Tetrahedron. Vol. 25, pp. 5295 to 5299 Pergamon Press 1969. Printed in Great Britain

POLYGALAXANTHONE A

A REVISED STRUCTURE

G. H. STOUT and J. L. FRIES

Department of Chemistry, University of Washington, Seattle, Wash. 98105

(*Received in USA* 12 *May* 1969; *Received in the UK for publication* 17 *July* 1969)

Abstract—Polygalaxanthone A has been shown by synthesis to have the structure 1,2,3-trimethoxy-6,7-methylenedioxyxanthone (**2a**) and not that originally proposed. The previous evidence for the structure is discussed, and the material is shown also to be identical with another xanthone previously isolated from *Polygala macradenia*.

POLYGALAXANTHONE A, isolated by Moron *et al.*[1] from *Polygala paenea* (Polygalaceae) was assigned the structure **1** on chemical and spectroscopic grounds. Such a compound is unique among natural products in bearing a single C-3 proton in an otherwise oxygenated xanthone ring. Although this pattern can be accommodated in proposed biosynthetic schemes,[2] it requires an uncommon reduction at C-3 of a phloroglucinol moiety sequentially with oxidation at the C-2 and C-4 positions. Such a series of transformations, although possible, appears unlikely, and to confirm or refute this structure and its implications we have undertaken a program of synthesis of **1** and its isomers **2a** and **3a**,* using the methods proved successful in the *Frasera* xanthones.[3]

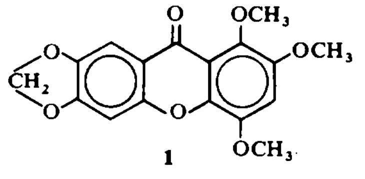
2-Methoxy-4,5-methylenedioxybenzoic acid was condensed with 1,2,3-trimethoxy-5-acetoxybenzene in $(CF_3CO)_2O$ for 9 days. Hydrolysis and ring closure of the crude products with $Me_4N^+OH^-$ in aq. pyridine gave 1,2,3-trimethoxy-6,7-methylenedioxyxanthone, **2a**, which showed three crystalline forms, m.ps 174, 178, and 187°. The spectral properties and the 174° m.p. of this material are very similar to those reported for Polygalaxanthone A (lit.[1] m.p. 170°) and direct comparison with a sample furnished by Dr. Polonsky (our m.p. 174°) showed the two to be identical by m.m.p., IR, UV, NMR, and mass spectra. Thus the true structure of Polygalaxanthone A is **2a** and it is quite consistent biosynthetically with the many other simple oxygenated xanthones.[2]

The structure **2a** has already been proposed for a xanthone m.p. 177° isolated from *Polygala macradenia* by Dreyer.[4] The similarity of its properties to those of Polygalaxanthone A was noted at that time; however, a direct comparison of the IR spectra of the two substances was reported to show small but distinct differences. Dr. Dreyer provided an IR spectrum as well as a sample of this material. His spectrum differed slightly from that of synthetic **2a**, but the sample was identical in NMR ($CDCl_3$, C_6H_6), R_f value, and mass spectra. Further purification by preparative

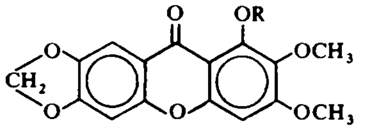
---

* Depending on the degree to which one wishes to disregard the published evidence, other structures may be advanced, but these appeared to us to represent the most reasonable alternatives.

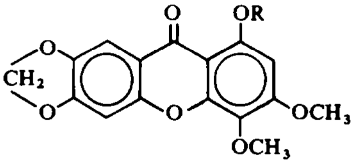
TLC gave a sample which was identical with **2a** by IR and m.m.p. (187°) as well, confirming that both species of *Polygala* yield the same product.



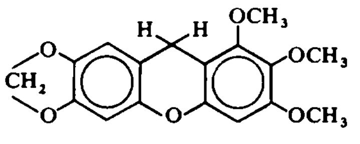
1



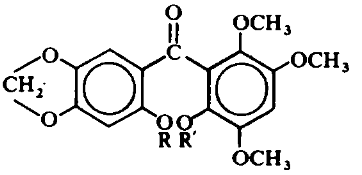
2a: R = CH_3
b: R = H



3a: R = CH_3
b: R = H



4



5a: R = CH_3, R′ = H
b: R = H, R′ = CH_3

The original investigation of Polygalaxanthone A involved the elimination of 4-unsubstituted structures such as **2** on the basis of a Gibbs test on the 1-demethyl derivative (i.e. **2b**). In view of the known uncertainties of this test[5] we have re-examined it, also using the qualitative spectrophotometric procedure of King *et al.*[6] Both the 1-hydroxy compound **2b** and 1-hydroxy-2,3,7-trimethoxyxanthone[3] fail to give absorption maxima in the region 500–700 nm, *but show pronounced maxima* ca. 745 nm. In contrast, both **3b** and 1-hydroxy-2,3,4,5-tetramethoxyxanthone show no peaking in this region. Thus for heavily oxygenated xanthones of this type the limits of examination must be extended if a false negative test is to be avoided.

The reduction of **2a** with LAH was carried out using the method described for natural Polygalaxanthone A,[1] but only the xanthene **4a** was obtained, no trace of the reported demethoxyxanthene being observed. It is not clear whether these differences in products reflect small differences in reaction conditions, or whether the reported product may have arisen from a small amount of an undetected impurity[3a] in the natural material.

To complete the series of isomers, the same acid was converted to its acid chloride and condensed with 1,2,3,5-tetramethoxybenzene using $AlCl_3$ in ether. Ring closure of the demethylated products with $Me_4N^+OH^-$ in pyridine led to a very small yield of **2a**, and, as would be predicted from the expected direction of demethylation,[3] a larger amount of **3a**, m.p. 218°. Demethylation with refluxing HCl/MeOH gave the 1-hydroxy derivative **3b**, m.p. 219–221°.

The synthesis of authentic **1** was more difficult, but was accomplished in very poor yield by the condensation of the same acid chloride with 1,2,4,5-tetramethoxybenzene using $ZnCl_2$ in $POCl_3$. The crude product was demethylated in refluxing THF/$AlCl_3$ and cyclized as usual in base. This yielded 1·8% of **1** after chromatography, m.p. 202–206°. A major cause of the poor yield was apparently the predominant formation in the demethylation reaction of the benzophenone **5b** in addition to the expected[3] **5a**. The latter closed readily in the usual basic medium, but the former proved unexpectedly inert even under forcing conditions.

EXPERIMENTAL

TLC was carried out on Merck silica gel HF_{254}. Elution was with various mixtures of EtOAc/pet. ether containing 1% HOAc, the ratios being given as (EtOAc–pet. ether) below. Preparative TLC used a continuous elution technique in which the solvent was allowed to evaporate from the upper end of the plate until sufficient migration could be observed with a UV lamp. Mass spectra were obtained on a AEI MS-9 spectrometer.

Methyl 2-hydroxy-4,5-methylenedioxybenzoate. The method of Arnold and Bordwell[7] was used to prepare this compound from piperonylic acid. The overall yield was 31%.

2-Methoxy-4,5-methylenedioxybenzoic acid. Methyl 2-hydroxy-4,5-methylenedioxybenzoate (7·6 g), 60 g anhyd K_2CO_3, 16 Me_2SO_4, 3 g $Na_2S_2O_4$, and 170 ml acetone were refluxed on a steam bath for 20 hr, after which most of the acetone was distilled off. The residue was made basic with 200 ml of 20% NaOH aq and refluxed for 1 hr to hydrolyze the ester. The cooled reaction mixture was acidified with conc HCl, and the off-white ppt filtered off, washed, and dried *in vacuo*. On thin layer plates, the product acid was accompanied by a less polar impurity which was easily removed by dissolving the acid in sat $NaHCO_3$ aq and extracting this with dichloromethane. Reprecipitation by acidification gave pure 2-methoxy-4,5-methylenedioxybenzoic acid, m.p. 153–153·2°, 5·48 g after drying *in vacuo*.

1,2,3-Trimethoxy-6,7-methylenedioxyxanthone (**2a**). In a previously flamed, dry N_2-gas-cooled flask were combined: 1·04 g (4·6 mmole) 1,2,3-trimethoxy-5-acetoxy-benzene,[3] 0·93 g (4·8 mmole) 2-methoxy-4,5-methylenedioxybenzoic acid, and 8 ml trifluoroacetic anhydride. The flask was stoppered, and the reaction mixture was allowed to stand at room temp for 9 days. The mixture was then poured into 100 ml ice-water and extracted with CH_2Cl_2 (3 × 25 ml). The combined organic layers were washed with 30 ml sat $NaHCO_3$aq, and the CH_2Cl_2 was evaporated. The residue was heated with 40 ml 10% NaOH for ½ hr on a steam bath to hydrolyze trifluoroacetate esters. After cooling, the alkaline soln was extracted with CH_2Cl_2 (4 × 60 ml), and the extracts evaporated to yield 0·55 g of viscous orange oil.

This oil was refluxed in 35 ml pyridine and 35 ml 10% aqueous $Me_4N^+OH^-$ for 1 hr. The reaction mixture was poured into 200 ml cold water, acidified, and extracted with CH_2Cl_2 (3 × 100 ml). Hexane (ca. 100 ml) was added to the combined extracts, which were washed with Claisen's alkali[8] (3 × 50 ml). The organic layer was dried ($MgSO_4$), filtered, and evaporated. The white crystalline product, 0·39 g (26%), was recrystallized from MeOH, giving two crystalline forms, m.p. 172·5–173·5 and 178·5–179°. A third modification, m.p. 186–187·5°, was obtained by seeding with crystals obtained from purified *P. macradenia* material (see below). A mixture with authentic Polygalaxanthone A (m.p. 173–173·9°) gave m.p. 173–178°. (Found: MW, 330·07396. $C_{17}H_{14}O_7$ requires: MW, 330·07396); NMR ($CDCl_3$): τ 2·40 (1H), 3·23 (1H), 3·33 (1H), 3·95 (2H), 6·01 (3H), 6·05 (3H), 6·12 (3H); UV; λ_{max}^{EtOH} 248 (37,000), 273 (10,100, sh), 314 (19,200), 339 (11,700), 353 (10,800, sh) nm. These spectra were essentially identical with those obtained from authentic Polygalaxanthone A and the *P. macradenia* product.

Purification of **2a** *from* P. macradenia. A sample of **2a** isolated by Dreyer[4] from *Polygala macradenia*, m.p. 175–178°, was purified by preparative TLC (1:2). The major band, which corresponded to synthetic **2a**, was isolated and crystallized from MeOH to yield material m.p. 187–188° with synthetic **2a** (high *or* low melting forms) 186–187·5°.

1-Hydroxy-2,3-dimethoxy-6,7-methylenedioxyxanthone (**2b**). A few milligrams of **2a** were refluxed in 2 ml MeOH and 4 ml conc HCl overnight. The diluted (H_2O) reaction mixture was extracted with CH_2Cl_2 (3 × 15 ml); the extracts were combined, dried ($MgSO_4$), and filtered. The crude material was purified by preparative TLC (1:8). The yellow solid obtained from the band of highest R_f was crystallized from MeOH, m.p. 234–238° (lit. m.p. 228–240°).[1] (Found: MW 316·05831, $C_{16}H_{12}O_7$ requires: MW 316·05831); UV: λ_{max}^{EtOH} (relative ε) 260 (1·0), 293 (0·38), 321 (0·52), 362 (0·28) nm.

1,3,4-*Trimethoxy*-6,7-*methylenedioxyxanthone* (**3a**). In a previously flamed, N_2-gas cooled 100 ml flask fitted with a drying tube were combined: 1·00 g 2-methoxy-4,5-methylenedioxybenzoic acid, 18 ml oxalyl chloride, and 10 ml benzene. The solution was allowed to stand at room temp overnight. The benzene and excess oxalyl chloride were removed by vacuum rotary evaporator, and to the solid beige acid chloride were added 1·10 g 1,2,3,5-tetramethoxybenzene[9] and 80 ml absolute ether (distilled from LAH). With ice-bath cooling, 4 g $AlCl_3$ was gradually dropped in and the resulting dark red soln was refluxed (drying tube) on the steam bath for 20 hr. It was then slowly poured into dilute ice-cold HCl and allowed to hydrolyze at room temp for ca. 3 hr. The soln was extracted with CH_2Cl_2 (4 × 10 ml). The combined CH_2Cl_2 extracts were washed with 10% NaOH (50 ml). Hexane (100 ml) was added to the CH_2Cl_2 soln and the total was extracted with Claisen alkali (3 × 75 ml). The Claisen alkali extracts were combined, acidified (conc HCl), and extracted with CH_2Cl_2. The organic extracts were combined, dried (Na_2SO_4), filtered, and evaporated. The bright yellow oily residue, 0·87 g, was refluxed for 2½ hr in 50 ml pyridine and 50 ml 10% aqueous $Me_4N^+OH^-$. Acidification of the reaction mixture with 400 ml dil HCl was followed by extraction with CH_2Cl_2 (5 × 100 ml). The combined organic extracts, with 100 ml added hexane, were washed with Claisen's alkali until the basic extract was colorless. The organic layer was dried ($MgSO_4$) and filtered. After removal of the solvent the residual white solid was chromatographed on preparative plates (1:3). The two major white fluorescent bands were each collected and separately rechromatographed (1:2). The more polar band yielded 0·45 g of white crystalline **3a**, m.p. 216·5–217·5° after recrystallizing from MeOH. (Found: MW, 330·074. $C_{17}H_{14}O_7$ requires: MW, 330·07396); NMR ($CDCl_3$): τ 2·37 (1H), 3·08 (1H), 3·57 (1H), 3·92 (2H), 5·99 (6H), 6·05 (3H); UV: λ_{max}^{EtOH} 239 (sh), 256 (41,500), ~295 (sh), 302 (11,300), ~355 (sh), 362 (13,900) nm.

The less polar white fluorescent band was collected and recrystallized from MeOH, giving 35 mg of **2a**, m.p. 178·3–178·8°.

1-*Hydroxy*-3,4-*dimethoxy*-6,7-*methylenedioxyxanthone* (**3b**). Partial demethylation of **3a** was accomplished as described above for **2a**. The product was purified by prep TLC (1:8). The least polar yellow band was collected, sublimed and recrystallized from MeOH, giving yellow needles, m.p. 219–221°. (Found: MW, 316·058. $C_{16}H_{12}O_7$ requires: MW, 316·05831); UV; λ_{max}^{EtOH} 260, 309, 372; $\lambda_{max}^{EtOH-AlCl_3}$ 228, 270–285, 316, 334, 410 nm.

1,2,4-*Trimethoxy*-6,7-*methylenedioxyxanthone* (**1**). The acid chloride was prepared from 1·00 g of 2-methoxy-4,5-methylenedioxybenzoic acid as previously described. To the acid chloride, in a flask fitted with drying tube and magnetic stirrer, were added 1·10 g 1,2,4,5-tetramethoxybenzene,[10] 20 ml $POCl_3$, and 9 g of hot but solidified freshly fused $ZnCl_2$. The mixture was stirred at room temp for 25 hr; then it was poured *very* gradually onto ca. 400 g ice and heated until the greenish tinge disappeared. After extraction with CH_2Cl_2 (4 × 75 ml), the combined organic layers with 100 ml added hexane were washed with Claisen's alkali (3 × 50 ml) and then dried ($MgSO_4$), filtered, and evaporated. The white solid residue, 1·38 g, was allowed to reflux for ca. 8 hr in a mixture of 40 ml anhyd THF and 5 g $AlCl_3$. The mixture was poured into 200 ml cold dil HCl and heated on a steam bath. Extraction with CH_2Cl_2 (3 × 50 ml) was followed by extraction of the combined CH_2Cl_2 layers (100 ml hexane added) with Claisen's alkali (3 × 50 ml). The combined, acidified base extracts were in turn extracted with CH_2Cl_2 (3 × 75 ml) which was dried ($MgSO_4$), filtered, and evaporated to give a bright oil, 0·40 g.

This material was refluxed 2 hr in a mixture of 40 ml each of 10% aqueous $Me_4N^+OH^-$ and pyridine. The diluted mixture (200 ml H_2O) was acidified, and extracted with CH_2Cl_2 (3 × 75 ml). The organic extracts plus 100 ml hexane were washed with Claisen's alkali (3 × 50 ml). The organic layer, after drying ($MgSO_4$), was filtered and the solvent was removed, yielding a very pale yellow solid, 40 mg.

The twice-chromatographed product (1:1) was recrystallized two times from MeOH, giving 32·3 mg of pale yellow prisms, 202–206°. (Found: MW, 330·074. $C_{17}H_{14}O_7$ requires: MW, 330·07396); NMR ($CDCl_3$): τ 2·37 (1H), 3·02 (1H), 3·06 (1H), 3·90 (2H), 5·99 (3H), 6·04 (6H); UV: λ_{max}^{EtOH} 254 (28,500), 277 (25,200), 327 (7540), 375 (6560) nm.

The Claisen's alkali soluble fraction from the cyclization reaction yielded a larger amount (ca. 100 mg) of pale yellow crystalline **5b**, m.p. 146–147°. (Found: MW, 362·106. $C_{18}H_{18}O_8$ requires: MW, 362·100); NMR ($CDCl_3$): τ −2·85 (1H), 3·31 (1H), 3·35 (1H), 3·50 (1H), 4·08 (2H), 6·12 (6H), 6·28 (6H).

Gibbs tests.[6] The substance to be tested (1–3 mg) was dissolved in 1·00 ml pyridine in a 25 ml graduated cylinder. To this was added 5·00 ml of a freshly prepared soln of 20–30 mg Gibb's reagent, N, 2,6-trichloro-*p*-benzoquinoneimine, in 15 ml pyridine. The mixture was diluted to 20 ml with sodium borate buffer (pH 9·2) and well stirred. A precisely similar blank soln (without unknown) was mixed at the same time.

The absorption spectrum was then determined between 10 and 20 min after making up the solns, using the blank in the reference cell. The spectrum from 550 to 800 nm was obtained.

Lithium aluminum hydride reduction of 1,2,3-*trimethoxy*-6,7-*methylenedioxyxanthone* (**2a**). To a soln of 0·53 g LAH in 40 ml anhyd ether was gradually added a soln of 126 mg xanthone dissolved in 10 ml anhyd benzene. When the addition was complete the reaction was refluxed (43°) for 2 hr, allowed to stand overnight, and refluxed 2 hr further. The cooled soln was cautiously added to 300 ml ice-water and extracted with ether (4 × 100 ml). The colorless aqueous layer was acidified with H_2SO_4 (yellow soln) and extracted with CH_2Cl_2 (100 ml). The organic portions were combined, dried ($MgSO_4$), filtered and the solvent was removed. The yellow residue was crystallized from MeOH, giving two crops of **4**, beige crystals of combined weight 80 mg (67%). A recrystallization gave m.p. 122–123·5°. (Found: MW, 316·093. $C_{17}H_{16}O_6$ requires: MW, 316·095); NMR ($CDCl_3$) τ 3·40 (1H), 3·48 (1H), 3·64 (1H), 4·10 (2H), 6·07 (2H), 6·17 (9H).

Analytical TLC of the dark brown mother liquors from the initial crystallizations showed much additional **4** and a multitude of minor spots. A NMR spectrum of the mother liquor in $CDCl_3$ showed primarily (> 70%) the absorptions due to **4**. A minor peak appeared at τ 3·80, and a broad absorption around τ 3·27. No bands could be seen at or near τ 3·72, although such an absorption was reported for the demethoxyxanthene.[1] Thus, if any of the demethoxylated product was produced, it was only in *very* minor amounts.

Acknowledgements—This work was supported in part by a grant (GM-12095) from the Public Health Service. One of us (JLF) thanks NDEA for a fellowship. We are particularly indebted to Drs. Polonsky and Dreyer for providing samples of their natural products.

REFERENCES

[1] J. Moron, J. Polonsky and H. Pourrat, *Bull. Soc. Chim. Fr.* 130 (1967).
[2] e.g. O. R. Gottlieb, *Phytochemistry* **7**, 411 (1968).
[3] [a] G. H. Stout and W. J. Balkenhol, *Tetrahedron* **25**, 1947 (1969);
[b] G. H. Stout, E. N. Christensen, W. J. Balkenhol and K. L. Stevens, *Ibid.* **25**, 1961 (1969).
[4] D. L. Dreyer, *Ibid.* in press.
[5] E. D. Burling, A Jefferson and F. Sheinmann, *Ibid.* **21**, 2653 (1965) and refs cited there.
[6] F. E. King, T. J. King and L. C. Manning, *J. Chem. Soc.* 563 (1957).
[7] R. T. Arnold and F. Bordwell, *J. Am. Chem. Soc.* **64**, 2983 (1942); E. Oertly and A. Pictet, *Ber. Dtsch. Chem. Ges.* **43**, 1336 (1910).
[8] L. Claisen, *Liebigs Ann.* **418**, 96 (1919).
[9] W. J. Baker, *J. Chem. Soc.* 2542 (1931).
[10] F. Benington, R. D. Marin and L. C. Clark, Jr., *J. Org. Chem.* **20**, 102 (1955).