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2D NMR STRUCTURE ELUCIDATION OF A NEW COUMARIN GLYCOSIDE FROM *XEROMPHIS SPINOSA*

S.P. SATI, D.C. CHAUKIYAL, O.P. SATI,*

Department of Chemistry, University of Garhwal, Srinagar (Garhwal) 246 174, India

F. YAMADA, and M. ONO

Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, 57301, Japan

Xeromphis spinosa Thunb. is an important Indian medicinal plant belonging to the Rubiaceae, and its pharmacognostic features have been described (1). We have reported (2) two molluscicidal triterpenoidal glycosides from the fruits of this plant. Two oleanolic-acid-based saponins (3) and an iridoid (4) have been reported from its piscicidal leaves. Tandon *et al.* (5) isolated two sapogenins, randialic acid A (19α-hydroxyursolic acid) and randialic acid B (19-dehydroursolic acid), from the bark. Repeated cc of an aqueous EtOH extract of the defatted bark afforded a new coumarin glycoside, 7-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-6-methoxycoumarin [**1**] and 7-*O*-(β-D-glucopyranosyl)-6-methoxycoumarin which is a known compound. The use of 2D nmr (COSY and NOESY) was particularly helpful in the structural elucidations.

The fdms of **1** showed peaks at *m/z* 509 $[M+Na]^+$ and 487 $[M+H]^+$ and cluster ions at 641 $[M+Na+132(pentosyl)]^+$ and 803 $[M+Na+132+162(hexosyl)]^+$. The peaks at *m/z* 354 corresponded to the loss of a pentosyl unit from the molecular ion, and the fragment at *m/z* 192 (base peak) arose from the subsequent loss of a hexosyl moiety. The uv spectrum in MeOH had maxima at 239, 254, 262, and 327 nm, and there was no bathochromic shift upon addition of NaOH, showing that the phenolic group was substituted. The ^1H nmr (normal mode, 400 MHz, 30°) was complex as several peaks overlapped. When the spectrum was recorded at 50°, some simplification was observed. It showed the characteristic coumarin C-3/C-4 proton doublet pair centered at δ 6.26 and 7.62 (1H, d, $J=9.5$ Hz each). Anomeric glycosidic protons appeared at δ 5.61 (d, $J=2.44$ Hz) and 5.59 (d, $J=7.63$ Hz). The interpretation of the complex spin spin coupling pattern of the glycone part of the molecule was done with the help of 2D nmr, namely homonuclear correlation spectroscopy (COSY), and is explained in Figure 1. Acid hydrolysis of **1** afforded scopoletin, D-glucose, and D-apiose (isolated by cc). The ^1H nmr of the acetate of **1** showed all typical signals for the scopoletin nucleus and those of the sugar portion. This spectrum had fewer overlapping signals than did that of the parent. The nature of the glycosidic linkages was defined as β,β by ^1H and ^{13}C nmr (6). The glucosyl C-6 occurred at δ 69.08 in the ^{13}C nmr shifted downfield (~7 ppm) from its position in methyl-β-D-glucoside (6) showing that it is glycosylated at C-1 of D-apiose. The 2D nmr (NOESY) spectrum (Figure 2) confirmed all the above conclusions. NOe's are observed between H-4 and H-5, H-5 and the protons of the methoxy group, H-8 and the H-1 of β-D-glucosyl unit, and between both H-6 protons of the glucosyl moiety and H-1 of D-apiose. The carbon hydrogen shift-correlated spectrum (2D) was helpful in the confirmation of ^{13}C- and ^1H-nmr assignments (Figure 3).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were recorded with a Boetius microscopic apparatus and are uncorrected. Fdms was on JEOL JMS-DX300 instrument using carbon emitter, cathode voltage 5 kV, accelerating voltage 3 kV. Nmr: 400 MHz was used for ^1H nmr

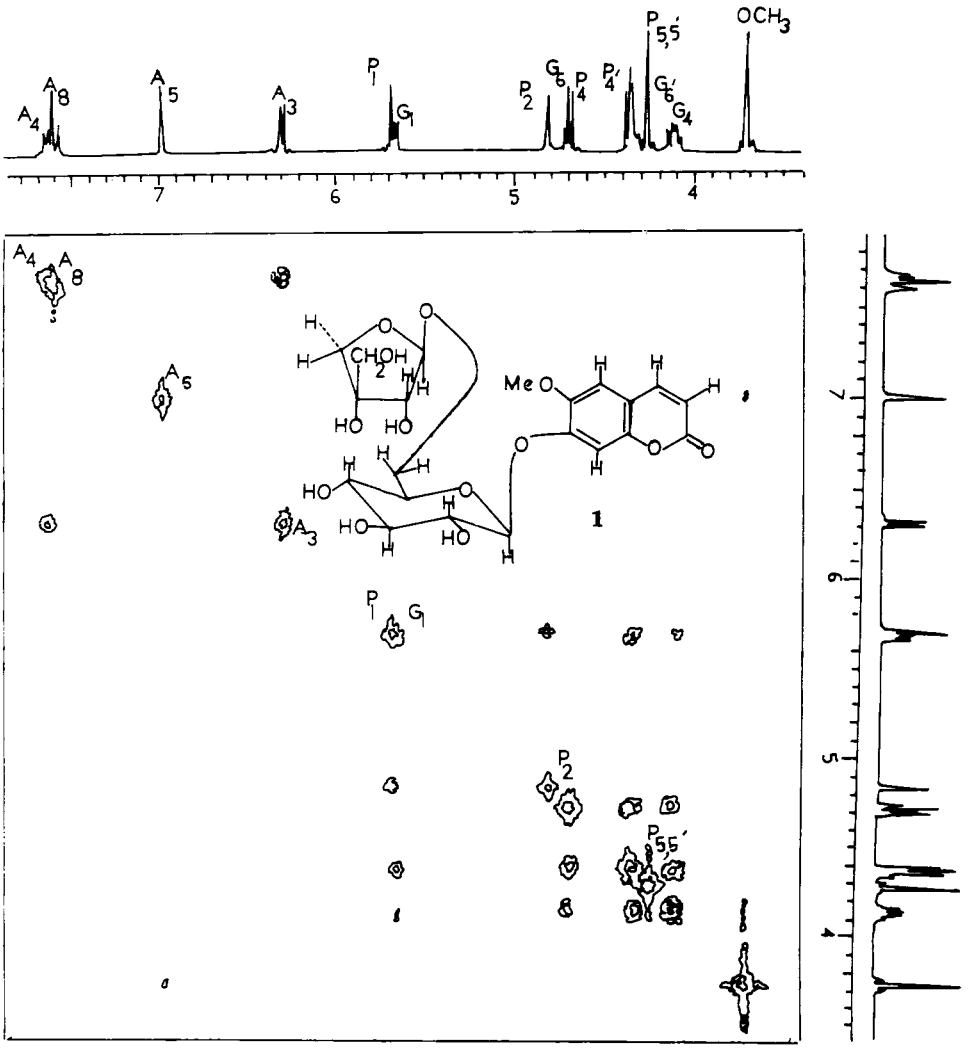

FIGURE 1. 2D *J*-correlated (COSY) spectrum of **1**. A = aglycone, P = D-apiose, G = glucose.

and 25 MHz for ^{13}C nmr, cc was on Si gel (Merck). Spots on tlc were visualized by spraying with 7% H_2SO_4 followed by heating.

ISOLATION.—The bark (3 kg) of wild *X. spinosa* was collected from Ratura, Uttar Pradesh; specimens were identified by Prof. R.D. Gaur, Department of Botany, University of Garhwal and are preserved in its Herbarium. The air-dried, coarsely powdered bark was defatted with light petroleum ether (bp 60–80°). The defatted material was exhaustively extracted with 90% aqueous EtOH and concentrated under reduced pressure. The extract was fractionated through cc using $CHCl_3$-MeOH (90:10) to afford 7-*O*-(β-D-glucopyranosyl)-6-methoxycoumarin (2.75 g, identified by its fdms, uv, ^1H-nmr normal mode, 2D COSY and NOESY, and ^{13}C-nmr spectra), compound **1** (3.95 g), and D-mannitol (20 g).

COMPOUND **1**.—Crystallized from MeOH, mp 238–240°; ir ν max (KBr) cm^{-1} 3500, 1723, 1630, 1575, 1520, 1440, 1290, 1085; eims *m/z* 192, 177, 164; fdms *m/z* 803, 641, 509, 487, 354, 192, 133; ^1H nmr (D_2O, 50°) 7.62 (1H, d, $J = 9.5$ Hz), 7.57 (s, H-8), 6.97 (s, H-5), 6.26 (1H, d, $J = 9.5$ Hz), apiofuranosyl protons (C-1 to C-5) 5.61 (1H, d, $J = 2.44$ Hz), 4.73 (1H, d, $J = 2.44$ Hz), 4.62 (1H, d, $J = 9.5$ Hz), 4.31 (1H, d, $J = 9.5$ Hz), 4.20 (2H, s), glucopyranosyl protons (C-1 to C-6) 5.59 (1H, d, $J = 7.63$ Hz), 4.26 (dd, $J = 9.77$, 7.63), 4.30 (dd, $J = 9.16$, 9.16), 4.01 (dd, $J = 9.16$, 9.16), 4.28 (ddd, 9.16, 7.32, 3.05), 4.63 (dd, $J = 11.0$, 3.05 Hz), 4.08 (dd, $J = 7.32$, 7.32 Hz), 3.71 (3H, s, -OMe); ^{13}C nmr (C_5D_5N) coumarin carbons (C-2 to C-10) 161.54, 113.99, 143.86, 109.91, 150.05, 151.39, 104.80, 147.12, 113.09, apiofuranosyl carbons (C-1 to C-5)

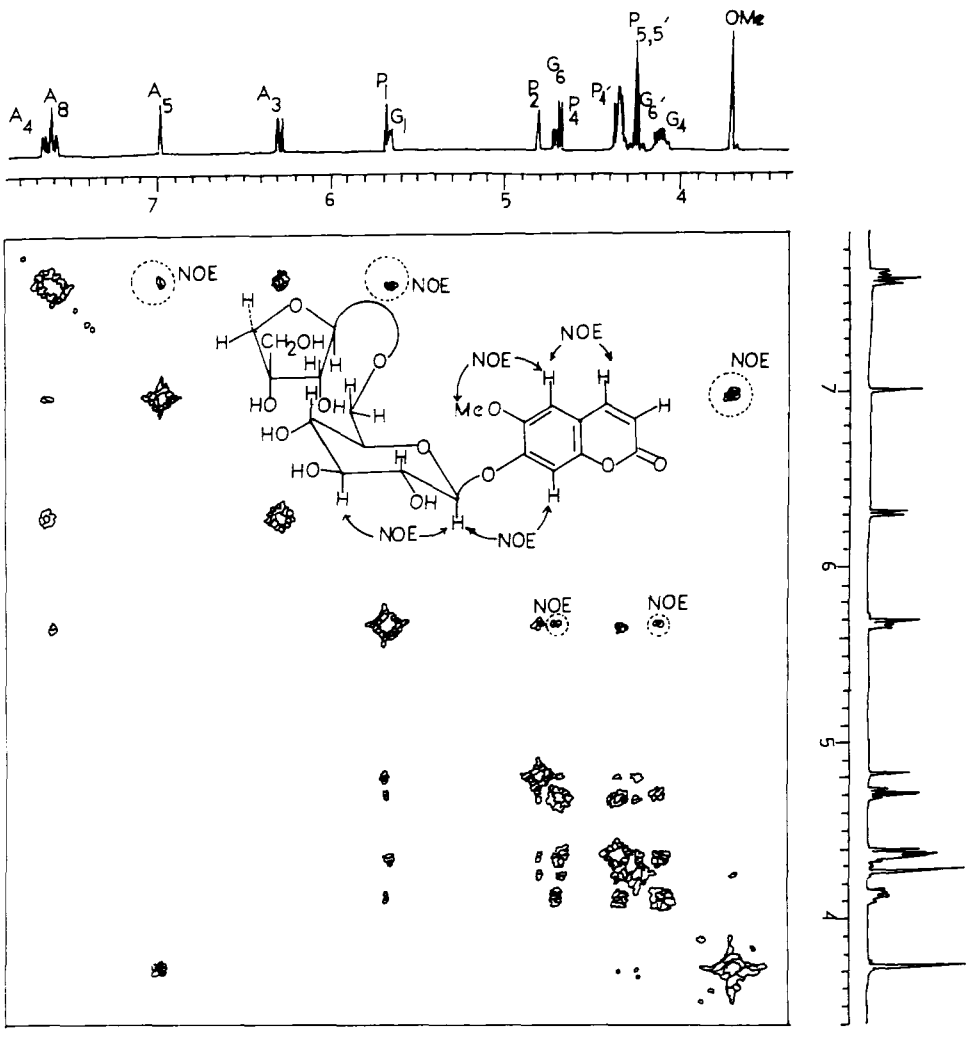

FIGURE 2. 2D NOESY spectrum of **1**. A = aglycone, P = D-apiose, G = D-glucose.

111.39, 78.03, 80.33, 75.14, 65.68, 56.31 (OCH_3), glucopyranosyl carbons (C-1 to C-6) 102.25, 74.61, 78.64, 71.56, 77.39, 69.08.

ACETYLATION OF **1**.—A mixture of **1** (49 mg), Ac_2O (4 ml), and pyridine (2 ml) was heated at 70° for 4 h. Usual workup and chromatographic purification on Si gel [*n*-hexane-EtOAc (1:2)] gave a powder (58 mg), mp 80–90°; ^1H nmr (CD_3OD, 50°) 7.70 (1H, d, J = 9.46, 4-H), 7.43 (1H, s, 8-H), 7.08 (1H, s, 5-H), 6.40 (1H, d, J = 9.46 Hz, 3-H), apiofuranosyl protons 5.81 (1H, d, J = 1.33 Hz), 5.37 (1H, d, J = 9.5 Hz), 5.08, 4.96 (AB doublet each, H-5,5'), 4.53, 4.42 (AB doublet each, H-4,4'), glucopyranosyl protons 5.89 (1H, dd, 3-H), 5.81 (1H, d, J = 7 Hz), 5.60 (1H, dd, H-4), 4.45 (1H, ddd, 5-H), 4.18 (1H, dd, 6-H), 3.98 (1H, dd, 6'-H), 3.75 (3H, s, -OMe), 2.1 (-OAc).

ACIDIC HYDROLYSIS OF **1**.—Compound **1** (60 mg) was heated with 3% H_2SO_4 on an H_2O bath for 1.5 h. The mixture was cooled and filtered, and the residue was crystallized from MeOH to afford fine needles, mp 202–207°. The filtrate was extracted with Et_2O, and the organic layer was concentrated to yield a residue that was crystallized with MeOH/H_2O affording plates (8 mg), mp 202–207°. The crystalline material was identified as scopoletin by mmp and ir. The filtrate was neutralized by passing through an anion exchange resin column, and the eluate was reduced and chromatographed over a Si gel column using $CHCl_3$-MeOH-H_2O (7:3:0.5) to give D-glucose (18.8 mg) as a syrup, $[\alpha]_D$ +56.9° (c = 1.25, H_2O), and D-apiose (16.6 mg) as a syrup, $[\alpha]_D$ +7.4° (c = 1.10, H_2O).

ACKNOWLEDGMENTS

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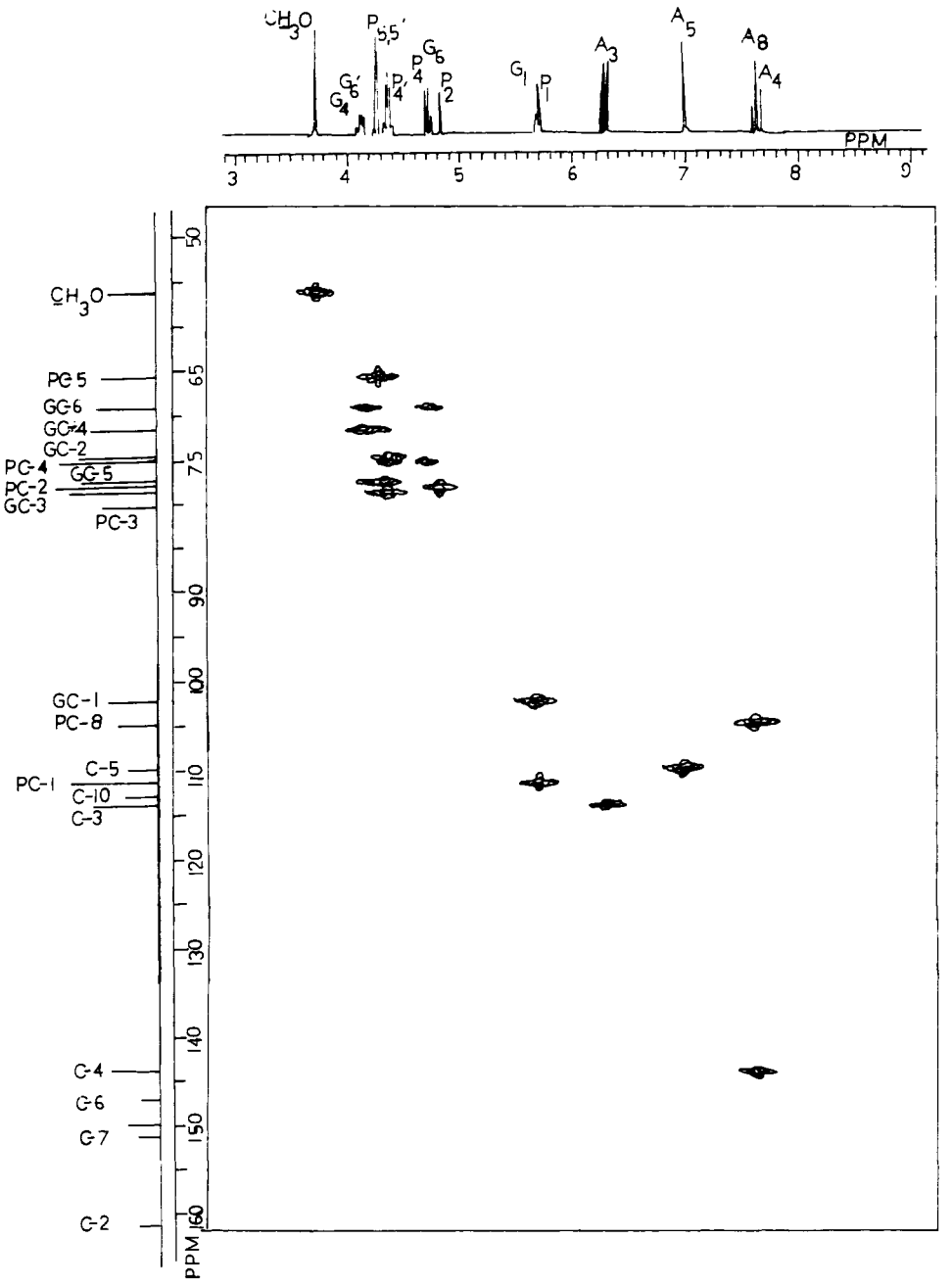

FIGURE 3. 2D Carbon-hydrogen shift correlated spectrum of **1**. A = aglycone, P = D-apiose, G = D-glucose, C = aglycone carbons, PC = D-apiose carbons, GC = D-glucose carbons.

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