Phytochemistry, Vol. 21, No. 12, pp. 2943–2946, 1982.
Printed in Great Britain.

0031-9422/82/122943-04$03.00/0
Pergamon Press Ltd.

THREE CHROMONES FROM BULBS OF *PANCRATIUM BIFLORUM**

SHIBNATH GHOSAL, SHRIPATI SINGH, MAHENDRA P. BHAGAT and YATENDRA KUMAR

Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India

(*Received* 8 *December* 1981)

Key Word Index—*Panacratium biflorum*; Amaryllidaceae; bulb; polyoxygenated chromones; 5,7-dihydroxy-2-methylchromone; 5,6-dihydroxy-7-methoxy-2-methylchromone; glucosyloxychromone; 7-glucosyloxy-5-hydroxy-2-methylchromone; chromone–metal complex.

Abstract—Two polyoxygenated chromones, 5,7-dihydroxy-2-methylchromone (**1**) and 5,6-dihydroxy-7-methoxy-2-methylchromone(**2**), and a glucosyloxychromone, 7-glucosyloxy-5-hydroxy-2-methylchromone (**3**), have been isolated from an extract of the bulbs of *Pancratium biflorum* collected when the plants were in flower. The compounds were characterized by chemical transformation, comprehensive spectral evidence and synthesis. **2** and **3** are new naturally occurring chromones, while **1** has been detected only once before in *Daucus carota* roots, as a stress metabolite.

INTRODUCTION

The literature dealing with Amaryllidaceae alkaloids shows no signs of abatement although practically nothing is known about the non-nitrogenous constituents of members of this family. The genus *Pancratium* (family Amaryllidaceae, sub-family Amaryllidoidae), for example, has attracted considerable attention due to the complex structural types of its alkaloids [2, 3] and the significant therapeutic properties of some of these compounds [3] but no information was available about the non-nitrogenous constituents of this genus until this investigation. In connection with our work on the anti-fungal principles from plants [4, 5], we investigated both alkaloidal [3] and phenolic constituents of the *P. biflorum* Roxb. for the first time. We propose to investigate both alkaloidal and phenolic constituents of *ca* 6 *Pancratium* species growing in India.

P. biflorum grows abundantly in the upper Gangetic Plain and is also cultivated in gardens as an ornamental plant and for medicinal purposes. Extracts of its flowers and bulbs are used in popular medicine in the treatment of earache, chest ailments and in fungal diseases. We report here the isolation and characterization of two polyoxygenated chromones (compounds 1 and 2) and a glucosyloxy chromone (compound 3) from the bulb extract of this species collected in flower. The investigation was repeated for two consecutive years to establish that the elaboration of chromones is a normal metabolic excursion of *P. biflorum* and is not under conditions of any stress. The biochemical significance of these chemical characters is also appraised.

*Part 2 in the series "Chemical constituents of Amaryllidaceae". For part 1 see ref. [1].

RESULTS AND DISCUSSION

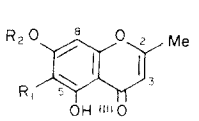
The phenolic constituents were obtained from the dried and powdered defatted bulbs by re-extracting the dark viscous EtOH extract with hot Me_2CO. The mixture in the Me_2CO-soluble fraction was then separated by CC and TLC over Si gel and polyamide. Two polyoxygenated chromones (compounds 1 and 2) and a glucosyloxychromone (compound 3) were obtained in quantities sufficient for their complete characterization.

Compound 1. This compound, $C_{10}H_8O_4$ (elemental analysis and M^+), mp 268–270°, gave a violet colour with EtOH–ferric chloride and showed UV absorption maxima in EtOH characteristic of 5, 7-dioxygenated chromones [6]. The bathochromic shift of the UV maxima observed by adding $AlCl_3$ indicated the presence of a free OH group *peri* (C-5 position) to the carbonyl group. Analogous behaviour was reported in flavonoids [7]. A bathochromic shift of the maxima was also observed by adding NaOAc which suggested [7] a free OH group at C-7. The IR spectrum showed bands characteristic of Ar–OH-benz-γ-pyrones. The structure of the compound followed from the mass and ^{1}H NMR spectra. The mass spectral fragmentation exhibited features characteristic of 5,7-dihydroxy-2-methylchromone. Thus, the compound exhibited fragment-ion peaks arising from pathway I and pathway II, commonly encountered in flavones [8]. The ^{1}H NMR spectrum of the compound showed signals due to two aromatic protons, as *m*-split doublets, one olefinic proton, as a vinylogously coupled doublet, a C–Me group coupled with the olefinic proton, and a strongly chelated proton associated with the C-5 OH. The compound on methylation with ethereal diazomethane afforded eugenin (**4**) [9]. These data suggest 5,7-dihydroxy-2-methylchromone (**1**) as its structure. The chromone has been

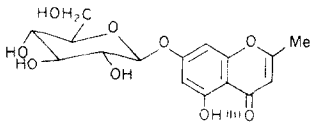
previously detected [9], in traces, in *Daucus carota* roots as an abnormal metabolite formed under conditions of stress [9]. It was identified before [9] on the basis of limited data, viz. UV maxima and TLC comparison with a synthetic sample, prepared by a published procedure [10]. This is the first demonstration of the occurrence of **1** as a normal metabolite, in appreciable quantity, in *P. biflorum*.

Compound 2. This compound, $C_{11}H_{10}O_5$ (elemental analysis and M^+), mp 242–244°, is a dihydroxy-monomethoxy-2-methylchromone. It showed UV maxima in EtOH and in EtOH–$AlCl_3$ closely similar to those of **1**. The position of the UV maxima remained unaltered by adding NaOAc. There was, however, a hyperchromic effect of the K-band [11] indicating the presence of a substituted oxygen function at C-7 and an additional OH function *ortho* to the C-5 OH. The UV chromophoric system was destroyed in EtOH–NaOH supporting the presence of an *ortho*-dihydroxy function [12]. The mass and ^{1}H NMR spectra of the compound suggested a dihydroxy-monomethoxy-2-methylchromone structure. The compound formed a diacetate, $C_{15}H_{14}O_7$, in which the aromatic proton singlet was shifted to *ca* 0.5 ppm downfield relative to its position in the corresponding permethyl ether. This suggested the presence of an acetoxy function *para* to the aromatic proton (H-8). This had precedents in flavones [7] and xanthones [13]. Thus, 5,6-dihydroxy-7-methoxy-2-methylchromone, structure **2**, was assigned to this compound. The compound has not been encountered before in nature nor has it been prepared synthetically.

Compound 3. This compound, $C_{16}H_{18}O_7 \cdot H_2O$, obtained as a hygroscopic solid, was optically active. It showed UV maxima similar to those of **1**. There was no change in the UV maxima in the presence of NaOAc, the C-7 oxygen function was, therefore, substituted. Addition of $AlCl_3$, however, produced a bathochromic shift of the UV maxima, thereby suggesting the presence of a free OH group *peri* (C-5 position) to the carbonyl function. As expected for an *O*-glycoside, the mass spectrum of the compound showed only the M^+ of the aglucone (*m/z* 192); hydrolysis with emulsin gave glucose and the aglucone which was identical with **1**. The negative sign of rotation ($[\alpha]_D$) of the compound and its acetate derivative, as also hydrolysis of the parent compound with emulsin, suggested a β-linkage between the aglucone and the sugar moiety. The ^{1}H NMR spectrum of the compound showed signals for two aromatic protons, as *m*-split doublets, one olefinic proton, one glucosyl anomeric proton, one Me group on a double bond, and a strongly chelated OH group. The compound formed a tetraacetate, under mild conditions of acetylation, which exhibited a M^+ in its mass spectrum at *m/z* 522. Apart from the M^+, significant fragment-ion peaks appeared due to tetraacetylglucose (*m/z* 331) and dihydroxy-2-methylchromone (*m/z* 192) moieties. The identity of the tetraacetate derivative was established as 5-hydroxy-7-*O*-(2′, 3′, 4′, 6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-methylchromone by its synthesis from **1** according to a published procedure of glucosidation as applicable to flavonoid and xanthone series [14]. Compound **3** is the first example of a naturally occurring glucosyloxychromone. It was present, in appreciable quantity, also in the roots of *P. biflorum*.



1 $R_1 = R_2 = H$
2 $R_1 = OH$, $R_2 = Me$
4 $R_1 = H$, $R_2 = Me$



3

The three chromones (**1–3**) appeared in appreciable quantity only during the flowering time (September–October) of *P. biflorum* when the vegetative growth of the species commences. At this time, the roots contain **3** as the major entity. The purpose of the elaboration of these compounds could be either prevention of ingress of predatory fungi from the rhizosphere or growth promotion of the species, or both. In order to examine these hypotheses, the three compounds were separately tested for their possible antifungal activity against a number of pathogenic *Fusaria* and *Aspergilli*, commonly encountered in the area of vegetation of *P. biflorum*. No antifungal activity was observed up to a concentration of 10^{-5} M of each compound. The growth-promoting property of the three chromones was indicated by the acceleration of seed germination and abundant root growth in lettuce and radish. Another role of the chromones could be associated with the transport of metal ions (micronutrients) and/or metal-ion buffering *in vivo*. All three compounds formed stable complexes with metal ions (Cu^{2+}, Zn^{2+}, Fe^{2+}). The uptake of Cu and Zn ions by the seedlings of *P. biflorum* was enhanced by the addition of the chromones in nutrient media in which the roots were submerged. The comcomitant increase in the concentration of the metal ions in the leaves of the chromone–metal-complex-treated plants was estimated by atomic absorption spectroscopy. The initial results are sufficiently impressive to warrant the expectation that the role of chromones in plant biochemistry will repay further and more detailed study.

EXPERIMENTAL

All mps are uncorr. UV spectra were recorded in aldehyde-free EtOH unless otherwise stated. The shift reagents were prepared according to [7]. IR spectra were determined in Nujol and only the major bands are quoted. ^{1}H NMR spectra were obtained at 90 MHz using TMS as int. standard. MS spectra were determined at 70 eV. Sepn by CC was carried out using Si gel (60–120 mesh, BDH) or polyamide (Macherey–Nagel SC_6) as adsorbents. TLC was carried out on Si gel G (Merck) using $CHCl_3$–HOAc (24:1, solvent 1) and EtOAc–MeOH–H_2O (20:5:3, solvent 2) as developers. I_2 vapour, EtOH–$FeCl_3$ and short-wave UV lamp (λ 254 nm) were used for staining and visualization purposes.

Ashes obtained from weighed quantities of leaves of control and chromone–metal-complex treated plants were leached with HCl. The metal ion (Cu^{2+}, Zn^{2+}) concns of aliquots of the acidic solns were determined by atomic absorption spectroscopy.

Plant materials were collected during September–October

of 1979–80, from the Banaras Hindu University campus and were properly identified. The species is now being cultivated in the garden of the Department of Pharmaceutics, Banaras Hindu University.

Extraction. In a typical expt, dried and milled bulbs (*ca* 200 g) were continuously extrd in a Soxhlet with petrol (60–80°) and then with EtOH (30 hr each). The two extracts were separately processed.

Treatment of EtOH extract. The extract was concd to a brown viscous liquid and then triturated with hot Me_2CO. The Me_2CO-soluble portion showed several $FeCl_3$ positive spots on TLC (solvents 1 and 2). The Me_2CO soln was concd and chromatographed over a column of Si gel (24 × 2 cm). Elution was carried out with C_6H_6 (1 l.), C_6H_6–$CHCl_3$ (1:1, 500 ml), $CHCl_3$ (1 l.), and $CHCl_3$–MeOH (49:1, 1 l.). Fractions (100 ml) were collected and monitored by TLC. Initial eluates afforded phytosterols and steryl esters followed by a major phenolic fraction from the middle $CHCl_3$ eluates (fractions 18–22). These were combined and evapd to give a light brown solid (62 mg). It showed two major $FeCl_3$ positive spots on TLC, R_f 0.35 and 0.5 (solvent 1), in the form of a streak. The two components were separated by repeated prep. TLC.

5,7-Dihydroxy-2-methylchromone (**1**). TLC scrapings of the upper R_f zone were extrd with $CHCl_3$–MeOH. The soln was evapd and the residue crystallized from Me_2CO as cream-coloured needles (28 mg), mp 268–270°; UV: λ_{max} nm (log ϵ) 228 (4.30), 250 (4.41), 255 (4.42), 295 (3.89), 325 *sh* (3.52); λ_{max} (EtOH–NaOAc) nm 260 *sh*, 268, 335; λ_{max} (EtOH–$AlCl_3$) nm 254 *sh*, 266, ~310, 365; IR: ν_{max} cm^{-1} 3400 (*br*), 1668, 1625, 1595; MS: *m/z* 192 (M^+, rel. intensity 100%), 164 (88), 163 (24), 152 (22), 124 (48), 69 (62), 43 (11), 39 (14); ^1H NMR ($CDCl_3$–DMSO-d_6): δ 12.70 (1H, *s*, exchangeable with D_2O, C-5 OH), 6.30 (1H, *d*, $J = 2$ Hz, H-8), 6.22 (1H, *d*, $J = 2$ Hz, H-6), 5.95 (1H, *d*, $J = 0.8$ Hz, H-3), 2.30 (3H, *d*, $J = 0.8$ Hz, C-2 Me). (Found: C, 62.12; H, 4.24. $C_{10}H_8O_4$ requires: C, 62.50; H, 4.16.) Methylation of **1** with Et_2O–CH_2N_2 gave a monoMe ether which crystallized from hexane–Me_2CO as plates, mp 117–118°; UV: λ_{max} nm 230, 250, 256, 294, 322 *sh*; MS: *m/z* 206 (M^+, 100%), 178 (5), 177 (42), 176 (11), 163 (14), 135 (5), 69 (7). The physical and spectral properties of the Me ether were consistent with those reported [9] for 5-hydroxy-7-methoxy-2-methylchromone (= eugenin) (**4**).

5,6-Dihydroxy-7-methoxy-2-methylchromone (**2**). From the TLC scrapings of the lower R_f zone **2** was obtained as a microcrystalline solid (18 mg), mp 242–244°; UV: λ_{max} nm (log ϵ) 230 (4.18), 250 (4.30), 258 (4.32), 292 (4.02), ~315 *sh* (3.02); λ_{max} (EtOH–NaOAc) nm (log ϵ) ~255 (4.36), 298 (4.18), ~320 (3.66); λ_{max} (EtOH–$AlCl_3$) nm 260, 265 *sh*, 315, 365; IR: ν_{max} cm^{-1} 3450, 1665, 1612, 1592, 1040; MS: *m/z* 222 (M^+, 100%), 204 (4), 194 (11), 193 (17), 182 (4), 179 (14), 167 (6), 151 (5), 150 (4), 139 (11); 222 → 194 transition, m* 170, 194 → 179 transition, m* 165.5; ^1H NMR ($CDCl_3$–DMSO-d_6): δ 12.68 (1H, *s*, exchangeable with D_2O, C-5 OH), 6.29 (1H, *s*, H-8), 5.94 (1H, *s*, H-3), 3.98 (3H, C-7 OMe), 2.26 (3H, *s*, C-2 Me). (Found: C, 59.28; H, 4.35. $C_{11}H_{10}O_5$ requires: C, 59.45; H, 4.50.) The diacetate of **2** (7 mg) prepared by treatment with Ac_2O (0.5 ml) and NaOAc (0.1 g), under reflux for 3 hr, crystallized from hexane–Me_2CO as colourless crystals, mp 202–203°; MS: *m/z* 306 (M^+, 42%), 264 (64), 222 (100); ^1H NMR ($CDCl_3$): δ 6.77 (1H, *s*, H-8), 2.35–2.30 (6H, C-5,6 OAc); the perMe ether prepared with NaH and MeI, in THF, according to [15] was obtained as a homogeneous gummy material, MS: *m/z* 250 (M^+, 100%), 235 (9), 233 (7), 207 (22); ^1H NMR ($CDCl_3$): δ 6.30 (1H, *s*, H-8), 3.98–3.90 (9H, C-5,6 OMe).

Following further elution of the Si gel column, a second major phenolic fraction was obtained from the later $CHCl_3$–MeOH eluates. Polyamide CC (50 × 2.5 cm) of this fraction using MeOH–H_2O (2:1) as the eluent afforded the glucosyloxychromone as a homogeneous gummy material (88 mg).

7-Glucosyloxy-5-hydroxy-2-methylchromone (**3**). The glucosyloxy chromone was re-ppted from a Me_2CO–MeOH soln by adding Et_2O as a hydroscopic solid, R_f 0.22 (solvent 2); $[\alpha]_D^{28}$ −48.4° (*c* 0.55, C_5H_5N); UV: λ_{max} nm (log ϵ) 228 (4.18), 248 (4.22), 255 (4.23), 290 (3.46); λ_{max} (EtOH–NaOAc) no change in UV maxima; λ_{max} (EtOH–$AlCl_3$) nm 255, ~265, 360; IR: ν_{max} cm^{-1} 3450 (*br*), 1665, 1620, 1605, 1590, 1020; ^1H NMR (DMSO-d_6): δ 12.68 (1H, *s*, exchangeable with D_2O, C-5 OH), 6.32 (2H, *d*, $J = 2$ Hz, H-8, H-6), 5.92 (1H, H-3), 5.08 (1H, *br d*, $J = 10$ Hz, glucosyl anomeric-H), 2.24 (3H, C-2 Me). (Found: C, 51.28; H, 5.04. $C_{16}H_{18}O_9 \cdot H_2O$ requires: C, 51.61; H, 5.38.) Hydrolysis of **3** with emulsin, according to [12] afforded glucose (PPC) and the aglucone **1**. Acetylation of **3** (21 mg) with Ac_2O (2 ml) and pyridine (0.5 ml), at room temp, afforded 5-hydroxy-7-*O*-(2′, 3′, 4′, 6′-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-methylchromone, which crystallized from Me_2CO as colourless crystals, mp 108–110°; $[\alpha]_D^{28}$ −54.8° (*c*0.44, $CHCl_3$); MS: *m/z* 522 (M^+, 5%), 480 (2), 463 (1), 331 (58), 289 (7), 193 (37), 192 (48), 169 (100), 137 (35), 109 (92); ^1H NMR ($CDCl_3$): δ 12.72 (1H, *s*, C-5 OH), 6.64 (1H, *d*, $J = 2$ Hz, H-8), 6.42 (1H, *d*, $J = 2$ Hz, H-6), 6.0 (1H, H-3), 5.3 − 4.9 (4H, *m*, glucosyl protons), 2.34 − 1.98 (15H, C-2 Me and C-2′, 3′, 4′, 6′ OAc).

Synthesis of tetraacetate of **3**. Condensation of **1** (22 mg) in pyridine (10 ml) with α-acetobromoglucose (55 mg) in the presence of Ag_2CO_3 (355 mg), according to [14], afforded a brown gummy material (42 mg). This was acetylated with Ac_2O (2 ml) and pyridine (0.5 ml), at room temp. for 24 hr. The product crystallized from Me_2CO–hexane as colourless crystals (18 mg), mp and mmp corresponding with the tetraacetate of **3**, 108–110°; co-TLC, R_f 0.44 (solvent 1), *m/z* 522 (M^+, 7%).

Acknowledgements—The authors are indebted to Dr. S. K. Roy, Department of Botany, Banaras Hindu University, Varanasi, for identification of the plant species, to Prof. A. W. Frahm, Pharmazeutisches Institut, Bonn, and Dr. B. C. Das, CNRS, Gif-sur-Yvette, for ^1H NMR and MS. S.S. and M.P.B. are grateful to the University Grants Commission, New Delhi, for research fellowships.

REFERENCES

1. Ghosal, S., Rao, P. H., Jaiswal, D. K., Kumar, Y. and Frahm, A. W. (1981) *Phytochemistry* **20**, 2003.
2. Fuganti, C. (1975) in *The Alkaloids* (Manske, R. H. F., ed.), p. 83. Academic Press, New York.
3. Ghosal, S., Saini, K. S., Rao, P. H., Kumar, Y., Jaiswal, D. K. and Chattopadhyay, S. (1981) *Proc. 6th Indo-Soviet Symp., Chemistry of Natural Products*, p. 71. N.C.L., Poone, India.
4. Ghosal, S., Banerjee, S., Chattopadhyay, B. K. and Chakrabarti, D. K. (1978) *Experientia* **34**, 1057.
5. Ghosal, S., Chakrabarti, D. K. and Srivastava, R. S. (1979) *Experientia* **35**,83.
6. Robeson, D. J., Ingham, J. L. and Harborne, J. B. (1980) *Phytochemistry* **19**, 2171.
7. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) in *The Systematic Identification of Flavonoids*. Springer, New York.

8. Mabry, T. J. and Markham, K. R. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds), p. 79. Chapman & Hall, London.
9. Coxon, D. T., Curtis, R. F., Price, K. R. and Levett, G. (1973) *Phytochemistry* **12**, 1881.
10. Gulati, R. C., Seth, S. R. and Venkataraman, K. (1934) *J. Chem. Soc.* 1765.
11. Barros Corrêa, D. De., Fonseca, L. G., Silva, E., Gottlieb, O. R. and Goncalves, S. J. (1970) *Phytochemistry* **9**, 447.
12. Ghosal, S., Sharma, P. V. and Chaudhuri, R. K. (1975) *Phytochemistry* **14**, 2671.
13. Ghosal, S., Biswas, K. and Chaudhuri, R. K. (1977) *J. Chem. Soc. Perkin Trans.* 1, 1597.
14. Chari, V. M., Klapfenberger, R. and Wagner, H. (1978) *Z. Naturforsch.* **336**, 946.
15. Ghosal, S., Srivastava, A. K., Srivastava, R. S., Chattopadhyay, S. and Maitra, M. (1981) *Planta Med.* **42**, 279.