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SOYASAPONIN I FROM *ROTHIA TRIFOLIATA*

G. VENKATESHWAR RAO, P.S. RAO,

Department of Chemistry, Kakatiya University, Warangal 506 009, AP, India

TSUYOSHI TOMIMORI, and HARUHISA KIZU

School of Pharmacy, Hokuriku University, 3, Ho Kanagawa-machi, Kanazawa 920-11, Japan

Rothia trifoliata Pers. (=*R. indica* L.) is a Papilionaceous annual herb and the only reported Indian species of the genus *Rothia* (1). Rothindin (pseudobaptigenin 7-*O*-glucoside) was earlier reported from this species (2). We now present the structure and chemistry of a saponin isolated from this species.

The air-dried, whole plant material of *R. trifoliata* was defatted with light petroleum and then exhaustively extracted with EtOH. The concentrated ethanolic extract was chromatographed over Sigel using eluants of increasing polarity. $CHCl_3$-MeOH (95:5) eluted a colorless compound which was identified as rothindin by direct comparison with an authentic sample. $CHCl_3$-MeOH (1:1) fractions, on concentration, yielded a colorless compound, crystallizing from MeOH as plates; color reactions suggested that it was a saponin.

The saponin was treated with weak acid to give the pure compound. Its ir spectrum showed the presence of hydroxyl (3380 cm^{-1}) and carboxyl (1725 cm^{-1}). The saponin was acid hydrolyzed to yield rhamnose, galactose, glucuronic acid, and a sapogenin, which was shown to be soyasapogenol B (mp, $[\alpha]_D$, eims, ^1H nmr; triacetate mp, $[\alpha]_D$) (3).

The ^{13}C-nmr spectrum of the sapogenin (Table 1, shift values in ppm) fully supported the revised structure of soyasapogenol B as olean-12-ene-3β,22β,24-triol, proposed by Kitagawa *et al.* (4). Assignments are based on the reported data of similar olean-18-ene derivatives (5) and supported by off-resonance decoupling studies. Resonances at 122.66 and 145.06 are for the C-12 and C-13 ethylenic carbons, respectively. The axial nature of C-24 is revealed by its signal at 64.69 (t) while C-3, having an equatorial hydroxyl, is seen at 80.27 (d). The C-22 carbon resonated at 75.68 because of its axial hydroxyl, and it influenced, through 1,3-diaxial interactions, the resonance of C-29 (deshielded and appearing at 33.30 (q)).

Partial hydrolysis of the saponin yielded prosapogenins I and II. Prosapogenin I retained the glucuronic acid moiety (ir 3400, 1720 cm^{-1}) and acid hydrolysis gave glucuronic acid and soyasapogenol B. The ^1H-nmr spectrum of prosapogenin I showed the ethylenic proton signal at 5.32 ppm as a broad singlet, while the anomeric proton of the glucuronic acid residue appeared at 5.16 ppm (d). The ^{13}C-nmr spectrum of prosapogenin I (Table 1, shifts in ppm) had resonances at 172.99 for the carboxylic carbon and at 106.64 for the anomeric carbon (6). The remaining sugar carbons are relatively deshielded compared with the genin carbons. The resonances at 89.21, 75.53, and 63.43 are all assigned to oxygenated carbons of the sapogenin residue viz., C-3, 22, and 23, respectively, and these assignments are supported by data of related saponins (6-8). Thus, prosapogenin I is 3-*O*-β-D-glucuronopyranosyl soyasapogenol B.

Prosapogenin II was acid hydrolyzed to give galactose and glucuronic acid besides soyasapogenol B. Its ^1H-nmr spectrum had the anomeric proton signal of galactose at 5.51 ppm (d), while the anomeric proton signal of glucuronic acid was broad and appeared at 4.96 ppm (9). The ^{13}C-nmr spectrum (Table 1, shifts in ppm) had well-marked signals for galactose, glucuronic acid, and

soyasapogenol B carbons (5-7). The C-2′ signal was more deshielded than in prosapogenin I and appeared at 81.30 ppm since it involved as inter sugar linkage to the galactose moiety. The above described evidence for prosapogenin II revealed its structure as 3-*O*-[β-D-galactopyranosyl (1↦2)-β-D-glucuronopyranosyl]-soyasapogenol B. Prosapogenin II is also naturally occurring and known as soyasaponin III, isolated from *Glycine max* (10). Prosapogenin II was identified as soyasaponin III by direct comparison

The ^{1}H-nmr spectrum of the saponin had a broad singlet at 5.30 ppm for the C-12 ethylenic proton (11), while the anomeric protons of rhamnose, galactose, and glucuronic acid (9, 10) resonated at 6.28, 5.79, and ca. 5.0 ppm, respectively. The saponin ^{1}H-nmr spectrum in CD_3OD had the same absorptions slightly upfield, and the rhamnose methyl protons resonated as distinct doublet at 1.27 ppm (J=6Hz), which appeared at 1.77 ppm (d, J=5.9 Hz) in the spectrum taken in pyridine-d_5. The ^{13}C-nmr spectrum of the saponin (Table 1) was conspicuous for the resonances of the genin and sugar carbons, and the assignments are made based on related saponins (5-8, 12). The sugar-linked oxygenated C-3 signal appeared at 91.35 and was more deshielded than the other oxygenated carbons, C-22 (75.68) and C-23 (63.67). The C-28 resonance appeared at 28.66 as it is *cis* relative to the adjacent C-22 hydroxyl group, and C-29 resonated at 33.3. The anomeric carbons involved in the intersugar linkage, C-2′ and C-2″, are relatively deshielded and produced signals at 77.8 and 76.7, respectively. The C-6‴ methyl carbon is highly shielded out of all the sugar carbons and conspicuously appeared at 18.9, while its shielding effect on C-5‴ placed it at 69.48.

The high resolution eims of the saponin did not show M^+; instead, the highest fragment was recorded at *m/z* 526.4058 for $C_{34}H_{54}O_4$. Its cracking pattern was typical of soyasapogenol B (*m/z* 458.3740 for $C_{30}H_{50}O_3$) (3).

From these findings, the structure of the saponin is considered to be 3-*O*-[α-L-rhamnopyranosyl (1↦2)-β-D-galactopyranosyl (1↦2)-β-D-glucuronopyranosyl]-soyasapogenol B, which is identical to soyasaponin I. This saponin was earlier reported from soybean, *Glycine max* (10). However, the present communication further confirms the assigned structure from detailed spectral information and also from partial hydrolytic studies.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. ^{1}H- and ^{13}C-nmr spectra were taken in pyridine-d_5 at 99.55 MHz and 25 MHz, respectively, with a JEOL JNM-FX-100 spectrometer, and chemical shifts are given as ppm with TMS as an internal standard. The ir spectra were obtained with a JASCO IR-A-2 spectrometer.

ISOLATION OF SAPONIN.—The air-dried, whole plant material (2 kg) of *R. trifoliata* was powdered and successively extracted in a Soxhlet apparatus with light petroleum and EtOH. The concentrated ethanolic extract was column chromatographed on Sigel with $CHCl_3$-MeOH (1:1); fractions 2-6 (each 500 ml) showed a single violet spot on tlc plates when they were sprayed with 10% H_2SO_4 and heated at 120°. These fractions were mixed and concentrated, and the separated colorless compound crystallized from MeOH as colorless plates (2.1 g), mp 252°.

CONVERSION OF CARBOXYLATE FORM OF SAPONIN INTO ACID FORM.—The above saponin was contaminated with the carboxylate form, which was hardly soluble in MeOH, H_2O, and pyridine. The saponin was dissolved in 0.02 N H_2SO_4 in 60% dioxane at room temperature and filtered to remove the insoluble portion. To this solution, two times its own volume of H_2O was added, and the total solution was concentrated to about half its volume to give a precipitate; the precipitate was recrystallized from aqueous EtOH to give colorless needles of pure saponin, mp 238-240°, ir (KBr) 3380 (OH), 1725 (COOH) cm^{-1}.

METHYL ESTER OF SAPONIN.—The saponin was treated with 0.05 N HCl-MeOH at room temperature for 20 h. The reaction mixture was neutralized with Ag_2CO_3 and the precipitate filtered. The filtrate was concentrated, and the residue was crystallized from MeOH to give colorless needles of a methyl ester, shown by ^{13}C-

TABLE 1. ^{13}C-nmr Data of Soyasapogenol B, Prosapogenins I and II, and Saponin

Carbon	Soyasapogenol B	Prosapogenin I	Prosapogenin II	Saponin
1	39.0	38.8	38.7	38.7
2	28.5	26.9	26.7	26.7
3	80.3	89.2	90.9	91.4
4	43.3	44.5	43.9	43.9
5	56.5	56.3	56.3	56.2
6	19.2	18.9	18.8	18.6
7	33.6	33.5	33.3	33.3
8	40.1	40.1	40.0	40.0
9	48.2	47.9	47.9	47.9
10	37.1	36.7	36.5	36.5
11	24.1	24.1	24.0	24.0
12	122.7	122.7	122.7	122.7
13	145.0	145.1	145.0	145.0
14	42.4	42.5	42.4	42.4
15	26.5[a]	26.5[a]	26.5[a]	26.5[a]
16	28.8[a]	28.7[a]	28.7[a]	28.7[a]
17	38.1	38.1	38.0	38.0
18	45.5	45.5	45.4	45.4
19	46.9	46.9	46.9	46.8
20	30.9	31.0	30.9	30.9
21	42.5	42.5	42.4	42.4
22	75.7	75.7	75.7	75.7
23	23.6	23.4	22.8	23.0
24	64.7	63.4	63.6	63.7
25	16.3	15.6	15.8	15.9
26	17.1	17.1	17.0	17.0
27	25.7	25.8	25.7	25.7
28	28.7	28.7	28.7	28.7
29	33.3	33.3	33.3	33.3
30	21.2	21.2	21.1	21.1
1′		106.6	105.1[d]	105.5
2′		75.5	81.3	77.8
3′		78.1	77.8	78.6[b]
4′		73.6	73.7	73.9
5′		78.3	78.3	77.7[b]
6′		173.0	172.5	172.6
1″			105.6[d]	102.5
2″			73.0	76.7[c]
3″			75.5	76.5[c]
4″			71.2	71.3
5″			77.3	76.9[c]
6″			62.8	61.8
1‴				101.9
2‴				72.4
3‴				72.9
4‴				74.4
5‴				69.5
6‴				18.9

[a-c]Assignments may be reversed in each vertical column.

[d]Assignments were confirmed by proton selective decoupling.

nmr spectroscopy to be soyasaponin I methyl ester.[1]

COMPLETE ACID-HYDROLYSIS OF SAPONIN.—A solution of saponin (50 mg) in 2 N H_2SO_4 in 60% dioxane (10 ml) was heated under reflux for 2 h, to which H_2O (20 ml) was added and concentrated to about half its original volume. The precipitates were collected by filtration and recrystallized from MeOH to give the aglycone, soyasapogenol B, as colorless needles (20 mg), mp 249-251°.

The aqueous hydrolysate was adjusted to pH 6 with aqueous saturated $Ba(OH)_2$ and centrifuged. The supernatant was concentrated and examined by tlc ($CHCl_3$-MeOH-H_2O, 25:17:3; *n*-BuOH-AcOH-H_2O, 4:1:2; *n*-BuOH-Me_2CO-H_2O, 4:5:1), revealing the presence of rhamnose, galactose, and glucuronic acid.

ACETYLATION OF SOYASAPOGENOL B.—Soyasapogenol B (20 mg) was heated with Ac_2O (1 ml) and pyridine (0.5 ml) on a steam bath for 2 h, left overnight, and poured over crushed ice. The separated compound was filtered, crystallized from $CHCl_3$-MeOH as colorless needles, mp 181-182°, and analyzed for $C_{36}H_{56}O_6$.

PARTIAL HYDROLYSIS OF SAPONIN, ISOLATION OF PROSAPOGENINS I AND II.—A solution of saponin (100 mg) in 0.1 N H_2SO_4 in 75% dioxane (10 ml) was heated under reflux for 1.5 h, to which H_2O (20 ml) was added, and the total solution was concentrated to about half volume and centrifuged. The precipitates were washed with H_2O and chromatographed over Sigel; elution with $CHCl_3$-MeOH-H_2O (25:3:0.3↦25:6:0.7↦25:8:0.9) gave prosapogenins I and II, which were contaminated with the carboxylate form. These prosapogenins I and II were treated with 0.02 N H_2SO_4 in 60% dioxane at room temperature and worked up as described for the conversion of the carboxylate form of the saponin into the pure form, to give prosapogenins I and II, respectively. Prosapogenin I, colorless needles (from MeOH), ir (KBr) 3400, 1720 cm^{-1}. ^1H nmr 0.85 (3H), 1.00 (6H), 1.23 (3H), 1.30 (6H), 1.57 (3H) (all s, tert-CH_3×7), 5.16 (1H, d, J=7.3Hz, H-1 of glucuronic acid unit), 5.32 (1H, broad singlet, H-12). Prosapogenin II, colorless needles from MeOH, mp 212-215°, ir (KBr) 3400, 1720 cm^{-1}, was identified as soyasaponin III by tlc, ir, ^1H and ^{13}C nmr.

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