Extractives of *Polygala*. Part 5.[1] New Trioxygenated Xanthones of *P. arillata*

By **Shibnath Ghosal,* Shanta Banerjee** (née Chakrabarti), **Rama Ballava P. S. Chauhan,** and **Radhey S. Srivastava,** Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India

The stems and roots of *Polygala arillata* have been shown to contain 1-hydroxy-2,3-dimethoxyxanthone (1), 1,2,3-trimethoxyxanthone (2), 1-hydroxy-2,3-methylenedioxyxanthone (3), 1-methoxy-2,3-methylenedioxyxanthone (4), and a compound that yields 1,3,4-trimethoxyxanthone (5) on methylation. Additionally, senegenic acid and large amounts of esters of glucose and of either protocatechuic acid or gallic acid have been isolated. Ring-B oxygen-free xanthones are comparatively rare, and this is the first report of the co-occurrence of the biogenetically related 1,2,3- and 1,3,4-trioxygenated xanthones in nature.

Polygala arillata Benth & Ham. (Polygalaceae), native to the mountains of the Western Himalayas, is a small plant used in the indigenous system of medicine for a variety of purposes.[1] About ten *Polygala* species have so far been chemically investigated, revealing a variety of chemical constituents of both chemical and biological significance.[1] No previous phytochemical investigation has been reported on *P. arillata*. The present paper describes the isolation and characterization of the xanthone constituents of the stems and roots of this plant.

Preliminary examination by t.l.c. of the petroleum

[1] Part 4, S. Ghosal, R. B. P. S. Chauhan, and R. S. Srivastava, *Plant Biochem. J. (India)*, 1974, **1**, 64.

extractives showed the presence of a number of yellow pigments. Extractions of a larger sample of powdered plant material with petroleum and with ethanol were followed by separation into carboxylic, phenolic, and neutral fractions. Repeated column and preparative layer chromatography (p.l.c.) of the last two fractions afforded four xanthones (1)—(4), characterized as follows.

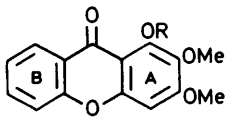
Compound (1), $C_{15}H_{12}O_5$ (M^+ 272), is 1-hydroxy-2,3-dimethoxyxanthone. The hydroxy-group is strongly chelated since in its ^1H n.m.r. spectrum it showed a broad one-proton signal at δ 12.74 (exchangeable with D_2O)[2] and the compound was unaffected by ethereal diazomethane. The u.v. absorption spectrum (neutral solution in ethanol) was characteristic of 1,2,3-trioxygenated xanthones.[3] The positions of the maxima remained unaltered in ethanolic sodium acetate. The 60 MHz ^1H n.m.r. spectrum (solvent $CDCl_3$) showed two methoxy-signals at δ 3.95 and 4.02, one aromatic proton singlet at δ 6.74 (H-4),[3] and two complex multiplets in the regions δ 7.72—7.18 and 8.3—8.1 associated with the four protons of ring B.[3] Methylation of compound (1) with dimethyl sulphate and potassium carbonate afforded 1,2,3-trimethoxyxanthone, previously reported as a derivative of 3-hydroxy-1,2-dimethoxyxanthone, which occurs in *Kielmeyera speciosa*.[3]

Compound (2), $C_{16}H_{14}O_5$ (M^+ 286), is 1,2,3-trimethoxyxanthone. It was identical (m.p., t.l.c. behaviour, and spectral properties) with the methyl ether of the xanthone (1), and with material synthesised from salicylic acid and 3,4,5-trimethoxyphenol.[4] This is the first report of the occurrence of 1,2,3-trimethoxyxanthone in nature.

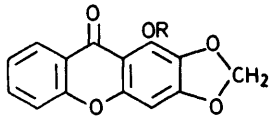
Compound (3), $C_{14}H_8O_5$ (M^+ 256), 1-hydroxy-2,3-methylenedioxyxanthone, also showed u.v. absorption spectrum characteristic of 1,2,3-trioxygenated xanthones.[3] It responded to the Labat test for a methylenedioxy-group, the presence of which was also indicated in its i.r. ($\nu_{max.}$ 933 cm^{-1}) and ^1H n.m.r. spectra (δ 6.02). The 60 MHz ^1H n.m.r. spectrum (solvent $CDCl_3$) showed additional signals due to one chelated hydroxy-group and five aromatic protons (H-4—8). The xanthone (3) was also obtained from demethylation of synthetic 1-methoxy-2,3-methylenedioxyxanthone.

Compound (4), $C_{15}H_{10}O_5$ (M^+ 270), 1-methoxy-2,3-methylenedioxyxanthone, was identical (m.p., t.l.c. behaviour, and spectral data) with an authentic sample prepared from 1,2,3-trihydroxyxanthone.[5]

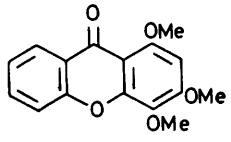
Chromatography of a large sample of the alcoholic extractives, from which no crystalline material was obtained directly, yielded a polar xanthone fraction which showed three major spots on t.l.c. The three components were separated by p.l.c. after permethylation. The least polar component was obtained as a pale yellow solid, $C_{16}H_{14}O_5$ (M^+ 286). The 60 MHz ^1H n.m.r. spectrum showed signals due to three methoxy-groups and five aromatic protons (H-2 and H-5—8). The compound was thus identified as 1,3,4-trimethoxyxanthone (5), and was identical with a synthetic sample.



(1) R = H
(2) R = Me



(3) R = H
(4) R = Me



(5)

The xanthone, of which (5) is the methylated derivative, is the first 1,3,4-trioxygenated xanthone to be found in nature.

The two other xanthone-bearing species of this genus, *viz. P. paeneae* and *P. macradenia*, contained xanthones [6,7] whose oxygenation patterns (1,2,3,4,7 and 1,2,3,6,7) are also taxonomically curious. Although ring-B oxygen-free xanthones of the 1,2,3-trioxygenated type were encountered before in the genus *Kielmeyera* (Guttiferae),[3] where they were found to co-occur with 2,3,4-trioxygenated xanthones, this is the first time that the co-occurrence of two biogenetically related series of trioxygenated xanthones (1,2,3- and 1,3,4-) has been reported.

In addition to the trioxygenated xanthones, senegenic acid [8] and large amounts of esters of glucose and of either protocatechuic acid or gallic acid were obtained from the polar fractions of the alcoholic extractives of this plant.

EXPERIMENTAL

U.v. spectra were recorded with a Cary 14 or Spektromom 203 spectrophotometer, i.r. spectra with a Perkin-Elmer 621 or 257 spectrophotometer, mass spectra with an A.E.I. MS9 instrument (at 70 eV), and 60 MHz ^1H n.m.r. spectra with a Varian A-60 spectrometer. T.l.c. was carried out on Kieselgel G (spot detection by u.v. fluorescence and treatment with iodine vapour). Two solvent systems, *viz.* benzene–chloroform (50 : 50; solvent 1) and chloroform–acetic acid (100 : 1; solvent 2), were used.

Isolation of Xanthones from P. arillata.*—Dried and powdered stems and roots (*ca.* 1 kg) were continuously extracted (Soxhlet) with light petroleum (b.p. 60—80 °C) for 30 h. The defatted plant material was then extracted with ethanol (30 h). The two extracts were processed separately.

* The plant material was supplied by Messrs United Chemical and Allied Products, Calcutta. A voucher specimen has been preserved at the Pharmaceutical Chemistry Research Laboratory, Banaras Hindu University, Varanasi-5, India.

[2] P. Arends and P. Helboe, *Acta Chem. Scand.*, 1972, **26**, 4180.
[3] O. R. Gottlieb, A. A. L. Mesquita, G. G. Oliveira, and M. T. de Melo, *Phytochemistry*, 1970, **9**, 2537.
[4] P. K. Grover, G. D. Shah, and R. C. Shah, *J. Chem. Soc.*, 1955, 3982.
[5] S. Ghosal, R. B. P. S. Chauhan, K. Biswas, and R. K. Chaudhuri, *Phytochemistry*, 1976, **15**, 1041.
[6] J. Moron, J. Polonsky, and H. Pourrat, *Bull. Soc. chim. France*, 1967, 130.
[7] D. L. Dreyer, *Tetrahedron*, 1969, **25**, 4415.
[8] S. W. Pelletier, N. Adityachaudhury, M. Tomaz, J. J. Reynolds, and R. Mechoulam, *J. Org. Chem.*, 1965, **30**, 4234.

Petroleum Extract.—The extract was processed for carboxylic, phenolic, and neutral fractions in the usual way. The residue from the neutral fraction was dissolved in petroleum (20 ml) and chromatographed on a column (22 × 1.8 cm) of silica gel (B.D.H.; 60—120 mesh). Elution was carried out with light petroleum (b.p. 40—60 °C) and benzene (5 l each). Fractions (100 ml) were collected.

The early benzene eluates afforded 1-*methoxy*-2,3-*methylenedioxyxanthone* (4) as needles (248 mg), m.p. 161—163° (from ethanol); R_F 0.62 (solvent 2; sea-green fluorescence under u.v. light); $\lambda_{max.}$ (EtOH) 212, 220, 245, 248, 277, 303, and 342 nm; $\nu_{max.}$ (KBr) 1 670, 1 650, 1 635, 1 620, 1 595, 1 522, 1 330, 1 305, 1 260, 1 218, 1 172, 1 110, 1 100, 1 062, 933, and 820 cm^{-1}; δ ($CDCl_3$) 8.30 (1 H, m, H-8), 7.7—7.2 (3 H, m, H-5—7), 6.74 (1 H, s, H-4), 6.02 (2 H, s, OCH_2O), and 3.98 (3 H, s, OMe); *m/e* 270 (M^+, 100%), 269 (12), 253 (12), 252 (14), 241 (18), 199 (6), 197 (9), 196 (22), 126 (14), 92 (11), and 77 (22) (Found: C, 66.45; H, 3.8. $C_{15}H_{10}O_5$ requires C, 66.65; H, 3.7%).

The benzene mother liquor was subjected to p.l.c. with solvent 1 as developer. The light blue fluorescent zone, R_F *ca.* 0.5 was eluted with chloroform and gave 1,2,3-*trimethoxyxanthone* as yellow needles (78 mg), m.p. 129—130° (from acetone); R_F 0.53 (solvent 1); $\lambda_{max.}$ (EtOH) 243, 255sh, 276, 300, and 336 nm; δ ($CDCl_3$) 8.32 (1 H, m, 8-H), 7.7—7.2 (3 H, m, H-5—7), 6.72 (1 H, s, H-4), and 4.05—3.94 (9 H, OMe); *m/e* 286 (M^+, 100%), 271 (88), 269 (12), 268 (8), 256 (8), 243 (28), 215 (6), 214 (12), 186 (24), 135 (17), and 77 (18) (Found: C, 67.1; H, 4.65. $C_{16}H_{14}O_5$ requires C, 67.15; H, 4.9%).

The residue from the phenolic fraction was dissolved in chloroform–benzene (1 : 1; 20 ml) and chromatographed over a column of silica gel. Elution was carried out with light petroleum (2 l), benzene (2 l), and benzene–ethyl acetate (95 : 5; 1 l). Fractions (100 ml) were collected.

The residue from the middle benzene eluates crystallized from methanol as fine yellow needles of 1-*hydroxy*-2,3-*dimethoxyxanthone* (1) (32 mg), m.p. 133—134°; R_F 0.20 (solvent 1); $\lambda_{max.}$ (EtOH) 216, 242, 255sh, 290, 305, and 362 nm; $\nu_{max.}$ (KBr) 3 480, 1 668, 1 645, 1 632, and 1 592 cm^{-1}; δ ($CDCl_3$) 12.74 (1 H, 1-OH), 8.28 (1 H, m, H-8), 7.7—7.2 (3 H, m, H-5—7), 6.72 (1 H, s, H-4), and 4.02—3.95 (6 H, OMe); *m/e* 272 (M^+, 98%), 257 (100), 244 (18), 243 (48), 229 (75), 215 (2), 186 (51), 141 (43), 121 (33), 120 (27), and 77 (31) (Found: C, 66.6; H, 4.25. $C_{15}H_{12}O_5$ requires C, 66.15; H, 4.4%). Methylation of the xanthone (1) (12 mg) with dimethyl sulphate (0.5 ml) and potassium carbonate (0.4 g) in anhydrous acetone (40 ml) under reflux (45 h) and the usual work-up gave 1,2,3-trimethoxyxanthone (8 mg) (m.p., mixed m.p., co-t.l.c.).

The early benzene–ethyl acetate eluates afforded a mixture of two xanthones which on p.l.c. (solvent 2) gave 1-*hydroxy*-2,3-*methylenedioxyxanthone* (3), R_F 0.28, as crystals (18 mg), m.p. 203—204°; $\lambda_{max.}$ (EtOH) 242, 253sh, 288, 300sh, and 365 nm; *m/e* 256 (M^+, 100%), 228 (12), 227 (14), 200 (5), 199 (7), 170 (3), and 77 (11) (Found: C, 65.2; H, 3.55. $C_{14}H_8O_5$ requires C, 65.6; H, 3.1%). Acetylation (acetic anhydride–pyridine under reflux, 2 h) yielded the 1-acetate, which crystallized from acetone–hexane as microcrystals, m.p. 178—180°; $\nu_{max.}$ (KBr) 1 785, 1 662, 1 625, 1 608, and 1 592 cm^{-1}. Methylation (dimethyl sulphate and potassium carbonate in anhydrous acetone under reflux; 45 h) gave the xanthone (4).

[9] M. A. E. Ansari, K. K. Reddy, K. N. Sastry, and Y. Nayudamma, *Phytochemistry*, 1971, **10**, 2239.

Alcoholic Extract.—The extract was concentrated to a syrup and poured into water (200 ml). The mixture was kept overnight at room temperature and then extracted with chloroform (3 × 200 ml) and ethyl acetate (3 × 200 ml). The two extracts were processed separately. The alcoholic residue left after the two extractions, on cellulose column chromatography (water as the eluant), gave senegenic acid [8] and esters of glucose and protocatechuic or gallic acid.[9] The chloroform extract gave further crops of the xanthones (1) (24 mg) and (3) (27 mg) in the usual way.

The residue from evaporation of the ethyl acetate extract was a dull yellow resin. Column chromatography over silica gel (24 × 1.8 cm) [benzene–ethyl acetate (90 : 10) as eluant (10 l); 1 l fractions] afforded, from the middle fractions, a brown gum which showed a major spot with tailing on analytical t.l.c. It was methylated with dimethyl sulphate and potassium carbonate in anhydrous acetone under reflux (45 h). After the usual work up the permethyl ether was subjected to p.l.c. (solvent 2). Extraction of the zone of 0.6 with methylene chloride–methanol yielded a *solid*, m.p. 92—95° (22 mg); δ ($CDCl_3$) 8.28 (1 H, m, H-8), 7.7—7.2 (3 H, m, H-5—7), 6.43 (1 H, s, H-2), 3.98—4.02 (9 H, OMe); *m/e* 286 (M^+, 100%), 271 (35), 269 (16), 256 (12), 243 (32), 215 (7), and 214 (5) (Found: C, 66.8; H, 4.55. $C_{18}H_{14}O_5$ requires C, 67.15; H, 4.9%), identical (co-t.l.c. and spectral data) with synthetic 1,3,4-trimethoxyxanthone (5).

1,2,3-*Trimethoxyxanthone*.—Salicylic acid (0.23 g), 3,4,5-trimethoxyphenol (0.18 g), phosphoryl chloride (2 ml), and freshly fused zinc chloride (0.5 g) were mixed and kept in a stoppered flask for 2 h at 20 °C. After 1.5 h under reflux, the solution was poured on to crushed ice. The precipitate was triturated several times with hot ethyl acetate to give an amorphous solid (0.11 g). This material on column chromatography [silica gel; benzene–ethyl acetate (100 : 5)] gave a product which showed two spots on t.l.c. The mixture was methylated with dimethyl sulphate and potassium carbonate in anhydrous acetone under reflux. The methylated product on p.l.c. (solvent 1) afforded 1,2,3-trimethoxyxanthone (70 mg), m.p. 129—130°, identical with the xanthone (2) (co-t.l.c., mixed m.p., and u.v. and i.r. spectra).

1-*Methoxy*-2,3-*methylenedioxyxanthone*.— 1,2,3-Trihydroxyxanthone (0.14 g), obtained from 1,2,3-trimethoxyxanthone by demethylation with hydrobromic acid, was refluxed with methylene iodide (0.28 g) and potassium carbonate (0.5 g) in anhydrous acetone (50 ml) for 12 h. The solution was then filtered and evaporated. Acidification of the residue and extraction with chloroform gave a brown gum. This was dissolved in acetone and filtered through a column of silica gel [benzene–ethyl acetate (90 : 10) as eluant (2 l)]. The product crystallized from methanol as cream-coloured micro-crystals (44 mg), m.p. 203—205°. Methylation with dimethyl sulphate and potassium carbonate gave 1-methoxy-2,3-methylenedioxyxanthone, m.p. 161—163, identical (spectra and chromatography) with the xanthone (4).

1,3,4-*Trimethoxyxanthone*.—2-Methoxybenzoyl chloride (2.38 g), 1,2,3,5-tetramethoxybenzene (2.02 g), and anhydrous aluminium chloride (5 g) in dry ether (100 ml) were kept at room temperature overnight. After 1.5 h under reflux, the product was worked up in the usual way [10] to give 2-hydroxy-2′,3,4,6-tetramethoxybenzophenone as a pale yellow flocculent precipitate (1.2 g). Cyclization of the

[10] S. Ghosal, R. K. Chaudhuri, and K. R. Markham, *J.C.S. Perkin I*, 1974, 2538.

benzophenone (0.92 g) in pyridine (9 ml) and tetramethylammonium hydroxide (10% aqueous solution; 20 ml) under reflux (48 h), acidification with hydrochloric acid, and extraction with ether gave 1,3,4-trimethoxyxanthone as a yellow gum (0.24 g) which crystallized from ethanol as micro-crystals, m.p. 88—92°, identical (u.v. and ^{1}H n.m.r. spectra and chromatography) with the xanthone (5).

We wish to thank Professor T. R. Govindachari, Ciba-Geigy Research Centre, Bombay, and Dr. B. C. Das, CNRS, Gif-Sur-Yvette, France, for the mass spectra. We also thank the Council of Scientific and Industrial Research, New Delhi, and the University Grants Commission, New Delhi, India, for financial support.

[6/1624 *Received*, 20*th August*, 1976]