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BIFLORIN, A CHROMONE-*C*-GLUCOSIDE FROM *PANCRATIUM BIFLORUM**

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Key Word Index—*Pancratium biflorum*; Amaryllidaceae; roots; chromone-*C*-glucoside; biflorin; 5,7-dihydroxy-2-methylchromone-C_6-β-D-glucopyranoside; chromone–metal ion complex; phosphodiesterase inhibitor.

Abstract—A new polyoxygenated chromone-*C*-glucoside, named biflorin, was isolated from the roots of *Pancratium biflorum* collected at flowering time. The structure of the compound was established as 5,7-dihydroxy-2-methylchromone-C_6-β-D-glucopyranoside on the basis of comprehensive spectral analyses (UV, IR, ^{1}H NMR, MS, $[\alpha]_D$) and crucial chemical transformations of the compound and its derivatives. The biochemical significance of the occurrence and ontogenic variations of biflorin and other polyoxygenated chromones in the title species is discussed.

INTRODUCTION

In a recent paper [1], we reported the isolation and characterization of two polyoxygenated free chromones (**2** and **3**) and a polyoxygenated glucosyloxy-chromone (**4**) in the flowering bulbs of *Pancratium biflorum* Roxb. This was the first report of the occurrence of chromones in a member of the family Amaryllidaceae and of **2** and **3** in nature. The glucosyloxy-chromone **4** has been reported once before in *Tecomella undulata* (Bignoniaceae) [2]. We now wish to report the natural occurrence of a new chromone-*C*-glucoside, named biflorin, in the root extracts of the title species. The possible role of biflorin in plant biochemistry is also appraised.

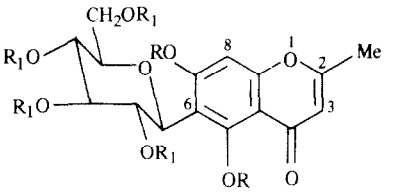
RESULTS AND DISCUSSION

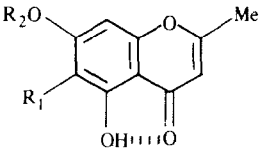
The MeOH extracts of dry and milled roots showed, on analytical TLC, three strongly polar phenolic spots. Column chromatography of the mixture over polyamide afforded biflorin as the major compound, mp 300–303°, $C_{16}H_{18}O_9 \cdot H_2O$ ($[M]^+$ and elemental analyses). Its UV and IR data were characteristic of a 5,7-dihydroxy-chromone derivative [1, 3]. Biflorin also responded to the benzidine–metaperiodate test for polyols. However, it resisted a conventional hydrolysis with dilute HCl, but refluxing with HI and phenol afforded 5,7-dihydroxy-2-methylchromone (**2**) [1]. Oxidation with $FeCl_3$ gave arabinose and glucose. Methylation of biflorin with Et_2O–CH_2N_2, in MeOH–DMF, followed by acetylation of the Me ether derivative afforded a di-*O*-Me ether tetra-acetate. Biflorin is, therefore, a chromone-*C*-glucoside. The ^{1}H NMR spectra of the compound and its di-*O*-Me ether tetra-acetate derivative (**5**) brought further clarity to its structure. Thus, the chemical shifts of the acetoxyl protons in **5** are in good agreement with those of the corresponding protons of acetylated flavonoid- and xanthone-*C*-β-D-glucopyranosides [4, 5]. Application of NOE to aromatic [6] and xanthone systems [7, 8] provided useful information for locating substituent groups. In the NOE studies of biflorin, saturation of the C-2 Me protons caused collapse of the H-3 resonance into a sharp singlet and area enhancements of both H-3 and the aromatic protons by *ca* 22 and 12%, respectively. No NOE was observed for any other proton. Likewise, NOE was observed for the C-2 Me when the aromatic proton was irradiated. The aromatic proton was thus located at C-8 and the glucoside linkage must therefore be at the C-6 position. Mass spectral data provided further supporting evidence to the above assignments. The relative abundance of the fragment ion and $[M]^+$ peaks of biflorin and its di-*O*-Me ether tetraacetate derivative (**5**) was similar to those observed for the corresponding compounds in C-2 glucosylated xanthones [9] and C-6 glucosylated flavones [10]. Consequently, biflorin is assigned the 5,7-dihydroxy-2-methylchromone-C_6-β-D-glucopyranoside structure (**1**). The compound has not been encountered before in nature nor has it been prepared synthetically.

Polyoxygenated chromones (fully acetate-derived) are sparsely distributed in higher plants in comparison to flavonoids (prephenate–acetate) and xanthones (shikimate/prephenate–acetate). Again, while a large number of flavone-*C*- [11] and xanthone-*C*-glucosides [12] have been encountered in nature, the natural occurrence of a chromone-*C*-glucoside has only been reported once before (aloesin from bitter aloes) [13]. Biflorin is thus the second example of a naturally occurring chromone-*C*-glucoside. The present investigation with several members of the Amaryllidaceae has also suggested their rare occurrence. It seems likely that polyoxygenated chromones are a typical taxonomic character in higher plants. Further work about their specificity (at species/genus/family level) in the Amaryllidaceae is currently being conducted.

Another important aspect of this investigation constituted the ontogenic variation of **1**–**4** in *P. biflorum*. These chromones appeared in detectable quantities only during the early flowering stage and were present in all parts of the plant. However, the glucosyl chromone **1** and the glucosyloxy-chromone **4** were the major phenolic constituents of the roots and bulbs, respectively. The

*Part 5 in the series "Chemical Constituents of Amaryllidaceae". For Part 4 see ref. [19].





1 R = R_1 = H

2 R_1 = R_2 = H

5 R = Me, R_1 = Ac

3 R_1 = OH, R_2 = Me

4 R_1 = H, R_2 = β-D-glc

chromones **1**–**4** formed stable metal ion complexes with divalent Cu, Zn and Mo, and were able to translocate these ions from the rhizosphere to all parts of the plant. This finding has relevance to two related observations. (1) Alkaloid production in *P. biflorum* and in many other Amaryllidaceae plants is highest in the early flowering stage. (2) Metal ions play an important role in the biomimetic synthesis of a number of Amaryllidaceae alkaloids. Thus, in the biomimetic synthesis of maritidine [14] and oxocrinine [15] the success of the oxidative stage was ascribed to the intramolecular complex formation between the metal ion (V^{5+}/Tl^{3+}) and the substrate (norbelladine equivalent).

Like khellin, biflorin exhibited inhibitory activity to phosphodiesterase and spared cyclic nucleotides when tested according to published procedures [16, 17]. This property of biflorin appears to reflect its general ability to activate phosphokinase enzymes which are important for the active growth of the producer organism.

EXPERIMENTAL

The general procedures were those reported recently [1].

Isolation of biflorin. Dried and milled roots (408 g) of *P. biflorum* Roxb., collected at flowering (August–September) from a garden of the Banaras Hindu University, were continuously extracted (Soxhlet) with MeOH (30 hr). The MeOH concentrate was processed for phenolic constituents in the usual way. The phenolic fraction showed, on analytical TLC (silica gel G), three ferric-positive spots, R_f 0.55 (minor), 0.62 (major), 0.78 (minor) ($CHCl_3$–MeOH–HOAc, 18:1:1). The mixture was dissolved in MeOH and chromatographed on a column of polyamide (Machery-Nagel SC_6) using MeOH–H_2O as eluant. From the middle fractions of MeOH–H_2O (1:1) eluates, biflorin was obtained as off-white micro-crystals (82 mg), mp 300–303° (dec.); R_f 0.62; $[\alpha]_D^{28}$ +24.4° (*c* 0.34; pyridine); UV λ_{max}^{MeOH} nm (log ε): 234 (4.35), 255 (3.70), 280 (3.62), 325 sh (3.20); $\lambda_{max}^{MeOH\ NaOMe}$ nm: 234, 255 sh, 278, 305 sh, 32, 396; $\lambda_{max}^{MeOH-AlCl_3}$ nm: 242, 262, 295, 335, 395; IR ν_{max}^{Nujol} cm^{-1}: 3400 (br, OH), 1660 (benz-pyrone CO), 1618, 1610, 1595, 1005; ^1H NMR (DMSO-d_6): δ13.2 (1H, *s*, exchangeable with D_2O, C-5 OH), 6.30 (1H, *s*, H-8), 5.88 (1H, *d*, *J* = 0.8 Hz, H-3), 4.85 (1H, *d*, *J* = 10 Hz, β-D-glucoside anomeric H), 2.3 (3H, *d*, *J* = 0.8 Hz, C-2 Me); MS *m/z* (rel. int.): 354 $[M]^+$ (42), 325 (100), 206 (38), 205 (44), 177 (7), 165 (5) (m* from 206 → 205 transition appeared at *m/z* 204.0) (Found: C, 51.0; H, 5.7. $C_{16}H_{18}O_9 \cdot H_2O$ requires: C, 51.6; H, 5.3%).

5,7-Di-O-methylbiflorin-2′,3′,4′,6′-tetra-acetate (**5**). Biflorin (21 mg), in DMF (5 ml) and MeOH (5 ml), was treated with Et_2O–CH_2N_2. The mixture was kept at room temp. overnight. After removing solvent, the process was repeated to ensure complete methylation of the C-5 OH. The major product, 5,7-di-*O*-methylbiflorin, crystallized from MeOH as colourless needles, mp 222–224°; *m/z* (rel. int.): 382 $[M]^+$ (62), 367 (100). It was acetylated with Ac_2O and pyridine at room temp. and the product crystallized from MeOH as colourless needles, mp 173–176°; ^1H NMR ($CDCl_3$): δ7.14 (1H, *s*, H-8), 5.90 (1H, *d*, *J* = 0.8 Hz, H-3), 5.0 (1H, *m*, anomeric H), 3.98 (3H, *s*, OMe), 3.95 (3H, *s*, OMe), 2.32 (3H, *d*, *J* = 0.8 Hz, C-2 Me), 2.04–1.70 (12H, OAc); MS *m/z* (rel. int.): 550 $[M]^+$ (16), 491 $[M-59]^+$, (100) (Found: C, 56.3; H, 5.5. $C_{26}H_{30}O_{13}$ requires: C, 56.7; H, 5.4%).

Treatment of biflorin with HI and phenol. A mixture of biflorin (52 mg), PhOH (0.22 g) and HI (specific gravity 1.7, 1 ml) was refluxed for 6 hr. The reaction mixture was cooled and an aq. soln of $NaHSO_3$ (10%, 5 ml) added when a ppt. appeared. The ppt. was collected by filtration, washed with H_2O and crystallized from Me_2CO–MeOH as colourless needles, mp 271–272°. The identity of the product with 5,7-dihydroxy-2-methylchromone was established by direct comparison (mp, mmp, co-TLC, IR) with a reference sample [1].

Oxidation of biflorin with $FeCl_3$. Biflorin (44 mg) was refluxed with an aq. soln (5 ml) of $FeCl_3$ (0.2 g). The reaction mixture was centrifuged and the supernatant was triturated with Dowex-1 (1 × 8-50, HCO_3^-, under 0.2 M $KHCO_3$) until effervescence ceased and the pH of the soln was neutral. The soln was then filtered and the filtrate passed through two successive columns of resin (Dowex-50W, dry mesh 100–200, H^+-form, and Dowex-1, 1 × 8-100, HCO_3^- in 0.02 M $KHCO_3$). The aq. eluate (*ca* 100 ml) from the second column was concd (*ca* 1 ml), under red. pres. and subjected to prep. PC (Whatman No. 1, EtOAc–pyridine–H_2O, 13:5:4; using $AgNO_3$ and aniline oxalate as the staining reagents) when arabinose (major) and glucose (minor) were detected. The identity of the two sugars was further confirmed by GC (5% Silar 10C on Gas-chrom Q, temp. 210°, flow rate 40 ml/min, N_2) of the corresponding mixture of the alditol acetates prepared according to a published procedure [18].

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REFERENCES

1. Ghosal, S., Singh, S. P., Bhagat, M. P. and Kumar, Y. (1982) *Phytochemistry* **21**, 2943.
2. Gujral, V. K., Gupta, S. R. and Verma, K. S. (1979) *Phytochemistry* **18**, 181.
3. Robeson, D. J., Ingham, J. L. and Harborne, J. B. (1980) *Phytochemistry* **19**, 2171.
4. Hillis, W. E. and Horn, D. H. S. (1965) *Aust. J. Chem.* **18**, 531.
5. Aritomi, M. and Kawasaki, T. (1970) *Chem. Pharm. Bull. (Tokyo)* **18**, 2327.
6. Bachers, G. E. and Schaefer, T. (1971) *Chem. Rev.* **71**, 617.
7. Arisawa, M., Morita, N., Kondo, Y. and Takemoto, T. (1973) *Chem. Pharm. Bull. (Tokyo)* **21**, 2562.
8. Ghosal, S. and Biswas, K. (1979) *Phytochemistry* **18**, 1029.
9. Aritomi, M., Komori, T. and Kawasaki, T. (1970) *Ann. Chem.* **734**, 91.
10. Prox, A. (1968) *Tetrahedron* **24**, 3697.
11. Chopin, J. and Bouillant, M. L. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.), p. 633. Chapman & Hall, London.
12. Ghosal, S., Sharma, P. V. and Chaudhuri, R. K. (1974) *J. Pharm. Sci.* **63**, 1286.
13. Haynes, J. L., Holdsworth, D. K. and Russell, R. (1970) *J. Chem. Soc. C* 2581.
14. Schwartz, M. A. and Holton, R. A. (1970) *J. Am. Chem. Soc.* **92**, 1090.
15. Schwartz, M. A., Rose, B. F. and Visnuvajjala, B. (1973) *J. Am. Chem. Soc.* **95**, 612.
16. Gilman, A. G. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 305.
17. Asakawa, T., Russell, T. R. and Ho, R. J. (1976) *Biochem. Biophys. Res. Commun.* **68**, 682.
18. Sloneker, J. H. (1972) in *Methods in Carbohydrate Chemistry* (Whistler, R. L. and BeMiller, J. N., eds.), Vol. VI, p. 20. Academic Press, London.
19. Chattopadhyay, S., Chattopadhyay, U., Mathur, P. P., Saini, K. S. and Ghosal, S. *Planta Med.* (in press).