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GENTIAN PIGMENTS—I

XANTHONES FROM *GENTIANA BELLIDIFOLIA*

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Abstract—Four pigments have been isolated from *Gentiana bellidifolia* and the structures of three shown to be I, VI and VII.

XANTHONES previously isolated from species of the genus *Gentiana* include gentisin[1] ($C_{14}H_{10}O_5$) and isogentisin ($C_{14}H_{10}O_5$).[2] These have been shown by synthesis to be 1,7-dihydroxy-3-methoxy-xanthone[3] and 1,3-dihydroxy-7-methoxy-xanthone[4] respectively. The isolation of corymbiferin ($C_{15}H_{12}O_7$) from *corymbifera* was reported in 1950[5] but structural investigations failed to reveal the oxygenation pattern.

In the present investigation xanthone pigments from *bellidifolia*[6] have been studied. Ethanol extraction of root material followed by acid hydrolysis of the extract gave a mixture which was separated by repeated chromatography on silica gel to give four yellow pigments, A, B, C, and D, each in about 0·3% yield. All four were provisionally assigned xanthone structures on the basis of their typically xanthone-like UV (Table 1) and IR absorption spectra (cf. refs 7 and 8).

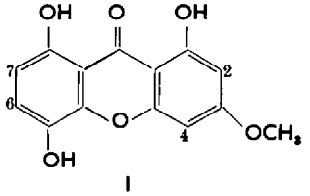
TABLE 1. ULTRAVIOLET ABSORPTION SPECTRA OF BELLIDIFOLIUM COMPOUNDS[9]
$m\mu$ (log ε)

A.	230(4·3),	254(4·4),	278(4·2),	300 sh.(3·8),	335(4·0)
B.	230–237(4·1),	255(4·2),	279(4·1),	302 sh.(3·7),	334(3·9)
C.	228(4·3),	248(4·2),	280(4·2),	—	366(4·2)
D.	231(4·3),	250(4·3),	274(4·2),	—	350(4·1)
(III)	236(4·4),	262(4·4),	266 sh.(4·3),	345 sh.(4·2)	363(4·2)

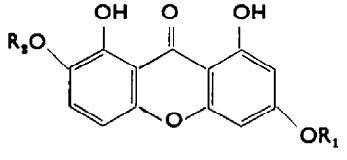
[1] A. G. Perkin, *J. Chem. Soc.* **73**, 666 (1898).
[2] L. Canonica and F. Pelizzoni, *Gazz. Chim. Ital.* **85**, 1007 (1955); *Chem. Abstr.* **50**, 9399 (1956).
[3] N. Anand and K. Venkataraman, *Proc. Indian Acad. Sci.* **25A**, 438 (1947); *Chem. Abstr.* **42**, 6810 (1948).
[4] J. Shinoda, *J. Chem. Soc.* 1983 (1927).
[5] D. J. Ross, *N.Z. J. Sci. and Tech.* **32**, No. 3, 39 (1950).
[6] Type sample: Dominion Museum, Wellington, New Zealand. W.E.L.T. 30847.
[7] R. C. Roberts, *Chem. Revs.* **61**, 592 (1961).
[8] F. Scheinmann, *Tetrahedron* **18**, 853 (1962).
[9] UV spectra were determined in ethanol on a Beckman D.K.2 spectrophotometer.

Bellidifolium (*Pigment-B*). Bellidifolium, m.p. 270-271°, $C_{14}H_{10}O_6$, formed a triacetate and a dimethyl ether, and gave an NMR spectrum (Table 2) showing methoxyl proton absorption at 233 c/s and hydroxyl at 592, 670 and 720 c/s. This suggests that bellidifolium is a monomethoxytrihydroxyxanthone. The absence of C_1 or C_8 carbonyl-deshielded protons (which should occur at about 490 c/s[10]) indicates that bellidifolium is oxygenated in the 1 and 8 positions. The presence of a 1,8-dihydroxy system is strongly suggested by the two hydrogen-bonded hydroxyl protons at 670 and 720 c/s. In such a system, methylation of one hydrogen-bonded hydroxyl group would be expected to increase the extent of hydrogen bonding of the other and thus produce a downfield shift of its signal (cf. ref. 11). This is found to be the case with dimethyl bellidifolium where the one remaining hydrogen-bonded hydroxyl proton has shifted downfield to 773 c/s, a value which is in accord with those observed for 1-hydroxyxanthones such as 1,3-dihydroxyxanthone (768 c/s) and celibixanthone (789 c/s).[12]

Coupled doublets centred at 436, 398 c/s (J = 9) and 393, 380 c/s (J = 3) represent two pair of aromatic protons which, in the absence of more complex signal splitting, can not be in the same ring. Splitting constants of 9 and 3 c/s indicate[13] the presence of "ortho" and "meta" proton pairs respectively; thus the only oxidation patterns tenable for bellidifolium are 1,3,5,8 and 1,3,7,8. The methoxyl group must be placed at position 3 since bellidifolium is insoluble in sodium carbonate (cf. ref. 14) and this reduces the structural possibilities to two only, i.e. I and II. These are consistent with all of the above evidence and contain the 5,8 or 7,8-dihydroxy systems implied[12,15] by the positive Tollens reagent test given by bellidifolium.



I



II $R_1 = CH_3$, $R_2 = H$.
III $R_1 = H$, $R_2 = CH_3$

In view of the established oxygenation patterns for xanthones earlier isolated from members of the genus *Gentiana*, structure (II) was considered the more probable for bellidifolium. A synthesis of dimethyl bellidifolium was therefore attempted.

1,3,8-Trihydroxy-7-methoxyxanthone (III) was prepared by a nine step synthesis outlined in a series of papers[16,17] and then methylated with diazomethane. The trimethyl ether so formed was decussatin (1,3,7-trimethoxy-8-hydroxyxanthone) and

[10] L. Crombie and D. Whiting, *Tetrahedron Letters* No. 18, 801 (1962).
[11] J. H. Chaudet, G. C. Newland, H. W. Patton and J. W. Tamblyn, *S.P.E. Transactions* **1**, No. 1 26 (1961).
[12] G. H. Stout, V. F. Stout and M. T. Welsh, *Tetrahedron* **19**, 667 (1963).
[13] L. M. Jackman, *Applications of NMR Spectroscopy in Organic Chemistry* p. 85. Pergamon Press, Oxford (1959).
[14] G. V. Rao and T. R. Sheshadri, *Proc. Indian Acad. Sci.* **37A**, 710 (1953); *Chem. Abstr.* **48**, 10017 (1954).
[15] R. C. Roberts, *J. Chem. Soc.* 785 (1960).
[16] W. Baker, *J. Chem. Soc.* 956 (1939); *Ibid.* 2439 (1953).

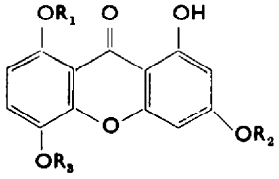
TABLE 2. NUCLEAR MAGNETIC RESONANCE SPECTRA OF XANTHONES*

	Hydroxyl Protons (c/s)		Methoxyl Protons (c/s)		Aromatic Protons (c/s)	
Methyl bellidifolium (A)	706, 667	(2)	233	(6)	450, 441, 405, 396, 393, 382, 379	(4)
Bellidifolium (B)	720, 670, 592	(3)	233	(3)	440, 431, 401, 395, 392, 382, 379	(4)
Desmethyl bellidifolium (D)	722, 675, 590	(3)			443, 434, 405, 396, 391, 388, 378, 375	(4)
Dimethyl bellidifolium	773	(1)	237, 239, 242	(9)	435, 426, 405, 398, 395, 383, 380	(4)
1,3,8-Trihydroxy-7-methoxyxanthone (III)	713, 707	(2)	233	(3)	458, 449, 425, 415, 387, 384, 379, 377	(4)
1,3-Dihydroxyxanthone	768	(1)	—		(499 — 448) 386, 383, 376, 373,	(6)
1-Hydroxy-3-methoxyxanthone	762	(1)	236	(3)		

* All NMR spectra were determined on a Varian D.P. 60 spectrometer at 60 Mc. As solvent, chloroform-*d* was used for dimethyl bellidifolium and dimethyl sulphoxide-*d* for the others. T.M.S. was used as internal standard and deuterium oxide was added to identify hydroxyl groups. The number of protons in each region is in parenthesis.

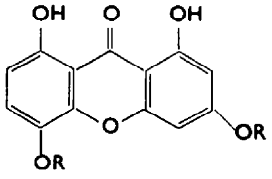
this differed spectrally and chromatographically from dimethyl bellidifolium. Other important differences between bellidifolium and the 1,3,7,8-oxygenated series occur in the UV absorption spectra (Table 1 and ref. 7) and in the aromatic proton region of the NMR spectra (Table 2). Bellidifolium should therefore be 1,5,8-trihydroxy-3-methoxyxanthone (I) containing the same oxidation pattern as swerchirin (IV)[17] and swertianol (V),[18] both of which have been isolated from the genus *Swertia* (*F. Gentianaceae*).

Since swerchirin and swertianol derivatives were unobtainable, a comparison of the melting points of several bellidifolium compounds with their analogues in this series was carried out.



IV $R_3 = CH_3$, R_1 or $R_3 = CH_3$

V $R_3 = H$, R_1 or $R_3 = CH_3$



VI R = CH_3.

VII R = H.

Desmethyl bellidifolium (VII) m.p. 315–320° and its tetra-acetate m.p. 242° compare well with desmethyl swertianol m.p. 315–320° and its tetra-acetate m.p. 244°[18] (cf. 1,3,7,8-tetrahydroxyxanthone m.p. 335°, tetra-acetate m.p. 208°),[19] and the melting points of the dimethyl ether and its mono-acetate at 206° and

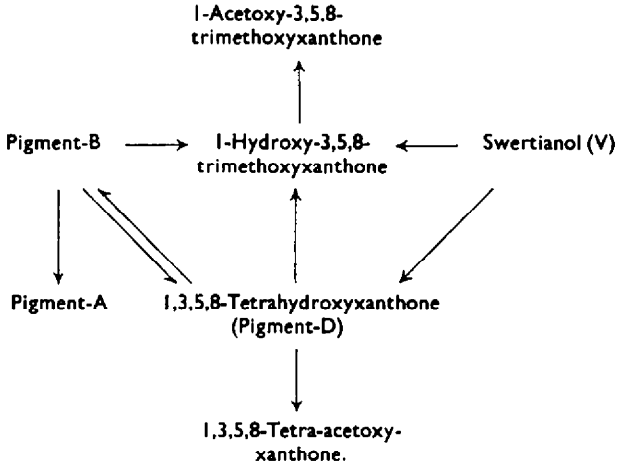


FIG 1

222–225° respectively are in accord with those of the swertianol equivalents at 205° and 223°.[18] These relationships (Fig. 1) provide convincing evidence that bellidifolium possesses structure I.

[17] S. R. Dalal and R. C. Shah, *Chem. & Ind.* 140 (1957).

[18] Y. Tanase, *J. Pharm. Soc. Japan* **61**, 341 (1941).

[19] A. B. Kulkarni and J. R. Merchant, *J. Sci. Ind. Res. India* **14B**, 153 (1955); *Chem. Abstr.* **50**, 7012 (1956).

Methyl bellidifolium (*Pigment-A*). Pigment-A, ($C_{15}H_{12}O_6$), m.p. 185–186°, gave an NMR spectrum (Table 2) which shows the presence of two hydroxyl, four aromatic and six methoxyl protons. On methylation it produced dimethyl bellidifolium and in view of its insolubility in sodium carbonate and its negative Tollens reagent test, pigment-A is assigned the methyl bellidifolium structure (VI). In confirmation of this, pigment-A was produced by methylation of bellidifolium.

The structure proposed for methyl bellidifolium is one of the two possible swerchirin structures (IV) and since the m.p. of swerchirin (186°) agrees well with that of methyl bellidifolium, it is reasonable to suppose, in the absence of authentic material or spectral data, that swerchirin is dimethyl bellidifolium.

Desmethyl bellidifolium (*Pigment-D*). Pigment-D, m.p. 315–320°, was found to be identical in all respects with the desmethyl bellidifolium produced from bellidifolium and in confirmation of its structure (VII) was soluble in sodium carbonate, gave a positive Tollens reagent test, formed a tetra-acetate and on methylation yielded both methyl and dimethyl bellidifolium.

It will be noticed that, as with 1,3-dihydroxyxanthone and 7-methoxy-1,3,8-trihydroxyxanthone (III), pigment-D shows no 3-hydroxyl proton in the NMR (Table 2) under the conditions of measurement. This is not unexpected in view of the acidic nature of the 3-hydroxyl group. Its presence in the molecule however is well established through such derivatives as the tetra-acetate and 1-hydroxy-3,5,8-trimethoxyxanthone.

Pigment-C. Pigment-C ($C_{15}H_{12}O_7$) was shown to be identical with the previously isolated corymbiferin.[5] The structure of this penta-oxygenated xanthone will be the subject of a later communication.

Biogenetic relationships The three new naturally occurring pigments, 1,3,5,8-tetrahydroxy-, 1,5,8-trihydroxy-3-methoxy-, and 1,8-dihydroxy-3,5-dimethoxyxanthones are the first 1,3,5,8-tetra-oxygenated xanthones to be isolated from genus *Gentiana*, although several have previously been obtained from other genera within *F. Gentianaceae*.[20–22] The proposed structures are biogenetically consistent with those previously established for xanthones in this genus, the 1,3,8-oxygenation pattern being present in five of the six known examples. It is to be noted in this context that the 5 and 7 positions are biogenetically equivalent if ring closure of the central γ-pyrone ring takes place at the ether oxygen.

EXPERIMENTAL

M.ps are uncorrected and all relevant UV and NMR data are presented in Tables 1 and 2. IR absorption spectra were determined as KBr discs on a Perkin Elmer model 21 recording spectrophotometer.

Extraction. Fresh root material (64 g) from *G. bellidifolium* was extracted for 4 days with boiling ethanol. Evaporation of this extract yielded 9 g of a yellow solid which lost ⅓ of its weight on hydrolysis with hot 3% HCl aq. Thin layer chromatography indicated that the product consisted largely of a mixture of 4 pigments, A, B, C and D, the separation of which was achieved using silica chromatography with eluants varying from 1:20 to 1:1 ethyl acetate:benzene. All fractions were analysed by thin layer chromatography and mixed fractions were rechromatographed using both thick-thin layer and column chromatography.

Approximate yields from 9 g of extract were as follows: A, 0·17 g; B, 0·25 g; C, 0·23 g; D, 0·5 g; ethanol washings, 0·6 g; insoluble residues, 0·45 g.

Thin layer chromatography. Kieselgel G. was used as adsorbent in all cases, and a solvent mixture of benzene:ethyl acetate:ethanol (50:43:7) was found satisfactory for the xanthones. Proportions

were changed to 72:25:3 for the methyl ethers and acetates. The spray reagent, ferric chloride in ethanol, gave a red colour with both B and D and grey with A and C. Iodine vapour was used to detect non-phenolic derivatives.

Bellidifolium (Pigment-B). Recrystallized from hot ethanol or acetone as yellow needles m.p. 270–271° (Found: C, 61·3; H, 3·8. $C_{14}H_{10}O_6$ requires: C, 61·3; H, 3·7%). ν_{max} 3400, 2900, 1670, 1635, 1616, 1583, 1512, 1498, 1474, 1431, 1373, 1323, 1306, 1243, 1205, 1189, 1158, 1096, 1052: 1000, 955, 879, 845, 828, 804, 769, 750, 730, 710, 682, 660, 640 cm^{-1}.

Bellidifolium, acetylated with acetic anhydride-pyridine at 20° for 6 days, gave triacetyl bellidifolium m.p. 240°. (Found C, 60·2; H, 3·6; OCH_3, 7·8. $C_{20}H_{16}O_9$ requires: C, 60·0; H, 4·0; OCH_3, 8·1%).

Methylation of bellidifolium. Bellidifolium (20 mg) in ethanol (20 ml) was left overnight with an excess of ethereal diazomethane at 20°. Dimethyl bellidifolium (20 mg) crystallized from ethanol–chloroform as yellow needles m.p. 206–207° (Found: C, 63·9; H, 4·8. Calc. for $C_{16}H_{14}O_6$: C, 63·6; H, 4·7%). ν_{max} 3440 (br), 2930, 2860, 1665, 1618, 1597, 1575, 1494, 1472, 1408, 1433, 1389, 1366, 1329, 1306, 1283, 1249, 1218, 1209, 1188, 1168, 1155, 1118, 1065, 986, 967, 925, 857, 820, 792, 742, 674, cm^{-1}. When insufficient diazomethane was employed A was isolated in good yield (mixed m.p. evidence).

Dimethyl bellidifolium on treatment with boiling acetic anhydride–pyridine yielded a monoacetate m.p. 222–224°, λ_{max} (ethanol) 238, 245, 268 sh, 302, 340 mμ (log ε, 4·2, 4·2, 3·8, 3·9, 3·5).

Demethylation of bellidifolium. Chromatographically pure bellidifolium (10 mg) was heated on a water-bath for 2 hr with glacial acetic acid (4 ml) and HBr (1 ml) and then refluxed 8 hr. Removal of the solvent, followed by silica chromatography in 15% ethyl acetate–benzene yielded bellidifolium (3 mg) and desmethyl bellidifolium (3 mg) m.p. 315–320°. Thin layer chromatography, IR absorption and a mixed m.p. determination proved this material identical with pigment-D.

Methyl bellidifolium (Pigment-A). Crystallization from hot ethanol produced yellow crystals m.p. 185–186° (Found: C, 62·1; H, 4·5, $C_{15}H_{12}O_6$ requires: C, 62·5; H, 4·2%). ν_{max} 3450 (br), 2930, 2860, 1671, 1638, 1607, 1583, 1505, 1461, 1359, 1338, 1279, 1236, 1221, 1178, 1158, 1105, 1055, 975, 942, 847, 820, 815, 770, 745, 735, 672 cm^{-1}. Methylation with diazomethane yielded dimethyl bellidifolium (IR absorption, m.p. and mixed m.p., identical with authentic material).

Desmethyl bellidifolium (Pigment-D). This crystallized poorly from a number of solvents and was therefore purified by repeated chromatography over silica-gel in 10–20% ethyl acetate–benzene. m.p. 315–320° ν_{max} 3320 (br), 2940, 1665, 1635, 1612, 1575, 1518, 1488, 1438, 1391 (w), 1351, 1298, 1237, 1234, 1186 (w), 1164, 1100, 1069, 1048, 991, 954, 925, 838, 819, 806, 800, 752, 737, 717, 691, 660 cm^{-1}.

Desmethyl bellidifolium was acetylated with acetic anhydride–pyridine at 100°. Dilution with water yielded white crystals which recrystallized from hot ethanol, m.p. 242° (Found: C, 59·3; H, 4.0. Calc. for $C_{21}H_{16}O_{10}$: C, 58·9; H, 3·8%.)

Methylation of desmethyl bellidifolium with excess diazomethane produced dimethyl bellidifolium whilst less than a 3 molar proportion gave methyl bellidifolium also. (IR and m.p. evidence.)

Comparative spot tests. (a) All three pigments were tested simultaneously at 20° for their solubility in 10% Na_2CO_3 aq. A and B remained insoluble for 10 min whilst D (like 1,3-dihydroxyxanthone) was immediately soluble.

(b) The sample in ethanol was treated with Tollens reagent on a white spotting plate. Alizarin, B and D all gave an immediate deposition of silver whilst 1,3-dihydroxyxanthone, dimethyl bellidifolium and A did not.

1,3-*Dihydroxyxanthone.* This was prepared according to Shah *et al.*[22] Diazomethane treatment gave 1-hydroxy-3-methoxy-xanthone m.p. 146°.

Attempted synthesis of dimethyl bellidifolium. 1,3,8-Trihydroxy-7-methoxyxanthone (III) was prepared according to the method referred to by Shah.[17] Methylation was carried out as for bellidifolium and the products were separated on a silica column in benzene. The major product (4 mg) crystallized as yellow needles m.p. 151° and formed an acetate m.p. 168–169°; λ_{max} (ethanol) 240,

[20] S. R. Dalal, S. Sethna and R. C. Shah, *J. Indian Chem. Soc.* **30**, 457 (1953).

[21] S. R. Dalal and R. C. Shah, *Chem. & Ind.* 664 (1956).

[22] Y. Asahina, J. Asano and Y. Uyeno, *Bull. Chem. Soc., Japan* **17**, 104 (1942); *Chem. Abstr.* **41**, 4488 (1947).

[23] P. K. Grover, G. D. Shah and R. C. Shah, *J. Chem. Soc.* 3982 (1955).

260, 313, 377 mμ (log ε, 4·6, 4·6, 4·2, 3·7). These values are identical with those published for decussatin.[7,30] A minor product (1·5 mg) crystallized as yellow needles m.p. 183° and depressed the m.p.s of both methyl bellidifolium and dimethyl bellidifolium.

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