Tetrahedron Vol. 25, pp. 4415 to 4420. Pergamon Press 1969 Printed in Great Britain

EXTRACTIVES OF *POLYGALA MACRADENIA* GRAY (POLYGALACEAE)

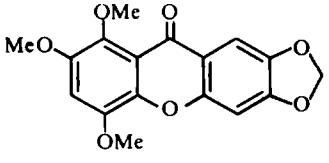
D. L. DREYER

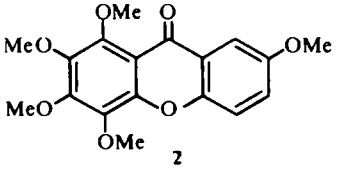
Department of Chemistry, San Francisco State College, San Francisco, California

(*Received in USA* 11 *February* 1969; *Received in the UK for publication* 23 *May* 1969)

Abstract—Four new, highly substituted xanthones have been isolated from the extracts of *Polygala macradenia* Gray (Polygalaceae). The assigned structures of 1,2,3,4-tetramethoxy-7-hydroxyxanthone (**3**), 1,2,3,4,6,7-hexamethoxyxanthone (**7**), 1-methoxy-2,3,6,7-dimethylenedioxyxanthone (**10**), and 1,2,3-trimethoxy-6,7-methylenedioxyxanthone (**13**) are based on spectroscopic properties of the parent compounds and some of their simple derivatives.

CHEMICAL extractives of the plant family Polygalaceae have been relatively little investigated. Previous reports of work in this family have been largely confined to the genus *Polygala* and most studies have resulted in the isolation of sapogenins.[1] Of special relevance to the present study is the report by Moron *et al.*[2] on the isolation and structure determination of two xanthones, polygalaxanthone A(**1**) and polygalaxanthone B(**2**), from *Polygala paenea* L. The present study reports the isolation and structure determination of four new, highly substituted xanthone derivatives from *P. macradenia* Gray.



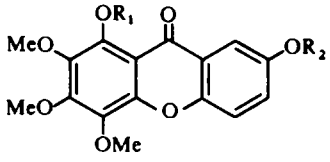


2

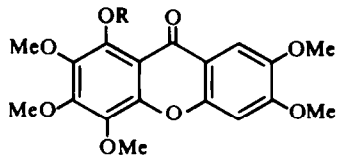
The extractives were isolated by employing classical isolation procedures. A yellow pigment, m.p. 197–198°, crystallized readily. It failed to give a positive Mg–HCl test for flavenoids or a positive $FeCl_3$ test. Moreover, the UV spectrum was quite unlike that of flavonoids, but similar to that of xanthones which generally show high intensity bands in the region 240–270 mμ and relatively low intensity long wave bands out to *ca.* 400 mμ.[3] The UV spectrum was unchanged with added sodium acetate but did shift with added sodium hydroxide. The IR spectrum confirmed the presence of a OH group and a CO group with considerable single bond character.

The NMR spectrum of the yellow pigment showed resonances for three aromatic protons and four OMe groups. The pigment readily formed a monoacetate and a monomethyl ether. The NMR spectra of these derivatives again showed resonances for three aromatic protons and an OAc and additional OMe resonances respectively. The aromatic resonances of the monomethyl ether showed a downfield doublet with a small (3 Hz) coupling constant. The downfield position of this resonance suggested the presence of a proton at the 1- (or 8-) position. The small coupling constant

indicated a substituent at the 2-position and that the coupling was to a proton in the *meta* (3-) position. The other resonance with 3 Hz coupling constant was a four line pattern with 3 and 9 Hz coupling constants. This four line pattern formed one half of an AB doublet with a 9 Hz coupling constant. These resonances are assigned to protons at the 3- and 4-positions respectively. Thus, the 4-proton appeared as a doublet (J = 9 Hz). The chemical shift of this ABX system changed significantly in the acetate so that the 3- and 4-resonances had the same chemical shift. All the positions in the other ring must be occupied in order to accommodate the remaining four substituents. The single substituent in the B-ring must be the OH group in order to account for the change in chemical shifts of the aromatic resonances upon acetylation and methylation. Structure 3 is thus indicated for the yellow pigment and structures 2 and 4 follow for the methyl ether and acetate respectively.



2: R_1 = Me, R_2 = Me
3: R_1 = Me, R_2 = H
4: R_1 = Me, R_2 = Ac
5: R_1 = H, R_2 = Me
6: R_1 = Ac, R_2 = Me



7: R = Me
8: R = H
9: R = Ac

It is generally accepted that isolated OMe groups are selectively solvated in benzene relative to chloroform or carbon tetrachloride and that resonances for such OMe groups suffer a 20–40 Hz upfield shift in benzene relative to chloroform.* In the acetate (**4**) none of the OMe resonances moved upfield in benzene, whereas in the methyl ethers (**2**) one of the OMe resonances occurs well upfield relative to the other OMe resonances.

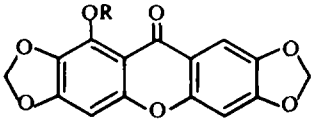
The upfield benzene shift of the OAc resonance in the acetate (**4**) further shows that the OH group is not located at the 1-position. These solvent shift results are only consistent with a structure in which the OH group of the yellow pigment occupies the isolated 7-position of the B-ring, as in 3.

The methyl ether (**2**) has been reported[2] as a constituent of *Polygala paenea* L. and the physical properties found for **2** compared well with those previously reported for polygalaxanthone B. Selective demethylation of the 1-OMe group of **2** to give **5** was achieved with refluxing 30% hydrochloric acid. The demethylated product (**5**) gave a strong green $FeCl_3$ test and showed physical properties consistent with the presence of a chelated OH group. Acetylation of **5** gave a monoacetate (**6**). The NMR spectrum of each of these derivatives showed an ABX aromatic pattern and were consistent with the assigned structures. The pigment (**5**) has been found naturally occurring in *Frasera caroliniensis* Walt. (Gentianaceae) and both **2** and **5** have been synthesized by Stout and Balkenhol.[5]

* For previous application of benzene solvent shifts to xanthones, see Ref 4a. Application of solvent shift techniques has led to ambiguous results on mangostin. However, the use of the method to compounds like mangostin which contain so many free hydroxy and C-isopentenyl groups which could also complex in an unexpected manner may well be unwarranted.

A second, colorless, relatively non-polar product, m.p. 156–157°, was isolated from the extracts by chromatography on alumina. Again, its UV spectrum suggested a xanthone chromophore. The IR spectrum again indicted the presence of a CO group with considerable single bond character. The NMR spectrum showed two aromatic singlets and six OMe resonances. The downfield position of one of the aromatic singlets indicated the presence of a proton at the 8-position. Two of the OMe resonances shifted upfield in benzene. The hexamethoxyxanthone could be selectively demethylated with 30% HCl to give the 1-demethyl derivative (**8**). The chemical shifts of the aromatic resonances were unchanged in **8** and its derived acetate (**9**), suggesting that both aromatic protons are in the other ring. These solvent shift data and lack of change in the chemical shifts of the aromatic resonances are consistent only with structure **7**. The UV spectrum of **8** is very similar to that reported for synthetic 1-hydroxy-3-methoxy-6,7-methylenedioxyxanthone.[2]

Smaller amounts of a colorless, high melting product, m.p. 250–252°, were recovered from the chloroform eluents. Again the UV spectrum indicated a xanthone chromophore. The NMR spectrum showed three aromatic singlets, two methylenedioxy resonances and one OMe resonance. Only one of the aromatic singlets occurred well downfield indicating the presence of a proton in the 8-position. In order to accommodate these groups on a xanthone system in a manner consistent with one group in the 1-position and three non-coupled aromatic protons, one of the rings must be substituted in the 8- and 5-positions by hydrogen and in the 6- and 7-positions by a



10: R = Me
11: R = H

methylenedioxy group. Both methylenedioxy resonances shifted upfield in benzene while the OMe resonance remained unaffected. The 5-OMe resonance in meliternatin[6] also does not undergo an upfield benzene shift.* These solvent shift data strongly support structure **10**.†

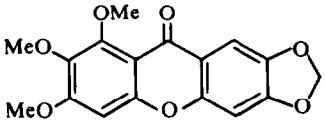
Chemical proof for the presence of the OMe group in the 1-position was obtained by selective demethylation with 30% HCl. The phenolic product (**11**) still retained both methylenedioxy groups. This fact, when considered with the hindered solvation of the OMe group, indicates that one methylenedioxy group must be in the 2,3-position. The demethylated product also gave a monoacetate and a positive Gibbs test[7] which further suggests the presence of a proton in the 4-position. Only one of the aromatic singlets moved upfield in the acetate relative to the parent methyl ether, further supporting structure **10**.

Rechromatography of the mother liquors gave additional amounts of **7** and **10** as well as small amounts of another minor xanthone. It showed m.p. 177° and gave

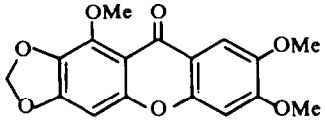
* Meliternatin (3,5-dimethoxy-6,7,3',4'-dimethylenedioxyflavone) shows δ 6·25, 6·25 O·CH_2O; 4·25, 3·99 OMe's ($CDCl_3$); δ 5·42, 5·25 O·CH_2O, 4·10, 3·89 OMe's (benzene).

† These NMR solvent shift data compare well with those of apiol (2,5-dimethoxy-3,4-methylenedioxypropenyl benzene); δ 5·82 O·CH_2O; 3·82, 3·77 OMe's (CCl_4); δ 5·62 O·CH_2O; 3·75, 3·55 OMe's (benzene) and safrole(3,4-methylenedioxypropenylbenzene); δ 5·92 O·CH_2O, ($CDCl_3$); δ 5·42 O·CH_2O (benzene).

a UV spectrum identical with that of **10**. The NMR spectrum showed three aromatic singlets, resonances for three OMe groups and one methylenedioxy group. The low field position of one of the aromatic singlets indicated that it must be due to a proton at H-8. The chemical shifts of the other two singlets were consistent with protons at the 3- or 4-positions, but not the 2-position.[8] The similarity of the UV spectrum with that of **10** indicates that one of the methylenedioxy groups in **10** is replaced by two OMe groups. This leads to two possible structures, **12** or **13**.



12



13

Distinction between these two possibilities could again be achieved by application of the benzene solvent shifts. Thus, in benzene, only one of the OMe resonances occurred well upfield. This is consistent with structure **12**. Two upfield OMe resonances would be expected for structure **13**. Compound **12** is an isomer of polygalaxanthone A(**1**), and the m.p. and UV spectrum found for **12** compare moderately well with those reported for polygalaxanthone A(**1**). However, comparison of the IR curve of **12** with that of polygalaxanthone A(**1**) established non-identity.*

The hexamethoxyxanthone found in this study is the most highly substituted alkoxyxanthone thus far reported. The permethylated xanthones found in *P. macradenia* are closely related to the xanthones reported in *P. paenea* both in the high degree of O-alkylation and in position of substitution.

The substitution patterns of the xanthones found in this study are unexceptional and parallel those previously reported in nature. They are consistent with ideas proposed for their biogenesis.[9]

In an effort to determine the general distribution of xanthones in other Polygalaceae species, the examination of extracts of several other *Polygala* species including *P. acanthoclada* A. Gray and *P. cornuta* Kell. have been undertaken. TLC of extracts of these species indicate they are devoid of xanthones.

EXPERIMENTAL

NMR spectra were taken at 60 MHz. The relative areas of the peaks were consistent with their assignments.

Isolation. Plant material was collected between Courtland and Gleesen on the southern edge of the Dragoon Mts., East of Tombstone, Arizona. The whole plants were ground and extracted with acetone. Solvent was removed from the extracts and the residue warmed with $CHCl_3$ and filtered through celite to remove $CHCl_3$ insoluble tar and sugars. $CHCl_3$ was removed from the filtrates and the residue was triturated with hexane. The hexane insoluble material was crystallized from EtOAc–hexane to give **3**. m.p. 197–198° when recrystallized from $CHCl_3$–EtOH; negative $FeCl_3$ and Mg–HCl test; ν 3360 (OH), 1620 (CO) cm^{-1} (Nujol); λ_{max}^{EtOH} 243 (27,200), 261 (35,800), 288 (8300), ~310, 370 (5600) mμ; $\lambda_{max}^{EtOH-NaOH}$ 260, 410 mμ; NMR δ 8·20 (s) aromatic, 4·80, 4·75, 4·43, 4·33 OMe's (CF_3COOH); (Found: C, 61·4; H, 4·84. $C_{17}H_{16}O_7$ requires: C, 61·44; H, 4·85%). Acetylation with Ac_2O–pyridine on a steam bath for 1 hr gave **4**; m.p. 88–90°, from MeOH; ν 1749 (acetate), 1652 (CO) cm^{-1} (Nujol); λ_{max}^{EtOH} 217, 239, 256, 287, ~300,

* The author is indebted to Dr. J. Polonsky for a comparison IR curve of **1**.

351 mμ; NMR δ 8·02 (broad singlet), 7·59 (s), 7·54 (s), 4·20, 4·06, 4·04, 4·04 OMe's, 2·38 OAc (CCl_4); δ 4·16, 4·03, 3·95, 3·95 OMe's, 1·98 OAc (benzene). (Found: C, 61·5; H 4·94. $C_{19}H_{18}O_8$ requires: C, 60·96; H, 4·85%).

Chromatography of the mother liquors on alumina and elution with hexane gave fractions (monitored by fluorescence on silicic acid TLC under UV light) which were concentrated, cooled and scratched with a little EtOAc–hexane to give **7**, m.p. 156–157°, after recrystallization from EtOAc–hexane; yellow fluorescence on TLC; ν 1645 (CO) cm^{-1} (Nujol); λ_{max}^{EtOH} ~247, 257(32,800), 280 (15,400), 312 (17,700), 344 (8100) mμ; NMR δ 7·50 (s) H-8, 6·70 (s) H-5, 4·22, 4·20, 4·20, 4·15, 4·12, 4·10 OMe's ($CDCl_3$); δ 4·25, 4·07, 3·99, 3·94, 3·58, 3·44 OMe's (benzene). (Found: C, 61·80; H, 5·77. $C_{19}H_{20}O_8$ requires: C, 60·63; H, 5·36%).

Further elution with benzene gave fractions which when concentrated and cooled gave a crop of 10; m.p. 250–252° after a further recrystallization from EtOAc; greenish-blue fluorescence on TLC; ν 1655 (CO), 1640, 930 (methylenedioxy) cm^{-1} (Nujol); λ_{max}^{EtOH} 248 (32,200), ~259, ~282, 321 (20,600), ~350 mμ; NMR δ 7·90 (s), 7·50 (s), 7·35 (s) aromatics, 6·57 (s) methylenedioxy, 4·80 OMe (CF_3COOH); δ 7·62 (s) H-8, 6·82 (s), 6·62 (s) H-4 and H-5, 6·13 (s), 6·08 (s) methylenedioxys, 4·20 OMe ($CDCl_3$); δ 5·48, 5·28 methylenedioxys, 4·01 OMe (benzene). (Found: C, 60·8; H, 3·29. $C_{16}H_{10}O_7$ requires: C, 61·15; H, 3·21%).

Further amounts of **3** were recovered from the $CHCl_3$ eluents. The mother liquors from the above isolation procedures were combined, solvent removed, and the residue rechromatographed on alumina. Fractions eluted with hexane yielded further amounts of **2**. Workup of fractions eluted with benzene gave **12**, m.p. 177° after recrystallization from EtOAc; ν 1635 (CO), 1597 (aromatic), 934 (methylenedioxy) cm^{-1} (Nujol); λ_{max}^{EtOH} 248 (34,500), ~272,313 (16,200), ~345 (8100) mμ; NMR δ 7·83 (s) H-8, 6·85 (s) 6.75 (s), H-4 and H-5, 6·15 (s) methylenedioxy, 4·10, 4·03, 3·99 OMe's ($CDCl_3$); δ 4·17, 3·85, 3·33 OMe's (benzene). (Found: C, 61·5; H, 4·36. $C_{17}H_{14}O_7$ requires: C, 61·82; H, 4·27%).

1,2,3,4,7-*Pentamethoxyxanthone* (**2**). The crude, yellow pigment was methylated with Me_2SO_4 and 10% NaOH aq. The mixture was extracted with benzene. The benzene extracts were dried and filtered through a short column of alumina. Removal of solvent and scratching with a little cold hexane gave **2**, m.p. 123·5–124°, (lit.[2] m.p. 120–121°) after recrystallization from EtOAc–hexane; ν 1660 (CO), 1610, 1590 cm^{-1} (Nujol); λ_{max}^{EtOH} 240 (18,500), 260 (24,000), 287 (9000), ~307, 365 (6700) mμ; NMR δ 7·70 (d) J = 3, H-8, 7·50 (d) J = 9 H-5, 7·30 (q) J = 9, J = 3 H-6, 4·20, 4·10, 4·05, 4·05, 4·05 OMe's (CCl_4); δ 4·25, 4·09, 4·00, 4·00, 3·62 OMe's (benzene); (Found: C, 62·7; H, 5·27. $C_{18}H_{18}O_7$ requires: C, 62·42; H, 5·24%). This material was identical in all respects with a synthetic sample of **2** provided by Professor G. H. Stout.

1-*Hydroxy*-2,3,4,7-*tetramethoxyxanthone* (**5**). Compound **4** was demethylated by refluxing with 30% HCl aq for 45 min to give, after workup, **5**, m.p. 111–113° (lit.[2] m.p. 115°), from MeOH; green $FeCl_3$ test; ν 1647 cm^{-1} (Nujol); λ_{max}^{EtOH} 236 (17,000), 269 (21,200), 305 (7500), 384 (3700) mμ; $\lambda_{max}^{EtOH-NaOH}$ 282, 422 mμ; $\lambda_{max}^{EtOH-AlCl_3}$ 214, 236, 285, 322, 447 mμ; NMR δ 12·62 (s) OH, 7·60 (d) J = 3 H-8, 7·50 (d) J = 9 H-5, 7·32 (q) J = 3, J = 9 H-6, 4·22, 4·03, 4·03, 3·97 OMe's ($CDCl_3$); δ 4·07, 4·03, 3·93, 3·47 OMe's (benzene).

Acetylation with Ac_2O–pyridine gave **6**, m.p. 147·5–148·5°, from EtOAc–hexane; ν 1755 (acetate), 1645 (CO), 1600 cm^{-1} (Nujol); λ_{max}^{EtOH} 240, 259, 280, 306, 360 mμ; NMR δ 7·63 (d) J = 3 H-8, 7·50 (d) J = 9 H-5, 7·30 (q) J = 9, J = 3 H-6, 4·20, 4·13, 4·02, 3·97 OMe's, 2·55 OAc ($CDCl_3$); δ 3·97, 3·97, 3·87, 3·53 OMe's, 2·57 OAc (benzene). (Found: C, 61·2; H, 4·90. $C_{19}H_{18}O_8$ requires: C, 60·96; H, 4·85%).

1-*Hydroxy*-2,3,4,6,7-pentamethoxyxanthone (**8**). A soln of 100 mg of **7** was refluxed 30 min with 30% HCl aq. The mixture was cooled, diluted with water and extracted with EtOAc. The dried EtOAc extracts were concentrated and hexane was added. After cooling, **8** was collected, m.p. 170–173°, after a second recrystallization from EtOAc; green $FeCl_3$ test; ν 1645 (CO) cm^{-1} (Nujol); λ_{max}^{EtOH} 242 (28,700), 263 (29,500), 318 (17,800), 365 (5100) mμ; $\lambda_{max}^{EtOH-NaOH}$ 281, 421 mμ; $\lambda_{max}^{EtOH-AlCl_3}$ 235, 275, 338, 410 mμ; NMR δ 13·93 (s) OH, 7·50 (s) H-8, 6·70 (s) H-5, 4·23, 4·23, 4·14, 4·10, 4·09 OMe's; ($CDCl_3$); δ 4·07, 4·07, 3·95, 3·50, 3·42 OMe's (benzene). (Found: C, 59·6; H, 5·01. $C_{18}H_{18}O_8$ requires: C, 59·66; H, 5·01%). Acetylation with Ac_2O–pyridine gave **9**, m.p. 168–170°, after recrystallization from EtOAc–hexane; ν 1755 (acetate), 1640 (CO) cm^{-1} (Nujol); λ_{max}^{EtOH} ~246, 255, ~272, 312, ~338 mμ; NMR δ 7·40 (s) H-8, 6·97 (s) H-5, 4·13, 4·13, 4·01, 4·00, 3·93 OMe's, 2·58 OAc ($CDCl_3$); δ 4·01, 3·98, 3·93, 3·62, 3·45 OMe's, 2·58 OAc (benzene). (Found: C, 59·2; H, 4·81. $C_{20}H_{20}O_9$ requires: C, 59·40; H, 4·99%).

1-*Hydroxy*-2,3,6,7-*dimethylenedioxyxanthone* (**11**). A soln of 70 mg of **10** was refluxed with conc HCl for 45 min. After workup, **11** was recrystallized from EtOAc, m.p. 282–284° (dec); green $FeCl_3$ test; ν 1660 cm^{-1} (Nujol); λ_{max}^{EtOH} 249, ~258, ~284, 324, ~353 mμ; $\lambda_{max}^{EtOH-AlCl_3}$ 353, 403 mμ; $\lambda_{max}^{EtOH-NaOH}$ 320, 386 mμ; NMR δ 7·70 (s) H-8, 7·32 (s), 7·12 (s) H-4 and H-5, 6·42 methylenedioxys (CF_3COOH). (Found: C, 59·6; H, 2·81. $C_{15}H_8O_7$ requires: C, 60·01; H, 2·69%).

Acknowledgements—The author is indebted to Austin Griffiths, Jr., Los Angeles County Arboretum, Arcadia, Calif. for identification of plant material, to Douglas Ripley for collection of *P. Cornuta*, and to Professor G. H. Stout for a sample of synthetic 2. This study was supported, in part, by an NSF Institutional grant to San Francisco State College.

REFERENCES

[1] See, for example, M. Shamma and L. P. Reiff, *Chem. & Ind.* 1272 (1960); J. S. Rondest, J. Polonsky, *C.R. Acad. Sci., Paris* **254**, 1298 (1962).

[2] J. Moron, J. Polonsky and H. Pourrat, *Bull. Soc. Chim. Fr.* 130 (1967).

[3] See, for example, D. L. Dreyer, Ph.D. Thesis, University of Washington, 1960; O. R. Gottlieb, M. Taveira Magalhaes, M. Camey, A. A. Lins Mesquita and D. de Barros Correa *Tetrahedron* **22**, 1777 (1966); O. R. Gottlieb, M. Taveira Magalhaes and G. M. Stefani, *Ibid.* **22**, 1785 (1966).

[4] [a] B. Jackson, H. D. Locksley and F. Scheinmann, *J. Chem. Soc.* (C) 2500 (1967);
[b] F. Scheinmann, *Chem. Comm.* 1015 (1967); G. H. Stout, M. M. Krahn, P. Yates and H. B. Bhat, *Ibid.* 211 (1968).

[5] G. H. Stout and W. J. Balkenhol, *Tetrahedron* **25**, 1947 (1969).

[6] L. H. Briggs and R. H. Locker, *J. Chem. Soc.* 2157 (1949); *Ibid.* 2376 (1950); *Ibid.* 3131 (1951).

[7] F. E. King, T. J. King and L. C. Manning, *J. Chem. Soc.* 563 (1957); E. D. Burling, A. Jefferson and F. Scheinmann, *Tetrahedron* **21**, 2653 (1965).

[8] K. Markham, *Tetrahedron* **21**, 3687 (1965).

[9] O. R. Gottlieb, *Phytochemistry* **7**, 411 (1968).