

### Fungal Metabolites. Part III.† Isolation of Secalonic Acids from *Phoma terrestris*

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Extraction of the dried mycelium of *Phoma terrestris* Hansen gave two yellow pigments to which are assigned the substituted 9,9'-dioxo-2,2'-bixanthen-10a,10a'-dicarboxylate structures (1a) and (2a). Compound (1a) has not been reported previously and we designate this secalonic acid E, although it appears to be enantiomeric with secalonic acid B. Compound (2a) has a structure already assigned to secalonic acid A but differs from the latter in some analytical details; reasons for these differences are suggested.

COMPOUNDS of the general structural types (1) and (2) have been termed secalonic acids<sup>1</sup> and four of these (A, B, C, and D) have been isolated and identified. All have been isolated as fungal metabolites of *Claviceps purpurea* (A, B, and C)<sup>2-4</sup> or *Penicillium oxalicum* (D)<sup>5</sup> and the compounds differ in stereochemical detail at positions 5,6,10a,5',6',10a'. Because of these possible stereochemical variations, a large number of isomers is possible within the framework of structures (1) and (2). In work reported here on the fungus, *Phoma terrestris* Hansen, we have isolated a new secalonic acid (E) (1a) and also secalonic acid A (2a).

Extraction of the dried mycelium from the fungus grown in shake culture afforded a yellow solid consisting mainly of two components (t.l.c. on silica impregnated with 6% oxalic acid<sup>2</sup>). The two components were readily separated by fractional crystallisation to give two yellow crystalline compounds (1a) and (2a). These structures were assigned on the basis of the analytical data and of chemical conversions described later. The results of many of the reactions of one of these secalonic acids were similar to those of the other, and the reactions are only discussed in detail for the new secalonic acid (1a). Analytical and spectral data are set out in the Table.

Compound (1a) was not identical with either secalonic

acid C or D.<sup>6</sup> Mass spectrometry showed that the pigment had the molecular composition C<sub>32</sub>H<sub>30</sub>O<sub>14</sub>, and the results of elemental analysis agreed with the formula C<sub>32</sub>H<sub>30</sub>O<sub>14</sub>.2H<sub>2</sub>O. The water was very difficult to remove but no evidence was obtained that it was chemically bound as labile hydroxy-functions (see n.m.r. data). The mass spectrum suggested that at least two aliphatic hydroxy-groups were present (two successive losses of H<sub>2</sub>O from the molecular ion) and a methoxycarbonyl or an acetoxy-group (loss of C<sub>2</sub>H<sub>3</sub>O<sub>2</sub> from molecular ion). The relatively few abundant fragment ions indicated the probability of an aromatic structure.

The i.r. spectrum of compound (1a) showed both free and hydrogen-bonded OH stretching frequencies, a carbonyl band characteristic of an aliphatic ester or similar grouping, and a broad absorption centred at 1600 cm<sup>-1</sup> which was interpreted as a β-dicarbonyl system [with iron(III) chloride, a red-brown colour was produced, typical of a β-diketone<sup>1</sup>].

From the simplicity of the n.m.r. spectrum and the fact that only 15 protons are accounted for, we deduced that the molecule possesses two identical halves; most of the following discussion refers therefore to half the molecule only. The spectrum of a solution in [2H<sub>6</sub>]dimethyl sulphoxide showed two very low field singlets at δ 14.85 and 12.7, assigned to phenolic and enolic

† Part II, R. A. W. Johnstone, *Phytochemistry*, in the press.

<sup>1</sup> J. W. ApSimon, J. A. Corran, N. G. Creasy, W. Marlow, W. B. Whalley, and K. Y. Sim, *J. Chem. Soc.*, 1965, 4144.

<sup>2</sup> B. Franck and E. M. Gottschalk, *Angew. Chem. Internat. Edn.*, 1964, 3, 441.

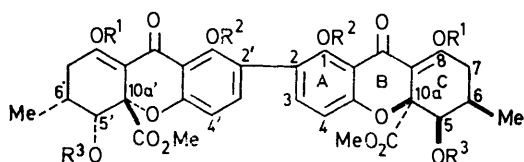
<sup>3</sup> B. Franck and G. Baumann, *Chem. Ber.*, 1966, 99, 3842.

<sup>4</sup> D. J. Aberhart, Y. S. Chan, P. de Mayo, and J. B. Stothers, *Tetrahedron*, 1965, 21, 1417.

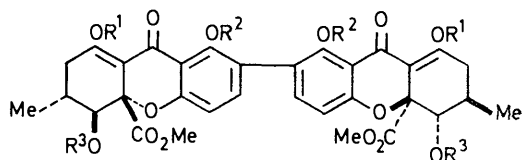
<sup>5</sup> P. S. Steyn, *Tetrahedron*, 1970, 26, 51.

<sup>6</sup> Small samples kindly supplied by Professor W. B. Whalley.

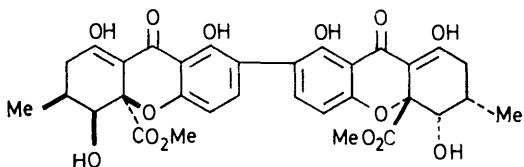
proton resonances, respectively. On treatment with diazomethane, compound (1a) gave a dimethyl ether



- (1) a;  $R^1 = R^2 = R^3 = H$   
 b;  $R^1 = Me, R^2 = R^3 = H$   
 c;  $R^1 = R^2 = Me, R^3 = H$   
 d;  $R^1 = R^3 = H, R^2 = Me$   
 e;  $R^1 = R^2 = R^3 = Ac$



- (2) a-e as in (1)



- (3)

(1b), which no longer showed the signal at  $\delta$  12.7. In keeping with the enolic nature of (1a), very mild treat-

which lacked both the signals at  $\delta$  14.85 and 12.7. With acetic anhydride, compound (1a) yielded a hexaacetate (1e); this indicated the presence of two further (aliphatic) hydroxy-groups in the molecule besides the two phenolic and two enolic systems. The n.m.r. spectrum of compound (1a) showed a doublet at  $\delta$  5.80 which disappeared on treatment with  $D_2O$  and was therefore ascribed to a secondary hydroxy-proton. On irradiation of compound (1a) at a frequency corresponding to  $\delta$  4.0, the doublet at  $\delta$  5.80 collapsed to a singlet, and *vice versa*. A pair of doublets centred at  $\delta$  7.44 and 6.56 was ascribed to *ortho*-oriented aromatic protons and a singlet at  $\delta$  3.65 to a methoxy-group. A doublet at  $\delta$  1.04 indicated a secondary methyl group and two peaks centred at  $\delta$  2.38 were ascribed to allylic methylene protons, with no protons on the double bond (absence of allylic coupling).

The foregoing information, coupled with the u.v. spectrum, suggested a secalonic acid structure for the pigment (1a), which could not be any one of those previously reported [B (opposite sign of optical rotation), C, or D (comparison by t.l.c.)]. Also the available data did not fit those reported for secalonic acid A.<sup>1,2,7</sup> It was necessary therefore to determine the stereochemistry at positions 5, 6, and 10a, assumed to be the same as at positions 5', 6', and 10a' because of the symmetry of the molecule (n.m.r. spectrum and later evidence).

Oxidation of the pigment (1a) with alkaline potassium permanganate gave only (+)-(R)-methylsuccinic acid, which provides evidence for the R-configuration at positions 6 and 6' and incidentally for the arrangement of carbon atoms at positions 5, 6, 7, and 8 (and 5', 6', 7', and 8').

#### Analytical and spectral data for compounds (1a) and (2a)

M.p.	206—208°	208—209°
Analysis	Found: C, 56.7; H, 4.9. $C_{32}H_{30}O_{14} \cdot 2H_2O$ requires C, 57.8; H, 5.05%	Found: C, 57.3; H, 5.1. $C_{32}H_{30}O_{14} \cdot 2AcOH$ requires C, 56.9; H, 5.0%
$M^+$	638.1620 ( $C_{32}H_{30}O_{14}$ )	638.1632 ( $C_{32}H_{30}O_{14}$ )
$[\alpha]_D^{22}$	-212° (in pyridine, $c$ 0.41)	-264° (in pyridine, $c$ 0.24)
$\lambda_{max.}/nm$ (log $\epsilon$ ) (in dioxan)	265 (4.37), 338 (4.64)	240 (4.19), 267 (4.18), 338 (4.53)
$\nu_{max.}/cm^{-1}$ (in KBr)	3600 (non-bonded OH), 3200 (H-bonded OH), 1740 (aliphatic C=O), 1600 (H-bonded C=O)	3500 (H-bonded OH), 1725 (aliphatic C=O), 1610 (H-bonded C=O)
$\delta^*$	1.04 (3H, d, $J$ 6 Hz, $CH \cdot CH_3$ ),  3.65 (3H, s, $OCH_3$ ), 3.96 (1H, d, $J$ 4 Hz, $CHOH$ ), 5.78 (1H, d, $J$ 4 Hz, $CHOH$ ), {6.56 (1H, d, $J$ 8 Hz), 7.44 (1H, d, $J$ 8 Hz),} 11.60 (1H, s, enolic OH), 13.85 (1H, s, phenolic OH)	1.16 (3H, d, $J$ 6 Hz, $CH \cdot CH_3$ ), 1.54br (disappeared with $D_2O$ ), 2.74br (d, $C=C-CH_2$ ), 3.68 (3H, s, $OCH_3$ ), 3.88br (1H, d, $J$ 10 Hz, $CHOH$ ),  <i>ortho</i> {6.60 (1H, d, $J$ 8 Hz), 7.74 (1H, d, $J$ 8 Hz),} 11.70 (1H, s, enolic OH), 13.78 (1H, s, phenolic OH)

\* The n.m.r. data are reported for half the molecule. Compound (1a) was run in  $(CD_3)_2SO$  and compound (2a) in  $CDCl_3$ . The values quoted here for the aromatic protons (100 MHz; Varian HA-100) are slightly different (by about 12 Hz) from those quoted in ref. 7 for secalonic acids A and B, which were obtained with a 60 MHz instrument. On running samples of secalonic acids C and D\* on our machine, we found identical differences; these differences are therefore simply instrumental.

ment of the dimethyl ether (1b) with acid gave back the original compound in quantitative yield. Treatment of the dimethyl ether (1b) with alkaline dimethyl sulphate produced a tetramethyl ether (1c), the spectrum of

The 5-proton is coupled to the adjacent hydroxylic proton but is not strongly coupled to the other adjacent

\* J. W. Hooper, W. Marlow, W. B. Whalley, A. D. Borthwick, and R. Bowden, *J. Chem. Soc. (C)*, 1971, 3580.

proton (H-6): irradiation at the frequency of the hydroxy-proton ( $\delta$  5.78) causes the signal for H-5 to collapse to a broad singlet. Also, in  $\text{CDCl}_3$ , the hydroxy-proton in the dimethyl ether (1b) appears as a very broad band centred at  $\delta$  2.30 and H-5 still appears as a broad singlet. From the width of this singlet we estimate  $J_{5,6}$  to be 0—2 Hz, and use of a modified<sup>8</sup> Karplus equation gives a torsion angle between them of *ca.* 70—90°. Accordingly, H-5 and H-6 must be *cis* to each other in ring A (1a), and position 5 is an *R*-centre. The resonance frequency for H-5 ( $\delta$  3.96) suggests that it is equatorial.

Assignment of stereochemistry at position 10a rests on several pieces of evidence. In the mass spectrum, the relative abundances of the ions at *m/e* 638 and 579 (*M* and *M* —  $\text{CO}_2\text{CH}_3$ ) are in a ratio close to 1 : 1 and, in view of earlier work on secalonic acids,<sup>3</sup> this suggests a *trans* relationship between the 5-hydroxy-group and the 10a-methoxycarbonyl group. In keeping with earlier studies on the c.d. of secalonic acids,<sup>3</sup> the large negative rotation of compound (1a) at the sodium D-line is consistent with this *trans* relationship [methoxycarbonyl group projecting below the general plane of ring B (1a)<sup>9</sup>]. In its i.r. spectrum, compound (1a) exhibits a sharp non-bonded OH stretching absorption. If the 5-hydroxy-group and the 10a-methoxycarbonyl group had had a *cis* relationship, strong hydrogen bonding could have been expected between them and therefore no free OH stretching absorption would have been observed (the phenolic and enolic groups are expected to be strongly hydrogen-bonded to the carbonyl group of ring B). In compound (2a), in which the hydroxy- and methoxycarbonyl groups are *cis* to each other, the i.r. spectrum shows no free OH stretching frequency (see later). The above evidence adduced in favour of the *trans* orientation makes position 10a an *S*-centre.

We then wished to confirm that the linkage between the two halves of the molecule was 2,2' rather than 4,4'. At one time, the Gibbs test was used in attempts to decide the presence or absence of a proton *para* to the phenolic hydroxy-group at position 1,<sup>2,4</sup> but ambiguous results have been obtained<sup>4,10</sup> with this test. The Gibbs test is actually performed on the phenolate anion with the reaction being carried out in alkaline solution and, for weakly phenolic substances, the low concentration of phenolate ion means that the test reagent itself (2,6-dichlorobenzoquinone chloroimide) has time to produce the characteristic blue-green colour. Recently, evidence has been presented<sup>7</sup> in favour of 2,2'-linkages in secalonic acids (A, B, C, and D) rather than the previously supposed 4,4'-linkages. On the basis of similar evidence we assign a 2,2'-linkage to secalonic acid

E (1a). Thus, for the hexa-acetate (1e), the n.m.r. spectrum shows that the signal of one of the protons ( $\delta$  6.88) in the aromatic ring C is shifted strongly downfield compared with that in the free phenol (1a) ( $\delta$  6.54), and this is characteristic of a proton *para* to a phenolic group.<sup>11</sup> Therefore, there is a proton at position 4 *ortho*-coupled to another aromatic proton; thus only a 2,2'-linkage is possible. The resonance frequencies of H-3 and H-4 protons are also characteristic of a 2,2'-linkage.<sup>7</sup>

Compound (1a) appears to be enantiomeric with secalonic acid B (3) although there is a slight doubt about this because optical activity could arise from restricted rotation about the 2,2'- (biphenyl-type) linkage. Such restricted rotation seems unlikely in a biphenyl system which is only substituted with two *ortho*-hydroxy-groups but, on these grounds, this probable enantiomer of secalonic acid B is here named independently as secalonic acid E. The enantiomer of secalonic acid A is secalonic acid D; thus two pairs of enantiomers of the secalonic acids are now known.

The reactions of secalonic acid E with diazomethane, dimethyl sulphate, and acetic anhydride gave complex mixtures, as was apparently the case for other secalonic acids.<sup>4,7</sup> In each case, the major products had to be separated from mixtures by thick-layer chromatography on silica.

Structure (2a) for the second pigment is identical with that reported for secalonic acid A, again except for a slight uncertainty concerning optical isomerism about the 2,2'-linkage. This structure was assigned on the basis of arguments similar to those already given for secalonic acid E. Thus, oxidation of the pigment with permanganate again gave (+)-(*R*)-methylsuccinic acid, and therefore demonstrated *R*-centres at positions 6 and 6'. The n.m.r. spectrum showed H-5 strongly coupled to H-6 (irradiation at the frequency of H-6 caused the H-5 doublet to collapse to a singlet). Use of the modified Karplus equation<sup>8</sup> and Dreiding models indicated a torsion angle of about 165° between H-5 and H-6 and therefore a *trans* relationship between the 5-hydroxy-group and the 6-methyl group. Position 5 is thus an *S*-centre.

Methyl and hydroxy-groups situated in a 1,2-relationship to each other are reported to exhibit marked solvent shifts.<sup>12</sup> Thus, on changing the solvent from [<sup>2</sup>H]-chloroform to pyridine a shift in the methyl resonance is observed of about 3 Hz when the torsion angle between the groups is near 180° and of about 16—28 Hz for angles of *ca.* 50°. In the case of secalonic acid A (2a) we observed a solvent shift of 11 Hz, qualitatively in keeping with the torsion dihedral angle calculated from the 5,6-proton coupling. Secalonic acid E (1a) was not sufficiently soluble in [<sup>2</sup>H]-chloroform for an n.m.r. spectrum

<sup>8</sup> R. U. Lemieux and J. W. Lown, *Canad. J. Chem.*, 1964, **42**, 893.

<sup>9</sup> For a discussion of Cotton effects in  $\alpha\beta$ -unsaturated ketones, see for example P. Crabbé, 'Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry,' Holden-Day, San Francisco, 1965, p. 191.

<sup>10</sup> F. E. King, T. J. King, and L. C. Manning, *J. Chem. Soc.*, 1957, 563.

<sup>11</sup> See Dun-Mei Yang, N. Takeda, Y. Itaka, U. Sankawa, and S. Shibata, *Tetrahedron*, 1973, **29**, 519, and references cited therein.

<sup>12</sup> For examples see P. V. Demarco, E. Farkes, D. Doddrell, B. L. Mylari, and E. Wenkert, *J. Amer. Chem. Soc.*, 1968, **90**, 5480; M. Fetizon, J.-C. Gramain, and P. Mourgues, *Bull. Soc. chim. France*, 1969, 1673; T. Nambara, H. Hosoda, and M. Usui, *Chem. and Pharm. Bull. (Japan)*, 1969, **17**, 1678.

to be obtained but a similar solvent shift (12 Hz) was observed in changing the solvent from  $[^2\text{H}_6]$ dimethyl sulphoxide to pyridine.

That the relationship between the 5-hydroxy-group and the 10a-methoxycarbonyl group is *cis* is shown by the mass spectrum, in which the ratio of abundances of the  $M$  and  $M - \text{CO}_2\text{CH}_3$  ions is 1 : 3.<sup>3</sup> This is confirmed by the large negative rotation, indicating that the methoxycarbonyl group projects below the plane of ring B. As discussed with respect to structure (1a), the absence of a free OH stretching absorption in the i.r. also agrees with a *cis*-orientation of hydroxy- and methoxycarbonyl groups. The resonance frequency for H-5 suggests that it has an axial orientation.

The two secalonic acids isolated in this study tenaciously held solvent of crystallisation which was difficult to remove by drying. The properties of a sample of secalonic acid A crystallised from carbon tetrachloride-dichloromethane (elemental analysis and mass and n.m.r. spectra) showed that these solvents were held in molecular proportions. A sample recrystallised from acetic acid retained 2 mol. equiv. of solvent, which were again difficult to remove. This strong clathrate effect is probably responsible for the slightly different analytical figures and optical rotation data reported here and earlier for secalonic acid A.<sup>4</sup>

## EXPERIMENTAL

*Isolation of the Secalonic Acids (1a) and (2a).*—*Phoma terrestris* Hansen was grown in shake culture for 17 days at 25° in a medium of the following composition: glucose (20 g), malt extract (Oxoid) (2 g), petone (Oxoid) (2 g),  $\text{KH}_2\text{PO}_4$  (2 g),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (2 g), and distilled water (1 l). The mycelium was separated from the aqueous phase by filtration (Beer filtration paper) and dried for 2 days at 60°. The dried and crushed mycelium (100 g) was continuously extracted with ethyl acetate for 48 h to yield an orange-red solution. The extract was concentrated to a small bulk and allowed to cool to give a yellow solid precipitate (200 mg), later shown to be mainly secalonic acid E. Complete removal of solvent from the extract gave a dark red viscous oil (24 g) which was triturated with methanol to yield an amorphous yellow solid (7 g). This solid was filtered off, washed with ether, and separated by fractional crystallisation from carbon tetrachloride-dichloromethane (2 : 1) into a more soluble component (2a) (2.5 g), m.p. 208–209° (bright yellow needles) and a less soluble component (1a) which was recrystallised from acetic acid-water (yield 3.25 g; m.p. 209–210°; yellow microcrystals). The two substances had  $R_F$  0.64 (1a) and 0.46 (2a) on t.l.c. on silica gel impregnated with 6% oxalic acid (elution with  $\text{CHCl}_3$ -MeOH-AcOH, 97 : 2 : 1) (secalonic acid C,  $R_F$  0.60; secalonic acid D,  $R_F$  0.53).

*Methyl Ethers of Secalonic Acids.*—To a stirred suspension of secalonic acid E (1a) (150 mg) in ethyl acetate (10 ml) at 0° was added a solution of diazomethane in ether (10 ml). The mixture after 30 min had given a clear yellow solution. The excess of diazomethane was destroyed by addition of glacial acetic acid and then the solvents were evaporated

off under reduced pressure to give a bright yellow glass (175 mg), which was chromatographed on thick-layer silica plates with ethyl acetate. The slowest running material ( $R_F$  0.38) was collected and recrystallised [ $\text{CCl}_4$ - $\text{CH}_2\text{Cl}_2$  (2 : 1)] to give 8,8'-di-*O*-methylsecalonic acid E, m.p. 180–184° (1b) (30 mg) (yellow microcrystals); this ether gave an intense green colour with iron(III) chloride in ethanol. Brief treatment of the ether with a trace of hydrochloric acid gave back secalonic acid E [reddish brown colour with iron(III) chloride]. Similarly, the secalonic acid (2a) with diazomethane afforded the 8,8'-di-*O*-methyl ether m.p. 193–195° (decomp.) (2b), as yellow prisms,  $[\alpha]_D^{24} -14.6^\circ$  ( $c$  0.21 in MeOH), which could be hydrolysed rapidly and quantitatively to the original secalonic acid with a trace of hydrochloric acid.

Treatment of the dimethyl ether (1b) (67 mg) with anhydrous potassium carbonate (3.5 g) and dimethyl sulphate (0.65 ml) in refluxing acetone (15 ml) for 1 h yielded a yellow oil which was taken up in hot aqueous methanol. On cooling, a yellow gum was obtained which had solidified after 3 days at 0° (yield 40 mg) and gave a brown colour with iron(III) chloride in ethanol. The n.m.r. spectrum showed  $\delta$  ( $\text{CDCl}_3$ ) 3.90 (6H, s,  $-\text{C}=\text{C}-\text{OCH}_3$ ) and 3.67 (6H, s,  $\text{ArOCH}_3$ ). Brief treatment with methanolic hydrogen chloride gave the 1,1'-di-*O*-methyl ether, in the n.m.r. spectrum of which the enol peak had reappeared.

*Acetylation of Secalonic Acids.*—A mixture of acetic anhydride (10 ml), pyridine (5 ml), and secalonic acid E (1a) (200 mg) was kept at room temperature for 24 h. Removal of solvent under reduced pressure gave a pale-brown oil (280 mg), which was dissolved in ether (30 ml) and washed successively with 5N-hydrochloric acid (15 ml), water (15 ml), saturated aqueous sodium hydrogen carbonate (15 ml), and water ( $2 \times 15$  ml). The resulting solution was dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to yield an off-white solid (265 mg), which was chromatographed on thick-layer silica plates. The material of  $R_F$  0.64, 1,1',5',8,8'-hexa-*O*-acetylsecalonic acid E (1e), was collected as an amorphous white solid, m.p. 150–154° (35 mg);  $\lambda_{\text{max}}$ , 244 (log  $\epsilon$  4.27), 275 (4.26), and 335 nm (3.58);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 1740 (Ac), 1680 (C=O), and 1610  $\text{cm}^{-1}$  (H-bonded C=O);  $\delta$  ( $\text{CDCl}_3$ ) 2.08 (s), 2.14 (s), 2.23 (s,  $\text{CH}_2$ ), 3.68 (s,  $\text{OCH}_3$ ), 5.67 (s,  $\text{CH}\cdot\text{OAc}$ ), and 6.87 (d,  $J$  8 Hz) and 7.26 (d,  $J$  8 Hz) (aromatic *ortho*-protons). Similarly, the secalonic acid (2a) yielded 1,1',5',8,8'-hexa-*O*-acetylsecalonic acid (2e) ( $R_F$  0.66) as an amorphous white solid, m.p. 130–134° (40 mg);  $\lambda_{\text{max}}$ , 240 (log  $\epsilon$  4.23), 277 (4.26), and 335 nm (5.55);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 1750 (Ac), 1690 (C=O), and 1610  $\text{cm}^{-1}$  (H-bonded C=O);  $\delta$  ( $\text{CDCl}_3$ ) 0.99 (d,  $J$  6 Hz;  $\text{CH}\cdot\text{CH}_3$ ), 2.01 (s), 2.13 (s), 2.19 (s, Ac), 3.67 (s,  $\text{OCH}_3$ ), 5.43 (d,  $J$  10 Hz,  $\text{CH}\cdot\text{OAc}$ ), and 6.91 (d,  $J$  8 Hz) and 7.29 (d,  $J$  8 Hz) (aromatic *ortho*-protons).

*Oxidation of Secalonic Acids.*—A solution of secalonic acid E (1a) (800 mg) in aqueous 2N-sodium hydroxide (30 ml) was slowly added to a stirred solution of potassium permanganate (5 g) in water (125 ml). The mixture was kept at 0° for 48 h and then clarified at 0° by bubbling in sulphur dioxide. The resulting solution was acidified to pH 1 with 6N-sulphuric acid and extracted with ethyl acetate ( $5 \times 100$  ml). The combined extracts were shaken with saturated aqueous sodium hydrogen carbonate. The aqueous layer was acidified and extracted with ethyl acetate to give (+)-(*R*)-methylsuccinic acid (58 mg), which was sublimed at 90–100° and 0.05 mmHg to afford a solid, m.p. 108–110° (lit.,<sup>3,5</sup> 105–108°),  $[\alpha]_D^{24} +12.0^\circ$  ( $c$  1.30 in

MeOH) (Found: C, 45.5; H, 6.1. Calc. for  $C_5H_8O_4$ : C, 45.5; H, 6.1%). Similarly, the secalonic acid (2a) also gave (+)-(R)-methylsuccinic acid, m.p. 111—112°,  $[\alpha]_D^{24} +12.1^\circ$  (*c* 0.74 in MeOH) (Found: C, 45.6; H, 6.2%).

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