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3′-PRENYL-4′-METHOXY-ISOFLAVONE-7-*O*-β-D-(2″-*O*-*p*-COUMAROYL) GLUCOPYRANOSIDE, A NOVEL PHYTOESTROGEN FROM *SOPUBIA DELPHINIFOLIA*

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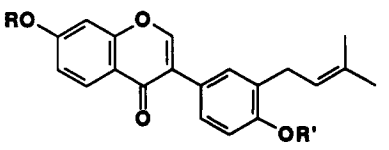
ABSTRACT.—A novel phytoestrogen compound, 3′-prenyl-4′-methoxy-isoflavone-7-*O*-β-D- (2″-*O*-*p*-coumaroyl)glucopyranoside [**1**], has been isolated from the EtOAc-soluble fraction of the stems of *Sopubia delphinifolia* and has been identified by chemical and spectral analysis. Pharmacological examination of the compound showed it to have estrogenic activity.

Sopubia delphinifolia G. Don (Scrophulariaceae) is a widely available hemiroot-parasite herbage in India (1–3). Its juice is reported to possess healing properties for sores caused by moisture and is also reported to be an abortifacient. In view of the latter reputed therapeutic value, attempts were made to isolate and identify estrogenic principles.

RESULTS AND DISCUSSION

Compound **1**, $C_{36}H_{36}O_{11}$, obtained as a colorless microcrystalline substance, gave a positive Na-Hg/HCl test, confirming its isoflavone nature; a peak in the ir at 3300 cm^{-1} indicated the presence of free OH group(s). Preparation of a tetra-acetyl derivative, $C_{44}H_{44}O_{15}$, suggested 4 acetylizable OH groups. Alkaline hydrolysis of **1** with 2% NaOMe yielded *p*-methyl coumarate (mmp, co-tlc, and superimposable ir and nmr spectra). The Et_2O-insoluble part obtained from alkaline hydrolysis gave the isoflavone glycoside **2**, $C_{27}H_{30}O_9$, as a colorless compound, which on acid hydrolysis (7% ethanolic H_2SO_4) gave an isoflavone **3**, $C_{21}H_{20}O_4$, and glucose (1 mol). Compound **3** formed a monoacetate, $C_{23}H_{22}O_5$. The uv spectrum of **3** exhibited a pronounced bathochromic shift in band II with the addition of NaOMe, suggesting a free OH at the 7 position of ring A (4). ^1H nmr of the acetyl derivative of **3** showed a singlet at δ 8.06, a characteristic feature of isoflavones. The doublets at δ 8.34 and 7.0 (J = 10 Hz) indicated ortho coupled protons on C-5 and C-6 of ring A. The signals appearing as a singlet δ 1.72, doublet δ 3.34 (J = 7 Hz), and triplet δ 5.24 integrated for prenylation (5,6); there was also strong absorption in the ir at 1370–1380 for a gem dimethyl group. A sharp singlet at δ 3.84 indicated the 4′-OMe in ring B (7). The aromatic proton of C-8 and protons of C-2, C-5, and C-6 appeared as a multiplet at δ 7.12–7.45.

These chemical shifts suggested the absence of an A_2B_2 system in the molecule and the presence of a prenyl unit at the 3′ position. The eims of **3** gave fragment ions at *m/z* 145 and 137, presumably $[B - C_4H_7]^+$ and $[A_1 + 1]^+$. Absence of prenylation at C-2′ and C-6′ was confirmed because there was no fragment available at *m/z* 268 by the loss of a 68 unit (8). The prenyl group at C-3′ was confirmed by demethylation, subsequent



1 R=glucose-(2″-*O*-*p*-coumaroyl), R′=Me
2 R=glucose, R′=Me
3 R=H, R′=Me
4 R=R′=H

cyclization, and alkaline oxidation of **3**, resulting in 2,2-dimethoxychroman-6-carboxylic acid (9).

Permethylation of **1** and **2** followed by acid hydrolysis led to the conclusion that the attachments of the isoflavone and *p*-coumaric acid were at C-1″ and C-2″ of D-glucose, respectively. The 7-*O*-β linkage and pyranose form of the sugar were confirmed by enzymatic hydrolysis and periodate oxidation of **2**. All evidence, when taken together, identified **1** as 3′-prenyl-4′-methoxy-isoflavone-7-*O*-β-D-(2″-*p*-coumaroyl)glucopyranoside.

The estrogenic activity of **1** was assessed on female albino rats by the uterine weight bioassay procedure (10). The estrogenic activity of the compound was measured by an increase in weight of uteri in comparison to that of a control group. The results revealed that there was an increase in weight of uteri at all the given levels of the compound. The maximum increase in uterine weight and 100% vaginal openings were noted on administration of 50 μg/rat of **1** twice a day.

EXPERIMENTAL

PLANT MATERIAL.—Plant material was collected from adjoining range lands in the Sagar district during November and identified by the Chairman, Botany Department of this University; an herbarium specimen (No. V-XXII) has been deposited at herbarium room No. 36 of the Chemistry Department. Dried and powdered stems were extracted exhaustively with hot 90% EtOH. The extract was concentrated under reduced pressure to a viscous mass. It was segregated into petroleum-ether (60–80°)-, C_6H_6-, EtOAc-, and Me_2CO-soluble fractions.

ISOLATION.—The concentrated EtOAc-soluble fraction showed a single spot on tlc [Si gel G, EtOAc-MeOH-H_2O (12:1:1), I_2 vapor visualization]. The fraction was purified over a Si gel column (60–120 mesh) and eluted with $CHCl_3$-MeOH-H_2O (13:7:2), providing compound **1**: colorless, microcrystalline solid; mp 158–160°, $C_{36}H_{36}O_{11}$ (found C 67.108, H 5.623, calcd C 67.080, H 5.590), eims $[M]^+$ 644; uv λ max (MeOH) 254 (sh), 259, 301 (sh) (log ε 4.51, 4.5, 3.80); λ max (MeOH + NaOMe) 256 (sh), 260, 301 (sh) (log ε 4.48, 4.50, 3.80); λ max (MeOH + $AlCl_3$) 256 (sh), 259, 308 (sh) (log ε 4.50, 4.51, 3.82); λ max (MeOH + $AlCl_3$ + HCl) 252 (sh), 259, 308 (sh) (log ε 4.1, 4.51, 3.82); λ max (MeOH + NaOAc) 261, 310 (sh) (log ε 4.60, 3.84); λ max (MeOH + NaOAc + H_3BO_3) 260, 308 (log ε 4.60, 3.82); eims *m/z* (%) $[M]^+$ 644 (2.4), 525 (10.0), 498 (28.7), 336 (100), 308 (40.3), 281 (80.2), 145 (11.7), 137 (67.9); ir ν max (KBr) 3300, 2980, 2910, 1760, 1640, 1600, 1500, 1400, 1390, 1368, 1280, 1215, 1150, 800 cm^{-1}. Tetraacetate: mp 143–144°; $C_{44}H_{44}O_{15}$ (found C 65.034, H 5.473, calcd C 65.024, H 5.418), eims $[M]^+$ 812; ^1H nmr (90 MHz, DMSO-d_6, δ ppm) 8.06 (1H, s, H-2), 7.71 (1H, d, J = 8 Hz, H-β), 7.57 (2H, d, J = 3.6 Hz, H-2‴ and H-6‴), 7.12–7.45 (4H, m, H-2′, H-5′, H-6′, and H-8), 6.87 (2H, d, J = 4.5 Hz, H-3‴ and H-5‴), 6.27 (1H, d, J = 7.8 Hz, H-α), 8.34, 7.0 (2H, 2d, J = 10 Hz, H-5 and H-6), 5.54 (1H, d, J = 7 Hz, H-1″ anomeric proton) 5.05 (1H, dd, J = 7, 7 Hz, H-2″ proton of sugar), 4.32–4.82 (5H, m, protons of sugar) 3.84 (3H, s, 4′-OMe), 2.52 (3H, s, OAc, H-6″), 2.45 (3H, s, OAc, H-4‴), 2.12 (3H, s, OAc, H-3″), 2.03 (3H, s, OAc, H-4″), 1.72 (6H, s, H-4⁗, H-5⁗), 3.34 (2H, d, J = 7 Hz, H-1⁗), 5.24 (1H, t, H-2⁗).

ALKALINE HYDROLYSIS.—Compound **1** was dissolved in MeOH and kept overnight after addition of 2% NaOMe. The reaction mixture was neutralized by diluted HOAc and concentrated under vacuum. An Et_2O-soluble part yielded silky needles, mp 138–139°.

The Et_2O-insoluble part furnished colorless amorphous substance **2**: mp 160–163°, $C_{27}H_{30}O_9$ (found C 65.084, H 6.120, calcd C 65.060, H 6.024), eims $[M]^+$ 498; tlc homogeneous; uv λ max (MeOH) 258 (sh), 302 (sh) (log ε 4.59, 3.04); λ max (MeOH + NaOMe) 251 (sh), 258, 302 (sh) (log ε 4.1, 4.6, 3.04); λ max (MeOH + $AlCl_3$) 253 (sh), 259, 302 (sh) (log ε 4.12, 4.52, 3.04); λ max (MeOH + $AlCl_3$ + HCl) 258 (sh), 260, 301 (sh) (log ε 4.52, 4.48, 3.04); λ max (MeOH + NaOAc) 259, 308 (sh) (log ε 4.52, 3.11); λ max (MeOH + NaOAc + H_3BO_3) 255, 302 (sh) (log ε 4.5, 3.04); ir ν max (KBr) 3500, 2970, 2910, 1660, 1645, 1600, 1500, 1400, 1385, 1370, 1282, 1216, 1150, 825 cm^{-1}. Compound **2** yielded an acetate: mp 169–170°, $C_{35}H_{38}O_{13}$ (found C 63.085, H 5.625, calcd C 63.063, H 5.705); ^1H nmr (90 MHz, $CDCl_3$, δ ppm) 8.06 (1H, s, H-2), 8.34 and 7.0 (2H, 2d, J = 10 Hz, H-5 and H-6), 3.34 (2H, d, J = 7 Hz, H-1‴), 5.24 (1H, t, H-2‴), 3.84 (3H, s, 4′-OMe), 1.72 (6H, s, H-4⁗, H-5⁗), 5.16 (1H, d, J = 7 Hz, H-1″ anomeric proton), 4.3–4.8 (6H, m, proton of glucose) 7.12–7.45 (4H, m, H-2′, H-5′, H-6′ and H-8), 2.14 (3H, s, OAc, H-3″), 2.03 (3H, s, OAc, H-4″), 2.52 (3H, s, OAc, H-6″), 2.03 (3H, s, OAc, H-2″).

ACID HYDROLYSIS.—Compound **2** was hydrolyzed (7% ethanolic H_2SO_4) by refluxing for about 8

h; after removal of EtOH it yielded aglycone **3**. The hydrolysate was neutralized with $BaCO_3$, $BaSO_4$ was filtered off, and the filtrate was concentrated under vacuum. It was examined on pc [*n*-BuOH–HOAc–H_2O (4:1:5)] and showed the presence of D-glucose. The quantitative estimation of sugar in the hydrolysate showed the presence of 1 mol of glucose (11).

IDENTIFICATION OF AGLYCONE **3**.—Colorless microcrystalline solid: mp 180–181°, $C_{21}H_{20}O_4$ (found C 75.023, H 5.974, calcd C 75.0, H 5.952); eims $[M]^+$ 336; tlc homogeneous [R_f 0.80, $CHCl_3$-MeOH-H_2O (13:7:2)]; uv λ max (MeOH) 245 (sh), 257, 270 (sh), 319 (log ε 4.59, 4.40, 4.41, 3.31); λ max (MeOH + NaOMe) 259, 278 (sh), 340 (log ε 4.48, 4.10, 4.03); λ max (MeOH + $AlCl_3$) 240 (sh), 250, 264 (sh), 304 (log ε 4.3, 4.6, 4.0, 3.0); λ max (MeOH + $AlCl_3$ + HCl) 243 (sh), 250, 264 (sh), 304 (log ε 4.1, 4.6, 4.4, 3.0); λ max (MeOH + NaOAc) 272, 318 (sh), 334 (log ε 4.43, 3.2, 3.6); λ max (MeOH + NaOAc + H_3BO_3) 274 (sh), 307 (log ε 4.30, 3.0); ir ν max (KBr) 3500, 2970, 2900, 1660, 1645, 1615, 1560, 1400, 1380, 1370, 1266, 1170, 825 cm^{-1}; eims *m/z* (%) $[M]^+$ 336 (100), 308 (41.0), 281 (80.5), 145 (11.7), 137 (67.9). It formed a monoacetate: mp 172°, $C_{23}H_{22}O_5$) found C 73.04, H 5.89, calcd C 73.015, H 5.82); eims $[M]^+$ 378; ^1H nmr (90 MHz, $CDCl_3$, δ ppm) 8.06 (1H, s, H-2), 8.34, 7.0 (2H, 2d, J = 10 Hz, H-5 and H-6 ortho coupled), 3.34 (2H, d, J = 7 Hz, H-1″), 5.24 (1H, t, H-2″), 1.72 (6H, s, H-4″, H-5″), 3.84 (3H, s, 4′-OMe), 7.12–7.45 (3H, m, H-6′, H-5′, H-2′, and H-8), 2.39 (3H, s, OAc).

DEMETHYLATION OF AGLYCONE **3**.—Compound **3** underwent demethylation when heated at 160° with HI and phenol for about 6 h in a round bottom flask. The content was cooled and poured into H_2O to form a dark-colored precipitate which was washed with KI solution followed by H_2O. On crystallization from MeOH it afforded compound **4**, mp 195–196°.

CYCLIZATION AND ALKALINE OXIDATION.—Compound **4** was heated with HCO_2H on an H_2O bath for 1 h. It afforded a solid which crystallized with MeOH. The resulting product was dissolved in 5% KOH (80% EtOH, 20 ml), and 12% H_2O_2 was added drop by drop till effervescence ceased. After some time, H_2O was added and the mixture was extracted with Et_2O. The aqueous layer was acidified with diluted HCl and re-extracted with Et_2O. The Et_2O layer on evaporation resulted in colorless needles, mp 176–177°, identified as 2,2-dimethylchroman-6-carboxylic acid, confirmed by co-tlc and mmp.

ATTACHMENT OF AGLYCONE AND *p*-COUMARIC ACID WITH SUGAR.—Compound **1** was treated with MeI and Ag_2O in DMF at room temperature. The reaction mixture was kept for 2 days and filtered. The residue was washed with DMF. The filtrate was dried in vacuo and hydrolyzed with 22% ethanolic H_2SO_4 for 4 h. After usual workup, the methylated sugar was identified by co-pc as 3,4,6-tri-*O*-methyl-D-glucose. A similar procedure was repeated with compound **2**, and the methylated sugar was identified as 2,3,4,6-tetra-*O*-methyl-D-glucose.

PERIODATE OXIDATION.—Compound **2** was dissolved in MeOH and treated with sodium metaperiodate for 2 days. The liberated HCOOH and consumed periodate were estimated by the Jones method (12).

ENZYMATIC HYDROLYSIS.—Compound **2** in MeOH was mixed with an equal volume of almond emulsion solution and left at room temperature for 24 h. The examination of hydrolysate on pc showed the presence of D-glucose.

ESTROGENIC ACTIVITY.—Twenty female albino rats (20–30 days of age, average wt 35 g) were selected. The rats were placed in four groups of 5 rats each. An aqueous solution of compound **1** was administered in varying doses (10 μg, 20μg, and 50 μg/rat) two times per day at 6-h intervals. One group was kept as control and was fed normal diet without any treatment. After 4 days the animals were killed. Their uteri were taken out and separated from vaginas by cutting through the cervix. The surrounding tissues were removed from uteri and weighed fresh after pressing out their intrauterine fluid on filter paper. The number of vaginal openings and mean uteri weights were noted (Table 1).

TABLE 1. Effect of Compound **1** on Uteri of Immature Rats.[a]

Group[b]	Dose (μg/rat twice a day)	Mean uterus weight after 4 days (mg)	Vaginal openings (no. of rats)
1	0	15.0 ±0.27	0
2	10	18.0 ±0.54	0
3	20	27.79±0.48	3
4	50	33.21±0.65	5

[a]Average wt was 35 g/rat.
[b]Five rats per group.

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