Chemistry of Natural Compounds, Vol. 46, No. 2, 2010

CHEMICAL CONSTITUENTS OF *Rhododendron lepidotum*

K. Ahmad,[1*] Shakeel-u-Rehman,[1] A. M. Chisti,[1]
A. S. Shawl,[1] and S. C. Taneja[2]

UDC 547.972

*Two new coumarin glycosides, 7-*O-β-*D-glucopyranosyl-8-methoxybenzopyranone (**1**) and 7-hydroxy-8-*O-β-*glycosylbenzopyranone (**2**), are reported along with the isolation of 7,8-dihydroxy coumarin (daphnetin) from* Rhododendron lepidotum *(aerial part).*

Keywords: coumarin glycosides, daphnetin, *Rhododendron lepidotum*.

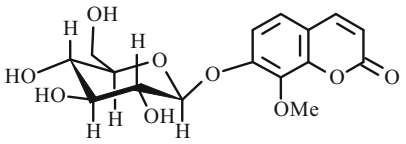
The genus *Rhododendron* comprises more than 900 species in the world, 600 of which are found in China [1]. *Rhododendron lepidotum* (Ericaceae) is mainly found in the Western Himalayas along with *R. campanulatum*, *R. arboretum*, and *R. anthopogon*. Chemical constituents such as triterpene and flavanone glycosides [2], anti-HIV principles rhodoaurichromanic acid A and rhodoaurichromanic acid B [3], dihydroflavonol glycoside [4], phloroacetophenone glycoside [5], terpenoids [6], iridiod glycosides [7], etc. have been isolated from different species of *Rhododendron* genus. Earlier we isolated two new compounds from *Rhododendron lepidotum* [8].

The aerial parts of *Rhododendron lepidotum* were collected from Sonamarg, Kashmir in September 2007. After proper identification, a voucher specimen (No. 1455/93) was deposited in the Herbarium of the Institute. The dried plant material, 800 g, was defatted with hexane and then extracted with methanol (cold extraction). After removal of the solvent under vacuum, the methanol extract (112 g) thus obtained was subjected to column chromatography over silica gel. Repeated column chromatography yielded two compounds, **1** (25 mg) and **2** (35 mg).

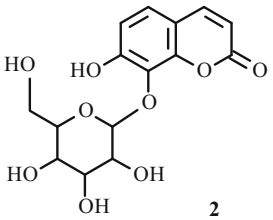
Compound **1** was obtained as a whitish amorphous powder, mp 185°C; $[\alpha]_D^{25}$ –62° (*c* 1.0, MeOH). It was assigned the molecular formula $C_{16}H_{18}O_9$ as determined from ESI-MS, *m/z* at 354, which is supported by its elemental analysis and NMR data. The IR spectrum showed the absorption bands of an OH group (3463 cm^{-1}), a CO group (1724 cm^{-1}), and an aromatic moiety (1610 and 1578 cm^{-1}). The ^{1}H NMR spectrum showed two doublets at δ 7.90 (1H, d, J = 9.5 Hz) and 6.28 (1H, d, J = 9.5 Hz) and another pair of doublets at 7.11 and 7.19 with J = 8.6 Hz, besides other signals in the nonaromatic range. In the aromatic region the ^{13}C NMR and DEPT spectra showed nine signals in the range of δ_C 113 to δ_C 160 (aromatic region) and six resonances at δ 103.3 (C-1′), 78.3 (C-3′), 76.7 (C-5′), 74.2 (C-2′), 70.9 (C-4′), and 61.8 (C-6′) [glycosidic carbons] and a methoxyl carbon at δ 55.3. From the above data, compound **1** can be taken as a coumarin glycoside, in which the two doublets at δ 6.28 and 7.90 account for H-3 and H-4, respectively. The proton resonances at δ 4.96 (1H, d, J = 7.15 Hz), 3.68–3.93 (3H, m), and 3.52 (5H, m) are attributed to hexose and a methoxyl group. The above spectral data are more or less similar to that of rhodonin except for the signals in the glycosidic region [8]. Two above doublets (J = 8.6 Hz) with the difference of chemical shifts ≈0.1 ppm correspond to the H-5 and H-6 protons. Unlike that of rhodonin, which bears a pentose moiety at C-7, the sugar part in compound **1** can be clearly characterized as glucose on the basis of the above ^{1}H and ^{13}C NMR spectrum. The coupling constant 7.1 Hz between H-1′ and H-2′ and the ^{13}C NMR value of the anomeric carbon at δ 103.3 indicate that the sugar moiety is attached to the coumarin skeleton through a *β*-linkage. Furthermore, acid hydrolysis of compound **1** afforded 7-hydroxy-8-methoxycoumarin, which was identified by comparison of its ^{13}C NMR spectrum and melting point with that reported in the literature [9]. Thus, the structure of compound **1** was elucidated as 7-*O*-*β*-D-glucopyranosyl-8-methoxybenzopyranone.

1) Indian Institute of Integrative Medicine (CSIR) Sanatnagar Srinagar, Jammu & Kashmir, India, 190005, e-mail: kabhat@iiim.res.in; 2) Indian Institute of Integrative Medicine (CSIR) Jammu, India, 180001. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 165–166, March–April, 2010. Original article submitted November 12, 2008.

0009-3130/10/4602-0195



1



2

Compound **2** was obtained as a yellow powder, mp 195°C; $[\alpha]_D^{25}$ –25° (*c* 1.0, MeOH). Its molecular formula was determined as $C_{15}H_{16}O_9$ on the basis of the ESI-MS peak at 363 $[M + Na]^+$, which was confirmed by elemental analysis. The IR (KBr, ν, cm^{-1}) spectrum showed the absorption bands of OH group (3535), a CO group (1723), and an aromatic moiety (1613, 1579). The UV spectrum with λ_{max} at 220 and 368 corresponds to a coumarin nucleus. The ^1H NMR spectrum showed four equivalent doublets in the aromatic region at δ 7.78 and 6.14 (J = 9.5 Hz) and δ 7.20 and 6.79 (J = 8.6 Hz). This ^1H NMR spectrum is quite similar to that of daphnetin except for a singlet at δ 4.68 (1H) and two multiplets at δ 3.63–3.73 and δ 3.34–3.38 (equivalent to 3H each). These signals can be attributed to a hexose moiety. The nature of the sugar part was determined by hydrolyzing compound **2** with 1.0 N HCl and comparing its ^1H NMR values with that of mannose.

EXPERIMENTAL

Materials and Methods. ^1H NMR spectra were recorded as δ values at 200 MHz and ^{13}C NMR at 50 MHz using deuterated DMSO-d_6 as a solvent and TMS as internal standard. Infrared spectra were recorded as KBr pellets in cm^{-1} on a Hitachi 270–30 spectrophotometer.

Melting points were determined on a BUCHI melting point apparatus. UV spectra were scanned in methanol on Specord S100. Mass spectra were recorded using a Bruker Daltonics electrospray ionization spectrometer. Column chromatography was run using silica gel (60–120 mesh). TLC plates were visualized under UV light and after exposure to iodine vapor in an iodine chamber.

Plant Material. The aerial parts of *Rhododendron lepidotum* were collected from Sonamarg, Kashmir in September 2007. After proper identification, a voucher specimen (No. 1455/93) was deposited in the Herbarium of the Institute.

Extraction and Isolation. Air-dried and coarsely powdered plant material (aerial part, 800 g) was defatted with hexane for 48 h. The defatted material was dried and extracted with methanol for 48 h. The methanolic extract thus obtained was concentrated under reduced pressure to give a crude extract, 112 g. This extract was dissolved in the minimum amount of methanol and adsorbed on silica gel to form a slurry. The dried slurry was subjected to column chromatography over silica gel. The column was eluted with different percentages of petroleum ether and ethyl acetate and finally with methanol. The following compounds were isolated.

7-*O*-β-D-Glucopyranosyl-8-methoxybenzopyranone (1). $C_{16}H_{18}O_9$. Mp 185°C; $[\alpha]_D$ –62° (*c* 1.0, MeOH); IR bands (KBr, ν, cm^{-1}): 3362, 2896, 1724, 1610, 1578, 1179, 1072, 1023, 832; ^1H NMR (200 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.90 (1H, d, J = 9.5, H-4), 7.19 (1H, d, J = 8.6, H-5), 7.11 (1H, d, J = 8.6, H-6), 6.28 (1H, d, J = 9.5, H-3), 4.96 (1H, d, J = 7.15, H-1′), 3.68–3.93 (3H, m, Glc-H), 3.52 {5H, m; OMe(3H) and Glc-H (2H)}; ^{13}C NMR (50 MHz, DMSO-d_6): 160.8 (C-2), 149.1 (C-7), 145.2 (C-4), 143.6 (C-9), 135.2 (C-8), 118.9 (C-5), 115.5 (C-10), 114.3 (C-6), 113.2 (C-3), 103.3 (C-1′), 78.3 (C-3′), 76.7 (C-5′), 74.2 (C-2′), 70.9 (C-4′), 61.8 (C-6′), 55.3 (OMe); ESI-MS: *m/z* 354 $[M^+]$.

7-Hydroxy-8-*O*-β-glycosylbenzopyranone (2). $C_{15}H_{16}O_9$. Mp 195°C; $[\alpha]_D^{25}$ –25° (*c* 1.0, MeOH); IR bands (KBr, ν, cm^{-1}): 3535, 3320, 1723, 1613, 1579, 1084, 1068, 1023, 972, 843; ^1H NMR (200 MHz, DMSO-d_6, δ, ppm, J/Hz): 7.78 (1H, d, J = 9.5, H-4), 7.20 (1H, d, J = 8.6, H-5), 6.79 (1H, d, J = 6, H-6), 6.14 (1H, d, J = 9.5, H-3), 4.68 (1H, s, H-1′), 3.63–3.73 (3H, m, Man-H), 3.34–3.38 (3H, m, Man-H); ^{13}C NMR (50 MHz, DMSO-d_6): 161.8 (C-2), 154.1 (C-7), 147.9 (C-9), 145.0 (C-4), 131.6 (C-8), 124.5 (C-5), 113.5 (C-3), 112.7 (C-10), 111.3 (C-6), 105.3 (C-1′), 77.1 (C-3′), 76.4 (C-5′), 74.1 (C-2′), 69.5 (C-4′), 60.8 (C-6′); ESI-MS: *m/z* 363 $[M + Na]^+$.

REFERENCES

1. C. Zhao, X. N. Li, Y. Z. Liang, H. Z. Fang, L. F. Huang, and F. Q. Guo, *Chemometr. Intel. Lab. Syst.*, **82**, 218 (2006).
2. H. Takahashi, S. Hirata, H. Minami, and Y. Fukuyama, *Phytochemistry,* **56**, 875 (2001).
3. Y. Kashiwada, K. Yamazaki, Y. Ikeshiro, T. Yamagishi, T. Fujioka, K. Mihashi, K. Mizuki, M. L. Cosentino, K. Fowke, L. S. Morris-Natschke, and L. Kuo-H, *Tetrahedron*, **57**, 1559 (2001).
4. E. Chosson, A. Chaboud, J. A. Chulia, and J. Raynaud, *Phytochemistry,* **49**, 1431 (1998).
5. J. Chosson, A. Chaboud, J. A. Chulia, and J. Raynaud, *Phytochemistry*, **47**, 87 (1998).
6. A. J. Kloocke, Y. H. Mei, C. F. Shin, and I. Kubo, *Phytochemistr*y, **25**, 1637 (1986).
7. C. Q. Fan, W. M. Zhao, B. Y. Ding, and W. G. Qin, *Fitoterapia*, **72**, 449 (2001).
8. R. Khan, A. S. Shawl, M. Tantray, and M. S. Alam, *Fitoterapia,* **79**, 232 (2008).
9. W. Zhang, Y. H. Shen, R. H. Liu, C. Zhang, H. S. Chen, P. Fu, L. Shan, and W. D. Zhang, *Chem. Nat. Comp*., **43**, 317 (2007).