Studies on the Syntheses of Heterocyclic Compounds containing Benzopyrone. Part 5.[1] Total Synthesis of Fulvic Acid

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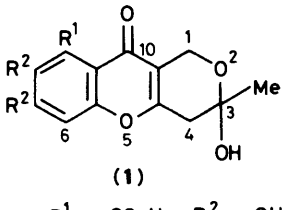
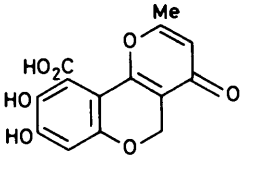
Total synthesis of fulvic acid (**1a**) is described. Regioselective cyclization of the enedione (**8f**), an equivalent of the proposed biogenetic intermediate (**5a**) for citromycetin (**2**), gave the pyrone (**11a**), which led to fulvic acid (**1a**) by a route involving debenzylation, selective ozonization, and hydration.

In 1935 Oxford *et al.*[2] isolated a yellow crystalline material, which they named fulvic acid, from cultures of *Penicillium griseofulvum* Dierckx. Since then this compound has been isolated from various kinds of fungi.[3] In 1957 Dean *et al.*[3a,b] showed that this metabolite has structure (**1a**), and that in spite of the presence of one asymmetric carbon this compound is optically inactive because of tautomerism of the hemiacetal group. It has been reported that fulvic acid (**1a**) stimulates respiration[4] or root formation[5] in some plants. Some approaches to its synthesis have been studied[6] but its total synthesis has not been established in spite of the simple structure. In contrast, the biosynthesis of fulvic acid (**1a**) has been explained by analogy with that of similar metabolites.[3,7]

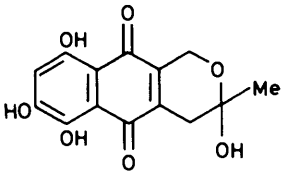


(**1**)

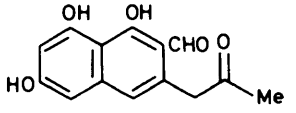


(**2**)

a; R^1 = CO_2H, R^2 = OH
b; R^1 = R^2 = H
c; R^1 = CH=CHMe, R^2 = OMe
d; R^1 = CHO, R^2 = OMe
e; R^1 = CO_2H, R^2 = OMe



(**3**)



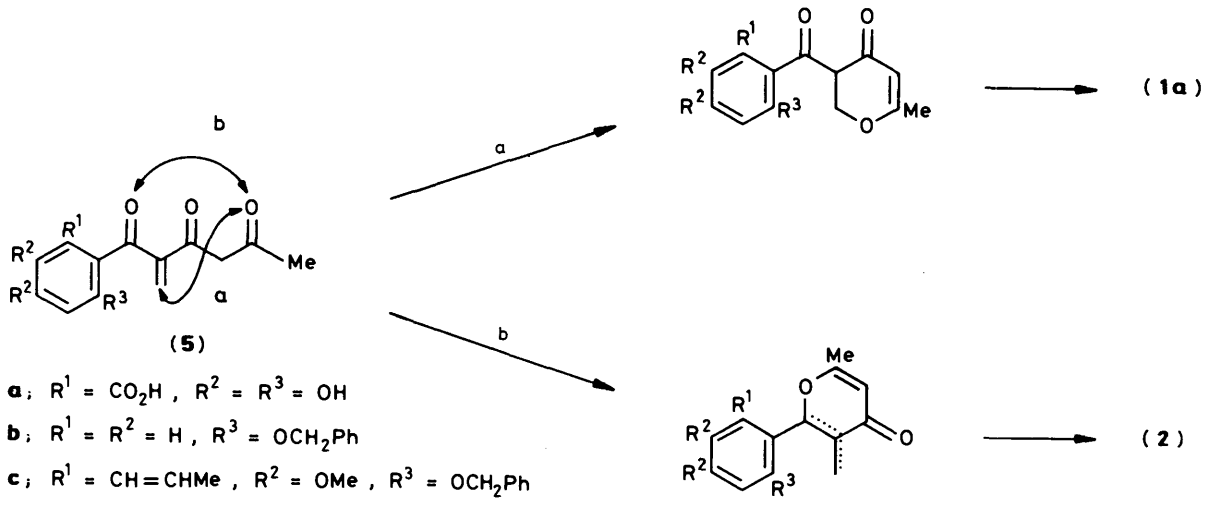
(**4**)

Most recently Hutchinson and co-workers[8] have proposed that fungal metabolites such as fulvic acid (**1a**), citromycetin (**2**), and fusarbin (**3**) are biosynthesized through common intermediate (**4**) formed from seven acetate units. We studied enetrione (**5a**), a known intermediate for citromycetin, and thought it could be a synthetic intermediate for both fulvic acid (**1a**) and citromycetin (**2**). If condensation were to occur between the ene and the acetyl ketone (path a), fulvic acid (**1a**) would be synthesized; and condensation between the benzoyl ketone and the acetyl ketone (path b) would lead to citromycetin (**2**) (Scheme). Although this regioselective cyclization was achieved by using the non-substituted enetrione (**5b**) in our preliminary studies,[1,9] the synthetic strategy for compound (**1b**) could not be applied to the enetrione (**5a**) because of the naked hydroxy and carboxy groups. We chose enetrione (**5c**) as a synthetic intermediate, for the propenyl and methoxy groups could be converted into carboxy and hydroxy groups respectively at the last stage of the total synthesis of fulvic acid (**1a**). In this paper we describe the first total synthesis[10] of fulvic acid (**1a**), *via* regioselective cyclization and selective ozonization as key steps.

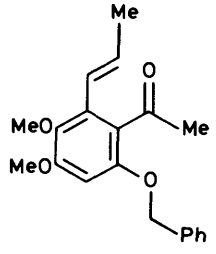
Results and Discussion

Synthesis of the Intermediate (**8f**).—The acetophenone (**6**), chosen as a starting material, was prepared from vanillin *via* 9 steps (38% total yield) by a slightly modified Dean method.[11] The aldol condensation of compound (**6**) with (2-methyl-1,3-dioxolan-2-yl)acetaldehyde (**7**)[12] in the presence of lithium diisopropylamide (LDA)–magnesium bromide ($MgBr_2$) in tetrahydrofuran (THF)[13] gave the hydroxy ketone (**8a**) in 85% yield despite the presence of two bulky *ortho* substituents in compound (**6**). Oxidation of the alcohol (**8a**) with chromic acid–sulphuric acid in dimethylformamide (DMF)[14] [the same conditions for oxidation of the non-substituted analogue (**9**)[9b]] did not give the diketone (**8b**). Although on treatment with 1—2 equiv. of *N*-chlorosuccinimide (NCS)–dimethyl sulphide (Me_2S) complex (Corey–Kim reagent)[15] a large amount of the starting material was recovered, treatment of compound (**8a**) with a large excess (>5 equiv.) of the complex gave diacyl-*S*-ylide (**8c**) in almost quantitative yield. The structure of compound (**8c**) was determined by the following spectral data: $\nu_{max.}$ 1 570 cm^{-1} (C=O); two downfield-shifted methyl signals (δ_H 2.71, 2.78) due to *S*-methyls; characteristic ^{13}C n.m.r. signal at δ_C 91.5 due to $\bar{C}$–$\overset{+}{S}$.[16] Reduction of diacyl-*S*-ylide (**8c**) with Zn–AcOH gave the desired diketone (**8b**) in 98% yield. This diketone (**8b**) is converted quantitatively back into the ylide (**8c**) by treatment with the same complex, and the oxidation pathway is explained as follows: first, oxidation of the hydroxy group takes place with the complex to give the diketone (**8b**) in a Corey–Kim oxidation manner,[15] and, since the reactivity of the resulting diketone (**8b**) toward the complex is higher than that of the starting material, this leads to accumulation of the diacyl-*S*-ylide (**8c**). Alkylation of the diketone (**8b**) with *N*-(methylthiomethyl)piperidine hydrochloride[17] gave thiomethyl dione (**8d**) (87%) and thiomethylpyrone (**10**) (7%). Pyrolysis of the sulphinylmethyl dione (**8e**), obtained by sodium metaperiodate oxidation of sulphide (**8d**), afforded the enedione (**8f**), which can be regarded as an equivalent of our planned synthetic intermediate (**5c**).

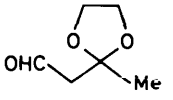
Synthesis of Fulvic Acid (**1a**) via *Regioselective Cyclization and Selective Ozonization.*—When the enedione (**8f**) was stirred



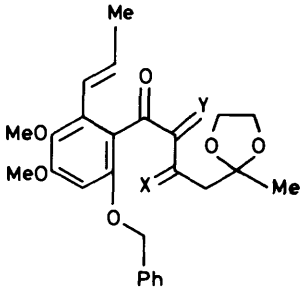
Scheme



(6)

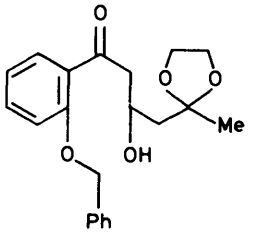


(7)

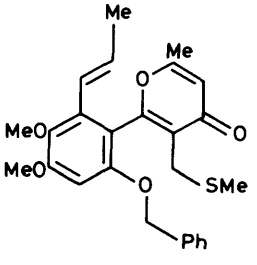


(8)

a; X = H, OH, Y = H, H
b; X = O, Y = H, H
c; X = O, Y = SMe_2
d; X = O, Y = H, CH_2SMe
e; X = O, Y = H, $CH_2S(O)Me$
f; X = O, Y = CH_2

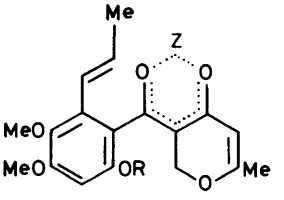


(9)



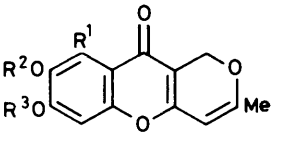
(10)

in 5% aqueous HCl–THF (1:2, v/v) for 24 h, the regioselectively formed product was the dihydropyrone (**11a**) (91% yield) resulting from condensation of the ene and the terminal acetyl ketone. (Under these conditions prior hydrolysis of the acetal group must take place.) This dihydropyrone (**11a**) was also obtained (79% yield) by refluxing the sulphinylmethyl dione (**8e**) in 5% aqueous H_2SO_4–AcOH–THF (1:1:4) for 3 h. The dihydropyrone (**11a**) was an unstable oil, but the structure was confirmed by a positive $FeCl_3$ test and characteristic ^1H n.m.r. signals for the 2,3-dihydro-4*H*-pyran-4-one portion of the molecule [δ 4.51 (2-H) and 5.42 (5-H)].[18] Application of Fujita's debenzylation method[19] to the BF_2-complex (**11b**), prepared from the dihydropyrone (**11a**) and BF_3·OEt_2, afforded the BF_2-complex of the phenol, (**11c**). Acid treatment of the (**11c**) resulted in deboronation and dehydration to yield the tricyclic pyranobenzopyrone (**12a**). From this stage, conversion of the substituents in the benzene ring into carboxy and hydroxy groups and hydration of the double bond of the vinyl ether would achieve the total synthesis of fulvic acid (**1a**).



(11)

a; R = CH_2Ph, Z = H
b; R = CH_2Ph, Z = BF_2
c; R = H, Z = BF_2



(12)

a; R^1 = CH=CHMe, R^2 = R^3 = Me
b; R^1 = CO_2H, R^2 = R^3 = Me
c; R^1 = CO_2H, R^2 = H, R^3 = Me
d; R^1 = CO_2H, R^2 = R^3 = H

Ozonization or osmium tetraoxide oxidation of compound (**12a**) gave unidentified complex products owing to the presence of the various double bonds as well as the propenyl group.

However, prior hydration [5% aqueous HCl–acetone (1:2), room temperature, 4 days][20] of the pyranobenzopyrone (**12a**) gave the hemiacetal (**1c**), whose ozonization in the presence of a dye (Oil Violet)* as an internal indicator,[21] followed by reduction with Me_2S, gave the aldehyde (**1d**) in 64% yield from compound (**12a**). Oxidation of the aldehyde (**1d**) with sulphamic acid and sodium chlorite[22] afforded *O,O*-dimethylfulvic acid (**1e**), which was slightly unstable and which was partly converted into the anhydro compound (**12b**) on chromatography on silica or on recrystallization. Thus we converted hemiacetal (**1e**) into anhydro-*O,O*-dimethylfulvic acid (**12b**) [74% yield from (**1d**)] by treatment with conc. H_2SO_4–acetone (1:250). During the conversion of the propenyl group into a carboxy group the hydroxy group played a role as a protecting group for the vinyl ether. Demethylation of bis(methyl ether) (**12b**) with boron tribromide gave unidentified products at ambient temperature, or the monomethyl ether (**12c**) at −78 °C. The structure (**12c**) was established from the fact that methoxy group *ortho* to the carbonyl group is easily demethylated.[23] However, treatment of compound (**12b**) with $AlCl_3$–Me_2S in CH_2Cl_2[24] furnished anhydrofulvic acid (**12d**) in 52% yield. When anhydrofulvic acid (**12d**) was treated with 5% aqueous HCl–acetone (1:2) as for reaction of the acid (**12a**) a considerable amount of the starting material (**12d**) was unexpectedly recovered, and this recovery was independent of reaction time. This is presumably because equilibrium would be established between hydration and dehydration under these conditions. Finally, conversion of compound (**12d**) into fulvic acid (**1a**) was achieved by treatment with acid [5% aqueous H_2SO_4–acetone (1:2)],† and proceeded in 68% yield. The synthetic fulvic acid is identical in all respects (mixed m.p., i.r., u.v., and n.m.r.) with a natural specimen.

Experimental

M.p.s were measured on a Yanako micro-melting-point apparatus and are uncorrected. Extracts were dried over anhydrous magnesium sulphate. I.r. spectra were recorded on a JASCO IR-810 spectrophotometer. The u.v. spectrum was recorded with a Hitachi Model 200-10 spectrophotometer. Mass spectra were taken on a Shimazu LKB-9000 mass spectrometer, and high-resolution mass spectra with a JEOL JMS-01SG instrument. N.m.r. spectra were obtained with a JEOL JMN-GX 270 spectrometer (tetramethylsilane as internal reference).

6′-Benzyloxy-3-hydroxy-3′,4′-dimethoxy-4-(2-methyl-1,3-dioxolan-2-yl)-2′-(prop-1-enyl)butyrophenone (**8a**).—A solution of 6-benzyloxy-3,4-dimethoxy-2-(prop-1-enyl)acetophenone (**6**) (28.0 g, 86 mmol) in THF (150 ml) was added to a solution of LDA [from di-isopropylamine (10.8 g, 107 mmol) and 15 w/w % Bu^nLi in n-hexane (65 ml)] in THF (200 ml) during 45 min at −78 °C under N_2. The resulting solution was stirred at −78 °C for 1 h and was then treated with a mixture of (2-methyl-1,3-dioxolan-2-yl)acetaldehyde (**7**) (14.0 g, 108 mmol) and $MgBr_2$ [from ethylene dibromide (21.0 g, 112 mmol) and Mg (10.0 g, 412 mg-atom)] in THF (50 ml) at −78 °C. The mixture was stirred for 3 h at the same temperature, and AcOH (15 ml) in Et_2O (150 ml) and then water (300 ml) were added to the mixture. The resulting solution as extracted with benzene (200 ml × 2) and the benzene layer was washed with water (100 ml × 3), dried, and evaporated. The residue was subjected to column chromatography (Florisil; Wako) to elute (n-pentane–Et_2O, 4:1) the *title butyrophenone* (**8a**). Recrystallization from n-pentane–Et_2O gave *needles* (33.3 g, 85%), m.p. 79—81 °C (Found: C, 68.4; H, 7.1. $C_{26}H_{32}O_7$ requires C, 68.23; H, 7.07%); *m/z* 456 (M^+); $\nu_{max.}$(KBr) 3 500 and 1 650 cm^{-1}; δ($CDCl_3$) 1.33 (3 H, s, Me), 1.75—1.81 [2 H, m, $CH_2C(O)O$], 1.85 (3 H, dd, *J* 6.6 and 1.5 Hz, =CMe), 2.88 (2 H, d, *J* 5.9 Hz, CH_2CO), 3.60 (1 H, d, *J* 1.5 Hz, OH), 3.69 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.92—3.95 (4 H, m, OCH_2CH_2O), 4.41 (1 H, m, CHO), 5.04 (2 H, s, CH_2O), 6.03—6.11 (1 H, m, =C*H*Me), 6.37—6.38 (1 H, m, =CHAr), 6.43 (1 H, s, ArH), and 7.36—7.38 (5 H, m, Ph).

Diacyl-S-ylide (**8c**).—A solution of 6′-benzyloxy-3-hydroxy-3′,4′-dimethoxy-4-(2-methyl-1,3-dioxolan-2-yl)-2′-(prop-1-enyl)butyrophenone (**8a**) (22.0 g, 48 mmol) in anhydrous CH_2Cl_2 (150 ml) was added to a suspension of NCS (40.0 g)–Me_2S (120 ml) in anhydrous CH_2Cl_2 (800 ml), and the mixture was stirred for 3 h at −20 °C under argon. Triethylamine (150 ml) was added to the mixture, which was then stirred for a further 3 h at same temperature. The reaction mixture was then poured into saturated aqueous NaCl (300 ml) and the separated organic layer was washed with saturated aqueous NaCl (100 ml × 3), dried, and evaporated. The residue was subjected to column chromatography (Silicic Acid; 100 mesh; Mallinckrodt) with AcOEt–$CHCl_3$ (2:8) as eluant to give diacyl-*S*-ylide (**8c**) (24.6 g, 99%) as a yellow oil, $\nu_{max.}$(neat) 1 570 cm^{-1}; δ($CDCl_3$) 1.55 [3 H, s, OC(Me)O], 1.83 (3 H, d, *J* 5.0 Hz, =CMe), 2.71 (3 H, s, SMe), 2.78 (3 H, s, SMe), 3.59 (2 H, ABq, *J* 14.0 Hz, CH_2), 3.69 (3 H, s, OMe), 3.82 (3 H, s, OMe), 3.92—4.12 (4 H, m, OCH_2CH_2O), 5.03 (2 H, ABq, *J* 12.0 Hz, CH_2O), 6.35—6.46 (2 H, m, CH=CH), 6.44 (1 H, s, ArH), and 7.27—7.39 (5 H, m, Ph); δ_C($CDCl_3$) 19.6 (=C*Me*), 24.5 [OC(*Me*)O], 26.8 (SMe), 26.9 (SMe), 48.8 (CH_2CO), 55.9 (OMe), 60.1 (OMe), 64.5 and 64.6 (OCH_2CH_2O), 72.0 (OCHPh), 91.5 (CS), 98.3 (arom. C-5′), 109.0 (OCO), 123.7 (=CHAr), 132.2 (=CMe), 185.3 (CO), and 191.7 (CO).

1-[6′-Benzyloxy-3′,4′-dimethoxy-2′-(prop-1-enyl)phenyl]-4-(2-methyl-1,3-dioxolan-2-yl)butane-1,3-dione (**8b**).—A mixture of diacyl-*S*-ylide (**8c**) (2.363 g, 4.6 mmol), Zn (3 g, 45.9 mg-atom), and AcOH (3 ml) in dioxane (30 ml) was stirred for 36 h at 50 °C. The reaction mixture was treated with saturated aqueous NaCl (30 ml) and extracted with Et_2O (100 ml × 3). The extract was washed with saturated aqueous NaCl (30 ml × 3), dried, and evaporated. The residue was subjected to column chromatography (Silicic Acid; 100 mesh; Mallinckrodt) with $CHCl_3$ as eluant to give the title dione (**8b**) (2.052 g, 98%) as an oil, *m/z* 454 (M^+); $\nu_{max.}$(neat) 1 585 cm^{-1}; δ($CDCl_3$) 1.40 (3 H, s, Me), 1.82 (3 H, dd, *J* 6.6 and 1.5 Hz, =CMe), 2.63 (2 H, s, CH_2CO), 3.69 (3 H, s, OMe), 3.81 (3 H, s, OMe), 3.88 (4 H, s, OCH_2CH_2O), 5.04 (2 H, s, CH_2O), 5.77 (1 H, s, C(OH)=CHCO–), 6.21 (1 H, dq, *J* 16.0 and 6.6 Hz, =C*H*Me), 6.39 (1 H, dq, *J* 16.0 and 1.5 Hz, =C*H*Ar), 6.44 (1 H, s, ArH), 7.27—7.35 (5 H, m, ArH), and 15.57 (1 H, s, enolic OH); δ_C($CDCl_3$) 19.5 (=C*Me*), 24.6 [OC(*Me*)O], 47.6 [$\overline{OC(CH_2)O_2}$], 55.9 (OMe), 60.2 (OMe), 64.7 (OCH_2CH_2O), 71.6 (CH_2O), 97.9 (arom. C-5′), 106.0 (OH)C=CHCO), 123.7 (=CHAr), 132.8 (=CMe), 141.1, 152.2, 154.0 (arom. C-3′, -4, -6′), 187.4 (CO), and 189.3 (CO).

1-[6′-Benzyloxy-3′,4′-dimethoxy-2′-(prop-1-enyl)phenyl]-4-(2-methyl-1,3-dioxolan-2-yl)-2-(methylthiomethyl)butane-1,3-dione (**8d**).—A suspension of compound (**8b**) (772 mg, 1.7 mmol)

* The name Oil Violet is a commercial name (Tokyo Kasei) for *N*-ethyl-1-{[4-(phenylazo)phenyl]azo}naphthalen-2-amine (Sudan Red 7B).

† In a preliminary communication (ref. 10) we reported the acid conditions as 5% HCl–acetone (1:2); 55 °C; 24 h. This should be amended to the conditions reported in this text. The interconversion of compounds (**1a**) and (**12d**) with 1M-H_2SO_4 has been reported (ref. 3*b*), but our attempts to use this method led to unsatisfactory results.

and *N*-(methylthiomethyl)piperidine hydrochloride (417 mg, 2.3 mmol) in anhydrous dioxane (50 ml) was stirred for 24 h at 70 °C under argon. After the mixture had been cooled, the precipitate was filtered off. The filtrate was concentrated to one quarter of its original volume and dissolved in benzene (100 ml); the solution as washed with saturated aqueous NaCl (10 ml × 3), dried, and evaporated. The residue was subjected to column chromatography (Silicic Acid; 100 mesh; Mallinckrodt) with $CHCl_3$ as eluant to give the title compound (**8d**) (758 mg, 87%) as an oil, *m/z* 514 (M^+); δ($CDCl_3$) 1.47 [3 H, s, OC(Me)O], 1.83 (3 H, SMe), 1.78—1.94 (3 H, m, =CMe), 3.04 (2 H, s, CH_2S or CH_2CO), 3.25 (2 H, s, CH_2CO or CH_2S), 3.70 (3 H, s, OMe), 3.83 (3 H, s, OMe), 3.88 (4 H, s, OCH_2CH_2O), 5.04 (2 H, s, CH_2O), 6.33—6.47 (2 H, m, CH=CH), 6.47 (1 H, s, 5′-H), 7.33 (5 H, s, Ph), and 16.86 (1 H, s, enolic OH); δ_C($CDCl_3$) 15.7 (SMe), 19.6 (=C*Me*), 25.1 [OC(*Me*)O], 32.0 (CH_2S), 45.5 (CH_2CO), 55.9 (OMe), 60.2 (OMe), 64.8 (OCH_2CH_2O), 71.3 (CH_2O), 97.5 (C-5′), 109.0 (=CCH_2S), 110.9 (OCO), 123.6 (=C*A*r), 127.1, 127.8, 128.5 (arom. C), 130.7 (=CMe), 140.9, 151.9, 154.0 (C-3′, -4′, -6′), 187.6 (CO), and 193.1 (CO); and 2-[6-*benzoyloxy*-3,4-*dimethoxy*-2-(*prop*-1-*enyl*)*phenyl*]-6-*methyl*-3-*methylthiomethyl*-4H-*pyran*-4-*one* (**10**) (55 mg, 7%) as a second fraction, *m/z* (M^+) 452.1649 ($C_{26}H_{28}O_5S$ requires *M*, 452.1656); $\nu_{max.}$(neat) 1 664, 1 627, and 1 605 cm^{-1}; δ($CDCl_3$) 1.77 (3 H, d, *J* 6.0 Hz, =CMe), 1.96 (3 H, s, SMe), 2.22 [3 H, s, =C(–O)Me], 3.30 (2 H, ABq, *J* 13.2 Hz, CH_2S), 3.72 (3 H, s, OMe), 3.87 (3 H, s, OMe), 5.06 (2 H, s, CH_2O), 6.12 (1 H, dq, *J* 15.5 and 6.0 Hz, =C*H*Me), 6.20 (1 H, s, 5-H), 6.23 (1 H, d, *J* 15.5 Hz, =C*H*Ar), 6.50 (1 H, s, ArH), and 7.29—7.33 (5 H, m, Ph).

1-[6′-*Benzyloxy*-3′,4′-*dimethoxy*-2-(*prop*-1-*enyl*)*phenyl*]-4-(2-*methyl*-1,3-*dioxolan*-2-*yl*)-2-*methylenebutane*-1,3-*dione* (**8f**).—To a solution of compound (**8d**) (13.1 g, 25.5 mmol) in methanol (1 l) at 0 °C was added a solution of $NaIO_4$ (50 g, 234 mmol) in water (600 ml) under argon. The resulting emulsion was stirred at ambient temperature for 24 h. The precipitate was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in benzene (300 ml) and the solution was washed with saturated aqueous NaCl (30 ml × 3), dried, and evaporated to give 1-[6′-benzyloxy-3′,4′-dimethoxy-2-(prop-1-enyl)phenyl]-4-(2-methyl-1,3-dioxolan-2-yl)-2-(methylsulphinylmethyl)butane-1,3-dione (**8e**) (12.86 g, 96%), δ($CDCl_3$) 1.47 [3 H, s, OC(Me)O], 1.78—1.90 (3 H, m, =CMe), 2.96 (2 H, s, CH_2CO), 3.03 (3 H, s, SOMe), 3.18 (2 H, s, CH_2SO), 3.68 (3 H, s, OMe), 3.80 (3 H, s, OMe), 3.86 (4 H, s, OCH_2CH_2O), 5.01 (2 H, s, CH_2O), 5.80—6.60 (2 H, m, CH=CH), 6.44 (1 H, s, ArH), and 7.32 (5 H, s, Ph).

This compound was used for the next step without further purification. A solution of the sulphinyl compound (**8e**) (154 mg, 0.27 mmol) and one drop of AcOH in toluene (0.5 ml) was stirred for 4 h at 100 °C under argon. The reaction mixture was poured into ice–water (3 ml) and extracted with Et_2O (10 ml × 3). The extract was washed with water (5 ml × 3), dried, and evaporated to give the title compound (**8f**) (133 mg, 95%) as an unstable yellow oil, *m/z* 466 (M^+); $\nu_{max.}$(neat) 1 665 cm^{-1}; δ($CDCl_3$) 1.43 [3 H, s, OC(Me)O], 1.80 (3 H, dd, *J* 6.6 and 1.6 Hz, =CMe), 3.08 (2 H, s, CH_2CO), 3.70 (3 H, s, OMe), 3.83 (3 H, s, OMe), 3.85—3.89 (4 H, m, OCH_2CH_2O), 5.01 (2 H, s, CH_2O), 5.91 (1 H, s, =CH), 6.08 (1 H, dq, *J* 15.9 and 6.6 Hz, =C*H*Me), 6.31 (1 H, dq, *J* 15.9 and 1.6 Hz, =C*H*Ar), 6.40 (1 H, s, =CH), 6.44 (1 H, s, ArH), and 7.36 (5 H, s, Ph); δ_C ($CDCl_3$) 19.3 (=C*Me*), 24.5 [OC(*Me*)O], 49.6 (CH_2CO), 55.7 (OMe), 60.1 (OMe), 64.3 (OCH_2CH_2O), 71.1 (CH_2O), 97.0 (C-5′), 107.6 (OCO), 123.1 (=CHAr), 127.0, 127.8, 128.1 (arom. C), 132.7 (=CH), 132.9 (=CHMe), 140.5, 152.0, 153.6 (arom. C-3′, -4′, -6′), 194.8 (CO), and 196.9 (CO).

3-[6-*Benzyloxy*-3,4-*dimethoxy*-2-(*prop*-1-*enyl*)*benzoyl*]-2,3-*dihydro*-6-*methyl*-4H-*pyran*-4-*one* (**11a**).—*Method A.* A solution of compound (**8f**) (105 mg, 0.23 mmol) in 5% aqueous HCl–THF (1:2) (75 ml) was stirred for 24 h at ambient temperature. The reaction mixture was poured into saturated aqueous NaCl (50 ml), and extracted with Et_2O (50 ml × 2). The extract was washed with saturated aqueous NaCl (25 ml × 3), dried, and evaporated to yield the title compound (**11a**) (86 mg, 91%), *m/z* (M^+) 422; $\nu_{max.}$(neat) 1 610 cm^{-1}; δ($CDCl_3$) 1.84 (3 H, br s, =CMe), 1.96 (3 H, s, 6-Me), 3.70 (3 H, s, OMe), 3.82 (3 H, s, OMe), 4.57 (2 H, s, 2-H_2), 5.07 (2 H, ABq, *J* 12.5 Hz, OCH_2Ph), 5.42 (1 H, s, =CH), 6.35—6.44 (2 H, m, CH=CH), 6.44 (1 H, s, ArH), and 7.35—7.36 (5 H, m, Ph); δ_C($CDCl_3$) 19.7 (=C*Me*), 21.0 (6-Me), 55.9 (OMe), 60.2 (OMe), 68.4 (C-2), 71.6 (OCH_2Ph), 97.9 (arom. C-5), 102.1 (C-3), 103.9 (C-5), 123.2 (=*C*HAr), 127.0, 127.9, 128.5 (arom. C), 133.0 (=*C*HMe), 142.0, 152.4, 154.3 (arom. C-3, -4, -6), and 169.7, 173.3, 186.5 (COAr, C-4, -6).

Method B. A mixture of the sulphinyl compound (**8e**) (10.95 g, 20.7 mmol), 5% aqueous H_2SO_4 (75 ml), and AcOH (75 ml) in THF (300 ml) was stirred for 2 h at 70 °C. Similar work-up as above gave compound (**11a**) (7.127 g, (79%). This compound was too unstable to be purified by column chromatography and distillation.

BF_2 *Complex of* 3-[6-*Benzoyloxy*-3,4-*dimethoxy*-2-(*prop*-1-*enyl*)*benzoyl*]-2,3-*dihydro*-6-*methyl*-4H-*pyran*-4-*one*, (**11b**).—To a solution of compound (**11a**) (9.73 g, 23.1 mmol) in anhydrous Et_2O (800 ml) at 0 °C was added BF_3·OEt_2 (28 ml) under argon. The mixture was stirred for 1 h at ambient temperature and then poured into ice–water (300 ml) and extracted with Et_2O–AcOEt (5:1)(600 ml × 2). The organic layer was washed with water (300 ml), dried, and evaporated to give the BF_2 complex of the pyrone compound (**11b**) (10.0 g, 97%), $\nu_{max.}$(neat) 1 590 cm^{-1}; δ($CDCl_3$) 1.83 (3 H, d, *J* 6.0 Hz, =CMe), 2.03 (3 H, s, 6-Me), 3.69 (3 H, s, OMe), 3.85 (3 H, s, OMe), 4.74 (2 H, ABq, *J* 13.0 Hz, 2-H_2), 5.04 (2 H, ABq, *J* 12.0 Hz, CH_2Ph), 5.57 (1 H, s, 5-H), 6.14 (1 H, dq, *J* 17.0 and 6.0 Hz, =C*H*Me), 6.38 (1 H, d, *J* 17.0 Hz, =C*H*Ar), 6.48 (1 H, s, ArH), and 7.35 (5 H, s, Ph); δ_C($CDCl_3$) 19.5 (=CH*Me*), 21.7 (6-Me), 55.9 (OMe), 60.4 (OMe), 67.8 (C-2), 71.8 (OCH_2Ph), 97.7 (arom. C-5), 100.0 (5-C), 122.6 (=*C*HAr), 127.3, 128.0, 128.6 (arom. C), 134.2 (=*C*HMe), 141.1, 152.8, 155.2 (arom. C-3, -4, -6), and 174.6, 178.6, 181.2 (COAr, C-4, -6).

BF_2 *Complex of* 3-[6-*Hydroxy*-3,4-*dimethoxy*-2-(*prop*-1-*enyl*)*benzoyl*]-2,3-*dihydro*-6-*methyl*-4H-*pyran*-4-*one*, (**11c**).—A solution of the BF_2 complex (**11b**) (10.00 g, 21.3 mmol) in anhydrous CH_2Cl_2 (50 ml) was added dropwise to a solution of Me_2S (45 ml) and BF_3·OEt_2 (25 ml) in anhydrous CH_2Cl_2 (150 ml) at 0 °C. The reaction mixture was stirred for 24 h at ambient temperature, and then poured into saturated aqueous NaCl (100 ml) and extracted with Et_2O (200 ml × 2). The extract was washed with saturated aqueous NaCl (100 ml × 3), dried, and evaporated to afford the BF_2 complex of the phenol, complex (**11c**) (7.52 g, 93%), δ($CDCl_3$) 1.80 (3 H, dd, *J* 7.0 and 1.5 Hz, =CH*Me*), 2.13 (3 H, s, 6-Me), 3.70 (3 H, s, OMe), 3.83 (3 H, s, OMe), 4.77 (2 H, s, CH_2O), 5.66 (1 H, s, 5-H), 5.98 (1 H, dq, *J* 17.0 and 7.0 Hz, =C*H*Me), 6.42 (1 H, dq, *J* 17.0 and 1.5 Hz, =CHAr), and 7.18 (1 H, s, ArH).

7,8-*Dimethoxy*-3-*methyl*-9-(*prop*-1-*enyl*)*pyrano*[4,3-b][1]-*benzopyran*-10(1H)-*one* (**12a**).—A mixture of the BF_2 complex (**11c**) (1.236 g, 3.25 mmol), AcOH (30 ml), and conc. HCl (1 ml) was stirred at ambient temperature for 24 h. The reaction mixture was poured into ice–water (100 ml), and extracted with benzene (100 ml × 2). The extract was washed with water (50 ml × 3), dried, and evaporated. The residue was subjected to column chromatography (Silicic Acid; 100 mesh; Mallinckrodt) with $CHCl_3$ as eluant to give the *title compound* (**12a**), which

was recrystallized from benzene–n-hexane (450 mg, 44%), m.p. 150—151 °C; *m/z* 314.1122 (M^+) ($C_{18}H_{18}O_5$ requires *M*, 314.1153); $\nu_{max.}$(KBr) 1 660 and 1 620 cm^{-1}; $\delta(CDCl_3)$ 1.95 (3 H, dd, *J* 6.0 and 1.5 Hz, =CH*Me*), 2.00 (3 H, s, 3-Me), 3.69 (3 H, s, OMe), 3.93 (3 H, s, OMe), 5.26 (2 H, s, 1-H_2), 5.34 (1 H, s, 4-H), 6.18 (1 H, dq, *J* 15.0 and 6.0 Hz, =C*H*Me), 6.72 (1 H, s, 6-H), and 7.24 (1 H, dq, *J* 15.0 and 1.5 Hz, =C*H*Ar); $\delta_C(CDCl_3)$ 19.5 (=CH*Me*), 20.3 (3-Me), 56.0 (OMe), 60.1 (OMe), 65.1 (C-1), 93.9 (C-4), 98.8 (C-6), 103.3, 116.0, 132.7 (C-9, -9a, -10a), 124.7 (=*C*HAr), 131.1 (=*C*HMe), and 144.5, 154.0, 156.8, 157.6, 166.3, 175.0 (C-5a, -7, -8, -4a, -3, -10).

3,4-*Dihydro*-3-*hydroxy*-7,8-*dimethoxy*-3-*methyl*-9-(*prop*-1-*enyl*)*pyrano*[4,3-b][1]*benzopyran*-10(1H)-*one* (**1c**).—A mixture of compound (**12a**) (1.45 g, 4.62 mmol), 5% aqueous HCl (60 ml), and acetone (120 ml) was stirred for 4 days at ambient temperature. The reaction mixture was poured into ice–water (120 ml), and extracted with AcOEt (200 ml × 2). The extract was washed with saturated aqueous NaCl (100 ml × 2), dried, and evaporated. The resulting residue was subjected to column chromatography (Silicic Acid; 100 mesh; Mallinckrodt) with $CHCl_3$ as eluant. Unchanged starting material (**12a**) (307 mg) was eluted as the first fraction. Further elution with $CHCl_3$ gave the *title compound* (**1c**) [1.135 g, 74% (94% from consumed (**12a**)], m.p. 152—155 °C (from benzene–n-hexane); *m/z* 332.1293 (M^+) ($C_{18}H_{20}O_6$ requires *M*, 332.1259); $\nu_{max.}$(KBr) 3 350, 1 670, and 1 620 cm^{-1}; $\delta(CDCl_3)$ 1.62 (3 H, s, Me), 1.98 (3 H, dd, *J* 7.0 and 2.0 Hz, =CH*Me*), 2.76 (2 H, ABq, *J* 17.0 Hz, 4-H_2), 3.03 (1 H, s, 3-OH), 3.67 (3 H, s, OMe), 3.92 (3 H, s, OMe), 4.69 (2 H, ABq, *J* 13.0 Hz, 1-H_2), 6.23 (1 H, dq, *J* 16.0 and 7.0 Hz, =C*H*Me), 6.64 (1 H, s, 6-H), and 7.17 (1 H, dq, *J* 16.0 and 2.0 Hz, =C*H*Ar); $\delta_C(CDCl_3)$ 19.5 (=CH*Me*), 29.2 (3-Me), 36.9 (C-4), 56.0 (OMe), 57.6 (C-1), 60.1 (OMe), 95.1 (C-3), 98.5 (C-6), 124.6 (=*C*HAr), 131.4 (=*C*HMe), and 144.4, 155.0, 156.4, 157.2, 174.0 (C-5a, -7, -8, -4a, -10).

4,10-*Dihydro*-3-*hydroxy*-7,8-*dimethoxy*-3-*methyl*-10-*oxo*-1H,3H-*pyrano*[4,3-b][1]*benzopyran*-9-*carbaldehyde* (**1d**).—A solution of compound (**1c**) (273 mg, 0.82 mmol) in ethanol (100 ml) containing Oil Violet (1 mg) was ozonolysed at −75 °C. When the red colour of the reaction mixture had been discharged, the introduction of ozone was stopped and nitrogen was flushed into the mixture. Dimethyl sulphide (10 ml) was added to the reaction mixture and the mixture was stirred for a further 1 h. The solvent was distilled off and the resulting residue was dissolved in AcOEt (80 ml). The organic layer was washed with water (20 ml × 3), dried, and evaporated. The residue was subjected to column chromatography (Silicic Acid; 100 mesh; Mallinckrodt) with $CHCl_3$ as eluant to give crystalline material, which was recrystallized from Et_2O–acetone to give the title compound (**1d**) (180 mg, 68%) as needles, m.p. 172—174 °C; *m/z* (M^+) 320; $\nu_{max.}$(KBr) 3 460, 2 860, 1 700, and 1 650 cm^{-1}; $\delta(CDCl_3)$ 1.62 (3 H, s, 3-Me), 2.82 (2 H, ABq, *J* 17.5 Hz, 4-H_2), 3.84 (3 H, s, OMe), 3.96 (3 H, s, OMe), 4.67 (2 H, s, 1-H_2), 6.89 (1 H, s, ArH), and 10.56 (1 H, s, CHO). This compound was slightly unstable, presumably owing to the presence of the formyl group, and could not be obtained in an analytically pure state.

4,10-*Dihydro*-3-*hydroxy*-7,8-*dimethoxy*-3-*methyl*-10-*oxo*-1H,3H-*pyrano*[4,3-b][1]*benzopyran*-9-*carboxylic Acid* (**1e**).—To a solution of the aldehyde (**1d**) (507 mg, 1.58 mmol) in THF (80 ml) was added a solution of sulphamic acid (330 mg) and sodium chlorite (330 mg) in water (24 ml), and the reaction mixture was stirred for 3 h at ambient temperature. The reaction mixture was poured into water (50 ml) and extracted with AcOEt (40 ml × 2). The extract was washed with saturated aqueous NaCl (20 ml × 2), dried, and evaporated to give the title compound (**1e**) (450 mg, 85%), *m/z* (M^+) 336; $\nu_{max.}$(KBr) 3 430—2 500, 1 715, and 1 650 cm^{-1}; $\delta(CDCl_3)$ 1.57 (3 H, s, 3-Me), 2.83 (2 H, ABq, *J* 17.0 Hz, 4-H_2), 3.83 (3 H, s, OMe), 4.05 (3 H, s, OMe), 4.57 (2 H, s, 1-H_2), and 7.20 (1 H, s, ArH). This compound was partly converted into the anhydro compound (**12b**) by recrystallization or column chromatography.

7,8-*Dimethoxy*-3-*methyl*-10-*oxo*-1H,10H-*pyrano*[4,3-b][1]-*benzopyran*-9-*carboxylic Acid* (**12b**).—A mixture of the aforementioned acid (**1e**) (450 mg, 1.34 mmol) and conc. H_2SO_4 (0.5 ml) in acetone (100 ml) was stirred for 30 min at ambient temperature. The reaction mixture was poured into water (200 ml), and extracted with AcOEt (150 ml × 2). The extract was washed with saturated aqueous NaCl (50 ml × 3), dried, and evaporated to give crystalline material. Recrystallization of this crystalline residue from EtOH gave the title carboxylic acid (**12b**) (372 mg, 87%), m.p. 145—148 °C; *m/z* 318.0722 (M^+) ($C_{16}H_{14}O_7$ requires *M*, 318.0738); $\nu_{max.}$(KBr) 3 600—2 500, 1 730, and 1 650 cm^{-1}; $\delta([^2H_6]$acetone) 2.01 (3 H, s, 3-Me), 3.88 (3 H, s, OMe), 3.96 (3 H, s, OMe), 5.23 (2 H, s, 1-H_2), 5.43 (1 H, s, 4-H), and 6.90 (1 H, s, 6-H); $\delta_C(CDCl_3)$ 20.3 (3-Me), 56.5 (OMe), 62.1 (OMe), 64.6 (C-1), 94.4 (C-4), 101.1 (C-6), 102.1, 114.4, 127.1 (C-9, -9a, -10a), and 144.3, 153.1, 157.2, 160.0, 168.2, 169.0, 172.3 (C-5a, -7, -8, -3, -4a, -10, CO_2H).

Demethylation of Compound (**12b**) *with* BBr_3.—Boron tribromide (1 ml) was added to a solution of the carboxylic acid (**12b**) (87 mg, 0.27 mmol) in anhydrous CH_2Cl_2 (50 ml) at −75 °C. The reaction mixture was stirred for 1 h at the same temperature, and then poured into saturated aqueous NaCl (30 ml) and extracted with AcOEt (30 ml × 2). The extract was washed with saturated aqueous NaCl (10 ml × 3), dried, and evaporated to give 8-hydroxy-7-methoxy-3-methyl-10-oxo-1*H*,10*H*-pyrano[4,3-*b*][1]benzopyran-9-carboxylic acid (**12c**) (68 mg, 82%), $\delta(CDCl_3)$ 2.11 (3 H, s, 3-Me), 4.05 (3 H, s, OMe), 5.40 (2 H, s, 1-H_2), 5.59 (1 H, s, 4-H), 7.09 (1 H, s, 6-H), 16.07 (1 H, s, OH), and 20.03 (1 H, s, CO_2H).

Anhydrofulvic Acid (**12d**).—To a solution of the carboxylic acid (**12b**) (57 mg, 0.18 mmol) in anhydrous CH_2Cl_2 (30 ml) at −10 °C were added Me_2S (15 ml) and aluminium chloride (695 mg). The reaction mixture was stirred for 24 h at ambient temperature. Water was added to the mixture and the separated aqueous layer was washed with Et_2O (30 ml × 2). The aqueous layer was treated with 5% aqueous HCl (30 ml) and extracted with THF–AcOEt (1:1) (100 ml × 2). The extract was washed with saturated aqueous NaCl (20 ml × 2), dried, and evaporated. The resulting yellow crystalline material was recrystallized from dioxane to yield *anhydrofulvic acid* (**12d**) (26 mg, 52%) as yellow needles, m.p. 236—239.5 °C; (Found: C, 57.8, H, 3.4. $C_{14}H_{10}O_7$ requires C, 57.93, H, 3.48%); $\nu_{max.}$(KBr) 3 550—2 500 and 1 635 cm^{-1}; δ [$CDCl_3$–$(CD_3)_2SO$, 1:1] 2.09 (3 H, s, Me), 5.36 (2 H, s, 1-H_2), 5.67 (1 H, s, 4-H), 7.90 (1 H, s, 6-H), 10.90 (1 H, s, OH), 15.78 (1 H, s, OH), and 19.80 (1 H, s, CO_2H); δ_C[$^2H_5$]pyridine) 20.3 (3-Me), 65.2 (C-1), 94.0 (C-4), 101.9 (C-10a), 106.9 (C-6), 111.6, 112.9 (C-9, 9a), 152.3, 154.4, 156.4 (C-5a, -7, -8), 160.5 (C-4a), 170.1 (C-3), and 172.7, 174.1 (CO_2H, C-10).

Fulvic Acid (**1a**).—A mixture of anhydrofulvic acid (**12d**) (35 mg, 0.12 mmol), 5% aqueous H_2SO_4 (20 ml), and acetone (40 ml) was refluxed for 48 h. The reaction mixture was concentrated to half its original volume, poured into water (25 ml), and extracted with THF–AcOEt (1:1) (50 ml × 2). The organic layer was washed with saturated aqueous NaCl (10 ml × 3), dried, and evaporated. The resulting residue was recrystallized from dioxane–benzene to give fulvic acid (**1a**) (25 mg, 68%) as yellow needles, m.p. 242—244 °C, mixed m.p. 242—243 °C (lit.,[2] 246 °C) (Found: C, 54.6, H, 3.9. Calc. for

$C_{14}H_{12}O_8$: C, 54.76, H, 3.84%); $\nu_{max.}$(KBr) 3 700—2 200 and 1 635 cm^{-1}; $\lambda_{max.}$(EtOH) 224 log ε (4.50), 317 (4.07), and 343 nm (4.06); δ[$(CD_3)_2SO$] 1.45 (3 H, s, Me), 2.72 (2 H, ABq, *J* 17.6 Hz, 4-H_2), 4.43 (2 H, s, 1-H_2), 6.22 (1 H, s, 3-OH), 6.89 (1 H, s, 6-H), 9.74 (1 H, br s, OH), 11.22 (1 H, br s, OH), and 13.27 (1 H, br s, CO_2H); δ([$^2H_5$]pyridine) 1.77 (3 H, s, Me), 2.99 (2 H, ABq, *J* 19.0 Hz, 4-H_2), 5.08 (2 H, ABq, *J* 15.0 Hz, 1-H_2), and 7.26 (1 H, s, 6-H); δ_C([$^2H_5$]pyridine) 29.2 (Me), 38.3 (4-C), 57.5 (C-1), 95.2 (C-3), 106.1 (C-6), 112.6, 113.1 (C-9, -9a), 115.2 (C-10a), 152.7, 154.0, 156.6 (C-5a, -7, -8), 160.9 (C-4a), 173.5 (CO_2H), and 175.5 (C-10).

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References

1 Part 4, M. Yamauchi, S. Katayama, Y. Nakashita, and T. Watanabe, *J. Chem. Soc., Perkin Trans. 1*, 1985, 183.
2 A. E. Oxford, H. Raistrick, and P. Simonart, *Biochem. J.*, 1935, **29**, 1102.
3 (*a*) F. M. Dean, R. A. Eade, R. Moubasher, and A. Robertson, *Nature (London)*, 1957, **179**, 366; (*b*) *J. Chem. Soc.*, 1957, 3497; (*c*) T. Sakaki, A. Ichihara, and S. Sakamura, *Agric. Biol. Chem.*, 1981, **45**, 1275.
4 V. A. Biber and K. M. Magaziner, *Dokl. Akad. Nauk SSSR*, 1951, **76**, 609 (*Chem. Abstr.*, 1951, **45**, 7198).
5 M. Schnitzer and P. A. Poapst, *Nature (London)*, 1967, **213**, 598.
6 F. M. Dean, S. Murray, and D. A. Smith, *J. Chem. Res.*, 1977, (*S*), 230; (*M*), 2656; F. M. Dean, M. Al-Sattar, and D. A. Smith, *J. Chem. Soc., Chem. Commun.*, 1983, 535.
7 R. Thomas, *Biochem. J.*, 1961, **78**, 748; T. Money, *Nature (London)*, 1963, **199**, 592.
8 I. Kurobane, C. R. Hutchinson, and L. C. Vining, *Tetrahedron Lett.*, 1981, **22**, 493.
9 (*a*) T. Watanabe, S. Katayama, Y. Nakashita, and M. Yamauchi, *J. Chem. Soc., Chem. Commun.*, 1981, 761; (*b*) M. Yamauchi, S. Katayama, Y. Nakashita, and T. Watanabe, *J. Chem. Soc., Perkin Trans. 1*, 1984, 503; (*c*) M. Yamauchi, S. Katayama, Y. Nakashita, and T. Watanabe, *J. Chem. Soc., Chem. Commun.*, 1983, 335.
10 Preliminary communication, M. Yamauchi, S. Katayama, T. Todoroki, and T. Watanabe, *J. Chem. Soc., Chem. Commun.*, 1984, 1565.
11 F. M. Dean, D. R. Randell, and G. Winfield, *J. Chem. Soc.*, 1959, 1071.
12 Z. Horii, H. Hakusui, T. Shigeuchi, M. Hanaoka, and T. Momose, *Yakugaku Zasshi*, 1972, **92**, 503.
13 H. O. House, D. S. Crumrine, A. Y. Teranishi, and H. D. Olmstead, *J. Am. Chem. Soc.*, 1973, **95**, 3310.
14 G. Snatzke, *Chem. Ber.*, 1961, **94**, 729.
15 E. J. Corey and C. V. Kim, *J. Am. Chem. Soc.*, 1972, **94**, 7586.
16 H. Nozaki, D. Tunemoto, Z. Morita, K. Nakamura, K. Watanabe, M. Takaku, and K. Kondo, *Tetrahedron*, 1967, **23**, 4279; H. Matsuyama, H. Minato, and M. Kobayashi, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 3393.
17 M. Yamauchi, S. Katayama, and T. Watanabe, *Synthesis*, 1982, 935.
18 P. Yates and D. J. MacGregor, *Can. J. Chem.*, 1973, **51**, 1267.
19 K. Fuji, T. Kawabata, and E. Fujita, *Chem. Pharm. Bull.*, 1980, **28**, 3662.
20 J. Ficini, P. Kahn, S. Falou, and A. M. Touzin, *Tetrahedron Lett.*, 1979, 67.
21 T. Veysoglu, L. A. Mitscher, and J. K. Swayze, *Synthesis*, 1980, 807.
22 B. O. Lindgren and T. Nilsson, *Acta Chem. Scand.*, 1973, **27**, 888.
23 A. Schoenberg and N. Badran, *J. Am. Chem. Soc.*, 1951, **73**, 2960; T. Watanabe, S. Katayama, Y. Nakashita, and M. Yamauchi, *J. Chem. Soc., Perkin Trans. 1*, 1978, 726.
24 M. Node, K. Nishide, M. Sai, K. Ichikawa, K. Fuji, and E. Fujita, *Chem. Lett.*, 1979, 97; M. Node, K. Nishide, M. Sai, K. Fuji, and E. Fujita, *J. Org. Chem.*, 1981, **46**, 1991.

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