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1,2,3-TRIOXYGENATED GLUCOSYLOXYXANTHONES FROM *POLYGALA TRIPHYLLA**

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Key Word Index—*Polygala triphylla*; Polygalaceae; xanthones; 1,2,3-trioxygenated xanthone; 1,2,3,6,7-pentaoxygenated xanthone; glucosyloxyxanthone; 1-glucosyloxy-2-hydroxy-3-methoxyxanthone; 1-glucosyloxy-2,3-methylenedioxyxanthone.

Abstract—Four B-ring oxygen-free trioxygenated xanthones, viz. 1-OH-2,3-$(OMe)_2$-, 1,2,3-$(OMe)_3$-, 1-OH-2,3-(OCH_2O)-, 1-OMe-2,3-(OCH_2O)-xanthone, two B-ring oxygen-free glucosyloxyxanthones, viz. 1-*O*-gl.-2-OH-3-OMe- and 1-*O*-gl.-2,3-(OCH_2O)-xanthone, and a pentaoxygenated xanthone, 1-OMe-2,3,6,7-$(OCH_2O)_2$-xanthone, have been isolated from the flowering top of *Polygala triphylla*. The xanthones have been characterized on the basis of chemical transformation, comprehensive spectral evidence, and by direct comparison where possible. This is the first report of occurrence of the glucosyloxyxanthones in nature. The biochemical significance of these chemical characters in higher plants is appraised.

INTRODUCTION

Polygala triphylla Buch.-Ham. (Polygalaceae) is a small herb which grows abundantly in the Kumaon region of the Western Himalayas up to 2133 m. Its extract is used as a tonic and in chest ailments in popular medicine. Phytochemical investigation of this species has not been reported before. The present paper deals with the isolation and characterization of the xanthone constituents of this species. Additionally, xanthone constituents of the *Polygala* species investigated so far have been recorded and biochemical significance of these chemical characters is appraised.

RESULTS AND DISCUSSION

Extensive column and layer chromatography of the petrol and EtOH extracts of the flowering top of *P. triphylla* afforded five known polyoxygenated xanthones, viz. 1-OH-2,3-$(OMe)_2$- (**1**), 1,2,3-$(OMe)_3$- (**2**), 1-OH-2,3-(OCH_2O)- (**3**), 1-OMe-2,3-(OCH_2O)- (**4**) [2], 1-OMe-2,3,6,7-$(OCH_2O)_2$-xanthone (**7**) [3], and two new glucosyloxyxanthones (**5** and **6**). The characterization of only the two new compounds is described here.

Compound **5**, $C_{20}H_{20}O_{10}\cdot H_2O$, showed UV and IR spectra characteristic of a 1,2,3-trioxygenated xanthone [2]. The changes in the UV maxima in presence of the usual shift reagents indicated the presence of a 2-OH with added oxygen functions at 1- and 3-position [4, 5]. As expected for an *O*-glucoside, the mass spectrum showed only the ion of the aglycone (*m/e* 258); hydrolysis with emulsin gave glucose, and the aglycone found to be identical with a synthetic sample of 1,2-dihydroxy-3-methoxyxanthone. The parent compound formed a pentaacetate which also did not exhibit any molecular ion peak in its mass spectrum but showed significant fragment ion peaks due to a dihydroxy-monomethoxyxanthone moiety and ions arising from an acetylated glucose moiety [6]. The compound was permethylated according to a published procedure [6], followed by hydrolysis when 1-hydroxy-2,3-dimethoxyxanthone and 2,3,4,6-tetra-*O*-methylglucose were obtained. Thus the compound was identified as 1-glucosyloxy-2-hydroxy-3-methoxy-xanthone (**5**). This glucosyloxyxanthone was not reported before in nature or synthetically.

Compound **6**, $C_{20}H_{18}O_{10}\cdot H_2O$, also showed UV and IR spectra characteristic of a 1,2,3-trioxygenated xanthone [2]. There was, however, no change in the UV maxima in presence of the usual shift reagents [4, 5]. As expected for an *O*-glucoside, the mass spectrum showed only the molecular ion of the aglycone (*m/e* 256); hydrolysis with emulsin gave glucose, and the aglycone which was identical with 1-hydroxy-2,3-methylenedioxyxanthone [2]. The parent compound formed a tetraacetate which also did not exhibit any molecular ion peak in its mass spectrum but showed significant fragment ion peaks due to a monohydroxy-methylenedioxyxanthone moeity (*m/e* 256) and ions arising from the acetylated glucose moiety. On the basis of the above evidence, the new compound was assigned 1-glucosyloxy-2,3-methylene-dioxyxanthone structure (**6**).

The concentration of the glucosyloxyxanthones of *P. triphylla* was maximum during the flowering time and declined rapidly at **1**–**4** the post-flowering stage. The plants collected in fruit contained **1**–**4** and **7** as the only xanthone constituents but their concentration was low. Similar observations were earlier made with a number of xanthone-bearing species, viz. *Swertia chirata*, *S. angustifolia* [7] and *Canscora decussata* [8] (Gentianaceae). These observations would seem to suggest that like flavonoids [9], the xanthones have a quick turnover and the degradation products play a major role in the metabolism of higher plants.

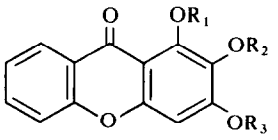
* Part 8 in the series 'Extractives of *Polygala*'. For Part 7 see ref. [1].

PHYTO 20:3 - I

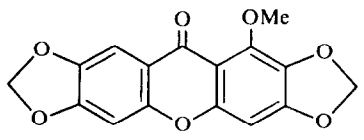
Table 1. Xanthones occurring in the genus *Polygala*

Species	Plant part(s) investigated	Xanthone present
P. arillata [2, 11]	stems, roots	1-OH-2,3-$(OMe)_2$, 1,2,3-$(OMe)_3$, 1-OH-2,3-(OCH_2O), 1-OMe-2,3-(OCH_2O), 1,3,4-$(OMe)_3$, 1,2,3,4,7-$(OMe)_5$
P. macradenia [3, 13]	roots	1,2,3-$(OMe)_3$, 1,2,4-$(OMe)_3$, 1-OH-2,3,4-$(OMe)_3$, 1,2,3,4-$(OMe)_4$, 1-OH-2,3,6,7-$(OCH_2O)_2$, 1-OMe-2,3,6,7-$(OCH_2O)_2$, 1,6,7-$(OMe)_3$-2,3-(OCH_2O), 1,2,3,4-$(OMe)_4$-7-OH, 1-OH-2,3,4,7-$(OMe)_4$, 1,2,3,4,7-$(OMe)_5$
P. paenaea [14, 15]	roots	1-OH-2,3,4,7-$(OMe)_4$, 1,2,3,4,7-$(OMe)_5$, 1-OH-2,4-$(OMe)_2$-6,7-(OCH_2O), 1,2,4-$(OMe)_3$-6,7-(OCH_2O)
P. spectabilis [16]	bark	1,3-$(OMe)_2$-2-OH-7,8-(OCH_2O), 1,2,3,7,8-$(OMe)_5$, 1,2,3-$(OMe)_3$-6,7-(OCH_2O)
P. tenuifolio [17]	roots	1,2,3,7-$(OMe)_4$, 1,2,3,7-$(OMe)_4$-6-OH, 1,2,3,6,7-$(OMe)_5$, 1,2,3-$(OMe)_3$-6,7-(OCH_2O), 1,2,3,4,7-$(OMe)_5$
P. triphylla	flowers and stalk, roots	**1–7**

Twenty-six species of the genus *Polygala* have so far been chemically investigated, revealing a variety of constituents of both chemical and biological significance [10, 11]. The members of this genus showed the following two broad patterns of distribution of chemical characters—(a) triterpene saponins, lactone-containing lignans and flavonols and (b) triterpene saponins, lactone lignans, and xanthones. Esters of cinnamic acid, methyl salicylate and esters of phenolic acids with oxygenation at the 2,4 or 2,4,5-position were also found to be associated with these patterns. The six xanthone-bearing *Polygala* species (Table 1) were found to be devoid of flavonol constituents. The oxygenation patterns of the xanthones are curious, frequently revealing the co-occurrence of oxygen-free B-rings and 7- or 6,7-oxygenated compounds (in lieu of flavonol derivatives). The substitution of flavonol constituents by 1,3,7-, 1,3,6,7-oxygenated xanthones, frequently with added oxygen functions at the 2- and 4-position, and the B-ring oxygen-free xanthones seems to suggest a 'switching' phenomenon as was observed in the case of *C*-glycosyl flavones by mangiferin [12]. On the basis of the above circumstantial evidence it would be tempting to conclude that the xanthones of the *Polygala*, like mangiferin, are formed by the condensation of a C_6–C_3 moiety with two acetate units, rather than by the coupling of condensation product of a C_6–C_1 unit with three acetate units.



1 R_1 = H, R_2 = R_3 = Me
2 R_1 = R_2 = R_3 = Me
3 R_1 = H, $R_2 + R_3$ = —CH_2—
4 R_1 = Me, $R_2 + R_3$ = —CH_2—
5 R_1 = Glc, R_2 = H, R_3 = Me
6 R_1 = Glc, $R_2 + R_3$ = —CH_2—



7

EXPERIMENTAL

The general methods have been reported in a recent paper [18]. The plant materials were collected 6 times during its growing period (July–September of 1976–77) from Ranikhet, Uttar Pradesh, and were properly identified. A voucher specimen has been preserved at the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.

Extraction of P. triphylla. In a typical experiment, dried and milled plant material (flowers and stalk, *ca* 200 g) was

continuously extracted in a Soxhlet with light petrol (60–80°) and then with EtOH (30 hr each). The 2 extracts were separately processed.

Treatment of the petrol extract. The extract was processed for carboxylic, phenolic, and neutral fractions in the usual way. The residue from the neutral fraction was dissolved in petrol (20 ml) and was chromatographed on a column (20 × 2 cm) of silica gel. Elution was carried out with light petrol (40–60°) and C_6H_6 (3 l. each). Fractions (100 ml) were collected and monitored by analytical TLC.

1-*Methoxy*-2,3-*methylenedioxyxanthone* (**4**). The residue from the early C_6H_6 eluates crystallized from EtOH to give the xanthone **4** as colourless needles (12 mg), mp 161–163°. The mp, mmp, UV, IR, and R_fs were found to be identical with those of 1-methoxy-2,3-methylenedioxy-xanthone [2].

1,2,3-*Trimethoxyxanthone* (**2**). The EtOH mother liquor, after separation of compound **4**, showed one major and one minor spot on TLC and was subjected to prep. TLC using C_6H_6–$CHCl_3$ (1:1) (solvent 1) as the developer. TLC scrapings of the less polar zone, corresponding to the R_f value of 1,2,3-trimethoxy-xanthone [2], was triturated with $CHCl_3$. The solvent was removed from the $CHCl_3$ solution and the residue crystallized from MeOAc to give xanthone **2** as yellow needles (7 mg), mp 129–30°. The identity of the compound was established by direct comparison (mp, mmp, co TLC, UV, IR) with an authentic sample of 1,2,3-trimethoxyxanthone [2].

1-*Methoxy*-2,3,6,7-*bismethylenedioxyxanthone* (**7**). The more polar component in the prep. TLC zone showed a blue fluorescence under short-wave UV light. The TLC scrapings were eluted with $CHCl_3$. The residue from the $CHCl_3$ eluate crystallized from AcMe–hexane as colourless needles (3 mg), mp 155°. The mp, mmp, UV, and R_fs were found to be identical with those of an authentic sample of 1-methoxy-2,3,6,7-bismethylene-dioxyxanthone [3].

1-*Hydroxy*-2,3-*dimethoxyxanthone* (**1**). The residue from the phenolic fraction was subjected to prep. TLC, as above, when compound **1** (4 mg) was obtained as yellow needles, mp 133–34°. The identity of the compound was established by direct comparison (mp, mmp, co TLC, UV) with an authentic sample of 1-hydroxy-2,3-dimethoxyxanthone [2].

1-*Hydroxy*-2,3-*methylenedioxyxanthone* (**3**). The component from the lower prep. TLC zone, from the separation of **1**, showed a light yellow streak. It was subjected to re-prep. TLC using $CHCl_3$–AcOH (99:1, solvent 2) as the developer. Compound **3** was obtained as the major component from the less polar zone, $R_f \sim 0.4$. It crystallized from AcMe as cream coloured crystals (5 mg), mp 204°. The mp, mmp, UV and R_fs were found to be identical with those of an authentic sample of 1-hydroxy-2,3,-methylenedioxyxanthone [2].

Treatment of the EtOH *extract.* The extract was concentrated (*ca* 100 ml) and kept at ordinary temperature overnight when a brown solid (64 mg) was separated. It was collected by filtration. It consisted essentially of xanthones **1**, **3**, and **7** as was revealed by analytical TLC (solvent 2). In addition, the solid suggested the presence of a number of steroidal constituents (Liebermann–Burchard test positive). The filtrate was further concentrated to a syrupy liquid (22 g) and was triturated with hot $CHCl_3$. A portion of the $CHCl_3$ insoluble residue (*ca* one-fourth of 8.3 g), which showed two major I_2 positive spots on TLC, was dissolved in MeOH (20 ml) and was chromatographed over a column of polyamide (50 × 2 cm). Different proportions of MeOH–H_2O (1:1, 3:2, 2:1), and MeOH, 1 l. each, were used as eluents. Fractions were collected (50 ml).

1-*Glucosyloxy*-2-*hydroxy*-3-*methoxyxanthone* (**5**). The early and middle MeOH–H_2O (1:1) fractions were combined and evapd under vacuum to give a brown powder. It was dissolved in $CHCl_3$–AcOEt (40 ml) and was passed through a short column of silica gel using the same mixture solvent as the eluent. Concentration of the eluate afforded **5** as a straw coloured solid (87 mg), softening at 100–10°; R_f 0.44 (AcOEt–MeOH–H_2O, 25:5:1, solvent 3); $[\alpha]_D^{30}$ – 98.3° (*c* 0.54, MeOH); UV: λ_{max} (EtOH) nm (log ε) 242 (4.39), 255 sh (4.01), 275 (3.99), 295 (4.04), 300 (4.05), ~325 (3.67); λ_{max} (EtOH–NaOAc) nm (log ε) 246 (4.41), 276 (4.08), 300–305 (4.06), ~330 (3.82); IR: ν_{max}^{KBr} cm^{-1}: 1668, 1650, 1620, 1592. (Found: C, 54.6; H, 5.2. $C_{20}H_{20}O_{10}.H_2O$ requires: C, 54.8; H, 5.0.) Hydrolysis of the compound (11 mg) with emulsin (8 mg), according to a published procedure [19], gave glucose (PPC) and 1,2-dihydroxy-3-methoxyxanthone, mp 189–91°; λ_{max} nm 248, 270 sh, 295, 320; *m/e* 258 (M^+, 100%), 240 (4), 230 (7), 228 (8), 215 (14), 213 (11), 201 (5), 200 (4); the aglucone on methylation with ethereal diazomethane gave 1-hydroxy-2,3-dimethoxyxanthone, mp and mmp 133–4° [2]. The above properties of the aglucone suggested its identity with 1,2-dihydroxy-3-methoxyxanthone. Direct comparison of the aglucone with a synthetic sample of 1,2-dihydroxy-3-methoxy-xanthone, prepared according to a published procedure [20], established its identity.

The pentaacetate of the glucosyloxyxanthone, prepared by treatment with Ac_2O and C_5H_5N at ordinary temprature overnight followed by heating on a steam bath (2 hr), crystallized from EtOH as microcrystals, mp 181–4°; δ ($CDCl_3$) 8.30 (1H, *m*, H-8), 7.75–7.30 (3H, *m*, H-5-7), 6.88 (1H, *s*, H-4), 5.4 (1H, *br.*, glucosyl anomeric proton), 3.98 (3H, OMe), 2.40 (3H, C_2–OAc), 2.1–2.0 (12H, glycosyl OAc); *m/e* 331 (78%), 271 (14), 258 (100), 257 (22), 229 (18), 228 (12), 226 (5), 215 (11), 211 (7), 169 (6).

The permethyl ether of the glucosyloxyxanthone (**5**), prepared with MeI and NaH in THF, under N_2 atmosphere, was obtained as a light brown semisolid, R_f 0.28 (solvent 2), blue fluorescence under UV light. It was hydrolysed with HCl (2%) on a steam bath (4 hr). The hydrolysate was extracted with $CHCl_3$. The residue from the $CHCl_3$ extract was crystallized from EtOH as pale yellow needles, mp and mmp (with 1-hydroxy-2,3-dimethoxyxanthone) 133°. The glucone fraction from the aq. hydrolysate was obtained as a syrup, $[\alpha]_D^{20}$ + 658° (*c* 033, H_2O); *m/e* 236 (M^+, 100%). The R_fs, spectral and optical properties of the compound were indistinguishable from those of 2,3,4,6-tetra-*O*-methyl-glucose [7].

1-*Glucosyloxy*-2,3-*methylenedioxyxanthone* (**6**). The residue from the MeOH–H_2O (2:1) fractions was dissolved in MeOH and was subjected to prep. TLC using solvent 3 as the developer. The TLC scrapings from the light brown zone, ~R_f 0.3, was eluted with $CHCl_3$–MeOH. Evapn of the solvent afforded the glucosyloxyxanthone as a light brown amorphous solid (44 mg); $[\alpha]_D^{30}$ – 108.4° (*c* 0.52, MeOH); λ_{max} (EtOH) nm 248, 276, 300, 325–30; ν_{max}^{KBr} cm^{-1}: 1670, 1645, 1618, 1594; *m/e* 256 (100%), 228 (7), 227 (8). (Found: C, 55.1; H, 4.6. $C_{20}H_{18}O_{10}.H_2O$ requires: C, 55.0; H, 4.6). Hydrolysis of the glucosyloxyxanthone (8 mg) with emulsin (5 mg) gave glucose (PPC) and 1-hydroxy-2,3-methylene-dioxyxanthone, mp and mmp 202–4°. The tetra-acetate of the parent compound, prepared by treatment with Ac_2O and C_5H_5N, as above, crystallized from aq. EtOH as colourless micro-crystals, mp 161–4°; *m/e* 331 (64%), 271 (7), 256 (100), 228 (12), 227 (11), 211 (5), 180 (4), 169 (3); δ ($CDCl_3$) 8.34 (1H, *m*, H-8), 7.75–7.3 (3H, *m*, H-5-7), 6.95 (1H, *s*, H-4), 6.04 (2H, *s*, OCH_2O), 2.1–2.0 (12H, OAc).

The $CHCl_3$ soluble solid from the EtOH extractives on analytical TLC showed the presence of xanthones **1**, **3**, **5**, **6** and triterpenes as major constituents. Further characterization of the latter compounds is in progress.

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