Constituents of the Higher Fungi. Part XIII.[1] 2-Aryl-3-methoxymaleic Anhydrides from Pulvinic Acid Derivatives. A Convenient Method for Determination of Structure of Fungal and Lichen Pulvinic Acid Derivatives

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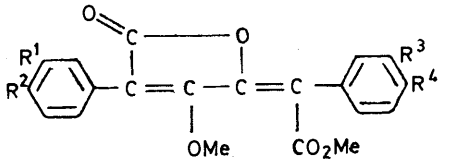
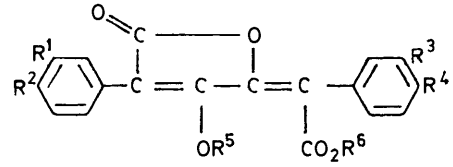
Fully methylated pulvinic acid derivatives are degraded by alkali to 2-aryl-3-methoxymaleic anhydrides and arylacetic acids. The products formed indicate the structure of the pulvinic acid.

SEVERAL methods for determining the relative orientation of the substituted aryl rings in pulvinic acid derivatives have been described; the two chemically sound methods involve identification of either the ozonolysis products or those produced by reduction with zinc and acetic acid. Both methods require relatively large quantities of material (0·5 g) and both give products which are either difficult to crystallise or not easily synthesised. More important, despite a report that methyl vulpinate can be ozonised successfully,[2] ozonolysis is only applicable to the enolic pulvinate esters; their *O*-methylated analogues *e.g.* (I), either resist degradation [1] or yield a large number of products which are of no value for structure determination. This limits application of this technique to only some lichen pigments, and it is not applicable to fungal pigments such as xerocomic acid (VIII) since no unambiguous route has been described for the synthesis of enolic pulvinate esters *e.g.* (XI), from the free acids; all the published methods proceed *via* the dilactone.



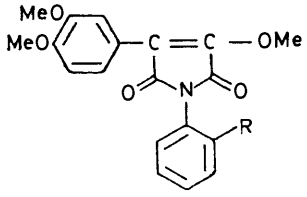
(I) $R^1 = R^2 = R^3 = R^4 = H$
(II) $R^1 = R^2 = R^3 = R^4 = OMe$
(III) $R^2 = R^3 = R^4 = OMe$, $R^1 = H$
(IV) $R^1 = R^2 = R^4 = OMe$, $R^3 = H$
(V) $R^1 = R^2 = H$, $R^3 = R^4 = OMe$
(VI) $R^1 = R^2 = OMe$, $R^3 = R^4 = H$
(VII) $R^1 = R^3 = OMe$, $R^2 = R^4 = H$



(VIII) $R^1 = R^2 = R^4 = OH$, $R^3 = R^5 = R^6 = H$
(IX) $R^1 = R^2 = R^3 = R^4 = OH$, $R^5 = R^6 = H$
(X) $R^1 = R^2 = R^3 = R^4 = OMe$, $R^5 = Me$, $R^6 = H$
(XI) $R^1 = R^2 = R^3 = R^4 = R^5 = H$, $R^6 = Me$
(XII) $R^1 = R^2 = R^3 = R^4 = R^5 = R^6 = H$

During our work on variegatic acid (IX),[3] the fully methylated compound (II) was subjected to alkaline hydrolysis in an attempt to prepare the acid (X). Treatment with aqueous barium hydroxide solution for 45 min gave a colourless suspension. Acidification yielded the highly crystalline, yellow 2-(3,4-dimethoxyphenyl)-3-methoxymaleic anhydride (XVIII), and extraction of the solution gave 3,4-dimethoxyphenylacetic acid. This degradation may be contrasted with the hydrolysis of vulpinic acid (XI) to pulvinic acid (XII) using aqueous calcium hydroxide solution,[4,5] and to oxalic acid and phenylacetic acid using barium hydroxide solution,[6] the rearrangement of pulvinic acid and vulpinic acid to dibenzylglycolic acid using potassium hydroxide,[4] and the rearrangement of methyl vulpinate (I) and methyl 4,4′-dimethoxyvulpinate to the corresponding diphenylcyclopentenediones with methanolic 4% potassium hydroxide.[7]

2-(3,4-Dimethoxyphenyl)-3-methoxymaleic anhydride (XVIII) dissolves in hot sodium hydroxide solution to yield a colourless sodium salt and is reprecipitated on acidification. The compound was unchanged after heating with methanol or with 2N-hydrochloric acid and attempts to methylate it with dimethyl sulphate or with methyl iodide were unsuccessful. In the ^1H n.m.r. spectrum the six-proton singlet at τ 6·04 is assigned to the two aromatic methoxy-groups and the three-proton singlet at τ 5·54 to the enolic methoxy-group on the five-membered ring. This latter absorption is at a similar chemical shift to that of the enolic methoxy-signal in methoxydiphenylcyclopent-4-ene-1,3-diones, *e.g.* 4-methoxy-2,5-diphenylcyclopent-4-ene-1,3-dione [1] absorbs at τ 5·48 and the isomeric trimethoxy-analogues absorb at τ 5·52 and 5·53. In the i.r. spectrum, anhydride carbonyl absorption bands occur at 1832 and 1765 cm^{-1} and the u.v. absorption at 243·5, 270, 329, and 382 nm is consistent with the proposed structure. Treatment with *o*-phenylenediamine gives a yellow crystalline imine, $C_{19}H_{18}N_2O_5$ (XIII), and aniline gives a

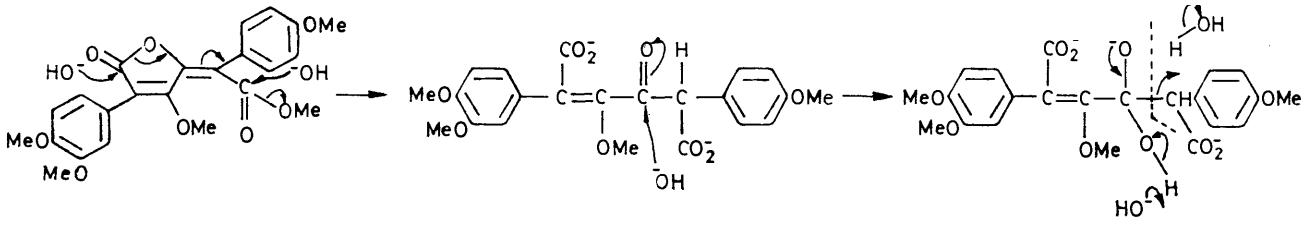


(XIII) R = NH_2
(XIV) R = H

[1] Part XII, R. L. Edwards and M. Gill, preceding paper.
[2] P. Karrer, K. A. Gehrckens, and W. Heuss, *Helv. Chim. Acta*, 1926, **9**, 446.
[3] P. C. Beaumont, R. L. Edwards, and G. C. Elsworthy, *J. Chem. Soc.* (*C*), 1968, 2968.
[4] A. Spiegel, *Ber.*, 1880, **13**, 1629.
[5] R. L. Frank, G. R. Clark, and J. N. Coker, *J. Amer. Chem. Soc.*, 1950, **72**, 1824.
[6] A. Spiegel, *Ber.*, 1881, **14**, 1686.
[7] F. Kögl, H. Becker, G. de Voss, and E. Worth, *Annalen*, 1928, **465**, 243.

similar yellow product, $C_{19}H_{17}NO_5$ (XIV). These reactions parallel those between *o*-phenylenediamine and diphenylmaleic anhydride [8] and between aniline and di-*m*-tolylmaleic anhydride.[9]

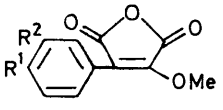
Free diarylmaleic acids are unknown, since they revert to the anhydride when liberated from their salts;[9,10] they are not hydrolysed by water, and methyl diarylmaleates are prepared from the anhydrides only with difficulty.[9,11] Monoarylmaleic acids are equally unstable and spontaneously dehydrate.[12,13] Mixed arylmethoxymaleic anhydrides have not been previously described.



SCHEME 1

The anhydride (XVIII) was readily synthesised. With diazomethane, ethyl 3-cyano-3-(3,4-dimethoxyphenyl)pyruvate gave ethyl β-cyano-α-methoxy-3,4-dimethoxycinnamate which on acidic hydrolysis gave (XVIII). Alkaline hydrolysis of the cinnamate gave 3,4-dimethoxyphenylacetic acid.

The formation of the 2-aryl-3-methoxymaleic anhydride and the arylacetic acid can be explained in terms of hydrolysis of the lactone and ester functions to produce a β-keto-acid which then undergoes hydrolytic fission (Scheme 1). This mechanism suggested that an unsymmetrically substituted pulvinic acid derivative should be cleaved by alkali to give a maleic anhydride bearing the aryl group which was originally adjacent to



(XV) $R^1 = R^2 = H$
(XVI) $R^1 = OMe$, $R^2 = H$
(XVII) $R^1 = H$, $R^2 = OMe$
(XVIII) $R^1 = R^2 = OMe$

the lactone function and an arylacetic acid containing the aryl group originally adjacent to the methoxycarbonyl group. This was confirmed when cleavage of methyl *O*-methyl-3',4',4-trimethoxypulvinate (III) gave 2-(4-methoxyphenyl)-3-methoxymaleic anhydride (XVI) and 3,4-dimethoxyphenylacetic acid, and cleavage of its isomer (IV) gave 2-(3,4-dimethoxyphenyl)-3-methoxymaleic anhydride (XVIII) and 4-methoxyphenylacetic acid. Similarly, methyl *O*-methyl-3',4'-dimethoxypulvinate (V) gave 2-phenyl-3-methoxymaleic anhydride (XV) and 3,4-dimethoxyphenylacetic acid, and its positional isomer (VI) gave 2-(3,4-dimethoxyphenyl)-3-methoxymaleic anhydride (XVIII) and phenylacetic acid. Cleavage of the symmetrical methyl *O*-methyl-3,3'-dimethoxypulvinate (VII) gave 2-(3-methoxyphenyl)-3-methoxymaleic anhydride (XVII) and 3-methoxyphenylacetic acid. The cleavage proceeds most rapidly when *para*-substituents are present on the aryl rings. Without such substituents up to 3 h is required to complete the reaction.

The mass spectra of the anhydrides are relatively simple, showing only four or five major fragment ions. The spectra of 2-phenyl-3-methoxymaleic anhydride (XV) and 2-(3-methoxyphenyl)-3-methoxymaleic anhydride (XVII) can be rationalised in terms of two modes of fragmentation which arise by initial electron abstraction from each of the carbonyl oxygen atoms (Scheme 2). The 4-methoxy- and 3,4-dimethoxy-phenyl derivatives [(XVI) and (XVIII)] show a different fragmentation pattern (Scheme 3). The presence of an intense ion at *m/e* 147 in (XVI) can be explained by assuming that the planarity of the hypothetical *m/e* 175 ion and the availability of electrons from the 4-methoxy-group facilitate fragmentation by the loss of CO.

The barium hydroxide cleavage of fully methylated pulvinic acid derivatives has distinct advantages over the previous techniques: (*a*) it is applicable to all naturally occurring pulvinic acid derivatives. Methylation of the natural product effectively prevents intermediate lactonisation and is a rapid route to the required intermediate from either hydroxypulvinic acids or hydroxypulvinate esters; (*b*) the products are highly crystalline and the arylmaleic anhydride is conveniently identified by comparison with readily prepared authentic material or by ^1H n.m.r. or mass spectral analysis; (*c*) characterisation of *either* of the degradation products establishes the orientation of the aryl residues in an unsymmetrical lactone; (*d*) crystallisable products can be isolated from less than 25 mg of starting material; and (*e*) characterisation of both products identifies the number and position of aryl methoxy-groups and makes chromium trioxide or permanganate oxidation of the pigment unnecessary.

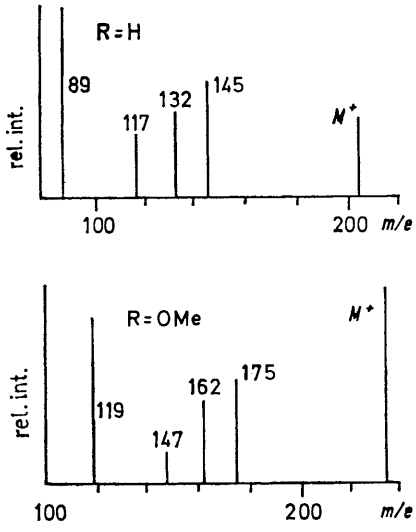
EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer 237 spectrophotometer for $CHCl_3$ solutions, u.v. spectra on a Unican SP 800 spectrophotometer for ethanolic solutions, ^1H n.m.r. spectra on a J.E.O.L. JNM-MH-100 spectrometer for $CDCl_3$ solutions

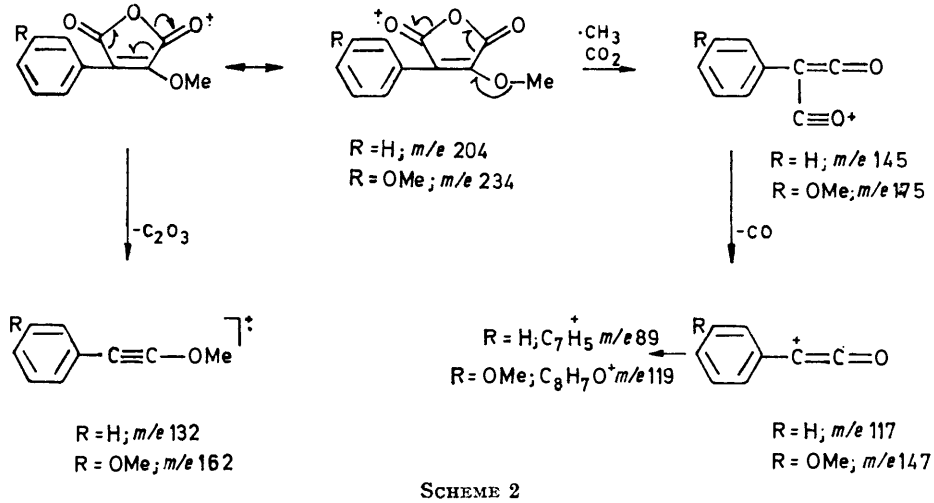
[8] A. Bistrzycki and K. Fassler, *Helv. Chim. Acta*, 1923, **6**, 519.
[9] Ramarte-Lucas and J. Hoch, *Ann. Chim.* (*France*), 1930, **13**, 385.
[10] R. Anschutz and P. Bendix, *Annalen*, 1890, **259**, 61.
[11] D. Biquard, *Ann. Chim.* (*France*), 1933, **20**, 97.
[12] H. Alexander, *Annalen*, 1890, **258**, 67.
[13] C. S. Rondestvedt and A. H. Filbey, *J. Org. Chem.*, 1954, **19**, 119.

(tetramethylsilane as internal standard), and mass spectra on an A.E.I. MS9 spectrometer.

Cleavage of Methyl O-Methylpulvinates.—A suspension of



methyl *O*-methyl-3,4,4′-trimethoxypulvinate (IV) (50 mg) in saturated barium hydroxide solution (1·5 ml) was heated under reflux for 1·5 h. The mixture was cooled and acidified with 2N-hydrochloric acid. Recrystallisation of the product from methanol gave 2-(3,4-*dimethoxyphenyl*)-3-*methoxymaleic anhydride* (19·7 mg), as yellow plates, m.p. 137°. The aqueous filtrate was extracted with ether (2 × 2·5 ml) and the extract was washed with water and dried. Evaporation, and recrystallisation of the residue from water gave 4-methoxyphenylacetic acid (5·6 mg), as plates, m.p. and mixed m.p.[14] 85—87°. Similarly, cleavage of methyl *O*-methyl-3′,4′,4-trimethoxypulvinate (III) (20 mg) gave 3-*methoxy*-2-(4-*methoxyphenyl*)*maleic anhydride* (XVI) (9 mg), as yellow rhombs (from methanol) and 3,4-dimethoxyphenylacetic acid (4·5 mg) as pale yellow plates from benzene–light petroleum, m.p. and mixed m.p.[15] 88—89°; methyl *O*-methyl-3′,4′-dimethoxypulvinate (V) (27 mg), after heating under reflux for 4 h, gave 3-*methoxy*-2-*phenylmaleic anhydride* (XV) (12 mg) as needles (from methanol), m.p. 115—116°, and 3,4-dimethoxyphenylacetic acid (5 mg); methyl *O*-methyl-3,4-dimethoxypulvinate (VI) (25 mg) gave 2-(3,4-*dimethoxyphenyl*)-3-*methoxymaleic anhydride* (XVIII) (14 mg) and phenylacetic acid (3 mg) (from light petroleum), m.p. and mixed m.p.[16] 76°; methyl *O*-methyl-3,3′-dimethoxypulvinate (VII) (40 mg) gave 3-*methoxy*-2-(3-*methoxyphenyl*(*maleic anhydride* (XVII) (15 mg) as pale yellow needles (from methanol), m.p. 95—97°, and 3-methoxyphenylacetic acid (4 mg) as plates (from water), m.p. and mixed m.p.[17] 67°.



SCHEME 2

TABLE 1

Physical properties of 2-aryl-3-methoxymaleic anhydrides derived from pulvinic acid derivatives

	(XV)	(XVI)	(XVII)	(XVIII)
M.p. /°C	115—116	140—142	95—97	136—137
$\nu_{max.}$($CHCl_3$)/cm^{-1}	1833, 1770sh, 1757	1835, 1765	1835, 1820sh, 1760	1935sh, 1818, 1763
$\lambda_{max.}$(EtOH)/nm (log ε)	227 (3·79), 335 (3·62)	239·5 (4·03), 368 (3·93)	234infl (3·85), 263 (3·60), 333 (3·74)	243·5 (3·91), 270infl (3·68), 329infl (3·53), 382 (3·75)
τ($CDCl_3$)				
Aromatic protons	1·89—2·51 (5H)	1·77, 2·84 (4H, 2*d*, *J* 9 Hz)	2·19—3·03 (4H)	2·18—3·05 (3H)
Enolic OCH_3	5·54	5·50	5·52	5·54
Phenyl OCH_3		6·04 (3H)	6·07 (3H)	6·02 (6H)
m/e	204, 145, 132, 117, 89	234, 162, 147, 119	234, 175, 162, 147, 119	264, 192, 177, 149, 119
Found (%)	C, 64·5; H, 4·0	C, 61·9; H, 4·4	C, 61·7; H, 4·4	C, 58·8; H, 4·6
Required (%)	C, 64·7; H, 3·9	C, 61·55; H, 4·3	C, 61·55; H, 4·3	C, 59·1; H, 4·55

[14] R. Pschorr, O. Wolfes, and W. Buckow, *Ber.*, 1900, **33**, 162.
[15] A. Kaufmann, *Ber.*, 1918, **51**, 116.
[16] R. Adams and A. F. Thal, *Org. Synth.*, Coll. Vol. I, 1961, 436.
[17] R. Pschorr, *Annalen*, 1912, **391**, 40.

The physical properties of the 2-aryl-3-methoxymaleic anhydrides are shown in Tables 1 and 2.

N-(2-Aminophenyl)-2-(3,4-dimethoxyphenyl)-3-methoxymaleimide (XIII).—2-(2,4-Dimethoxyphenyl)-3-methoxymaleic anhydride (52 mg) in ethanol (5 ml), was heated on a water-bath for 15 min and then cooled. The solid was filtered off to yield the *product* (59 mg) as yellow needles (from ethanol), m.p. 192° (Found: C, 64·7; H, 5·3; N, 8·0. $C_{19}H_{18}N_2O_5$ requires C, 64·4; H, 5·1; N, 7·9%), $\nu_{max.}$ 1770 and 1709 cm^{-1} (C=O), $\lambda_{max.}$ 239, 287sh, and 396 nm (log ε 4·33, 3·70, and 3·64), τ 2·08—3·02 (7H), 5·50 (3H), 5·95 and 5·96 (each 3H), and 6·49 (2H).

A similar condensation of anhydride (XVIII) with aniline (18 mg) gave *N-phenyl-2-(3,4-dimethoxyphenyl)-3-methoxymaleimide* (XIV) (49 mg), as yellow needles (from ethanol), m.p. 134—137° (Found: C, 67·3; H, 4·9; N, 3·8. $C_{19}H_{17}NO_5$ requires C, 67·55; H, 5·0; N, 4·1%), $\nu_{max.}$ 1766, 1718sh, and 1705 cm^{-1} (C=O), $\lambda_{max.}$ 249, 297infl, and 400 nm (log ε 4·26, 3·54, and 3·65), τ 2·11—2·91 (8H), 5·52 (3H), and 5·95 (6H).

Ethyl β-Cyano-α-methoxy-3,4-dimethoxycinnamate.—Ethyl 3-cyano-3-(3,4-dimethoxyphenyl)pyruvate (250 mg) was methylated with an excess of ethereal diazomethane.

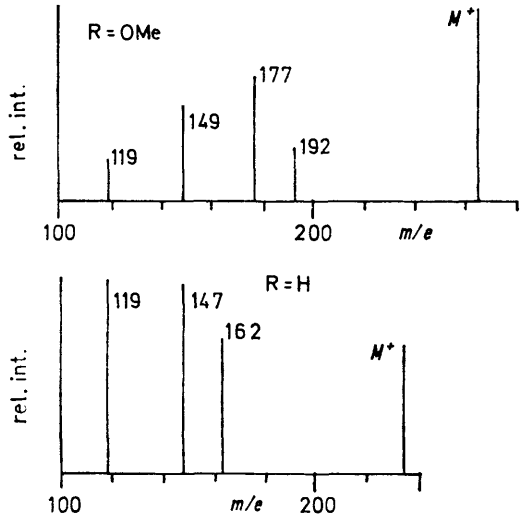
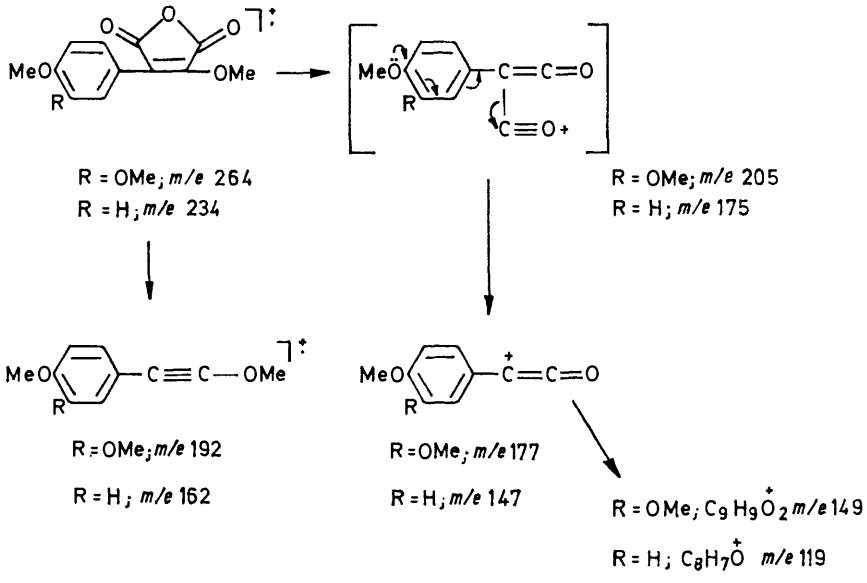


TABLE 2

High resolution mass measurements

	Ion	Found	C	H	O	Requires
2-Phenyl-3-methoxymaleic anhydride (XV)	145	0·029932	9	5	2	0·028952
	132	0·058284	9	8	1	0·057511
	117	0·033224	8	5	1	0·034037
2-(3-Methoxyphenyl)-3-methoxymaleic anhydride (XVII)	175	0·040140	10	7	3	0·039515
	162	0·067187	10	10	2	0·068075
	147	0·044520	9	7	2	0·044601
	119	0·049840	8	7	1	0·049687
2-(3,4-Dimethoxyphenyl)-3-methoxymaleic anhydride (XVIII)	117	0·055839	10	9	3	0·055165
	149	0·059088	9	9	2	0·060250
	119	0·049837	8	7	1	0·049687
2-(4-Methoxyphenyl)-3-methoxymaleic anhydride (XVI)	162	0·067187	10	10	2	0·068075
	147	0·045096	9	7	2	0·044601
	119	0·049840	8	7	1	0·049687

Evaporation of the ether gave a gummy residue which crystallised from light petroleum (b.p. 40—60°; twice) to give the *ester* (133 mg), as needles, m.p. 56—59° (Found: C, 62·2; H, 5·7; N, 4·8. $C_{15}H_{17}NO_5$ requires C, 61·9; H, 5·8; N, 4·8%); $\nu_{max.}$ 2209 (CN) and 1723 cm^{-1} (ester C=O);



SCHEME 3

τ 2·38—2·86 (3H), 5·42 (2H, q), 5·97, 5·98, and 6·02 (each 3H), and 8·53 (3H, t).

Hydrolysis of Ethyl β-Cyano-α-methoxy-3,4-dimethoxycinnamate.—(i) *With Alkali.* The ester (80 mg) was heated under reflux for 1·5 h with 2N-sodium hydroxide (15 ml). The mixture was cooled, acidified, and extracted with ether (2 × 25 ml). The extracts were combined, washed with water, dried, and evaporated. Crystallisation of the residue from water gave 3,4-dimethoxyphenylacetic acid (45 mg), m.p. and mixed m.p.[15] 88—89°.

(ii) *With acid.* The ester (40 mg) was heated under

reflux with a mixture of sulphuric acid (60%; 3 ml) and acetic acid (2 ml) for 1 h. The mixture was diluted with water (15 ml) and extracted with ether (2 × 10 ml). The extract was shaken with 2N-sodium hydroxide (10 ml) and acidified with 2N-sulphuric acid. Crystallisation of the product from methanol gave 2-(3,4-dimethoxyphenyl)-3-methoxymaleic anhydride (8 mg), identical with the product from the barium hydroxide cleavage of methyl *O*-methyl-3,4,4′-trimethoxypulvinate.

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