.0 (C-5), 18.3 (C-6), 33.0 C-7), 40.0 (C-8), 48.5 (C-9), 37.1 (C-10), 23.9 (C-11), 122.5 (C-12), 145.2 (C-13), 42.5 (C-14), 28.5 (C-15), 23.9 (C-16), 47.0 (C-17), 42.5 (C-18), 47.0 (C-19), 30.9 (C-20), 33.5 (C-21), 33.0 (C-22), 65.2 (C-23), 13.6 (C-24), 16.2 (C-25), 17.5 (C-26), 26.3 (C-27), 180.6 (C-28), 33.5 (C-29), 23.9 (C-30), 104.8 (C-1′), 75.5 (C-2′), 78.2 (C-3′), 71.0 (C-4′), 75.0 (C-5′), 64.8 (C-6′), 170.8 (CO), 19.3 (Ac); eims *m/z* 472, 248 (100), 222, 203, 191, 189, 180, 172, 162, 133.

ACID HYDROLYSIS OF **1**.—A solution of **1** (10 mg) in 10% aqueous HCl (2 ml) was heated under reflux on an H_2O bath for 4 h to afford the aglycone, hederagenin [**2**]: eims *m/z* [M]$^+$ 472; identified by comparison with an authentic sample [co-tlc (solvents B, C, D), mmp, ir, ^{1}H- and ^{13}C nmr] (4). The neutralized (Ag_2CO_3) and concentrated aqueous hydrolysate showed the presence of D-glucose [co-tlc (solvent E, F)] by comparison with an authentic D-glucose sample.

ACETYLATION OF **1**.—A solution of **1** (35 mg) and Ac_2O/pyridine was allowed to stand at room temperature for 24 h. The crude product was chromatographed over Si gel to give an amorphous powder **4**: ir ν max (KBr) 1748, 1695 cm^{-1}; ^{1}H nmr (C_5D_5N) δ 0.64, 0.75, 0.80, 1.10 × 2, 1.15 (each 3H, s, tert, Me × 6), 1.80, 1.85 × 2, 1.90, 2.03 (each 3H, S, -OAc), 3.67–5.55 (H-2′, -6′, -23), 4.52 (1H, d, J = 7 Hz, H-1′), 5.25 (1H, m, H-12); ^{13}C nmr (C_5D_5N) 38.5 (C-1), 24.8 (C-2), 83.2 (C-3), 42.0 (C-4), 46.8 (C-5), 17.4 (C-6), 33.0 (C-7), 39.9 (C-8), 48.5 (C-9), 36.9 (C-10), 23.8 (C-11), 122.1 (C-12), 145.1 (C-13), 42.0 (C-14), 27.4 (C-15), 23.8 (C-16), 46.6 (C-17), 42.0 (C-18), 46.4 (C-19), 31.0 (C-20), 34.4 (C-21), 33.2 (C-22), 68.5 (C-23), 13.6 (C-24), 15.8 (C-25), 17.4 (C-26), 25.0 (C-27), 180.0 (C-28), 33.2 (C-29), 23.8 (C-30), 102.5 (C-1′), 72.2 (C-2′), 72.8 (C-3′), 70.5 (C-4′), 73.0 (C-5′), 62.8 (C-6′), 168.0, 170.0 × 2, 170.5, 171.0 (CO), 19.3, 20.5 × 2, 20.7, 20.8 (Ac); eims *m/z* 331 [glucosyl (Ac)$_4$]$^+$.

ALKALINE HYDROLYSIS OF **1**.—A solution of **1** (45 mg) in 0.1 N aqueous KOH (3 ml) was al-

lowed to stand at room temperature for 50 h. The reaction mixture was neutralized with 0.1 N H_2SO_4 and extracted with *n*-BuOH saturated with H_2O. The *n*-BuOH layer was washed with H_2O and concentrated. The residue was dissolved in MeOH and then poured into Me_2CO to give a deacetyl product **3** (25 mg) as a white powder. The product was identified as HN-saponin D1 by comparison of mp, ir, ^1H nmr, and ^{13}C nmr with those of an authentic specimen (7).

ACID HYDROLYSIS OF **3**.—A solution of **3** (20 mg) in 2 ml of 10% aqueous HCl was hydrolyzed in the same manner as for **1** above, which gave hederagenin [**2**] as aglycone and D-glucose as sugar.

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LITERATURE CITED

1. S.K. Srivastava and D.C. Jain, *Phytochemistry*, **28**, 644 (1989).
2. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry", Holden-Day, San Francisco, 1964, Vol. II, p. 122.
3. G.A. Miana and Hassan M.G. Al-Hazimi, *Phytochemistry*, **26**, 225 (1987).
4. S. Seo, Y. Tomita, K. Tori, and Y. Yoshimura, *J. Am. Chem. Soc.*, **100**, 3331 (1978).
5. O. Tanaka and R. Kasai, in: "Progress in the Chemistry of Organic Natural Products." Ed. by W. Herz, H. Grisewach, G.W. Kirby, and Ch. Tamm, Springer, Vienna, 1984, Vol. 46, p. 26.
6. Y.N. Shukla and R.S. Thakur, *Phytochemistry*, **27**, 3012 (1988).
7. H. Kizu, S. Kitayama, F. Nakatani, T. Tomimori, and T. Namba, *Chem. Pharm. Bull.*, **33**, 3324 (1985).
8. K. Yamassaki, R. Kasai, Y. Masaki, M. Yokihara, O. Tanaka, H. Oshio, S. Takaga, M. Yamaki, K. Masuda, G. Nonaka, M. Tsuboi, and D. Nishioka, *Tetrahedron Lett.*, 1231 (1977).
9. T.C. Jain and C.M. Banks, *Can. J. Chem.*, **46**, 2325 (1968).
10. G.M. Robinson, *J. Chem. Soc.* 1543 (1934).
11. "Dictionary of Organic Compounds" (4th ed.). Eyre and Spottiswoode, Publishers Ltd., London, 1978, Vol. 5, p. 2970.

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