

## Ochnaflavone and its Derivatives: a New Series of Diflavonyl Ethers from *Ochna squarrosa* Linn.

By Masayoshi Okigawa and Nobusuke Kawano,\* Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki, Japan 852

Mohammad Aqil and Wasiur Rahman, Department of Chemistry, Aligarh Muslim University, Aligarh, India

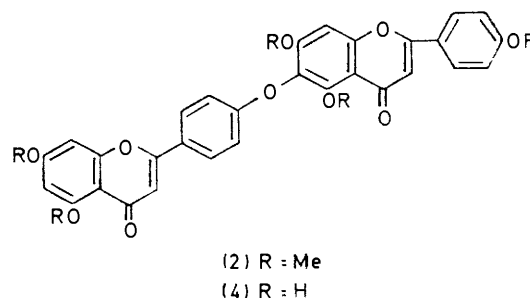
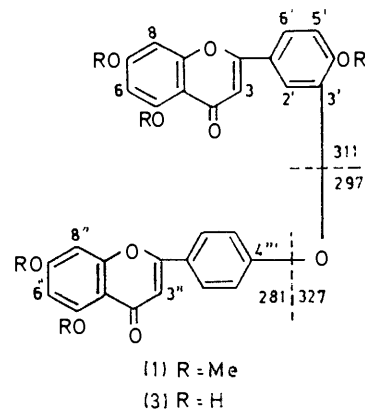
Ochnaflavone (4',5,5'',7,7''-pentahydroxy-3',4'''-oxydiflavone) (3) and its methyl ethers [(12) and (13)] isolated from the leaves of *Ochna squarrosa* Linn. (Ochnaceae), were identified from their spectral data and by synthesis of penta-*O*-methylochnaflavone (1). They are diflavonyl ethers linked *via* the two phenyl rings.

ALTHOUGH most known naturally occurring biflavanoids are found in the leaves of Gymnosperms,<sup>1</sup> the heartwood, bark, latex, and leaves of certain Angiosperms<sup>1</sup> such as Caprifoliaceae, Casuarinaceae, Euphorbiaceae, and Guttiferae<sup>2</sup> have also been reported to contain them. *Ochna squarrosa* Linn. belongs to the family Ochnaceae, closely related to Guttiferae, and a dimeric proanthocyanidin has been isolated from another Ochnaceae plant (*Ouratea* sp.).<sup>3</sup> In our study, extraction of the plant leaves with acetone gave three diflavonyl ethers, termed OSI (C<sub>30</sub>H<sub>18</sub>O<sub>10</sub>), OSII (C<sub>31</sub>H<sub>20</sub>O<sub>10</sub>), and OSIII (C<sub>32</sub>H<sub>22</sub>O<sub>10</sub>).

On methylation with dimethyl sulphate these three compounds all gave the same methyl ether (OSM), C<sub>35</sub>H<sub>28</sub>O<sub>10</sub>, whose mass spectrum showed a parent peak (*m/e* 608) and fragment peaks characteristic of a penta-methoxydiflavonyl ether.<sup>4</sup> The u.v. spectra of these compounds are similar to those of apigenin (5,7,4'-trihydroxyflavone) derivatives. These findings suggested a diflavonyl ether structure for OSI, which we have named ochnaflavone. Hinokiflavone (4) is the only naturally occurring diflavonyl ether reported previously.<sup>1</sup>

The n.m.r. spectrum of the methyl ether (OSM) showed the presence of five methoxy groups, six protons at the 3-, 3'',- 6-, 6''-, 8-, and 8''-positions, and seven phenyl protons (Table 1). The phenyl proton signals were assigned by double resonance experiments: when the 2H doublet at  $\delta$  7.01 was irradiated the other 2H doublet at  $\delta$  7.81 changed to a singlet. Irradiation of

the 1H doublet at  $\delta$  7.11 changed the quartet at  $\delta$  7.72 (*J* 9.0 and 2.5 Hz) to a doublet (*J* 2.5 Hz). These



phenyl proton signals are very similar to those of amentoflavone derivatives,<sup>5</sup> indicating a 3'-substituted pattern for one of the apigenin molecules.

\* A. Pelter, R. Warren, M. Ilyas, J. N. Usmani, S. P. Bhatnagar, R. H. Rizvi, M. Ilyas, and W. Rahman, *Experientia*, 1969, **25**, 350.

<sup>1</sup> H. D. Locksley, *Fortschr. Chem. org. Naturstoffe*, 1973, 207.

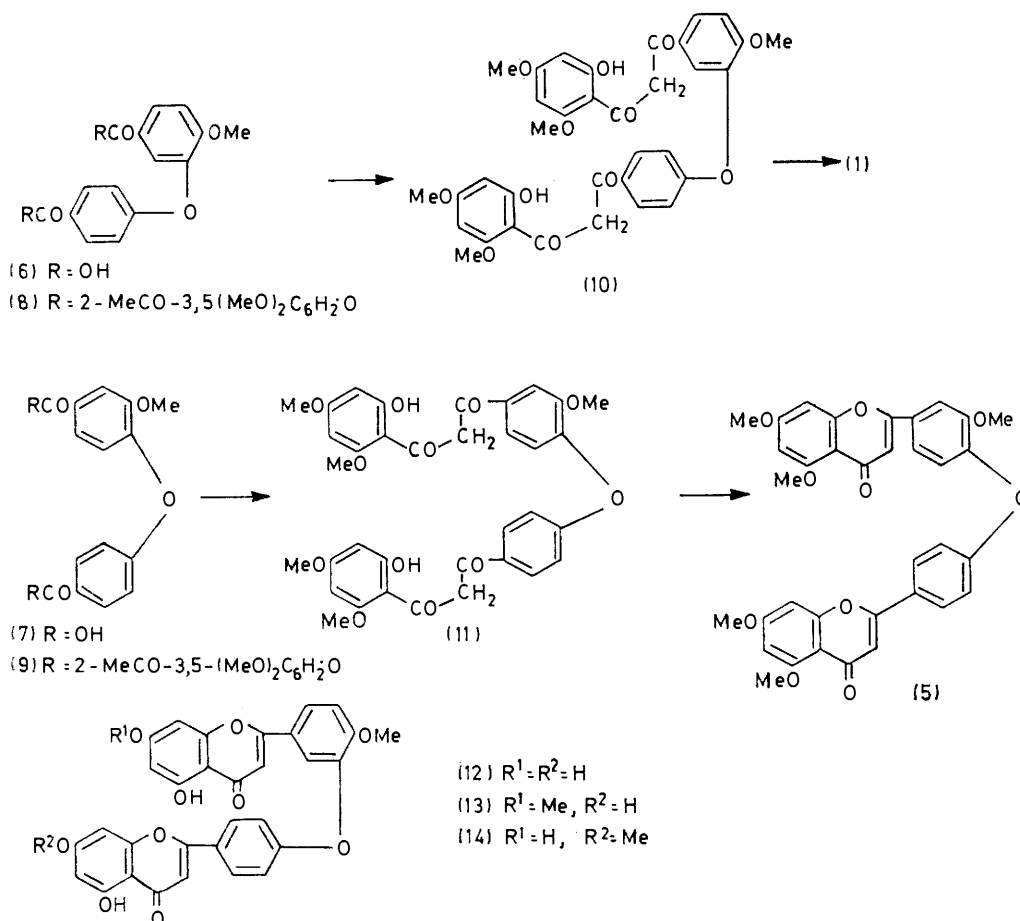
<sup>2</sup> B. Jackson, H. D. Locksley, and F. Scheinmann, *J. Chem. Soc. (C)*, 1971, 3791.

<sup>3</sup> P. D. Monache, I. L. d'Albuquerque, F. Ferrari, and G. B. M. Bettolo, *Tetrahedron Letters*, 1967, 4211.

<sup>4</sup> S. Natarajan, V. V. S. Murti, and T. R. Seshadri, *Indian J. Chem.*, 1969, **7**, 754.

It was recently reported<sup>6</sup> that when the shift reagent  $\text{Eu}(\text{fod})_3$  [tris (1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)europium(III)] was added the n.m.r. signals of the 5- and 5''-methoxy-groups of methylated biflavones showed much larger downfield shifts than those

shown in the Figure. Two pairs of steeply sloping lines assigned to 5- and 5''-MeO and H-6 and 6'' show that coordination between  $\text{Eu}(\text{fod})_3$  and the two carbonyl groups occurs almost to the same extent, without disturbance by the other flavone unit, which is therefore



of 7- and 7''-methoxy-groups, and that H-6 and -6'' showed a considerable downfield shift in comparison with H-8 and -8'' and H-3 and -3''. Chemical shift changes for the methyl ether OSM due to addition of  $\text{Eu}(\text{fod})_3$  are

not linked at the 3- nor the 6-position.<sup>6</sup> The slopes of the other lines are all compatible with those reported. Accordingly, the ether linkage between the two flavone units must be confined to the 3'- and 4'-positions of the two phenyl groups, and two possible structures, (1) and (5) can be proposed for penta-*O*-methylochnaflavone.

TABLE I

N.m.r. data ( $\delta$  values) of ochnaflavone derivatives

Assigned position	Nature of signal	$J$ /Hz	OSIA	OSIIA	OSIIIA	OSIVA	OSM (1)
4'	3 H, s		2.20	3.88	3.88	{3.87	3.88
7	3 H, s		{2.32	{2.31	{3.88	{3.88	3.88
7''	3 H, s		{2.35	{2.33	{2.34	{3.88	3.88
5	3 H, s		{2.42	{2.43	{2.43	{2.42	3.92
5''	3 H, s		{2.44	{2.43	{2.43	{2.42	3.92
3	1 H, s	2.5	{6.62	{6.54	{6.49	{6.47	{6.55
3''	1 H, s	2.5	{6.57	{6.58	{6.57	{6.51	{6.59
6	1 H, d	2.5	{6.83	6.82	{6.58	{6.57	6.34
6''	1 H, d	2.5	{6.84	6.82	{6.82	{6.59	6.34
8	1 H, d	2.5	{7.32	{7.29	{6.82	{6.81	{6.51
8''	1 H, d	2.5	{7.34	{7.31	{7.30	{6.83	{6.54
3'', 5''	2 H, d	9.0	7.13	7.02	7.01	7.01	7.01
5'	1 H, d	9.0	7.35	7.13	7.12	7.10	7.11
5''	1 H, d	2.5	7.58	7.60	7.61	7.59	7.60
6'	1 H, q	2.5 & 9.0	7.71	7.72	7.72	7.7	7.72
2'', 6''	2 H, d	9.0	7.86	7.81	7.79	7.79	7.81

From the corresponding oxydibenzoic acids (6) and (7)<sup>7</sup> compounds (1) and (5) have been synthesized by a route involving Baker-Venkataraman rearrangement<sup>8</sup> of the diesters (8) and (9) and subsequent ring closure of the  $\beta$ -diketones (10) and (11). The synthetic compound (1) was identical with the pentamethyl ether of natural ochnaflavone (OSM).

The three compounds OSI, OSII, and OSIII gave acetates, termed OSIA, OSIIA, and OSIIIA, respectively. The n.m.r. data of the acetates (Table 1) show that

<sup>6</sup> M. Okigawa, N. U. Khan, N. Kawano, and W. Rahman, *J.C.S. Perkin I*, 1975, 1563.

<sup>7</sup> E. Späth and J. Píkl, *Ber.*, 1929, **62**, 2251.

<sup>8</sup> W. Baker, *J. Chem. Soc.*, 1933, 1381; H. S. Mahal, and K. Venkataraman, *ibid.*, 1934, 1767.

OSIA has five acetoxy-groups and that OSIIA and OSIIIA have one and two methoxy-groups respectively. A relatively large upfield shift ( $\delta$  7.35 to 7.13) of the H-5' signal of OSIIA with respect to that of OSIA suggested the presence of a 4'-methoxy-group in the former. Therefore structures (3) and (12) are assigned to OSI and OSII, respectively.

The chemical shift differences of H-6 or -6" ( $\delta$  6.82 to 6.58) and H-8 or -8" ( $\delta$  7.29 to 6.82) between OSIIIA and OSIIA suggested the presence of a 7- or 7"-methoxy-group in OSIIIA, indicating structure (13) or (14) for OSIII. In order to distinguish between these possibilities, trideuteriomethylation of OSIII was carried out for mass spectrometric studies (Table 2). Comparison with

tained from a JEOL JMS-OISG double-focus high resolution instrument. Column chromatography was carried out with Mallinckrodt silicic acid (100 mesh) unless otherwise noted. Layer chromatography was carried out with silica gel (National Chemical Laboratory, Poona) and the solvent system benzene-pyridine-formic acid (36:9:5) unless otherwise noted. U.v. and i.r. spectral data are given in Supplementary Publication No. SUP 21650 (2 pp.).\*

**Isolation.**—The fresh leaves of *Ochna squarrosa* Linn. (10 kg) procured from the Horticulture Research Centre, Saharanpur, and Forest Research Institute, Dehradun, U.P., India, were exhaustively extracted with acetone. The combined extracts were concentrated under diminished pressure to give a dark green gummy mass (200 g), which was purified by extraction with petroleum (b.p. 40–60°), benzene, and chloroform successively. The insoluble residue (100 g) was treated with boiling water and the solution was filtered. The remaining insoluble mass was taken up in ethanol; the solution was filtered and evaporated under diminished pressure to give a yellowish brown residue (30 g), which was dissolved in acetone and put on a column of silica gel (N.C.L., Poona). The column was eluted successively with petroleum, benzene, chloroform, and benzene-ethyl acetate (1:1 and then 1:2). The benzene-ethyl acetate eluates were combined and the solvent was distilled off; the yellowish brown residue (6 g) was separated by p.l.c. into three bands,  $R_F$  0.56, 0.72, and 0.81. These were extracted separately with acetone-methanol to give OSI (1 g), OSII (2 g), and OSIII (0.4 g), respectively.

**Ochnaflavone(4",5,5",7,7"-Pentahydroxy-3',4"-oxydiflavone)** (3).—OSI (1 g) was recrystallized from pyridine-methanol to give yellow *needles* (700 mg), m.p. 233–235° (Found: C, 66.55; H, 3.4.  $C_{30}H_{18}O_{10}$  requires C, 66.9; H, 3.35%). A mixture of OSI (100 mg), pyridine (1.5 ml), and acetic anhydride (1 ml) was warmed on a steam-bath for 2 h. The cooled mixture was poured into ice-water and the precipitate was collected to give needles of the *penta-acetate* (70 mg) (from ethyl acetate-chloroform), m.p. 239–241° (Found: C, 64.2; H, 3.8.  $C_{40}H_{26}O_{15}$  requires C, 64.17; H, 3.77%).

**4'-O-Methylchnaflavone** (OSII). This gave yellow *needles* (1.5 g) (from pyridine), m.p. 297–299° (Found: C, 67.0; H, 3.7.  $C_{31}H_{20}O_{10}$  requires C, 67.4; H, 3.65%). OSII (100 mg) was acetylated with pyridine and acetic anhydride to give needles of the *tetra-acetate* (67 mg) (from ethyl acetate-chloroform), m.p. 248–250° (Found: C, 63.75; H, 3.95.  $C_{39}H_{28}O_{14}$  requires C, 63.4; H, 4.1%).

**4',7-Di-O-methylchnaflavone** (OSIII). This gave brownish yellow *prisms* (0.2 g) (from pyridine-methanol), m.p. 288–290° (Found: 67.45; H, 3.8.  $C_{32}H_{22}O_{10}$  requires C, 67.85; H, 3.9%). Acetylation of OSIII (100 mg) gave needles of the *triacetate* (46 mg) (from ethyl acetate), m.p. 212–214° (Found: C, 65.95; H, 4.15.  $C_{38}H_{28}O_{13}$  requires C, 65.9; H, 4.05%).

**Penta-O-methylchnaflavone** (OSM).—A mixture of OSI (100 mg), dimethyl sulphate (1.0 ml), dry acetone (400 ml), and anhydrous potassium carbonate (10 g) was refluxed on a steam-bath for 8 h. The mixture was filtered and the residue was washed with acetone. The filtrate and washings were combined and the solvent was distilled off. The residue was washed with petroleum and recrystallized from ethanol to give *needles* (60 mg), m.p. 167–169° (Found: C, 68.9; H, 4.65.  $C_{35}H_{28}O_{10}$  requires C, 69.05; H, 4.65%). OSII (300 mg) was similarly methylated to give needles (220 mg) (from ethanol), m.p. and mixed m.p. 167–169°, identical (u.v., i.r., and n.m.r. data) with the above methyl

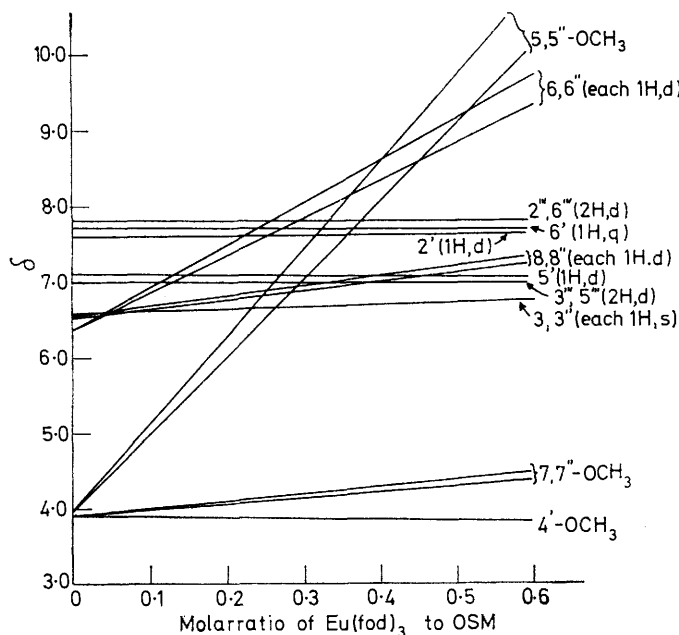


TABLE 2

Mass spectral data of OSIII trideuteriomethyl ether

<i>m/e</i>	617 ( <i>M</i> <sup>+</sup> )	330	314	303	287
Found	617.2229	330.1060	314.1108	303.1161	287.1209
Calc.	617.2247	330.1057	314.1108	303.1139	287.1190
for	$C_{35}H_{18}D_3O_{10}$	$C_{18}H_{12}D_3O_8$	$C_{18}H_{12}D_2O_8$	$C_{17}H_7D_2O_8$	$C_{17}H_7D_3O_8$

the mass spectral data (see Experimental section) for penta-*O*-methylchnaflavone (OSM) shows that the trideuteriomethyl groups are at positions 5, 5", and 7" of OSIII, which was thus identified as 4',7"-di-*O*-methylchnaflavone (13). On methylation with diazomethane, a mixture of OSI, OSII, and OSIII gave 4',7,7"-tri-*O*-methylchnaflavone (OSIV), whose acetate (OSIVA), gave the expected n.m.r. data (Table 1).

## EXPERIMENTAL

N.m.r. spectra were recorded with a JEOL PS-100 instrument (tetramethylsilane as internal reference) for solutions in deuteriochloroform. Mass spectra were ob-

\* For details of Supplementary Publications see Notice to Authors No. 7, *J.C.S. Perkin I*, 1975, Index issue.

ether.  $S$  Values (p.p.m.)<sup>9</sup> from addition of  $\text{Eu}(\text{fod})_3$  were: 11.56, 10.26, 0.96, 0.80, and  $-0.14$  (each OMe), 0.32 and 0.24 (each 1 H, s), 5.58, 4.86, 1.26, 1.14, 0, and  $-0.12$  (each 1 H, d),  $-0.10$  and  $-0.12$  (each 2 H, d), and  $-0.10$  (1 H, q). Mass spectral fragments were observed at  $m/e$  608.1694 ( $M^+$ ), 327.0884, 311.0938, 297.0776, and 281.0800 ( $\text{C}_{35}\text{H}_{32}\text{O}_{10}$ ,  $\text{C}_{18}\text{H}_{15}\text{O}_6$ ,  $\text{C}_{18}\text{H}_{15}\text{O}_5$ ,  $\text{C}_{17}\text{H}_{13}\text{O}_5$ , and  $\text{C}_{17}\text{H}_{13}\text{O}_4$  require 608.1683, 327.0869, 311.0920, 297.0763, and 281.0814, respectively). Methylation of OSIII (50 mg) in the same way gave the same (mixed m.p. and u.v., i.r., and n.m.r. data) penta-*O*-methylchnaflavone (23 mg) (from ethanol).

**Trideuteriomethylation of OSIII.**—Ethereal diazomethane (10 ml) [from nitrosomethylurea (5 g)] was mixed with dioxan (15 ml) and  $\text{D}_2\text{O}$  (1 ml) and kept for 3 h cooled in ice. OSIII (10 mg) was dissolved in dioxan (10 ml), mixed with two drops of  $\text{D}_2\text{O}$  (99%; Merck), and added to the diazomethane solution. After 2 days the solvent was distilled off and the residue was purified by p.l.c. (Merck silica gel G; chloroform-methanol, 97:3). The band corresponding to penta-*O*-methylchnaflavone was collected to give needles (4 mg) (from ethanol), m.p. 160–165° (for mass spectral data see Table 2).

**Tri-*O*-methylchnaflavone Diacetate (OSIVA).**—A mixture (400 mg) of OSI, OSII, and OSIII obtained from the benzene-ethyl acetate eluate was mixed with methanol (5 ml) and an excess of ethereal diazomethane (50 ml) and kept at 0 °C for 12 h. The solvent was evaporated off; t.l.c. of the residue showed the presence of one major ( $R_F$  0.87) and three minor components ( $R_F$  0.81, 0.72, and 0.56) on t.l.c. The major component (OSIV) was obtained by p.l.c. as brown plates (150 mg) (from methanol), m.p. 250–252°. Methylation with dimethyl sulphate and potassium carbonate in boiling acetone gave the same penta-*O*-methylchnaflavone (OSM). On acetylation with acetic anhydride and pyridine, OSIV (100 mg) gave needles of the *diacetate* (60 mg) (from ethyl acetate-chloroform), m.p. 170–172° (Found: C, 66.6; H, 4.3.  $\text{C}_{37}\text{H}_{28}\text{O}_{12}$  requires C, 66.85; H, 4.25%).

**Bis-(2-acetyl-3,5-dimethoxyphenyl) 4-Methoxy-3,4'-oxydibenzoate (8).**—2-Methoxy-3,4'-oxydibenzoic acid (6) (2.93 g) and thionyl chloride (5 ml) were mixed and refluxed for 1.5 h. The excess of thionyl chloride was distilled off under reduced pressure and the viscous residue was mixed with 2'-hydroxy-4',6'-dimethoxyacetophenone (4 g) and pyridine (20 ml) and kept for 15 min in an oil-bath at 100 °C. The cooled mixture was poured into ice-water and extracted with chloroform ( $2 \times 100$  ml). The extracts were combined, washed with 10% hydrochloric acid and water, dried, and evaporated under reduced pressure. The residue was chromatographed on silica gel (30 g). The fraction eluted with chloroform-methanol (98:2) gave the non-crystalline ester (8) (3.2 g),  $\delta$  2.45, 3.78, and 3.83 (each 6 H, s), 3.86 (3 H, s), 6.32 and 6.39 (each 2 H, d,  $J$  2.5 Hz), 6.95 (2 H, d,  $J$  9 Hz), 7.08 (1 H, d,  $J$  9 Hz), 7.86 (1 H, d,  $J$  2.5 Hz), 8.06 (1 H, q,  $J$  2.5 and 9 Hz), and 8.08 (2 H, d,  $J$  9 Hz).

**2,2''-Bis-(2-hydroxy-4,6-dimethoxybenzoyl)-4'-methoxy-3',4'''-oxydiacetophenone (10).**—The ether (8) (1 g) dissolved in pyridine (5 ml) was mixed with powdered potassium hydroxide (1 g) and kept for 10 min at 120 °C. The cooled mixture was poured into ice-water and extracted with chloroform. The extract was washed with 10% hydrochloric acid and water and evaporated under reduced

pressure to give yellow crystals (10) (650 mg) (from chloroform-methanol), m.p. 123–129° (Found: C, 65.0; H, 4.75.  $\text{C}_{35}\text{H}_{32}\text{O}_{12}$  requires C, 65.2; H, 5.0%).

**Penta-*O*-methylchnaflavone (1).**—The ether (10) (500 mg) dissolved in acetic acid (5 ml) was added to a mixture (0.5 ml) of concentrated sulphuric acid and acetic acid (1:4 w/w) and kept for 20 min on a steam-bath. The mixture was poured into ice-water to yield a precipitate, which was collected, washed with water, and purified by p.l.c. to give needles (280 mg) (from ethanol), m.p. and mixed m.p. 169–171° (Found: C, 69.15; H, 4.6.  $\text{C}_{35}\text{H}_{28}\text{O}_{10}$  requires C, 69.05; H, 4.65%), identical with OSM (spectral data).

**Bis-(2-acetyl-3,5-dimethoxyphenyl) 3-Methoxy-4,4'-oxydibenzoate (9).**—3-Methoxy-4,4'-oxydibenzoic acid (7) (1.2 g) and thionyl chloride (3 ml) were refluxed for 3 h. After the excess of thionyl chloride had been removed under diminished pressure the resulting acid chloride was mixed with pyridine (10 ml) and 2'-hydroxy-4',6'-dimethoxyacetophenone (1.7 g) and kept for 15 min in an oil-bath at 100 °C. The cooled mixture was treated as in the case of compound (8) to give the non-crystalline ester (9) (2.3 g) (Found: C, 66.85; H, 2.3.  $\text{C}_{35}\text{H}_{32}\text{O}_{12}$  requires C, 65.2; H, 5.0%),  $\delta$  2.43 and 2.46 (each 3 H, s), 3.91–3.97 (15 H), 6.45–6.51 (4 H), 7.11 (2 H, d,  $J$  9 Hz), 7.02 (1 H, d,  $J$  9 Hz), 7.88 (1 H, d,  $J$  2.5 Hz), 7.90 (1 H, q,  $J$  2.5 and 9 Hz), and 8.20 (2 H, d,  $J$  9 Hz).

**2,2''-Bis-(2-hydroxy-4,6-dimethoxybenzoyl)-3'-methoxy-4',4'''-oxydiacetophenone (11).**—To the ether (9) (1.5 g) dissolved in pyridine (10 ml) was added powdered potassium hydroxide (1.5 g) and the mixture was kept for 10 min in an oil-bath at 120 °C. The cooled mixture was poured into ice-water (30 ml), acidified with hydrochloric acid, and extracted with chloroform. The extract was washed with water, dried, and evaporated under diminished pressure to give a resinous substance, which was chromatographed on silica gel. A fraction eluted with chloroform-methanol (95:5) gave yellow crystals (11) (1.2 g), m.p. 130–135° (from chloroform-methanol) (Found: C, 65.1; H, 4.8.  $\text{C}_{35}\text{H}_{32}\text{O}_{12}$  requires C, 65.2; H, 5.0%).

**3',5,5'',7,7''-Pentamethoxy-4',4'''-oxydiflavone (5).**—The ether (11) (500 mg) dissolved in acetic acid (5 ml) was added to a mixture (1 ml) of concentrated sulphuric acid and acetic acid (1:4 w/w) and kept for 20 min at 100 °C. After cooling the mixture was poured into ice-water to afford a precipitate, which was collected, washed with water, and purified by column chromatography on silica gel. A fraction eluted with chloroform-methanol (98:2) gave needles, m.p. 154–156° (310 mg) (Found: C, 63.15; H, 5.15.  $\text{C}_{35}\text{H}_{28}\text{O}_{10}, 3\text{H}_2\text{O}$  requires C, 63.45; H, 5.15%),  $\delta$  3.80 (3 H, s), 3.83 (12 H, s), 6.27 and 6.30 (each 1 H, d,  $J$  2.5 Hz), 6.47 and 6.50 (each 1 H, d,  $J$  2.5 Hz), 6.53 and 6.59 (each 1 H, s), 7.01 (2 H, d,  $J$  9 Hz), 7.09 (1 H, d,  $J$  9 Hz), 7.41 (1 H, d,  $J$  2.5 Hz), 7.46 (1 H, q,  $J$  2.5 and 9 Hz), and 7.79 (2 H, d,  $J$  9 Hz).

We thank Dr. Sahni, FRI Dehradun, and Mr. Ch. Maqsood and others, HRC Saharanpure, India, for procuring the plant material. M. A. thanks the CSIR for financial assistance.

[5/1864 Received, 26th September, 1975]

<sup>9</sup> A. F. Cockerill and D. M. Rackham, *Tetrahedron Letters*, 1970, 5149.