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A NEW MONODESMOSIDIC TRITERPENOID SAPONIN FROM THE SEEDS OF *VIGNA UNGUICULATA* SUBSP. *UNGUICULATA*

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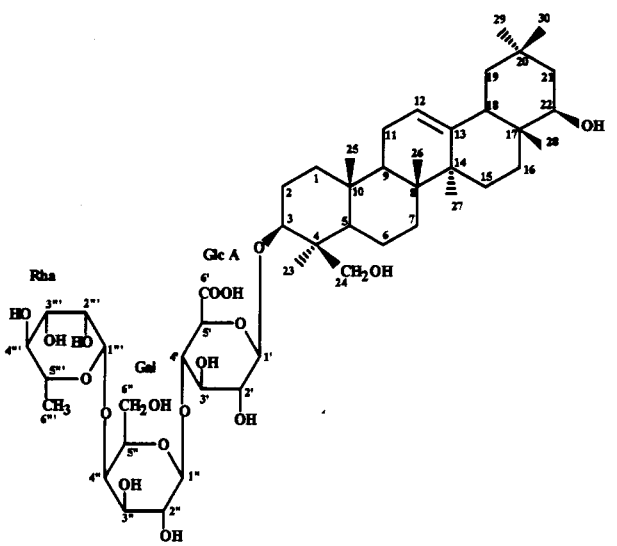
ABSTRACT.—A new triterpenoid saponin, 3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucuronopyranosyl]-soyasapogenol B [**1**] was isolated along with cycloartenol, stigmasterol, 3-*O*-acetyloleanolic acid, and sitosterol 3-β-D-glucoside from a methanolic extract of the seeds of *Vigna unguiculata* subsp. *unguiculata*. The structure of **1** was elucidated by spectroscopic and chemical means.

The seeds of *Vigna unguiculata* (L.) Walp. subsp. *unguiculata* [syn. *V. sinensis* (Linn.) Hassk.] (Leguminosae) are reported to have anthelmintic and diuretic properties. They are boiled and eaten as a staple food. Seeds are prescribed for liver complaints with jaundice (1,2). These medicinal properties prompted us to investigate the seeds for their chemical constituents. No prior work has been carried out on the chemical constituents of this plant. This paper deals with the isolation and structural characterization of a new pentacyclic triterpenoidal saponin, **1**, and the known compounds cycloartenol, stigmasterol, 3-*O*-acetyloleanolic acid, and sitosterol 3-*O*-β-D-glucoside from the seeds of *V. unguiculata* subsp. *unguiculata*. The aglycone of this saponin has been established by ^{1}H- and ^{13}C-nmr spectroscopy of the acid hydrolysate of the saponin [**1**] as soyasapogenol B (3,4). The interglycosidic linkages, the position of attachment of the sugar chain to the aglycone, and sequencing of the sugars in compound **1**, have been determined by the ^{1}H- and ^{13}C-nmr spectra, interpreted with the aid of COSY, HMQC, and negative-ion fabms data.

The crude saponin mixture obtained from a MeOH extract of the seeds of *V. unguiculata* subsp. *unguiculata* was subjected to Si gel cc. The fractions eluted with $CHCl_3$-MeOH (50:50) contained a mixture of compound **1** with some minor impurities which, on repeated Si gel chromatography, yielded pure compound **1** (mp 268–270° dec). Its ir spectrum ex-



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hibited characteristic absorption bands at 3400 cm^{-1} (OH) and 1700 cm^{-1} (COOH). The negative-ion fabms of **1** gave the mol wt and established the sugar sequence. It showed a pseudomolecular ion peak at *m/z* 941 $[M-H]^-$ and fragment ions at *m/z* 795 $[M-H-176]^-$, 633 $[M-H-(176+162)]^-$, 457 $[M-H-(176+162+146)]^-$. These fragment ions suggested the sequential loss of three hexose units. It further indicated that the terminal deoxyhexose is rhamnose and the innermost monosaccharide unit is glucuronic acid. The negative-ion fabms, together with the ^1H- and ^{13}C-nmr data, established a molecular formula of $C_{48}H_{78}O_{18}$ for compound **1**, and the presence of 10 double-bond equivalents in the molecule.

Acid hydrolysis of **1** yielded three sugars and the aglycone which was identified as soyasapogenol B by comparison of its physical and spectral data with literature values (5–7). The sugars obtained from the hydrolysates were identified as rhamnose, galactose, and glucuronic acid by comparison with authentic samples through paper chromatography as well as Si gel tlc.

The configurations at the anomeric centers of the sugar moieties were deduced from the ^1H-nmr spectrum (8) (DMSO-d_6, 400.13 MHz). The signals of the anomeric protons appeared at δ 4.18 (d, J=7.69 Hz, H-1′) and 4.75 (d, J=7.15 Hz, H-1″) showing a 1,2-diaxial relationship, while the anomeric proton appearing at δ 4.94 (d, J=1.21 Hz, H-1‴) showed a 1,2-diequatorial relationship, indicating the β-configuration for glucuronic acid and galactose and the α-configuration for rhamnose, respectively. These configurations were confirmed by ^{13}C-nmr assignments (δ 103.9, 99.9, and 100.2, respectively) of the sugar moieties (9,10). In the HMQC experiment these signals showed coupling with their respective anomeric protons at δ 4.18, 4.75, and 4.94. The ^1H-nmr spectrum showed seven tertiary methyl singlets at δ 0.75, 0.80, 0.84, 0.88, 0.96, 1.06, and 1.14. A doublet at δ 1.09 (J=6.22 Hz) was attributed to the secondary methyl of the rhamnopyranosyl unit.

The ^{13}C-nmr spectrum of **1** in DMSO-d_6 corroborated the presence of 45 carbon atoms in the molecule. Fifteen carbon signals were seen for the 18 carbon atoms of three sugar moieties: the signal at δ 70.6 was assigned to C-2 and C-3 of the rhamnose moiety, the signal at δ 74.0 was due to both C-3″ and C-4‴, and the signal at δ 74.6 was assigned to C-5′ and C-5″, confirming the presence of three monosaccharide (hexose) units. The remaining 30 carbon signals were attributed to the triterpenoid aglycone. The multiplicity assignments of each carbon atom were made from the DEPT nmr spectrum which revealed the presence of eight methyl, 11 methylene, and 21 methine carbon atoms. The chemical shift value at δ 90.0 was attributed to the C-3 of the aglycone, which is indicative of the presence of a β-hydroxyl group. A downfield shift (+9.9 ppm) as compared to soyasapogenol B, indicated that the sugar chain was attached at C-3 (3). An upfield shift of the C-2 signal by 3.2 ppm by comparison with the above sapogenol also confirmed the site of glycosidation.

The glycosidic linkages among the sugars were determined by ^{13}C-nmr glycosidation shifts. The C-4 signal of glcA and gal showed peaks at δ 79.1 and 77.0, corresponding to glycosidation shifts of ca. +6.8 and +6.9 ppm as compared with methyl glucuronoside and methyl galactoside, respectively (9,10). The upfield shifts of C-3 (−0.8, −1.2) and C-5 (−1.0, −2.2) of the glucuronic acid and galactose units also supported the (1→4) linkage of these sugar moieties (9,10). The (1→4) linkage of galactose to glucuronic acid was supported by comparing the ^{13}C-nmr literature data for similarly linked sugar moieties (11–13).

The structure of **1** was further supported by ^1H-^1H correlated spectroscopy (COSY–45°). COSY interactions were

observed between the anomeric H-1′ (δ 4.18) and the vicinal methine H-2′ (δ 3.50). The possibility of a (1→2) linkage between the inner galactose and glucuronic acid was eliminated, on the basis of the reported ^{13}C-nmr data, because in the HMQC spectrum the methine proton at δ 3.50 was coupled with the carbon at δ 72.5. It is known that when galactose is attached to C-2 of glucuronic acid, the ^{13}C-nmr chemical shift of this carbon is observed in the range of 78.1–81.6 ppm (14,15). Again the possibility of (1→2) linkage between rhamnose and galactose was eliminated, since the COSY-45° nmr spectrum showed cross-peaks between the anomeric methine H-1″ proton (δ 4.75) and H-2″ (δ 3.40). COSY interactions between the H-1‴ (δ 4.94) and H-2‴ (δ 3.30) methines were also observed. Finally, H-5‴ (δ 3.90) showed cross-peaks with the secondary methyl H-6‴ (δ 1.09).

Based on the above data the structure of **1** was established as 3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucuronopyranosyl]-soyasapogenol B.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Büchi-535 mp apparatus and are uncorrected. Optical rotations were determined on a Jasco DIP-360 polarimeter. Eims and fabms were determined on a Finnigan-MAT 312 mass spectrometer connected to a PDP 11/34 computer system. ^{1}H-Nmr (400.13 MHz) and ^{13}C-nmr (100.61 MHz) spectra were recorded on a Bruker AM-400 spectrometer in DMSO-d_6, and chemical shifts are given in the δ (ppm) scale, with coupling constants (*J*) in Hz with TMS as internal standard. Cc was performed on Merck Si gel 60 (70–230 mesh). Precoated Kieselgel 60, F_{254} cards (thickness 0.2 mm, Riedel de Haën, Seelze 1, Germany, Art No. 37360) were used for tlc using the solvent systems $CHCl_3$-MeOH-H_2O (75:25:2) and *n*-BuOH-HOAc-H_2O (12:3:5). The final purity of the compounds was checked using Si gel PF-254 precoated glass plates (Merck 70–230 mesh ASTM). Spot detection was conducted by spraying with a 10% solution $Ce(SO_4)_2$ in 1 M H_2SO_4 followed by heating at 80° for 5 min.

The 2D COSY–45° experiment was carried out at 400.13 MHz with a sweep width of 4065 Hz (1K data points in ω_2) and 2032 Hz (256 t_1 values 1K) in ω_1. A 1.5-sec relaxation delay was used and 16 transients were accumulated for each t_1 value. The 2D ^{1}H-^{13}C heteronuclear multiple quantum coherence (HMQC) experiment was carried out at 500.13 MHz with a sweep width of 4201.68 Hz (2K data points in ω_2) and 180.703 Hz (256 t_1 values zero filling 512) in ω_1. A 1.0-sec relaxation delay was used and 64 transients were performed for each t_1 value. In the HMQC experiment interpulse delays were optimized for a $^1J_{CH}$ of 0.0040 Hz [D_2O=(1/2*J*)×H, *J*=135 Hz].

PLANT MATERIAL.—The seeds of *Vigna unguiculata* subsp. *unguiculata* were purchased from the local market of Karachi in June 1988, and were identified by Prof. Dr. S.I. Ali, Department of Botany, University of Karachi. A voucher specimen (KU-023) is deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi.

EXTRACTION AND ISOLATION.—Dried, ground seeds (7.0 kg) were extracted with MeOH in a Soxhlet apparatus three times. The combined MeOH extract was evaporated under reduced pressure to afford a gummy residue, which was partitioned between hexane and H_2O. The H_2O layer was extracted with EtOAc followed by *n*-BuOH. The *n*-BuOH extract was evaporated *in vacuo* and the residue (34.0 g) was subjected to Si gel cc using hexane, $CHCl_3$, and MeOH in ratios of increasing polarity. The fractions eluted with $CHCl_3$-MeOH (1:1) were further purified by cc [$CHCl_3$-MeOH, 6:4] to give compound **1** (0.035 g, 0.1%). The fractions eluted in hexane-$CHCl_3$ (0.5:9.5) were further chromatographed on Si gel (hexane-$CHCl_3$, 1:9) to afford cycloartenol (0.18 g, 0.5%). The $CHCl_3$-eluted fractions contained a mixture of stigmasterol and 3-*O*-acetyloleanolic acid and were subjected to repeated Si gel chromatography to give stigmasterol (0.10 g, 0.3%) and 3-*O*-acetyloleanolic acid (0.13 g, 0.4%) in pure form. The fractions obtained in $CHCl_3$-MeOH (9.0:1.0) were combined and washed with MeOH to yield sitosterol 3-β-D-glucoside (0.20 g, 0.6%).

Compound **1**.—Mp 268–270° (dec); $[\alpha]^{28}_D$ −29.63° (*c*=0.054, DMSO); ir ν max 3400 (OH), 1700 (CO_2H) cm^{-1}; ^{1}H nmr (DMSO-d_6, 400.13 MHz) δ 5.16 (1H, t, *J*=3.55 Hz, H-12), 4.94 (1H, d, *J*=1.21 Hz, H-1‴), 4.75 (1H, d, *J*=7.15 Hz, H-1″), 4.18 (1H, d, *J*=7.69 Hz, H-1′), 1.14 (3H, s, Me-23), 1.09 (1H, d, *J*=6.22 Hz, H-6‴), 1.06 (3H, s, Me-27), 0.96 (3H, s, Me-28), 0.88 (3H, s, Me-26), 0.84 (3H, s, Me-29), 0.80 (3H, s, Me-25), 0.75 (3H, s, Me-30); ^{13}C nmr (DMSO-d_6, 100.61 MHz) δ 172.6 (C-6′), 144.0 (C-13), 121.5 (C-12), 103.9 (C-1′), 100.2 (C-1‴), 99.9 (C-1″), 90.0 (C-3), 79.1 (C-4′), 77.0 (C-4″), 75.7 (C-3′), 75.2 (C-22), 74.6 (C-5′, C-5″), 74.0 (C-3″, C-4‴), 72.5 (C-2′), 72.4 (C-2″), 70.6 (C-2‴, C-3‴), 69.3 (C-5‴), 62.4 (C-24), 59.8 (C-6″), 55.2 (C-5), 46.9 (C-9),

46.0 (C-19), 44.6 (C-18), 43.0 (C-4), 41.7 (C-14), 41.2 (C-21), 38.9 (C-8), 38.1 (C-1), 36.9 (C-17), 35.8 (C-10), 32.6 (C-29), 32.5 (C-7), 30.1 (C-20), 28.3 (C-28), 27.9 (C-16), 25.5 (C-15), 25.2 (C-2), 25.0 (C-27), 23.2 (C-11), 22.3 (C-23), 20.3 (C-30), 17.9 (C-6‴), 17.8 (C-6), 16.6 (C-26), 15.4 (C-25); fabms (negative-ion) *m/z* 941 $[M-H]^-$, 795 $[M-H-$rhamnose$]^-$, 633 $[M-H-$rhamnose-galactose$]^-$, 457 $[M-H-$rhamnose−galactose−glucuronic acid$]^-$; (positive-ion) *m/z* 1073 $[M+K+$glycerol$]^+$, 981 $[M+K]^+$, 943 $[M+H]^+$.

ACIDIC HYDROLYSIS OF COMPOUND **1**.—Saponin **1** (25 mg) was hydrolyzed with 2 N HCl in aqueous MeOH (10 ml) on a boiling H_2O bath for 3 h. The solvent from the reaction mixture was evaporated under reduced pressure, the mixture diluted with H_2O, and extracted with EtOAc to yield the aglycone (7 mg), whose physical and spectroscopic data corresponded to literature data (5–7). The aqueous layer thus separated was evaporated under reduced pressure with repeated addition of H_2O to remove HCl. The residue obtained was compared with standard sugars purchased from Sigma Chemical Company, St. Louis, MO, on Si gel tlc using the solvent system EtOAc-HOAc-H_2O-MeOH (6:1:1:2), which led to the tentative identification of the sugars as glucuronic acid, galactose, and rhamnose. The identities of the monosaccharides were further confirmed by comparison with standard sugars on paper chromatography (Whatman Filter paper No. 1, serrated edges along the lower descending end) using a solvent system consisting of *n*-BuOH-pyridine-H_2O (10:3:3) and a development time of 48 h. Three spots whose R_f's were identical to the R_f of L-rhamnose, D-galactose, and D-glucuronic acid, respectively, were detected by spraying with freshly prepared aniline phthalate sugar reagent followed by heating (16).

Cycloartenol.—Mp 113°; $[\alpha]_D$ +52° ($CHCl_3$); the physical and spectral data coincided to those reported in the literature for cycloartenol (17).

Stigmasterol.—Mp 168°; $[\alpha]_D$ −50° ($CHCl_3$); the physical and spectral data matched exactly with those reported in literature for stigmasterol (18–20).

3-O-Acetyloleanolic acid.—Mp 254°; $[\alpha]^{23}_D$ +72° ($CHCl_3$), physical and spectral data were in complete agreement with those for 3-*O*-acetyloleanolic acid (21–24).

Sitosterol β-D-glucoside.—Mp 287–288°; $[\alpha]^{26}_D$ −40° (pyridine); physical and spectral data coincided with those for sitosterol β-D-glucoside (25).

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