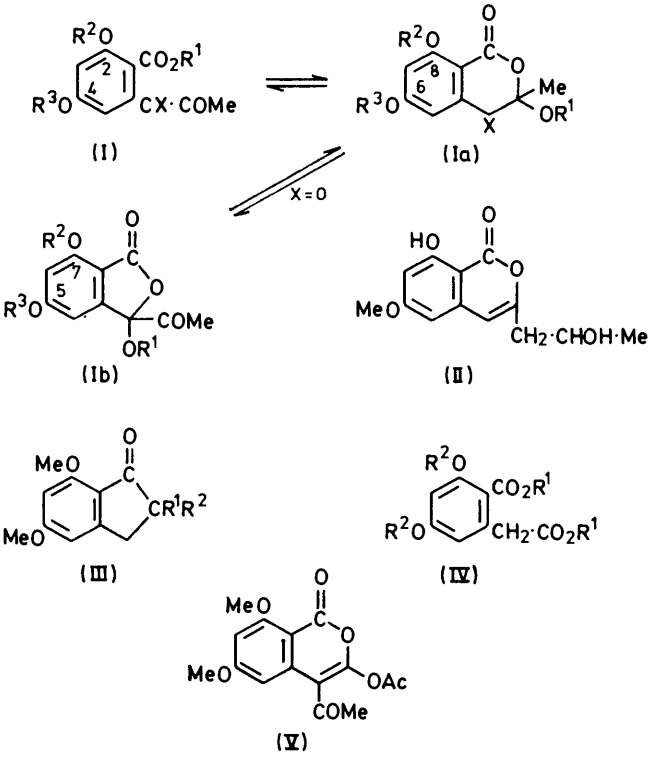
The Synthesis and Reactions of Some Derivatives of *C*-Acetylorsellinic Acid

By **John Frederick Grove** * and (in part) **Michael Pople**, A.R.C. Unit of Invertebrate Chemistry and Physiology, University of Sussex, Falmer, Brighton BN1 9QJ

The interconversion of the phytotoxic *C*-acetylorsellinic acid analogues produced by *Ceratocystis ulmi* is described and a number of derivatives of these compounds have been prepared. The 4-methyl and 2,4-dimethyl ethers of the natural products have been synthesised from the readily available 5,7-dimethoxyindan-1-one.

THE ketol-acid (I; $R^1 = R^2 = R^3 = H$, X = H,OH), originally isolated from *Penicillium brevicompactum*,[1,2] has recently[3] been identified as a major metabolic product of the elm pathogen *Ceratocystis ulmi*. The ketol-acid was accompanied by two minor metabolites, the corresponding diketone (I; $R^1 = R^2 = R^3 = H$, X = O) and ketone (I; $R^1 = R^2 = R^3 = H$, X = H_2) (*C*-acetylorsellinic acid). The latter has been shown[4,5] to have phytotoxic properties, and phytotoxicity to elm shoots has been demonstrated[6] for these compounds and for the methyl ether (I; $R^1 = R^2 = H$, $R^3 = Me$, X = H_2), diaporthic acid, a degradation product of the phytotoxin diaporthin (II).[7] Although separation of the naturally occurring solid solution of the *C. ulmi* co-metabolites into its components by preparative t.l.c. is satisfactory on a relatively small scale,[3] larger quantities of these compounds, and of their methyl ethers were required for biological testing. They have now been obtained by synthesis, both from the readily available, 5,7-dimethoxyindan-1-one, and from fermentation-derived ketol (I; $R^1 = R^2 = R^3 = H$, X = H,OH).



Previously reported[7-9] syntheses of (I; X = H_2) have started from esters of the homophthalic acid (IV; $R^1 = R^2 = H$), obtained in low yield by the self-condensation of acetonedicarboxylic ester.[8] An alternative, more attractive, route was outlined in 1967[10] without publication of experimental details. 5,7-Dimethoxyindan-1-one (III; $R^1 = R^2 = H$) was converted to the 1,2-dione (III; $R^1R^2 = O$), oxidative fission of which gave the acid (IV; $R^1 = H$, $R^2 = Me$) and thence, *via* the diacetylisocoumarin (V), the keto-acid (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H_2).

We have followed this route except that the acid (IV; $R^1 = H$, $R^2 = Me$) was obtained from the indanone by a conventional glyoxylic ester synthesis,[11] followed by oxidation of the product (III; $R^1 = H$, $R^2 = CO{\cdot}CO_2Et$) with alkaline hydrogen peroxide.[12]

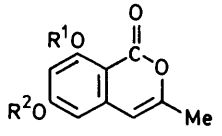
Oxidation of the keto-acid with selenium dioxide gave the diketone (I; $R^1 = H$, $R^2 = R^3 = Me$, X = O), hydrogenation of which, over palladium–charcoal in sodium hydrogencarbonate, afforded the ketol (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H,OH). In organic solvents the diketone is present, on spectroscopic evidence (see below), as the five-ring lactol (Ib; $R^1 = H$, $R^2 = R^3 = Me$) and attempted catalytic reduction in ethyl acetate failed, as did an attempted reduction with benzopinacol[13] in decahydronaphthalene at 170°. This behaviour contrasts with that of the diketone (I; $R^1 = R^2 = R^3 = H$, X = O), present in organic solvents as the open-chain tautomer, where catalytic reduction to the corresponding ketol occurred normally.[2]

Demethylation of the two dimethyl ethers (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H_2 and O) with boron trichloride was regiospecific giving the 4-methyl ethers (I; $R^1 = R^2 = H$, $R^3 = Me$, X = H_2 and O), respectively, of the *C. ulmi* metabolites. Attempted demethylation of the ketol (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H,OH) with 1 mol. equiv. of boron trichloride gave the 3-acetylphthalide (VII; $R^1 = H$, $R^2 = R^3 = Me$). Because of the lability of 3-acylphthalides to base (see below) this route was abandoned, and catalytic hydrogenation of the diketone (I; $R^1 = R^2 = H$, $R^3 = Me$, X = O), as described above, gave the desired product.

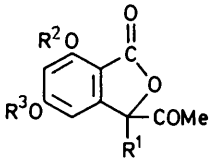
The second route to the keto-acid (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H_2) started from fermentation-derived ketol and proceeded *via* the corresponding diketone. Although ustic acid (VIII; X = H,OH) was readily oxidised by copper(II) sulphate to dehydroustic acid (VII; X = O),[14] the method virtually failed[2] with

the ketol (I; $R^1 = R^2 = R^3 = H$, X = HO,H). No satisfactory explanation [2] has been advanced for this failure, for we consistently obtained a near quantitative yield of the diketone by this method. Clemmensen reduction of the diketone gave the isocoumarin (VI; $R^1 = R^2 = H$) which afforded the keto-acid (I; $R^1 = R^2 = R^3 = H$, $X = H_2$) on treatment with sodium hydroxide.

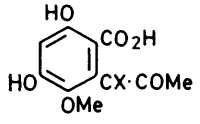
In the methylation of orsellinic acid derivatives, diazomethane, in the absence of alcohols, is usually regiospecific, giving the methyl ester 4-methyl ether, whilst



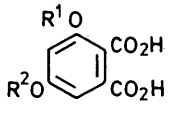
(VI)



(VII)



(VIII)

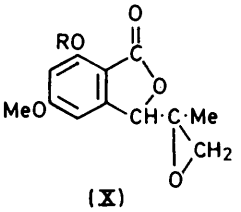


(IX)

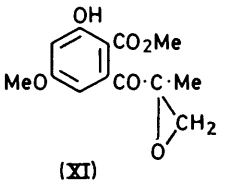
complete methylation results from the use of methyl iodide. Although methylation of the ketone (I; $R^1 = R^2 = R^3 = H$, $X = H_2$) to the esters (I; $R^1 = R^3 =$ Me, $R^2 =$ H and Me, $X = H_2$) and subsequent alkaline hydrolysis to the ethers (I; $R^1 = H$, $R^2 =$ H and Me, $R^3 =$ Me, $X = H_2$) was straightforward,[2,4] the preparation of the 4-methyl ethers of the ketol and diketone (I; $R^1 = R^2 = R^3 = H$, X = H,OH and O) presented a number of unexpected problems. Methylation of the ketol with diazomethane was reported [2] to give a complex mixture of products from which a dimethyl derivative, considered to be the ester (I; $R^1 = R^3 =$ Me, $R^2 = H$, X = H,OH), was obtained, in very low yield. We have obtained this derivative, together with the fully methylated ester (I; $R^1 = R^2 = R^3 =$ Me, X = H,OH), in acceptable yield and have confirmed the structural assignment. However, alkaline hydrolysis, under mild conditions, gave, as sole product, the phthalic acid (IX; $R^1 = H$, $R^2 =$ Me).

This acid was obtained by demethylation of 3,5-dimethoxyphthalic acid with boron trichloride. On a small scale, the preparation of the acid (IX; $R^1 = R^2 =$ Me) from the keto-acid (I; $R^1 = H$, $R^2 = R^3 =$ Me, $X = H_2$) is most easily achieved if the latter is first converted to the isocoumarin (VI; $R^1 = R^2 =$ Me), which is then oxidised with permanganate. This step avoids the formation of the highly water soluble phthalonic acid.[15]

To eliminate the effect of the tautomeric system [(I) ⇌ (Ia)], believed to be responsible, in part, for the multiplicity of products from the action of diazomethane on the ketol it was first converted into the phthalide (VII; $R^1 = R^2 = R^3 = H$). However, diazomethane readily attacked the side-chain carbonyl group of this compound, in preference to the 7-hydroxy group,

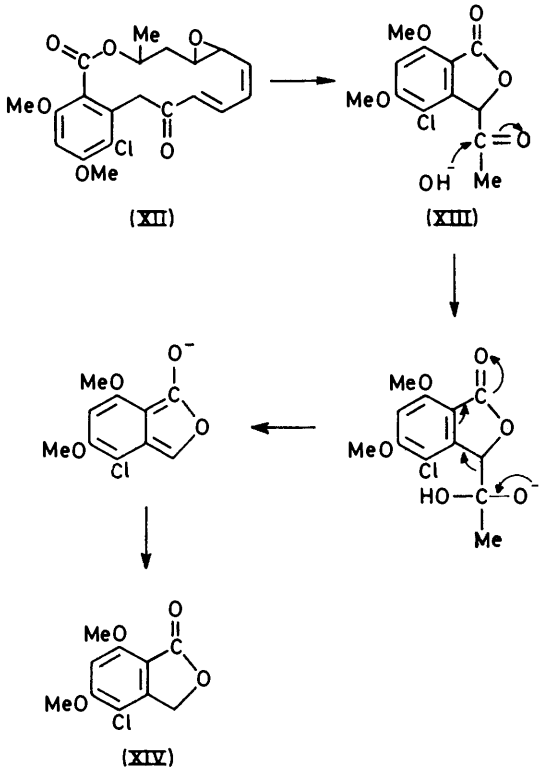


(X)



(XI)

giving the epoxide (X; R = H), whereas methyl iodide gave a $C_{13}H_{14}O_5$ compound, shown by n.m.r. spectroscopy to be the *C*-methyl derivative (VII; $R^1 = R^2 = R^3 =$ Me). This route to the ketol-acid 4-methyl ether was abandoned when it was found that the phthalide (VII; $R^1 = R^2 = R^3 = H$) underwent the same type of degradation as the ester (I; $R^1 = R^3 =$ Me, $R^2 = H$, X = H,OH), yielding, quantitatively, in 0.1N-sodium hydroxide at room temperature, the phthalic acid (IX; $R^1 = R^2 = H$). Interest in the lability of 3-acylphthalides to base was initiated by the ready conversion [16,17] of radicicol dimethyl ether (XII), by 0.4N methanolic potassium hydroxide, to the phthalide (XIV) in which the 3-acetylphthalide (XIII) is presumed to be an intermediate and to undergo decomposition by the mechanism outlined in Scheme 1. In these reactions the fragmentation product was a phthalide even though,



(XII) (XIII) (XIV)

SCHEME 1 Hypothetical mechanism for the degradation of 3-acetyl-4-chloro-5,7-dimethoxyphthalide [16]

in the reaction with radicicol dimethyl ether, air was not excluded. It is believed that a similar mechanism

must apply to the decomposition of the 3-acetylphthalide (VII; $R^1 = R^2 = R^3 = H$) and the ester (I; $R^1 = R^3 = Me$, $R^2 = H$, X = H,OH) oxidation taking place either during the reaction or in the subsequent work-up. The acid (I; $R^1 = R^2 = R^3 = H$, X = H,OH) did not undergo the reaction and was recovered unchanged from 2N-sodium hydroxide at room temperature.

The epoxidation and *C*-methylation reactions of the phthalide (VII; $R^1 = R^2 = R^3 = H$) also occur during methylation of the ketol (I; $R^1 = R^2 = R^3 = H$, X = H,OH) and both diastereoisomers of the epoxide (X; R = Me) and the *C*-methyl derivative (VII; $R^1 = R^2 = R^3 = Me$) were subsequently identified among the reaction products from the action of diazomethane and methyl iodide respectively. Likewise, the only isolable product from the action of diazomethane on the diketone (I; $R^1 = R^2 = R^3 = H$, X = O) was the epoxide (XI). Methyl iodide yielded a product believed on spectroscopic evidence to be the ester (I; $R^1 = R^2 = R^3 = Me$, X = O), but alkaline hydrolysis of this gave, as expected, a complex mixture of products containing little, if any, of the desired acid (I; $R^1 = H$, $R^2 = R^3 = Me$, X = O).

Ring–chain tautomerism in *C*-acetylorsellinic acid (I; $R^1 = R^2 = R^3 = H$, X = H_2) and its 4-methyl ether has been studied by several workers [4,7] and the compounds have been shown to exist in the lactol form (Ia) in the solid state and in organic solvents. With certain exceptions, outlined below, we have found n.m.r. to be the most reliable technique for the assignment of structure in this group of compounds as the ring (Ia) and open-chain (I) tautomers cannot always readily be identified on the basis of i.r. solution spectra.

Although the i.r. spectrum of the dimethyl ether (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H_2) showed it to be in the lactol form in the solid state, the n.m.r. data (Table 1) revealed that the open-chain form (I) was adopted in chloroform and in acetone. The proton signals for the 4-position and of the Me group are seen at τ 6.8 and 8.3 respectively in a typical lactol structure, compared with τ 6.3 and 7.9 for the corresponding signals in (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H_2). In methanol both tautomers were present in the ratio (I) : (Ia) of *ca.* 3 : 2.

The downfield shift of the proton signals of the Me group and for the 4-position, attributed to the free CO group, is also apparent in the ketol series (I; X = H,OH) if the normal ester (I; $R^1 = R^3 = Me$, $R^2 = H$, X = H,OH) is compared with the ketol-acid (I; $R^1 = R^2 = R^3 = H$, X = H,OH) and its ethers. The acid (I; $R^1 = R^2 = R^3 = H$, X = H,OH) and its 4-methyl ether were in the lactol form in the solvents used, but the dimethyl ether (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H,OH) was in the open-chain form in the chloroform and in methanol. Similar effects due to the free CO group apply to the phthalide derivatives (Table 2).

In the diketone series (I; X = O) n.m.r. showed that neither the acid (I; $R^1 = R^2 = R^3 = H$, X = O) nor its ethers existed in the six-ring lactol form (Ia) but the technique did not distinguish between the open-chain and five-ring lactol (Ib) structures. However, u.v. spectra readily made this distinction. In the solid state the acid (I; $R^1 = R^2 = R^3 = H$, X = O) existed in two crystalline modifications, one of which was the five-ring lactol, $\nu_{max.}$ 1 740 cm^{-1}. U.v. spectra, $\lambda_{max.}$ 295 and 348 nm, showed that this compound and its 4-methyl ether were in the open-chain form in methanol,

TABLE 1

Chemical shifts (τ) for protons in *C*-acetylorsellinic acid analogues (I) [a]

Compound										
R^1	R^2	R^3	X	Solvent	Tautomer	4-H	5-H [b]	7-H [b]	Me	OMe
H	H	H	H_2	$(CD_3)_2CO$	(Ia)	6.72	3.62	3.68	8.24	
H	H	Me	H_2	$(CD_3)_2CO$	(Ia)	6.8	3.65	3.65	8.28	6.17
H	Me	Me	H_2	$(CD_3)_2CO$	(I)	6.3	3.42	3.52	7.9	6.08, 6.12
				$CDCl_3$	(I)	6.2	3.56	3.64	7.82	6.02, 6.15
				CD_3OD	(I) + (Ia)	6.15, 6.82	3.44	3.54	7.84, 8.38	6.10
H	H	H	H,OH	$(CD_3)_2CO$	(Ia)	5.30	3.36	3.65	8.32	
H	H	Me	H,OH	CD_3OD	(Ia)		3.40	3.55	8.35	
H	Me	Me	H,OH	$CDCl_3$	(I)	4.48	3.38	3.52	7.90	6.04, 6.10
Me	H	Me	H,OH	$CDCl_3$	(I)	4.50	3.55	3.55	7.92	6.15, 6.22
H	H	H	O	CD_3OD	(I)		3.55	3.45	7.80	
H	H	Me	O	CD_3OD	(I)		3.24	3.52	7.84	6.10
H	Me	Me	O	$CDCl_3$	(Ib)		3.50	3.61	7.92	6.08, 6.18
				CD_3OD	(Ib)		3.25	3.30	7.75	6.00, 6.05

[a] For structure (Ia) or the corresponding protons in structures (I) or (Ib) [b] Singlet or doublet, $J_{5,7}$ 2 Hz.

TABLE 2

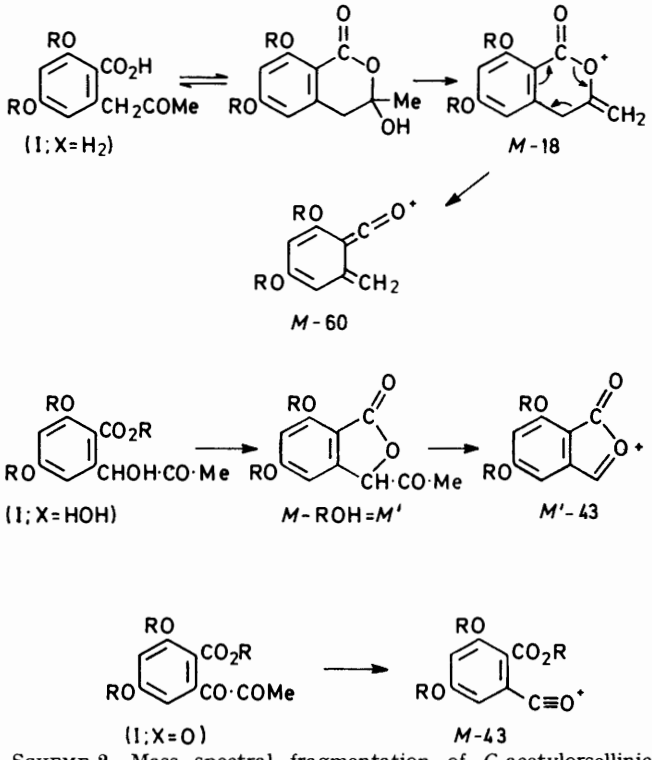
Chemical shifts (τ) for protons in phthalide derivatives

Compound	Solvent	3-H	4-H [a]	6-H [a]	Me	OMe	Other
(VII; $R^1=R^2=R^3=H$)	$(CD_3)_2CO$	4.24	3.40	3.52	7.78		
(VII; $R^1=R^2=R^3=Me$)	$CDCl_3$		3.52	3.58	7.86	6.08, 6.15	8.30 (3-Me)
(X; R=Me), m.p. 162°	$CDCl_3$	5.20	3.28	3.46	8.90	6.00, 6.04	7.00, 7.15 (CH_2) [b]
(X; R = Me), amorphous	$CDCl_3$	4.80	3.45	3.45	8.57	6.00, 6.05	7.28 (CH_2) [b]

[a] Singlet or doublet, $J_{4,6}$ 2 Hz. [b] Singlet or AB, *J* 4 Hz.

but the dimethyl ether (I; R = H, $R^2 = R^3$ = Me, X = O), $\lambda_{max.}$ 259 and 296 nm, existed as the five-ring lactol, the form preferred in the solid state, $\nu_{max.}$ 1 760 and 1 737 cm^{-1}. From these results it appears that dimethyl ethers in this group of *C*-acetylorsellinic acid analogues tend not to adopt the six-ring lactol structure.

Mass spectral fragmentation patterns were sometimes useful in structural diagnosis. Derivatives of the keto-acid (I; X = H_2) showed fragment ions at $M - 18$ and base peaks at $M - 60$ due to the consecutive loss of H_2O and C_2H_2O (see Scheme 2). Mass spectra of



SCHEME 2 Mass spectral fragmentation of *C*-acetylorsellinic acid analogues

derivatives of the ketol (I; X = H,OH) were similar to those of the corresponding phthalides and showed, after an initial loss of ROH mass units, base peaks corresponding to the further loss of 43. Derivatives of the diketone (I; X = O) showed base peaks at $M - 43$, whilst epoxides at the 2-position of the three-carbon side-chain showed base peaks at $M - 57$.

EXPERIMENTAL

M.p.s were taken on a Kofler hot-stage apparatus and are corrected. Unless stated otherwise, i.r. spectra were determined on mulls in Nujol, u.v. spectra for methanol solutions and R_F values are for the solvent system di-isopropyl ether–formic acid–water (90 : 7 : 3). Molecular weights were taken from the low resolution mass spectra. Mass spectra at high resolution were recorded on a Varian CH5D (double focusing) mass spectrometer coupled to a Varian 620L computer. Merck silica gel HF_{254} was used for t.l.c. and 0.075 cm layers were used in preparative t.l.c. Light petroleum had b.p. 60—80°. Tetramethylsilane was used as internal standard in n.m.r. spectra obtained at 100 MHz.

Ethyl 5,7-Dimethoxy-1-oxoindan-2-glyoxylate (III; R^1 = H, R^2 = CO·CO_2Et).—To sodium (0.3 g) in ethanol (5 ml) was added, dropwise, with stirring, a mixture of 5,7-dimethoxyindan-1-one (2.5 g) [18] and diethyl oxalate (3 g) in ethanol (15 ml), with cooling to maintain the temperature below 20°. The yellow solution was then stirred at room temperature for 24 h. After concentration to small bulk *in vacuo*, benzene was added and the solution was extracted with cold N-sodium hydroxide. Starting material (0.93 g), recovered from the neutral fraction, was recycled. The aqueous extract was acidified at 5—10° with concentrated hydrochloric acid, set aside at 0°, and filtered. The yellow precipitate was combined (1.11 g) with similar material obtained by extraction of the filtrate with ethyl acetate and recrystallised from ethanol giving needles or prisms, m.p. 135°, of the *ester* (III; R^1 = H, R^2 = CO·CO_2Et) (Found: C, 61.1; H, 5.3%; *M*, 292. $C_{15}H_{16}O_6$ requires C, 61.6; H, 5.5%; *M*, 292); $\nu_{max.}$ 1 720, 1 655, 1 600, and 1 585 cm^{-1}. It gave a green colour with iron(III) chloride in ethanol.

Oxidation of the Ester (III; R^1 = H, R^2 = CO·CO_2Et).—The ester (100 mg) in saturated sodium carbonate (5 ml) and water (5 ml) was treated with hydrogen peroxide (30%; 0.8 ml). After 5 h at room temperature, the solution was acidified and extracted with ethyl acetate. Concentration afforded the acid (IV; R^1 = H, R^2 = Me) as prisms (38 mg), m.p. 170—173° (lit.,[10] 172—173°); $\nu_{max.}$ 2 650, 2 550, 1 710, 1 660, 1 610, and 1 578 cm^{-1}.

Acetylation of the Acid (IV; R^1 = H, R^2 = Me).—The acid (70 mg) in pyridine (0.5 ml) and acetic anhydride (1.0 ml) was heated at 50° for 30 min. The product crystallised from ethyl acetate giving the isocoumarin (V) as needles (68 mg), m.p. 163° (decomp.) [lit.,[10] 156—160° (decomp.)]; $\nu_{max.}$ 1 775, 1 750, 1 620, and 1 600 cm^{-1}; τ($CDCl_3$) 3.12 (1 H, d, *J* 2 Hz), 3.55 (1 H, d, *J* 2 Hz), 6.10 (OMe), 6.16 (OMe), 7.54 (Me), and 7.82 (Me).

Hydrolysis of the Isocoumarin (V).—The isocoumarin (68 mg) was heated at 100° for 30 min with potassium hydroxide (2%; 2 ml). Crystallisation of the product from ethyl acetate gave the acid (I; R^1 = H, $R^2 = R^3$ = Me, X = H_2) as prisms (37 mg), m.p. 140° (lit.,[10] 139—140°); R_F 0.15, $\nu_{max.}$ 3 360, 1 680, 1 605, and 1 585 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1 720 cm^{-1}, $\lambda_{max.}$ 261 and 290 nm (log ε 3.94 and 3.67).

2,4-Dimethoxy-6-pyruvylbenzoic Acid (I; R^1 = H, $R^2 = R^3$ = Me, X = O).—The acid (I; R^1 = H, $R^2 = R^3$ = Me, X = H_2) (100 mg) was heated under reflux with selenium dioxide (50 mg) in dioxan (4 ml) for 6 h. The cooled solution was filtered and the solvent was removed at 50° *in vacuo*. The product crystallised from ethyl acetate in prisms (37 mg), m.p. 204°, of the *acid* (I; R^1 = H, $R^2 = R^3$ = Me, X = O) (Found: C, 56.8; H, 4.7. $C_{12}H_{12}O_6$ requires C, 57.1; H, 4.8%); $\nu_{max.}$ 3 355, 3 110, 3 030, 1 760, 1 737, 1 630, and 1 605 cm^{-1}, $\lambda_{max.}$ 259 and 296 nm (log ε 4.16 and 3.85); $\lambda_{max.}$ (Na salt in water) 285 and 350 nm.

Preparative t.l.c. of the residue from the mother liquors afforded the same acid (15 mg), R_F 0.24, and starting material (8 mg), R_F 0.15.

The *methyl ester* (I; $R^1 = R^2 = R^3$ = Me, X = O), prepared with diazomethane in ether at 0° during 30 min, formed prisms, m.p. 95—99° (from benzene–light petroleum) (Found: *M*, 266.079 5. $C_{13}H_{14}O_6$ requires *M*, 266.079 0); $\nu_{max.}$ 1 715, 1 688, 1 665, and 1 600 cm^{-1}; $\lambda_{max.}$ 252, 297, and 350 nm; R_F 0.68 [chloroform–methanol (95 : 5)].

6-(1-Hydroxyacetonyl)-2,4-dimethoxybenzoic Acid (I; R^1 =

H, $R^2 = R^3 = Me$, X = H,OH).—The acid (I; $R^1 = H$, $R^2 = R^3 = Me$, X = O) (16 mg) in sodium hydrogen carbonate (0.1M, 4 ml) at 25° was hydrogenated (uptake 0.92 mol) in the presence of palladium–charcoal (5%; 12 mg). The catalyst was filtered off and the filtrate was acidified and extracted with ethyl acetate. The product (15 mg) crystallised from ethyl acetate in prisms, m.p. 145—147°, of the *acid* (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H,OH), R_F 0.07 (Found: C, 56.7; H, 5.5. $C_{12}H_{14}O_6$ requires C, 56.7; H, 5.5%); $\nu_{max.}$ 3 470, ~3 340, 3 270, 1 682, 1 600, and 1 575 cm^{-1}, $\nu_{max.}$ ($CHCl_3$) 1 758, 1 718, and 1 605 cm^{-1}, $\lambda_{max.}$ 260 and 296 nm.

The *methyl ester* (I; $R^1 = R^2 = R^3 = Me$, X = H,OH), crystallised from benzene–light petroleum in prisms, m.p. 107—109° (Found: *M*, 268.094 7. $C_{13}H_{16}O_6$ requires *M*, 268.094 7); $\nu_{max.}$ 3 450, 1 727, 1 712, 1 605, and 1 585 cm^{-1}.

No uptake of hydrogen was observed over 2 h in the attempted reduction of the acid (I; $R^1 = H$, $R^2 = R^3 = Me$, X = O) in ethyl acetate.

Partial Demethylation of the Dimethyl Ethers with Boron Trichloride.—(1) The keto-acid (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H_2) (250 mg), suspended in dichloromethane (25 ml) at 0° was treated with boron trichloride (0.65 g) in dichloromethane (2.5 ml). The clear solution was set aside at 0° for 2 h when the solvent was removed *in vacuo*. The residue was triturated with warm water for 10 min and then extracted with ethyl acetate. The product crystallised from ethyl acetate in rhombs (223 mg), m.p. 125—127°, of diaporthic acid (I; $R^1 = R^2 = H$, $R^3 = Me$, X = H_2) (lit.,[7] 128—129°) (Found: C, 59.4; H, 5.6. Calc. for $C_{11}H_{12}O_5$: C, 58.9; H, 5.4%); R_F 0.55; $\nu_{max.}$ 3 350, 1 650, 1 622, and 1 580 cm^{-1}; $\lambda_{max.}$ 265 and 301 nm (log ε 4.02 and 3.76).

(2) The diketone (I; $R^1 = H$, $R^2 = R^3 = Me$, X = O) (12 mg) in dichloromethane (1 ml) was treated, as described above, with boron trichloride (21 mg) in dichloromethane (0.2 ml). The gummy product was subjected to preparative t.l.c.[3] giving the *4-methyl ether* (I; $R^1 = R^2 = H$, $R^3 = Me$, X = O), R_F 0.76 (4 mg), prisms, m.p. 90° (from ethyl acetate) (Found: *M*, 238.048 0. $C_{11}H_{10}O_6$ requires, *M*, 238.047 7); $\nu_{max.}$ 3 320, 3 230, 1 715, 1 660, and 1 615 cm^{-1}; $\lambda_{max.}$ 294 and 348 nm. It gave a reddish brown colour with iron(III) chloride.

(3) The ketol (I; $R^1 = H$, $R^2 = R^3 = Me$, X = H,OH) (3 mg) was stirred 1 h at 0° with boron trichloride (1.5 mg) in dichloromethane (1 ml). Trituration of the product with water furnished *3-acetyl-5,7-dimethoxyphthalide* (VII; $R^1 = H$, $R^2 = R^3 = Me$) as an amorphous powder, m.p. 152° (Found: *M*, 236.068 7. $C_{12}H_{12}O_5$ requires *M*, 236.068 5); $\nu_{max.}$ 1 765, 1 705, 1 620, and 1 600 cm^{-1}; R_F 0.61 [chloroform–methanol (95 : 5)].

(4) 3,5-Dimethoxyphthalic acid (6 mg), stirred 1 h at 0° with boron trichloride (10 mg) in dichloromethane (1 ml), furnished 3-hydroxy-5-methoxyphthalic acid (2 mg) identical with the acid obtained by the action of sodium hydroxide on the ester (I; $R^1 = R^3 = Me$, $R^2 = H$, X = H,OH) (see below).

2-Hydroxy-6-(1-hydroxyacetonyl)-4-methoxybenzoic Acid (I; $R^1 = R^2 = H$, $R^3 = Me$, X = H,OH).—The diketo-acid (I; $R^1 = R^2 = H$, $R^3 = Me$, X = O) was hydrogenated as described above for the acid (I; $R^1 = H$, $R^2 = R^3 = Me$, X = O) giving the *acid* (I; $R^1 = R^2 = H$, $R^3 = Me$, X = H,OH) prisms, m.p. 150—152° (decomp.) (from ethyl acetate); R_F 0.36 (Found: *M*, 240.063 4. $C_{11}H_{12}O_6$ requires *M*, 240.063 4); $\nu_{max.}$ 3 470, 3 340, 1 650, and 1 625 cm^{-1}; $\lambda_{max.}$ 262 and 301 nm.

Oxidation of the Ketol (I; $R^1 = R^2 = R^3 = H$, X = H,OH).—To the ketol[3] (57 mg) and hydrated copper(II) sulphate (0.15 g) in water (5 ml) at room temperature was added 0.1N-sodium hydroxide (15 ml) and the solution (pH 8.5) was set aside for 1 h. The precipitate was filtered off, and the filtrate was acidified to pH 2.5 and extracted with ethyl acetate giving the diketone (I; $R^1 = R^2 = R^3 = H$, X = O) (50 mg) as prisms, m.p. 166° (from dry ethyl acetate) (Found: C, 53.3; H, 3.6%; *M*, 224. $C_{10}H_8O_6$ requires C, 53.6; H, 3.6%; *M*, 224); $\nu_{max.}$ 3 300, 3 150, 1 710, 1 645, and 1 615 cm^{-1}; $\nu_{max.}$ ($CHCl_3$) 3 560, 3 180br, 1 725, 1 682, and 1 625 cm^{-1}. Sometimes the known[1] diketone hydrate, prisms, double m.p. 125—135 and 166°; $\nu_{max.}$ 3 465, 3 315, 3 150, 1 740, and 1 615 cm^{-1}, was obtained. When the hydrate was heated at 120—130° for 5 min and allowed to resolidify, the i.r. spectrum changed to that of the anhydrous diketone.

Reduction of Diketone (I; $R^1 = R^2 = R^3 = H$, X = O).—The diketone hydrate (50 mg) in water (1.5 ml) and concentrated hydrochloric acid (2 ml) was added to amalgamated zinc (0.5 g) and heated under reflux for 1 h. The cooled, diluted mixture was decanted and extracted with ethyl acetate. The extract was washed with sodium hydrogencarbonate and combined with a white sublimate from the condenser. Recovery gave the isocoumarin (VI; $R^1 = R^2 = H$) (21 mg), needles, m.p. 248° (lit.,[2] 247—249°); $\nu_{max.}$ 3 250, 1 685, 1 650, 1 632, and 1 580 cm^{-1}.

The acid fraction (11 mg) was shown by preparative t.l.c.[3] to consist mainly of the keto-acid (I; $R^1 = R^2 = R^3 = H$, X = H_2).

The isocoumarin (VI; $R^1 = R^2 = H$) (21 mg) was heated at 100° for 1 h with 0.1N-sodium hydroxide under nitrogen. The cooled solution was acidified to pH 3 with hydrochloric acid and extracted with ethyl acetate giving, the keto-acid (I; $R^1 = R^2 = R^3 = H$, X = H_2) (20 mg), m.p. 154°, identified by the i.r. spectrum.[3]

Methylation of the Ketol-acid (I; $R^1 = R^2 = R^3 = H$, X = H,OH).—(A) *Diazomethane.* (i) The ketol (100 mg) in ether was treated with a small excess of ethereal diazomethane during 1 h at room temperature. The recovered product was crystallised from benzene giving needles (32 mg), m.p. 78—81° (lit.,[2] 82°), of the *ester* (I; $R^1 = R^3 = Me$, $R^2 = H$, X = H,OH) (Found: C, 56.7; H, 6.0%; *M*, 254. $C_{12}H_{14}O_6$ requires C, 56.7; H, 5.55%; *M*, 254); $\lambda_{max.}$ 262 and 302 nm (log ε 4.19 and 3.88); $\nu_{max.}$ 3 370, 3 320, 1 722, 1 662, 1 625, and 1 588 cm^{-1}; R_F 0.50 [chloroform–methanol (95 : 5)]. It gave a reddish-brown colour with iron(III) chloride, and was unstable on storage at room temperature.

(ii) The ketol (250 mg) was treated as before, but the product (255 mg) in benzene–methanol (98 : 2; 25 ml) was chromatographed on a column of silica gel (Merck 7734; 15.3 g; 16 × 2 cm) in u.v. light. Elution of a blue fluorescent band with benzene gave a gum (109 mg) which crystallised from benzene in needles (61 mg) of the ester (I; $R^1 = R^3 = Me$, $R^2 = H$, X = H,OH).

Elution of a second blue band with benzene–methanol (200 : 1; 125 ml) gave a gum which did not crystallise, but the i.r. spectrum, $\nu_{max.}$ 3 400br, 1 720br, 1 610, and 1 590 cm^{-1}, of the amorphous solid product obtained by trituration with light petroleum was very similar to that of the ester (I; $R^1 = R^2 = R^3 = Me$, X = H,OH). It gave no colour with iron(III) chloride.

Further elution of the column with benzene–methanol (20 : 1; 500 ml) gave a gum (105 mg), τ 6.1 (3 H), which was remethylated in ether with diazomethane. Preparative t.l.c. of the product (110 mg) in chloroform–methanol (95 : 5) gave well defined bands at R_F 0.71 and 0.64. Gum (33 mg) from the band R_F 0.71 was triturated with light petroleum giving a solid which crystallised from ethyl acetate in prisms (15 mg), m.p. 162°, of *5,7-dimethoxy-3-(1-methylepoxyethyl)phthalide* (X; R = Me) (Found: *M*, 250.084 5. $C_{13}H_{14}O_5$ requires *M*, 250.084 1); $\nu_{max.}$ 1 755, 1 615, and 1 600 cm^{-1}, $\lambda_{max.}$ 258 and 293 nm (log ε 4.27 and 3.83).

The band R_F 0.64 yielded a *diastereoisomer* of the epoxide (X; R = Me) as an amorphous solid (15 mg) (Found: *M*, 250. $C_{13}H_{14}O_5$ requires *M*, 250); $\lambda_{max.}$ 258 and 294 nm; $\nu_{max.}$ 1 750br and 1 615br cm^{-1}. The mass spectral fragmentation pattern was identical with that of the compound R_F 0.71, but the n.m.r. spectrum (Table 2) was significantly different.

(B) *Methyl iodide.* The ketol (50 mg) in acetone (5 ml) was heated under reflux with methyl iodide (0.5 ml) in the presence of potassium carbonate (0.1 g) for 9 h, and the mixture was then set aside at room temperature for 3 days. After concentration *in vacuo* to small bulk, water (5 ml) was added and the emulsion was extracted with ethyl acetate. The neutral fraction (40 mg), which gave a negative iron(III) reaction, was subjected to preparative t.l.c. [chloroform–methanol (95 : 5)]. The gum (30 mg) recovered from a band R_F 0.66 crystallised from benzene in prisms (8 mg), m.p. 127°, of the phthalide (VII; $R^1 = R^2 =$ Me) (see below).

Methylation of the Diketone (I; $R^1 = R^2 = R^3 =$ H, X = O).—(A) *Diazomethane.* The diketone (8 mg) in ether was treated with ethereal diazomethane as described above, and the recovered gum was submitted to preparative t.l.c. [chloroform–methanol (95:5)]. Material (6 mg) from the major band, R_F 0.57 [benzene–methanol (95 : 5)], was extracted with light petroleum. Concentration of the extract afforded the *epoxide* (XI) as rosettes of needles, m.p. 92—95° (Found: *M*, 266.079 5. $C_{13}H_{14}O_6$ requires *M*, 266.079 0); $\nu_{max.}$ 1 682, 1 610, and 1 572 cm^{-1}; $\lambda_{max.}$ 256, 301, and 350 nm. It gave a reddish brown colour with iron(III) chloride.

(B) *Methyl iodide.* The diketone (7 mg) treated as described above, afforded a neutral gum (8 mg) which was subjected to preparative t.l.c. [chloroform–methanol (95 : 5)]. Recovery of material R_F 0.68 gave the ester (I; $R^1 = R^2 = R^3 =$ Me, X = O) (3 mg), $\lambda_{max.}$ 252, 297, and 350 nm.

Hydrolysis of the ester in methanol (0.1 ml) with 2N-sodium hydroxide (0.1 ml) at room temperature during 4 h and isolation of the acid fraction in the usual way gave a tar with constituents at R_F 0.15, 0.20, 0.23, 0.29, and 0.40. The spot R_F 0.23 corresponded to the diketone (I; $R^1 =$ H, $R^2 = R^3 =$ Me, X = O) but was only a minor component of the mixture.

3-Acetyl-5,7-dihydroxyphthalide (VII; $R^1 = R^2 = R^3 =$ H).—The ketol (I; $R^1 = R^2 = R^3 =$ H, X = H,OH) (20 mg) was dissolved in concentrated sulphuric acid (0.2 ml) at 0°. After 10 min the solution was poured into water (2 ml) at 0° and, after extraction with ethyl acetate and washing with water, the product (14 mg) was recovered. It crystallised from ethyl acetate in prisms, m.p. 213° (decomp.), of the *phthalide* (VII; $R^1 = R^2 = R^3 =$ H) (Found: C, 57.3; H, 4.0%; *M*, 208. $C_{10}H_8O_5$ requires C, 57.7; H, 3.9%; *M*, 208); $\nu_{max.}$ 3 300, 3 080br, 1 710, and 1 610 cm^{-1}; $\lambda_{max.}$ 258 and 295 nm (log ε 4.03 and 3.62); R_F [chloroform–methanol (95 : 5)] 0.18.

Methylation of the Phthalide (VII; $R^1 = R^2 = R^3 =$ H).—(A) *Diazomethane.* The phthalide (32 mg) was treated as described above and the product (26 mg) was submitted to preparative t.l.c. in chloroform–methanol (95 : 5). Gummy material from a band R_F 0.48 crystallised from methanol in prisms, m.p. 208°, of *7-hydroxy-5-methoxy-3-(1-methylepoxyethyl)phthalide* (X; R = H) (Found: *M*, 236.069 6. $C_{12}H_{12}O_5$ requires *M*, 236.068 5); $\nu_{max.}$ 1 710, 1 635, and 1 605 cm^{-1}, $\lambda_{max.}$ 258 and 294 nm.

(B) *Methyl iodide.* The phthalide (7 mg) afforded a yellow gum (8 mg). Crystallisation from benzene gave *3-acetyl-5,7-dimethoxy-3-methylphthalide* (VII; $R^1 = R^2 = R^3 =$ Me) as prisms, m.p. 125—127° (Found: C, 62.1; H, 5.3%; *M*, 250.084 8. $C_{13}H_{14}O_5$ requires C, 62.4; H, 5.6%; *M*, 250.084 1), $\nu_{max.}$ 3 100w, 1 755, 1 730, 1 627, and 1 602 cm^{-1}, $\lambda_{max.}$ 259 and 295 nm (log ε 4.13 and 3.75).

Action of Sodium Hydroxide on the Ester (I; $R^1 = R^3 =$ Me, $R^2 =$ H, X = H,OH).—The ester (26 mg) in 2N-sodium hydroxide (1 ml) was set aside at room temperature for 18 h. Recovery gave a solid product (17 mg), which was soluble in sodium hydrogencarbonate and showed only one spot, R_F 0.38, on t.l.c. It was crystallised from ethyl acetate and then from water giving 3-hydroxy-5-methoxyphthalic acid, m.p. 170—175° (decomp.) [lit.,[19] 177—179° (decomp)] (Found: C, 50.9; H, 4.2. Calc. for $C_9H_8O_6$: C, 51.0; H, 3.8%), $\nu_{max.}$ 3 100—2 500, 1 705, 1 655, and 1 625 cm^{-1}, identical with a specimen prepared by the oxidation of 6,8-dimethoxy-3-methylisocoumarin and demethylation of the product.

Under the same conditions the ketol-acid (I; $R^1 = R^2 = R^3 =$ H, X = H,OH) (20 mg) was recovered unchanged.

Action of Sodium Hydroxide on the Phthalide (VII; $R^1 = R^2 = R^3 =$ H).—The phthalide (6 mg) in methanol (0.5 ml) and 0.1N-sodium hydroxide (2.0 ml) was set aside at room temperature for 6 h. The product crystallised from ethyl acetate in prisms, m.p. 185—190° (decomp.), R_F 0.55, of 3,5-dihydroxyphthalic acid, the i.r. spectrum of which, $\nu_{max.}$ 3 280, 2 520, 1 710, 1 655, and 1 615 cm^{-1}, was identical with that of an authentic specimen.[1]

The same result was obtained when the reaction was carried out using boiled-out reagents under nitrogen.

6,8-Dimethoxy-3-methylisocoumarin (VI; $R^1 = R^2 =$ Me).—The keto-acid (I; $R^1 =$ H, $R^2 = R^3 =$ Me, X = H_2) (50 mg) was dissolved in concentrated sulphuric acid (0.2 ml) at 0°. After 10 min the solution was poured into water (3 ml) at 0° and the precipitated isocoumarin (35 mg), m.p. 149—150° (lit.,[2] 149—151°); $\nu_{max.}$ 1 710, 1 662, 1 600, and 1 568 cm^{-1}, was collected.

3,5-Dimethoxyphthalic Acid.—The isocoumarin (VI; $R^1 = R^2 =$ Me) (15 mg) was suspended in sodium hydrogencarbonate (1 ml) at 100° and potassium permanganate (5%; 1 ml) was added dropwise with vigorous stirring until the purple colour persisted for 15 min. The cooled mixture was filtered and the filtrate was acidified and extracted with ether. The product (10 mg) crystallised from water in prisms of 3,5-dimethoxyphthalic acid, $\nu_{max.}$ 3 440, 2 630, 2 510, 1 700, 1 685, and 1 600 cm^{-1}; m.p. 145° (anhydride: lit.,[2] 147—149°).

Part of this work, supported by the Agricultural Research Council, was carried out between 1968 and 1970 at the University Chemical Laboratory, Cambridge during the

tenure, by J. F. G., of a Comyns Berkeley Bye-Fellowship from Gonville and Caius College. Technical assistance with the large-scale preparation of some intermediates was given by Mr. R. Poppi, Tropical Products Institute, London, to whom we are greatly indebted. We thank Messrs. B. A. J. Alexander and P. E. Meadows for the n.m.r. spectra, Dr. F. A. Mellon for the mass spectra, and A. Olney for microanalyses.

[8/208 *Received*, 8*th February*, 1978]

REFERENCES

[1] P. W. Clutterbuck, A. E. Oxford, H. Raistrick, and G. Smith, *Biochem. J.*, 1932, **26**, 1441.
[2] A. E. Oxford and H. Raistrick, *Biochem. J.*, 1933, **27**, 634.
[3] N. Claydon, J. F. Grove, and M. Hosken, *Chem. and Ind.*, 1974, **344**; *Phytochemistry*, 1974, **13**, 2567.
[4] K. Kameda, H. Aoki, H. Tanaka, and M. Namiki, *Agric. Biol. Chem.*, 1973, **37**, 2137.
[5] S. Iwasaki, H. Muro, K. Sasaki, S. Nozoe, S. Okuda, and Z. Sato, *Tetrahedron Letters*, 1973, 3537.
[6] N. Claydon and J. F. Grove, unpublished results.
[7] E. Hardegger, W. Rieder, A. Walser, and F. Kiegler, *Helv. Chim. Acta*, 1966, **49**, 1283.
[8] H. Nogami, *J. Pharm. Soc. Japan*, 1941, **61**, 56.
[9] R. F. Curtis, P. C. Harries, and C. H. Hassall, *J. Chem. Soc.*, 1964, 5382.
[10] H. L. Slates, S. Weber, and N. L. Wendler, *Chimia* (*Switz.*), 1967, **21**, 468.
[11] R. Robinson and R. C. Shah, *J. Chem. Soc.*, 1933, 610.
[12] J. Eichenberger, *Helv. Chim. Acta*, 1948, **31**, 1663.
[13] M. B. Rubin and J. M. Ben-Bassat, *Tetrahedron Letters*, 1971, 3403.
[14] H. Raistrick and C. E. Stickings, *Biochem. J.*, 1951, **48**, 53.
[15] J. F. Grove, *J.C.S. Perkin I*, 1972, 2400.
[16] R. N. Mirrington, E. Ritchie, C. W. Shoppee, S. Sternhell, and W. C. Taylor, *Austral. J. Chem.*, 1966, **19**, 1265.
[17] D. G. Buckley, E. Ritchie, and W. C. Taylor, *Austral. J. Chem.*, 1969, **22**, 577.
[18] R. Huisgen, G. Seidl, and I. Wimmer, *Annalen*, 1964, **677**, 21.
[19] A. J. Birch and J. J. Wright, *Austral. J. Chem.*, 1969, **22**, 2635.