Chemical Constituents of the Gentianaceae. Part XII.† Structure of the Pentaoxygenated Xanthones of *Canscora decussata* Schult ‡

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The oxygenation pattern of the major pentaoxygenated xanthones of *Canscora decussata* is shown by synthesis and reassessment of spectrsocopic evidence, to be 1,3,5,6,7- and not 1,3,6,7,8- as previously reported. The structures of three of these xanthones are revised to 1-hydroxy-3,5,6,7-tetramethoxy-1,7-dihydroxy-3,5,6-trimethoxy-, and 1,6,7-trihydroxy-3,5-dimethoxy-xanthone, and that of a new xanthone is shown to be 1,3,7-trihydroxy-5,6-dimethoxyxanthone. The presence of minor amounts of 1,3,6,7,8-oxygenated xanthones has also been established.

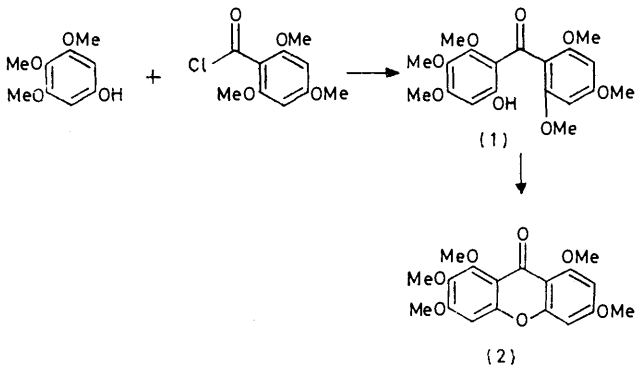
THE pentaoxygenated xanthones of *Canscora decussata* [1] which are of potential therapeutic importance,[2] were previously assigned structures based on the 1,3,6,7,8-oxygenated pattern.[1] Further analysis [3] of the spectral evidence however, has demonstrated that the 1,3,5,6,7-oxygenation pattern is not excluded by the published data. In particular, in the ^{1}H n.m.r. spectra of xanthones IX, XII, and XIII (compound numbers as in ref. 1) the singlet at δ 7·2—7·28 was assigned to 5-H but could equally as well have been assigned 8-H. It was previously thought that a C-8 proton in this environment would give a signal at much lower field, but subsequent calculations based on the method of Barraclough *et al.*,[4] suggest that such a proton should appear as a singlet at δ [$CDCl_3$—$(CD_3)_2SO$] 7·13—7·42. Unambiguous synthesis of either of these xanthones was therefore considered necessary to distinguish the two possibilities.

1,3,6,7,8-Pentamethoxyxanthone (2) was prepared by the route outlined in Scheme 1. Acylation of 3,4,5-trimethoxyphenol with 2,4,6-trimethoxybenzoyl chloride gave 2'-hydroxy-2,4,4',5',6,6'-hexamethoxybenzophenone (1), the structure of which followed from its ^{1}H n.m.r. and mass spectra. Thus the former showed the presence of six methoxy-groups, two phloroglucinol protons [δ 6·18 (2H, s)], and C-3' proton [δ 6·29 (1H, s)]. The mass spectrum showed *m/e* 378 (M^+), 211 [$(MeO)_3(OH)C_6H{\cdot}CO^+$], 210 (due to transfer of the phenolic proton to the adjacent ring to give the keten-type fragment [5]), and 195 [$(MeO_3)C_6H_2{\cdot}CO^+$]. The benzophenone (1) was cyclized with tetramethylammonium hydroxide and pyridine to give 1,3,6,7,8-pentamethoxyxanthone (2) in good yield.

† Part XI, S. K. Bhattacharya, S. Ghosal, R. K. Chaudhuri, A. K. Singh, and P. V. Sharma, *J. Pharm. Sci.*, 1973, **63**, 1341.
‡ Part of this work was reported by S. Ghosal and R. K. Chaudhuri, at the 9th I.U.P.A.C. Symposium on the Chemistry of Natural Products, Ottawa, Canada, 1974, Abstracts, p. 508.

[1] R. K. Chaudhuri and S. Ghosal, *Phytochemistry*, 1971, **10**, 2425.

This product, m.p. 145—146°, was distinctly different from the permethyl ether, m.p. 171—172°, obtained from the natural pentaoxygenated xanthones IX, XII, XIII, and XVI.[1]

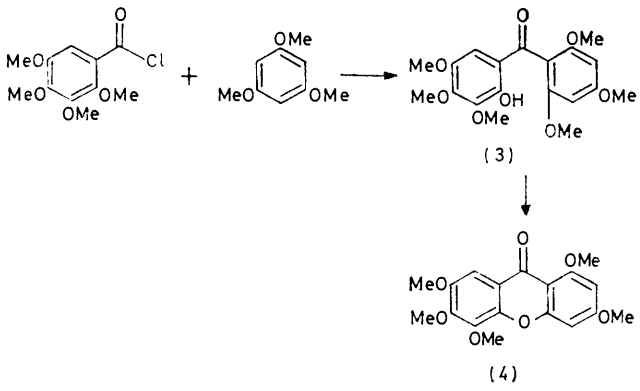


SCHEME 1

1,3,5,6,7-Pentamethoxyxanthone (4) was synthesized as outlined in Scheme 2. 1,3,5-Trimethoxybenzene was acylated with 2,3,4,5-tetramethoxybenzoyl chloride to give 2'-hydroxy-2,3',4,4',5',6-hexamethoxybenzophenone (3), demethylation of the 2'-methoxy-group occurring during the reaction. The mass spectrum of the product exhibited the molecular ion at *m/e* 378 and fragment ions at 211, 210, and 195, similar to benzophenone (1), while the ^{1}H n.m.r. spectrum showed the presence of six methoxy-groups (δ 3·58—4·0), two phloroglucinol protons [δ 6·20 (s)], and a C-6' proton [δ 6·52 (s)]. Cyclization of this product with tetramethylammonium hydroxide and

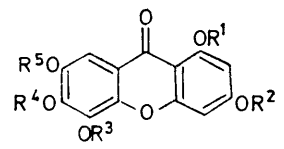
[2] S. K. Bhattacharya, S. Ghosal, R. K. Chaudhuri, and A. K. Sanyal, *J. Pharm. Sci.*, 1972, **61**, 1838.
[3] R. K. Chaudhuri, Ph.D. Thesis, Banaras Hindu University, 1972.
[4] D. Barraclough, H. D. Locksley, F. Scheinmann, M. Taveira Magalhães, and O. R. Gottlieb, *J. Chem. Soc.* (*B*), 1970, 603.
[5] J. A. Ballantine and C. T. Pillinger, *Org. Mass Spectrometry*, 1968, **1**, 425.

pyridine gave 1,3,5,6,7-pentamethoxyxanthone (4),* m.p. 174—175°, which was identical with the permethyl ether prepared from the *Canscora decussata* penta-oxygenated xanthones IX, XII, XIII, and XVI.



SCHEME 2

In view of the revised oxygenation pattern of the major pentaoxygenated xanthones of *C. decussata*, a reallocation of hydroxy- and methoxy-substituents in xanthones IX, XII, and XIII is required, and this is outlined below for each compound. The structure of xanthone XVI follows as 1,3,5,6,7-pentahydroxyxanthone (9).



(5) R^1 = H; R^2 = R^3 = R^4 = R^5 = Me
(6) R^1 = R^5 = H; R^2 = R^3 = R^4 = Me
(7a) R^1 = R^4 = R^5 = H; R^2 = R^3 = Me
(7b) R^1 = R^3 = R^4 = H; R^2 = R^5 = Me
(8) R^1 = R^2 = R^5 = H; R^3 = R^4 = Me
(9) R^1 = R^2 = R^3 = R^4 = R^5 = H

Xanthone IX.—Selective demethylation [6] of synthetic xanthone (4) with aluminium trichloride in ether gave 1-hydroxy-3,5,6,7-tetramethoxyxanthone (5), identical with xanthone IX.

Xanthone XII.—This compound, m.p. 240—243°, is a dihydroxytrimethoxyxanthone which was originally assigned as 1,8-dihydroxy-3,6,7-trimethoxyxanthone. However, it is converted into the tetra- and penta-methylated xanthones (4) and (5) on methylation. It is insoluble in 5% aqueous Na_2CO_3 and its u.v. spectrum is unaffected by sodium acetate. Thus two of the three methoxy-groups must be sited at C-3 and -6. The third methoxy-group is probably at C-5 since, in the ^1H n.m.r. spectrum of the diacetate, the C-8 proton signal is shifted downfield by 0·22 p.p.m. (relative to its position in the permethyl ether) thus suggesting [7] the presence of an *ortho*-hydroxy-group, *i.e.* at C-7. On this basis xanthone XII is 1,7-dihydroxy-3,5,6-trimethoxyxanthone (6).

Xanthone IV.—This compound, m.p. 278—281°, forms a triacetate and on methylation produces the tetra- and penta-methylated xanthones (4) and (5). It is thus a trihydroxydimethoxyxanthone (M^+ 304) with a 1,3,5,6,7-oxygenation pattern and a free hydroxy-group at C-1. A free hydroxy-group must also be present at C-3 and/or C-6 to account for the solubility in aqueous Na_2CO_3 and for the NaOAc-induced bathochromic shift of the longer wavelength absorption maxima from 335 and 355 to 374 nm. This hydroxy-group is considered to be at C-3 for the following reason. Xanthone IV lacks an *ortho*-dihydroxy-group (u.v. spectrum unchanged by NaOAc–H_3BO_3 [8]) and if the C-3 hydroxy-group were methylated, the 6-hydroxy-group would also have to be methylated to exclude this grouping from the structure. However, the acidity of the compound requires that at least one of these hydroxy-groups be unsubstituted, so that the 3-hydroxy-group cannot be methylated. Two methoxy-groups are thus present in the trisubstituted ring. These are probably sited at C-5 and -6 since acetylation of the hydroxy-group in this ring shifts the 5-H signal 0·26 p.p.m. downfield (relative to its position in the permethyl ether) suggesting, as for xanthone XII, that the hydroxy-group is *ortho*- to the C-8 proton.[7] Xanthone IV is therefore 1,3,7-trihydroxy-5,6-dimethoxyxanthone (8).

Xanthone XIII.—This compound, m.p. 290—291°, is a trihydroxydimethoxyxanthone (M^+ 304) which was previously assigned the structure 1,3,8-trihydroxy-6,7-dimethoxyxanthone. Like xanthone XII, however, it is converted into the tetra- and penta-methylated xanthones (4) and (5) on methylation thus confirming the 1,3,5,6,7-oxygenation pattern, and the presence of the 1-hydroxy-group is indicated by the ^1H n.m.r. signal at δ 12·69.[9] It is soluble in aqueous Na_2CO_3 and its long wavelength absorption maximum in the u.v. is shifted bathochromically from 335 to 375 nm in the presence of NaOAc. Free hydroxy-groups are thus present at C-3 and/or C-6. The presence of an *ortho*-dihydroxy-system is indicated by a positive Tollens reagent test and by bathochromic shifts of 23 and 77 nm induced in the 335 nm absorption band by NaOAc–H_3BO_3 [8] and $AlCl_3$ [8] respectively. As expected, the latter shift was reduced (to 26 nm) in the presence of HCl. One methoxy-group must therefore be at C-3 and the other at C-5 or -7 thus defining the structure of xanthone XIII as (7a) or (7b). Acetylation of this xanthone caused a 0·15 p.p.m. downfield shift of the 8-H signal in the ^1H n.m.r. spectrum (relative to its position in the permethyl ether) suggesting that as in xanthones XII and IV, a free hydroxy-group is present at C-7. Although the shift is somewhat below the range reported by Massicot *et al.*[7] for protons *ortho* to the introduced acetoxy-group (0·18—0·53 p.p.m.), this could well be accounted for by steric interaction between the *ortho*-related acetoxy-groups which would result in a

* Since the completion of our work, the synthesis of xanthone (4), following a similar route has been described (A. J. Quillinan and F. Scheinmann, *J.C.S. Perkin I*, 1973, 1329).

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

[7] J. Massicot, J. P. Marthe, and S. Heitz, *Bull. Soc. chim. France*, 1963, 2712.

[8] T. J. Mabry, K. R. Markham, and M. B. Thomas, in 'The Systematic Identification of Flavonoids,' Springer-Verlag, New York, 1970, p. 271.

[9] P. Arends and P. Helboe, *Acta Chem. Scand.*, 1972, **26**, 4180.

considerably reduced shift.[7] Present evidence therefore favours 1,6,7-trihydroxy-3,5-dimethoxyxanthone (7a) as the structure for xanthone XIII, although as with xanthones XII and IV, final confirmation of structure, in particular with respect to the assignment of methoxy-groups to C-5 or -7, must await synthesis.

Evidence was also found for the existence of 1,3,6,7,8-oxygenated xanthones in *C. decussata*. When synthetic 1,3,6,7,8-pentamethoxyxanthone (2) was chromatographed on a t.l.c. plate with the permethyl ethers of the crude natural mixture of xanthones, a blue fluorescent spot was observed at the same R_F value. The compound was isolated by preparative t.l.c. in small yield and its identity with the synthetic xanthone was established by mixed m.p., t.l.c., u.v. and mass spectrometry.

The isolation of 1,3,5,6,7-oxygenated xanthones from a higher plant substantiates the prediction of Carpenter *et al.*[10] that such xanthones should occur in nature if the phenol oxidative coupling biosynthetic mechanism is operative. This oxygenation pattern represents one of the few remaining ' standard ' patterns predicted to occur but as yet not found in nature. The expression ' standard ' refers to the fact that the oxygenation pattern originates directly from the shikimate- and acetate-derived precursors [11] without further nuclear oxidation. The xanthones of *C. decussata* provide a good example of the range of xanthones obtainable *via* the phenol oxidative coupling mechanism, and, as required if derived by this mechanism,[10] all contain oxygen functions at positions 1 and 5, or 1 and 7. Although the *C. decussata* xanthones are all based on a phloroglucinol oxygenation pattern in the acetate-derived ring, a representative has been found for each of the ' standard ' shikimate-derived oxygenation patterns. Thus the basic shikimic acid oxidation pattern is represented by the currently reported 1,3,5,6,7-pentaoxygenated xanthones, the 5-deoxyshikimate pattern by the 1,3,5,6- and 1,3,5,7-tetraoxygenated xanthones, and the 4,5-deoxyshikimate pattern by the 1,3,5-trioxygenated xanthones. The biosynthesis of the 1,3,6,7,8-pentaoxygenated and 1,3,5,6,7,8-hexaoxygenated xanthones [12] would appear to involve additional nuclear oxygenation.

EXPERIMENTAL

U.v. spectra were recorded on a Cary-14 or Spektromom-203 spectrophotometer, i.r. spectra on Perkin-Elmer 621 or 257 spectrophotometers, mass spectra on an AEI MS-9 (at 70 eV), and 60 MHz ^{1}H n.m.r. spectra on a Varian A-60 spectrometer. T.l.c. was carried out on Kieselgel G (spot detection by u.v. fluorescence and I_2 vapour). Satisfactory combustion analyses were obtained for all compounds reported. Some physical data relating to the naturally occurring xanthones has been reported previously (see ref. 1).

The isolation of the 1,3,5,6,7-pentaoxygenated xanthones (5)—(9) was described earlier.[1]

Isolation of 1,3,6,7,8-*Pentaoxygenated Xanthones as the Permethyl Ether* (2).—The crude ethanolic extract, after separation of mangiferin,[1] was concentrated to a syrup, and 4% HOAc (200 ml) was added. The mixture was kept at 20° overnight and then extracted with $CHCl_3$ (3 × 200 ml). The combined $CHCl_3$ extracts were washed, dried, and evaporated to dryness, when a brown solid (*ca.* 1·2 g) was obtained. A portion of the solid (0·5 g) was methylated with dimethyl sulphate and K_2CO_3 in acetone under reflux (48 h). The product was subjected to preparative t.l.c. (p.l.c.) on acidified SiO_2 using $CHCl_3$ as solvent. Five blue-green fluorescent zones, R_F 0·1—0·7, were observed. The two lower bands were each eluted from the SiO_2 with MeOH–$CHCl_3$, washed, after evaporation, with $NaHCO_3$ solution, and subjected to repeated p.l.c. on neutral plates. The solid from the R_F zone *ca.* 0·2 crystallized from EtOH as pale yellow needles, m.p. and mixed m.p. with synthetic, 1,3,6,7,8-pentamethoxyxanthone, m.p. 145—146°; co-t.l.c., R_F 0·23 ($CHCl_3$), 0·51 ($CHCl_3$–C_6H_6–HOAc, 100 : 20 : 1); 0·78 ($CHCl_3$–HOAc, 100 : 1).

2′-*Hydroxy*-2,4,4′,5′,6,6′-*hexamethoxybenzophenone* (1).—2,4,6-Trimethoxybenzoic acid (1·0 g), benzene (20 ml), and oxalyl chloride (5 ml) were mixed and refluxed for 30 min. The excess of oxalyl chloride and benzene was removed *in vacuo* and the acid chloride obtained was used without further purification. It was treated with 3,4,5-trimethoxyphenol (0·9 g) and $AlCl_3$ (2 g) in anhydrous ether (50 ml), refluxed (1 h), and left overnight at 20°. The product was hydrolysed with water (200 ml) and conc. HCl (4 ml), and extracted with ether (4 × 100 ml). The extracts were washed with a saturated $NaHCO_3$ solution and then extracted with 10% NaOH (4 × 50 ml). The NaOH extract was acidified and extraction with ether gave a dull yellow solid (0·31 g), which crystallized from EtOH as yellow *cubes* (0·26 g), m.p. 90—91°, R_F 0·62 (C_6H_6–$CHCl_3$, 1 : 2), $\lambda_{max.}$ (EtOH) 235—240 (ε 58·4), 254infl (20·5), 288 (53·2), and 345 nm (23·2), $\nu_{max.}$ (Nujol) 3280br, 1612, 1414, 1288, 1214, 1164, 1134, 1102, 1042, 1004, 958, and 824 cm^{-1}, δ ($CDCl_3$) 6·29 (1H, s), 6·18 (2H, s), and 3·45—4·0 (18H).

1,3,6,7,8-*Pentamethoxyxanthone* (2).—The foregoing benzophenone (0·08 g), pyridine (3 ml), and tetramethylammonium hydroxide (10% aqueous solution; 3 ml) were refluxed for 48 h. Acidification with dilute HCl, and ether extraction gave 1,3,6,7,8-pentamethoxyxanthone, which crystallized from benzene as pale yellow *needles* (0·052 g), m.p. 145—146°, R_F 0·23 ($CHCl_3$), $\lambda_{max.}$ (EtOH) 215 (ε 27·8), 242 (52·5), 248 (54·4), 270sh (13·1), 300 (28·2), and 332 nm (11·4), $\nu_{max.}$ (Nujol) 1660, 1634, 1612, 1580, 1430, 1278, 1224, 1212, 1144, 1120, 1100, 1068, 984, 814, and 802 cm^{-1}, δ ($CDCl_3$) 6·58 (1H, s), 6·34 (1H, d, *J* 2·5 Hz), 6·28 (1H, d, *J* 2·5 Hz), and 3·88—4·04 (15H), *m/e* 346 (M^+, 100%), 331 (14), 317 (18), 316 (8), and 303 (46).

2′-*Hydroxy*-2,3′,4,4′,5′,6-*hexamethoxybenzophenone* (3).—2,3,4,5-Tetramethoxybenzoyl chloride, prepared from the acid (1 g) in the usual way, was treated with 1,3,5-trimethoxybenzene (0·75 g) and $AlCl_3$ (2 g) in anhydrous ether (50 ml). The mixture was refluxed (1 h) and kept overnight at 20° before hydrolysis with water (150 ml) and conc. HCl (3 ml). Work-up as outlined for (2) yielded a yellow solid (0·37 g) which crystallized from EtOH as yellow *needles* (0·22 g), m.p. 108—111°, R_F 0·58 (C_6H_6–$CHCl_3$, 1 : 2), δ ($CDCl_3$) 12·42 (1H), 6·52 (1H, s), 6·29 (2H, s), and 3·58—4·0 (18H).

1,3,5,6,7-*Pentamethoxyxanthone* (4).—The above benzo-

[10] I. Carpenter, H. D. Locksley, and F. Scheinmann, *Phytochemistry*, 1969, **8**, 2013.

[11] S. Ghosal, P. V. Sharma, R. K. Chaudhuri, and S. K. Bhattacharya, *J. pharm. Sci.*, 1973, **62**, 926.

[12] S. Ghosal, R. K. Chaudhuri, and A. Nath, *J. Indian Chem. Soc.*, 1971, **48**, 589.

phenone (0·098 g), pyridine (3 ml), and tetramethylammonium hydroxide (4 ml) were mixed and refluxed for 48 h. Acidification with dilute HCl, extraction with ether, and washing with NaOH solution gave 1,3,5,6,7-pentamethoxyxanthone which crystallized from EtOH as orange-yellow *needles* (0·064 g), m.p. 175°, R_F 0·44 ($CHCl_3$), $\lambda_{max.}$ (EtOH) 242 (ε 36·2), 255 (46·4), 305 (20·2), 346sh (10·2), and 355 nm (10·1), $\nu_{max.}$ (Nujol) 1650, 1628, 1602, 1580, 1430, 1302, 1280, 1256, 1222, 1172, 1144, 1098, and 828 cm^{-1}, δ ($CDCl_3$) 7·42 (1H, s), 6·48 (1H), d, *J* 2·5 Hz), 6·25 (1H, d, *J* 2·5 Hz), and 3·88—4·02 (15H).

1-*Hydroxy*-3,5,6,7-*tetramethoxyxanthone* (5).—1,3,5,6,7-Pentamethoxyxanthone (0·045 g) in anhydrous ether (100 ml) was treated with $AlCl_3$ (1 g) at 20° for 3 h. Dilute HCl was added and the product was extracted with ether. The extract was washed with 10% NaOH and evaporated to give starting material (14 mg). The alkaline extracts were acidified and extracted with ether to yield a fawn solid (18 mg). This was chromatographed in benzene on a short column of silica gel, the combined benzene eluates yielding on evaporation pale yellow *needles* (12 mg), m.p. 174—175°, mixed m.p. with the natural hydroxy-tetramethoxyxanthone undepressed; co-t.l.c. in three different solvents also indicated their identity; R_F 0·32 ($CHCl_3$), $\lambda_{max.}$ (EtOH) 240 (ε 65·8), 260 (94·2), 275infl (23·7), 308—310 (42·8), and 365 nm (19·4), $\nu_{max.}$ (Nujol) 3380br, 1664, 1605, 1580, 1256, 1218, 1200, 1135, 1092, 1042, 1025, 940, and 810 cm^{-1}, δ ($CDCl_3$) 12·5 (1H), 73·8 (1H, s), 6·45 (1H, d, *J* 2·5 Hz), 6·25 (1H, d, *J* 2·5 Hz), and 3·85—4·0 (12H), *m/e* 332 (M^+, 100%), 317 (42), 302 (22), 271 (38), and 260 (14).

1,3,7-*Trihydroxy*-5,6-*dimethoxyxanthone* (8) (*Xanthone IV*).—The isolation of this xanthone has been previously reported,[1] m.p. 278—281°, R_F ($CHCl_3$–HOAc, 120 : 10), $\lambda_{max.}$ (EtOH) 253 (ε 15·0), 280infl (4·7), 335 (5·2), and 355 nm (4·4), $\lambda_{max.}$ (EtOH–NaOAc) 255 (ε 75·2) and 374 nm (43·2), $\nu_{max.}$ (Nujol) 3386, 1662, 1608, 1580, 1322, 1292, 1218, 1200, 1160, 1098, 1060, 1032, 960, 882, 812, 800, and 780 cm^{-1}, *m/e* 304 (M^+, 100%), 289 (14), 275 (31), 274 (14), 261 (63), and 233 (24). The dimethyl ether, prepared with CH_2N_2, crystallized from EtOH as yellow *needles*, m.p. 171—173°, was identical with synthetic 1-hydroxy-3,5,6,7-tetramethoxyxanthone by mixed m.p. and co-t.l.c. in three solvents. The permethyl ether (prepared with dimethyl sulphate and K_2CO_3 in acetone, 46 h) crystallized from EtOH as orange-yellow needles, m.p. and mixed m.p. 175°, co-t.l.c. (with synthetic 1,3,5,6,7-pentamethoxyxanthone) gave a single spot with the same R_F value.

We thank Professor G. B. Singh, Banaras Hindu University, Dr. Nitya Nand, C.D.R.I., Lucknow, and Dr. B. C. Das, C.N.R.S., Gif-Sur-Yvette, France, for obtaining some of the spectra and for analytical data, and Dr. F. Scheinmann for helpful discussions. Technical assistance from Mr. R. C. Bepin, Department of Chemistry, Banaras Hindu University, is gratefully acknowledged.

[4/956 *Received*, 16*th May*, 1974]