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FREE AND GLUCOSYLOXY ACETOPHENONES FROM *PANCRATIUM BIFLORUM**

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Key Word Index—*Pancratium biflorum*; Amaryllidaceae; flowering bulbs; pseudo-stem fluid; dimethoxy acetophenones; dimethoxy acetophenone-*O*-glucosides; effects on cell growth, viability; prostaglandin synthetase inhibitor.

Abstract—Two new dimethoxy-acetophenone-*O*-glucosides and the known 2,4,6-trimethoxyacetophenone were isolated from the flowering bulbs and pseudo-stem fluid of *Pancratium biflorum*. The structures of the new compounds were established as 4,6-dimethoxyacetophenone-2-*O*-β-D-glucoside and 2,6-dimethoxyacetophenone-4-*O*-β-D-glucoside on the basis of chemical transformation, comprehensive spectroscopic analyses, and synthesis of the aglucones. The biological activity profile of the glucosides and their aglucones is also appraised.

INTRODUCTION

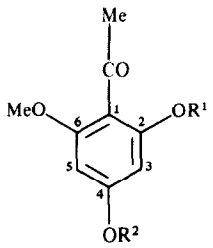
Previously, we reported the occurrence and biochemical significance of three chromones, 5,7-dihydroxy-2-methylchromone, 5,6-dihydroxy-7-methoxy-2-methyl-chromone 5,6,7-trimethoxy-2-methylchromone and two chromone glucosides, 7-glucosyloxy-5-hydroxy-2-methylchromone and 5,7-dihydroxy-2-methyl-6-*C*-glucosylchromone, in the flowering bulbs and roots of *Pancratium biflorum* Roxb. [1, 2]. The Ar-oxygenated 2-methylchromones, according to tenets of biogenesis, are derived from five acetate units via a poly-β-ketide intermediate. Yet another class of acetogenins, the Ar-oxygenated acetophenones, of sparse distribution in the plant kingdom [3, 4], are believed to be derived from four acetate units. These compounds, like the chromones, elicit significant biological activities [3, 4]. However, only one such polyoxygenated acetophenone, 2,4,6-trimethoxyacetophenone, has been reported before in the Amaryllidaceae in *Lycoris sanguinea* Maxim. [5]. Acetogenins and their metabolites never occur alone as they serve a number of significant biochemical purposes for the producer plants [2–4]. It was therefore thought worthwhile to examine in detail the previously partially explored Amaryllidaceae species for Ar-oxygenated acetophenones. In this paper, we report the isolation and characterization of three polyoxygenated acetophenones (free and conjugated) from the flowering bulbs and pseudo-stem fluid of *P. biflorum*. Additionally, their biological activity profile in the producer organism and in animal cells is evaluated.

*Part 31 in the series 'Chemical Constituents of Amaryllidaceae'. For part 30 see ref. [18].

RESULTS AND DISCUSSION

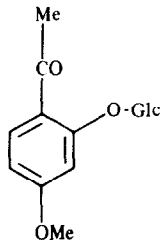
Gradient-solvent separation, TLC and prep. TLC of the fresh methanol extracts of flowering bulbs and pseudo-stem fluid of *P. biflorum* afforded two new acetophenone-*O*-glucosides (**1** and **2**) along with the previously reported 2,4,6-trimethoxyacetophenone [5].

Compound **1**, $C_{16}H_{22}O_9$ (elemental analyses and $[M]^+$), was optically active. Its UV spectrum in methanol and in the presence of acidic and basic shift reagents (see Experimental) was similar to that of a synthetic sample of 2,4,6-trimethoxyacetophenone. It gave a positive benzidine-metaperiodate test for sugars. Enzymic hydrolysis of the compound with β-glucosidase, in pH 5 buffer, liberated glucose (identified as the alditol acetate) [6] and 2-hydroxy-4,6-dimethoxyacetophenone (in equimolar proportion). The ^1H and ^{13}C NMR spectra of compound **1** suggested the structural features of an *O*-glucoside of an Ar-dimethoxylated arylmethylketone. The aglucone showed a bathochromic shift in its UV maxima in the presence of dry aluminium chloride and its IR absorption bands were characteristic of a chelated *ortho*-hydroxyketone. The aglucone was synthesized according to a previously described procedure [7]. Friedel–Crafts acylation of phloroglucinol-tri-*O*-methylether with acetyl chloride, in the presence of dry aluminium chloride, afforded 2-hydroxy-4,6-dimethoxyacetophenone by concomitant *O*-demethylation of the product [7]. It was identical with the aglucone of compound **1**. Treatment of the aglucone with an excess of ethereal-diazomethane afforded 2,4,6-trimethoxyacetophenone. Selective *O*-demethylation of this product with aluminium chloride, in ether, gave 2-hydroxy-4,6-dimethoxyacetophenone which was identical with the aglucone. Hence the compound was assigned the structure of 4,6-dimethoxyacetophenone-2-*O*-β-D-glucoside (**1**). The stereochemistry of the glucosidic linkage was settled as β from the coupling constant of the anomeric proton signal (δ4.88, $J = 7.5$ Hz).



1 R^1 = β-D-Glucoside
R^2 = Me

2 R^1 = Me
R^2 = β-D-Glucoside



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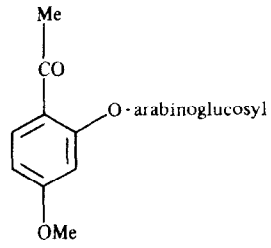

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Compound **2**, $C_{16}H_{22}O_9$, was closely similar to compound **1** in respect of its physical and spectral properties. However, the two compounds differed considerably in their polarity on analytical TLC and in optical rotations. On acidic and enzymic hydrolysis, compound **2** liberated glucose and a phenolic (non-chelated) aglucone (in 1:1 molar ratio). The aglucone was identified as 4-hydroxy-2,6-dimethoxyacetophenone by selective *O*-demethylation of 2,4,6-trimethoxyacetophenone with hydrochloric acid according to a previously described procedure [8]. Hence the compound was assigned the 2,6-dimethoxyacetophenone-4-*O*-β-D-glucoside structure (**2**). To our knowledge, the two Ar-dimethoxyacetophenone-*O*-glucosides (**1** and **2**) have neither been reported before in nature nor prepared synthetically.

In spite of the small molecular structures and sparse distribution in nature, Ar-oxygenated acetophenones have attracted considerable attention because of their significant biological effects in both plant and animal cells. Thus, paeonoside (**3**) and paeonolide (**4**) from *Paeonia suffruticosa* Andl. [9, 10], produced inhibition of blood platelet aggregation and were shown to be responsible for the haemostatic effect of the extract of this herb [11]. The corresponding aglycone, paeonol, was also shown to produce a similar response [12]. Likewise, Ar-(3,4)-dihydroxy acetophenone, one of the main constituents of *Ibex pubescens*, was reported to decrease the membrane fluidity of platelets thereby causing inhibition of their aggregation [13]. It was, therefore, thought worthwhile to examine the biological activity profile of the two new compounds (**1** and **2**) and their aglucones in producer and non-producer cells [14]. The bulbs of *P. biflorum* and *Zephyranthes flava* Roem. & Schult when treated with solutions of **1** or **2** (10^{-3} to 10^{-4} M) showed accelerated formation and linear growth of primary roots. The corresponding aglucones, on the other hand, inhibited the emergence of roots for a prolonged period of time. Similar observations of growth promotion by the *O*-glucosides (**1** and **2**) and inhibition by their aglucones were made in respect of viability and growth of some tumour cells. Thus, both **1** and **2** (10^{-4} M) *in vitro*, markedly potentiated the viability of Ehrlich ascites tumour cells [per cent viability of cells in treated samples (mean ± s.d.): **1**, 98.12 ± 3.0; **2**, 95.64 ± 3.34; control, 78.08 ± 4.07]. The corresponding aglucones, on the other hand, significantly inhibited the viability of these cells. The percent viability in the treated samples, *in vitro*, ranged from 55 to 65 depending on the concentration of the inhibitor (aglucones of **1** and **2**). The aglucones were also examined for their ability to modulate prostaglandin synthetase activity (as determined in terms of PGE_2 and $PGF_{2\alpha}$ production) and inhibition of 5-lipoxygenase (as determined in terms of cytosol 5-hydroxyeicosatetraenoic acid, 5-HETE). Both the aglucones, at a concentration of 10^{-3} to 10^{-4} M, significantly inhibited the production of prostaglandin synthetase and 5-lipoxygenase. The corresponding *O*-glucosides (**1** and **2**) had no detectable inhibitory activity on the synthesis of either enzyme in these concentrations. The details of the biological findings will be reported elsewhere.

The above observations would seem to suggest that the acetophenone glucosides and their aglucones, when in equilibrium, serve as an interlocked system to regulate growth and resting period of the producer organism. The balance is shifted to either direction (acetophenone-*O*-glucoside $\rightleftharpoons$ aglucone + glucose) according to the demand and requirement of the producer plants. Such interconversions in the case of phytosteroid glycosides and their aglycones is well documented [15]. Another interesting observation is that the Ar-oxygenated-2-methylchromones were produced in large quantities in *P. biflorum* bulbs when infested with the partial parasite, *Imperata cylindrica* [16]. In healthy bulbs, on the other hand, the Ar-oxygenated-acetophenones (both free and conjugated) were the major acetogenins. This observation is consistent with an earlier report of the occurrence of 5,7-dihydroxy-2-methylchromone in *Daucus carota* as an abnormal metabolite formed only under conditions of stress [17]. The details of the significance of these two types of closely related acetogenins (the acetophenones and the 2-methylchromones) in plant biochemistry are currently being investigated.

EXPERIMENTAL

Isolation procedure. In a typical experiment, the pseudo-stem fluid (*ca* 22 g) from freshly cut plant material was collected when the plants came into flowering in July. The viscous exudate was taken up in MeOH containing traces of EDTA. The MeOH soln was warmed on a water-bath at 60° for 15 min to deactivate the

enzymes present in the fluid, diluted with an equal vol. of water and concd to one-third vol. under red. pres. The concentrate was partitioned between EtOAc–H_2O (1:1) and the EtOAc (fraction A) and H_2O (fraction B) layers sepd. Fraction B then was exhaustively extracd with *n*-BuOH (fraction C).

Treatment of fraction A. The solvent was removed from this fraction and the residue (4.5 g) triturated, successively, with hot *n*-hexane (fraction a_1), $CHCl_3$ (fraction a_2), and MeOH (fraction a_3). The MeOH-insoluble residue (fraction a_4) was also preserved. Analytical TLC, using C_6H_6–EtOAc (9:1), showed closely similar patterns for fraction a_1 and a_2 and these were, therefore, combined. Likewise, the residue from fraction a_4 and fraction C showed closely similar TLC patterns and were combined before further processing.

2,4,6-*Trimethoxyacetophenone.* From the combined fractions a_1 and a_2, this compound was obtained by prep. TLC on silica gel G (E. Merck) using $CHCl_3$ as solvent. The TLC scraping at R_f ~0.4 was eluted with $CHCl_3$ and the solvent evapd from the $CHCl_3$ soln to give the acetophenone as a micro-crystalline solid (9 mg), mp 100–103°; UV: λ_{max}^{MeOH} nm (log ε): 209 (4.22), 224 (4.14), 273 (3.78); no shift in presence of either NaOAc or dry $AlCl_3$; IR ν_{max}^{Nujol} cm^{-1}: 1678 (CO), 1612 (Ar-OMe); EIMS *m/z* (rel. int.): 210 $[M]^+$ (100), 195 (22), 193 (11), 180 (14), 167 (18), 103 (9); ^1H NMR ($CDCl_3$): δ_H 6.55 (2H, *br*, H-3, 5), 3.9–3.95 (9H, 3 x OMe), 2.3 (3H, *s*, COMe).

The extractives from the combined fractions a_4 and -C were redissolved in aq. MeOH and the soln subjected to prep. TLC using *n*-BuOH–AcOH–H_2O (4:1:2) as the solvent. The different TLC zones were scraped off the plate and worked-up for the acetophenone *O*-glucosides.

4,6-*Dimethoxyacetophenone-2-O-β-*D-*glucoside* (**1**). The TLC scraping from R_f zone ~0.5 was eluted with *n*-BuOH and the solvent evapd from the extract under red. pres. This process was repeated x 5 to give the glucoside as a brown hygroscopic solid (25 mg), mp 177–181°; positive benzidine–metaperiodate test for polyols; $[\alpha]_D^{28}$ −37.8° (H_2O; *c* 0.75); UV λ_{max}^{MeOH} nm (log ε): 226 (4.02), 277 (3.47); no shift in UV maxima in presence of NaOAc or dry $AlCl_3$; IR ν_{max}: 3300 (br., chelated OH), 1668 (CO), 1615 (OMe); FABMS *m/z*: 397 $[M+K]^+$, 381 $[M+Na]^+$; therefore, the $[M]^+$ was deduced as 358. In the EIMS, the molecule fragmented before formation of the $[M]^+$ peak into the aglucone (*m/z* 196) and the glucone (*m/z* 162) moieties; ^1H NMR [$(CD_3)_2SO$]: δ_H 6.82 (1H, *d*, *J* = 2.5 Hz, H-3), 6.69 (1H, *d*, *J* = 2.5 Hz, H-5), 4.88 (1H, *d*, *J* = 7.5 Hz, H-1′, glucosyl anomeric proton), 3.90–3.92 (6H, 2 x OMe), 3.1–3.7 (*m*, glucosyl H + H_2O), 2.3 (3H, *s*, COMe); ^{13}C NMR: 26.6 (C-Me), 103.8 (C-3), 107.5 (C-5), 111.1 (C-1), 154.5 (C-4), 158.3 (C-2), 60.7 (C-6′), 70.2 (C-2′), 71.5 (C-4′), 78.1 (C-3′), 80.2 (C-5′), 92.8 (C-1′), 55.62 (C-6-OMe), 55.68 (C-4-OMe); [Found: C, 50.8; H, 6.5. $C_{16}H_{22}O_9 \cdot H_2O$ requires C, 51.1; H, 6.4]; the tetra-*O*-acetyl derivative of the glucoside, prepared by treatment with Ac_2O–pyridine on a steam bath for 4 hr, crystallized from *n*-hexane–Me_2CO as colourless crystals, mp 103–107°; EIMS *m/z*: 526 $[M]^+$ (7), 331 (100), 271 (12), 211 (7), 169 (24) (tetraacetyl glucosyl moiety); ^1H NMR ($CDCl_3$): δ_H 6.97 (1H, *d*, *J* = 2.5 Hz, H-3), 6.94 (1H, *d*, *J* = 2.5 Hz, H-5), 3.95 (6H, OMe), 2.32 (3H, *s*, COMe), 1.84–2.04 (12 H, 4 x OAc).

2,6-*Dimethoxyacetophenone-4-O-β-*D-*glucoside* (**2**). Prep. TLC scraping from R_f zone ~0.3 were processed as before to give the glucoside as a brown gummy material (37 mg); $[\alpha]_D^{28}$ −12.5° (H_2O; *c* 0.8); UV λ_{max} nm (log ε): 227 (4.03), 280–282 (4.13); FABMS *m/z*: 358 $[M]^+$; tetra-*O*-acetyl derivative, mp 114–116°; EIMS *m/z*: 526 $[M]^+$ (3).

Enzymic hydrolysis of **1**. Compound **1** (*ca* 5 mg) was emulsified with emulsin (3 mg) in NaOAc–HOAc buffer (pH 5). The reaction mixture was kept at room temp overnight and then worked-up in the usual way to give the aglucone as the $CHCl_3$ soluble component. The aq. hydrolysate, remaining after separation of the aglucone, was processed for the glucone component.

2-*Hydroxy*-4,6-*dimethoxyacetophenone.* This compound, the aglucone from the enzymic hydrolysis of **1**, crystallized from alcohol as needles, mp 83–84°; UV λ_{max}^{MeOH} nm (log ε): 225 (4.19), 288 (4.24), 320 (sh); $\lambda_{max}^{MeOH-AlCl_3}$ 244, 330, 398 nm (the shift in the UV maxima was consistent with the presence of *ortho*-hydroxyaryl ketones); IR ν_{max}^{Nujol} cm^{-1}: 3350 (OH, chelated), 1637 (CO, chelated), 1610 (Ar-OMe); MS *m/z*: 196 $[M]^+$ (100), 181 (78), 167 (5), 153 (5). The glucone component in the aq. hydrolysate was processed and converted to the alditol acetate according to a previously described procedure [6]. GC analysis of the product showed the presence of only glucitol acetate. The ratio of the aglucone: glucose was determined as 1:1. Enzymic hydrolysis of the glucoside **2**, in a similar fashion, afforded glucose and 4-hydroxy-2,6-dimethoxyacetophenone (in 1:1 ratio).

4-*Hydroxy*-2,6-*dimethoxyacetophenone.* This compound was obtained as a glassy solid, mp 75–78°; UV: λ_{max}^{MeOH} 224, 284, 324 nm; $\lambda_{max}^{MeOH-NaOAc}$ 242, 288, 348 nm; MS *m/z*: 196 $[M]^+$ (74), 181 (100), 179 (9), 167 (6), 166 (12), 153 (5), 138 (7), 110 (9).

Synthesis of 2-*hydroxy*-4,6-*dimethoxyacetophenone.* A mixture of acetylchloride (1 g), 2,4,6-tri-*O*-methylphloroglucinol (1 g) and dry $AlCl_3$ (2 g), in dry Et_2O (50 ml), was refluxed (1 hr) and left overnight at room temp. The product was hydrolysed with H_2O (200 ml) and conc HCl (4 ml) and extracted with Et_2O. The Et_2O extract was washed with a satd $NaHCO_3$ soln and then further extracted with cold NaOH (1%, 50 ml). The NaOH soln was acidified and again extracted with Et_2O. The Et_2O extract was worked-up in the usual way to give 2-hydroxy-4,6-dimethoxyacetophenone as a light brown solid (0.82 g). The concomitant 2-*O*-demethylation during Friedel–Crafts acylation of *peri*-methoxy aromatic compounds is well known [7, 8]. It crystallized from EtOH as needles, mp 83–85°. Methylation of the compound by repeated treatment with Et_2O–CH_2N_2 afforded 2,4,6-trimethoxyacetophenone, which was purified by CC over silica gel (C_6H_6–EtOAc, 9:1), mp 101–103°. The mmp and spectral properties of this compound were identical to those observed for the naturally occurring 2,4,6-trimethoxyacetophenone in *P. biflorum*.

Selective 4-*O-demethylation of* 2,4,6-*trimethoxyacetophenone.* 2,4,6-Trimethoxyacetophenone (14 mg) was refluxed with HCl (30%, 25 ml) for 16 hr. The product was extracted with Et_2O and the residue was chromatographed over a column of silica gel, using C_6H_6–EtOAc (9:1) as eluent. The later eluates efforded 4-hydroxy-2,6-dimethoxyacetophenone (3.5 mg). The compound was identical with the aglucone of **2** in all respects (mp, mmp, co-TLC, UV, MS).

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