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SOYASAPONIN A3, A NEW MONODESMOSIDIC SAPONIN ISOLATED FROM THE SEEDS OF *GLYCINE MAX*

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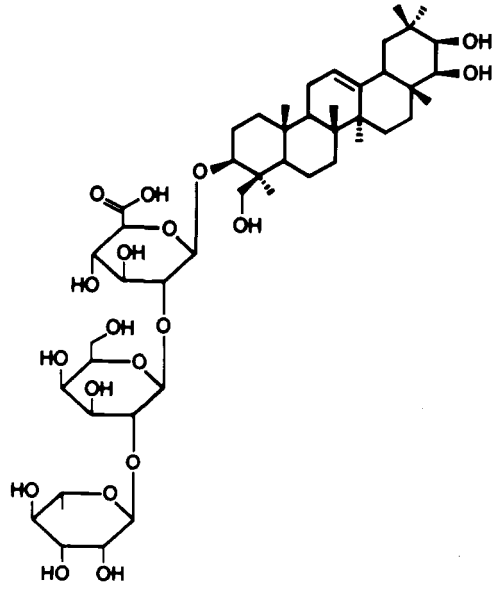
ABSTRACT.—A new triterpenoid saponin, named soyasaponin A3, has been isolated from soyabean and identified as 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]olean-12-en-21β,22β,24-triol on the basis of chemical and spectral evidence.

The presence of saponins in foodstuffs has attracted considerable interest over the past few years (1,2) due to their physiological properties, including hypocholesterolemic activity (3), gut wall permeabilization (4), and bitter taste (5). During the course of our studies in this area, we have isolated and characterized three additional saponins from soyabean [*Glycine max* (L.) Merrill, Leguminosae] in addition to those previously reported [soyasaponins I, II, III, and A_1 and A_2, which have been fully characterized by Kitagawa and co-workers (6–8)]. Two of these additional saponins, soyasaponins IV and V, the monodesmosides of soyasapogenol B, have been previously reported (9,10) while the third, tentatively named soyasaponin A_3 [**1**], a monodesmoside of soyasapogenol A, is described here.

RESULTS

The saponin **1** was eluted from a Si gel column as a mixture with soyasaponins I and V as described previously (9). It was purified by further chromatography using a reversed-phase (KC_{18}) flash chromatography column to give a product homogeneous on both normal and reversed-phase tlc.

Fabms of the intact saponin **1** in the negative mode gave a molecular ion at *m/z* 957 $[M-H]^-$ and ions at *m/z* 811 [M−H− deoxyhexose]$^-$, 649 [M−H− deoxyhexose − hexose]$^-$, and 473 [M−H− deoxyhexose − hexose − hexuronic acid]$^-$. The spectrum



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obtained in the positive mode gave a protonated molecular ion at *m/z* 959 together with a sodium adduct at *m/z* 981 and ions at *m/z* 813 $[M+H-$ deoxyhexose$]^+$, *m/z* 651 $[M+H-$ deoxyhexose $-$ hexose$]^+$, and at *m/z* 457, 439, and 421, indicative of losses of one, two, and three molecules of H_2O, respectively, from a saponin containing the aglycone, soyasapogenol A (mol wt 474). Gc of the trimethylsilyl derivative of the acid hydrolysis product of the saponin confirmed the presence of soyasapogenol A.

The elution characteristics of the saponin on normal phase tlc, where it coeluted with soyasaponin I, were indicative of a monodesmosidic structure. On reversed-phase tlc, **1** (R_f 0.35) eluted between soyasaponin I (R_f 0.17) and the bisdesmosidic soyasaponin A_2 (R_f 0.48) showing an intermediate polarity consistent with the structure suggested.

Standard sugar analysis and gc of the resultant alditol acetates showed the presence of rhamnose and galactose in a ratio of 2:1. ^{1}H nmr of the permethylated saponin gave signals at δ 4.28 (d, J=7.5 Hz), δ 4.65 (d, J=7.8 Hz), and δ 5.24 (s) indicating the presence of β-linked glucuronic acid and galactose and an α-linked rhamnose, respectively.

Coupled gc-ms of the partially methylated alditol acetates gave three components characteristic of a terminal rhamnose (Rt=79 min and *m/z* 175, 162, 131, 118), a 1,2 galactose (Rt=202 min and *m/z* 234, 205, 190, 161), and a deuterated 1,2-glucose (Rt=257 min and *m/z* 235, 191, 190). On the basis of these results the structure of the saponin was concluded to be 3-*O*-[α-L-rhamnopyranosyl(1↦2)-β-D-galactopyranosyl (1↦2)-β-D-glucuronopyranosyl]olean-12-en-21β,22β,24-triol. The existence of **1** comprising the carbohydrate moiety of soyasaponin I and the aglycone soyasapogenol A common to both soyasaponins A_1 and A_2 is consistent with previously suggested biosynthetic pathways (11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Reversed-phase flash chromatography column packing was obtained from J.T. Baker Chemical Co. Ltd., Phillipsburg, New Jersey. Reversed phase tlc plates were supplied by Whatman Separation Ltd. Fabms were obtained using a KRATOS MS80 RFA instrument (having a potential of 5–8 kV applied to the xenon gun). The ^{1}H nmr (400 MHz) was obtained on a JEOL GX400 spectrometer in C_5D_5N. Gc of the aglycone-trimethylsilyl derivative was carried out on a Carlo Erba HRGC 5300 chromatograph with FID using a 3% OV-1 column at 270°. Gc separation of the alditol acetates was carried out on a Carlo Erba HRGC 5300 with FID, and combined gc-ms of the partially methylated alditol acetates was carried out on a Perkin-Elmer Sigma 2 instrument coupled to an MS30 mass spectrometer, both using a 3% OV-225 on Diatomite C2 (100–200 mesh, 1.5 m×2.3 mm i.d.) column isothermally at 210° or programmed between 150° and 220° at 1°/min. Tlc was performed using normal phase Si gel plates (*n*-BuOH-EtOH-NH_3; 7:2:5), Si gel plates modified by oxalic acid ($CHCl_3$-MeOH-H_2O; 65:35:10 lower layer), and reversed-phase KC_{18} octadecylsilane bonded to Si gel (MeOH-H_2O; 3:2).

EXTRACTION PROCEDURES.—Soyabean meal (100 g) was defatted by Soxhlet extraction with $CHCl_3$ (800 ml, 16 h). The air-dried, defatted meal was then Soxhlet-extracted with redistilled MeOH (900 ml, 30 h). The MeOH extract was concentrated to dryness, dissolved in H_2O (50 ml), and eluted through a column of octadecylsilane (C_{18}) bonded Si gel (100 g) with H_2O (500 ml) and MeOH (500 ml). The MeOH eluate was evaporated to dryness (1 g) and treated with 5% methanolic KOH by reflux (30 min) to remove any acetyl groups. The reaction mixture was neutralized with ion exchange resin (Dowex 50W), filtered, evaporated to dryness, and applied to a Si gel column (100 g) in $CHCl_3$-MeOH-H_2O (7:3:1) and eluted with the same solvent. Fractions were monitored by tlc on Si gel $(COOH)_2$ ($CHCl_3$-MeOH-H_2O; 65:35:10, lower layer) and on Si gel (C_{18}) (MeOH-H_2O; 3:2). A chromatographically homogeneous sample of **1** (10 mg) was obtained as a noncrystalline glass.

ACID HYDROLYSIS OF SAPONIN.—The saponin (1 mg) was refluxed with 5% methanolic HCl (5 ml) for 3 h. The neutralized product was partitioned between EtOAc and H_2O. The aglycone-containing organic phase was evaporated to dryness, silylated with BSTFA (0.1 ml) and pyridine (0.1 ml) at 50° for 20 min, and analyzed by gc.

SUGAR ANALYSIS.—The saponin (2 mg) was refluxed with HCOOH (90%, 1.0 ml) for 1 h at 100°, evaporated to dryness, and then refluxed in H_2SO_4 (0.25 M, 1.5 ml) for 12 h at 100°. Deoxyhexose (200 μl, 1 mg/ml) was added as internal standard, the mixture neutralized [$Ba(OH)_2$, saturated solution], filtered, and evaporated to ca. 2 ml. The mixture was made alkaline (NH_4OH, 2 M, 2 drops), then reduced with $NaBH_4$ (15 mg) at room temperature for 3 h. Dowex (H^+ form) ion exchange resin (5 ml) was added to neutralize the product, and, after filtration, the product was evaporated to dryness and acetylated with pyridine (0.1 ml) and Ac_2O (0.1 ml) at 120° for 20 min and analyzed by gc.

PERMETHYLATIONS.—Compound **1** (6 mg) was dissolved in DMSO (0.5 ml) and treated with dimsyl sodium anion (2 M in DMSO, 0.5 ml) for 12 h under argon. MeI (0.5 ml) was added dropwise at 0°. After agitation in an ultrasonic bath for 1 h at room temperature, the mixture was added to H_2O (5 ml), and any excess MeI was removed by passing a stream of argon through the solution. After extraction with CH_2Cl_2 (4×3 ml) and washing with H_2O (4×3 ml), the dried organic phase was evaporated to dryness and dissolved in C_5D_5N for ^1H nmr.

After ^1H nmr, the permethylated product was dissolved in CH_2Cl_2-Et_2O (1:4, 2 ml) and reduced by refluxing the $LiAlD_4$ (15 mg) for 4 h. The cooled mixture was diluted with EtOAc-EtOH (1:1, 2 ml) and neutralized with H_3PO_4 (2 M); it was then hydrolyzed, reduced, and acetylated as for sugar analysis (except that $NaBD_4$ was used in place of $NaBH_4$) and the product analyzed by gc-ms.

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