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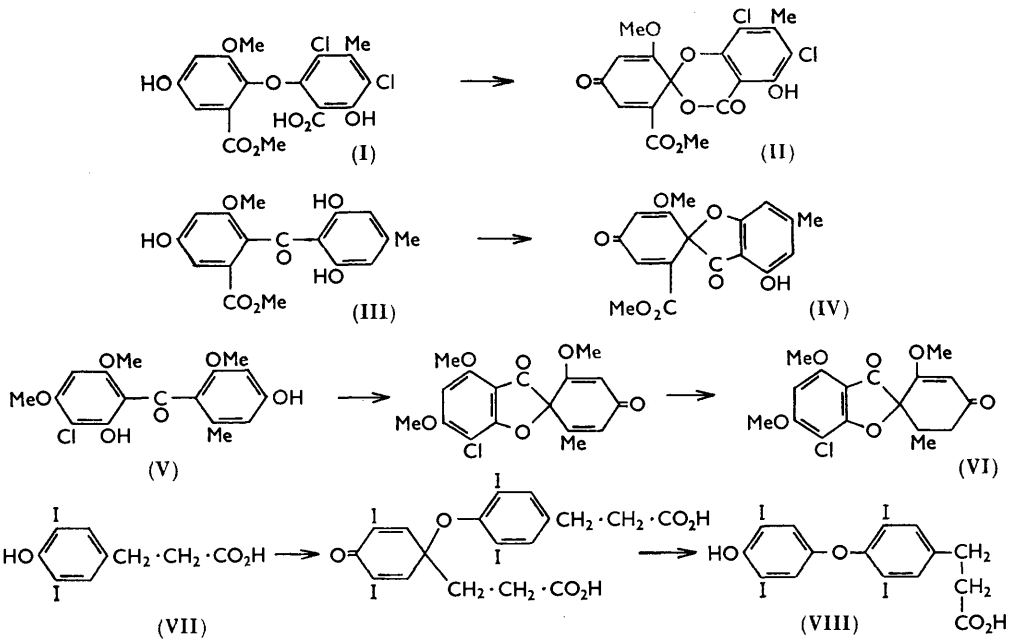
596. *The Biosynthesis of Phenols. Part VI.*[1] *Model Studies Relating to the Conversion of Tyrosine into Homogentisic Acid.*

By J. S. Davies, C. H. Hassall, and J. A. Schofield.

The spiro-lactone (XIV), prepared by oxidation of phloretic acid, undergoes dienone–phenol rearrangement with carbon migration. Acylation of (XIV) with acetic anhydride or diacetyl sulphide yields β-(2,4-diacetoxyphenyl)propionic acid and 7-acetoxy-3,4-dihydrothiocoumarin, respectively. The relationship of these results to the oxidation process leading through tyrosine to homogentisic acid is discussed.

It has been shown [1] that geodin hydrate (I) is converted into geodoxin (II) *in vivo* by an oxidation process that can be simulated *in vitro* with excellent yield.[2] This reaction



may be likened to the oxidation of sulochrin (III) to dechlorogeodin (IV),[3] to the intramolecular oxidative coupling process leading through the phenol (V) to griseofulvin (VI),[4] and to the intermolecular oxidation of 3,5-di-iodophloretic acid (VII) to the thyroxine

[1] Part V, Curtis, Harries, Hassall, and Levi, *Biochem. J.*, 1963, **90**, 43.
[2] Hassall and Lewis, *J.*, 1961, 2312.
[3] Curtis, Hassall, Jones, and Williams, *J.*, 1960, 4838.
[4] Day, Nabney, and Scott, *J.*, 1961, 4067.

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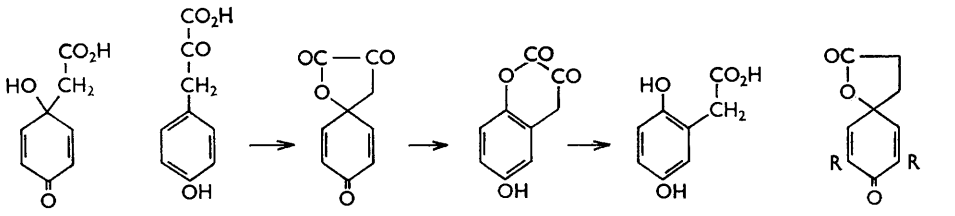
analogue (VIII).[5] Each of these *in vitro* reactions is modelled on an oxidation process known to occur *in vivo*.

It is common knowledge that phenylalanine, in mammalian liver tissue, is converted through tyrosine into 2,5-dihydroxyphenylacetic acid (homogentisic acid, XIII).[6] 4-Hydroxyphenylpyruvic acid (X) is an intermediate in this process; carbons 2 and 3 of the pyruvic acid chain are incorporated into homogentisic acid [7] by a molecular rearrangement and an oxidation process that involves the uptake of two atoms of oxygen and release of one mol. of carbon dioxide.[6] 2,5-Dihydroxyphenylpyruvic acid does not serve as a precursor of homogentisic acid.[8] As yet, the intermediate which undergoes rearrangement into homogentisic acid has not been defined, although hemiquinones such as (IX) have been suggested.[9] There is another alternative that deserves consideration. A spiro-dienone lactone such as (XI) could undergo rearrangement into (XII) followed by oxidation of the α-dicarbonyl function to yield homogentisic acid. We have studied the reactions of the spiro-lactone (XIV) in this connection.



(IX) (X) (XI) (XII) (XIII) (XIV: R = H) (XV: R = Br)

The 3,5-dibromo-spiro-lactone (XV) has been prepared in connection with studies relating to the action of *N*-bromosuccinimide on tyrosyl peptides [10] but the procedure could not be applied to the synthesis of the analogue (XIV). This compound has been obtained from phloretic acid by the action of lead tetra-acetate, peracetic acid, or hydrogen peroxide, or by anodic oxidation.* The constitution follows from the observation of characteristic bands in the infrared spectrum at 1779 (lactone CO), 1689 (conjugated CO), and 1639 cm.$^{-1}$ (conjugated C=C), and from hydrogenolysis to phloretic acid.

Treatment of the spiro-lactone (XIV) with N-sulphuric acid gave a mixture of β-(2,5-dihydroxyphenyl)propionic acid (XVII) and 3,4-dihydro-6-hydroxycoumarin (XVI). As the lactone (XVI) could be hydrolysed under the reaction conditions, whereas cyclisation of the acid (XVII) did not occur, evidently the 6-hydroxycoumarin was the first product of a dienone–phenol rearrangement initiated by protonation of the carbonyl group of the dienone system. No β-(2,4-dihydroxyphenyl)propionic acid or 3,4-dihydro-7-hydroxy-coumarin, products of the corresponding oxygen migration, were detected.

When Witkop and his co-workers studied the rearrangement of the dibromo-spiro-lactone (XV) using 4N-sulphuric acid,[10b] they identified β-(3,5-dibromo-2,4-dihydroxy-phenyl)propionic acid (XVIII) as the product. This suggested that oxygen, rather than carbon, migration occurred with such a spiro-lactone. Their experiment has been repeated using 4N- and N-sulphuric acid. In both cases, β-(3,5-dibromo-2,4-dihydroxyphenyl)-propionic acid and β-(2,4-dibromo-3,6-dihydroxyphenyl)propionic acid were identified in

* A recent report (Scott, Dodson, McCapra, and Meyers, *J. Amer. Chem. Soc.*, 1963, **85**, 3072) describes a preparation of compound XIV by electro-oxidation.

[5] Cahnmann and Matsuura, *J. Amer. Chem. Soc.*, 1960, **82**, 2055.

[6] La Du, " The Metabolic Basis of Inherited Disease," ed. Stanbury, Wyngaarden, and Fredrickson, McGraw-Hill, New York, 1960, p. 386.

[7] Lerner, *J. Biol. Chem.*, 1949, **181**, 281; Dische and Rittenberg, *ibid.*, 1954, **211**, 199; Weinhouse and Millington, *ibid.*, 1949, **181**, 645; Schepartz and Gurin, *ibid.*, 1949, **180**, 663.

[8] La Du and Zannoni, *J. Biol. Chem.*, 1955, **217**, 777.

[9] Goodwin and Witkop, (*a*) *J. Amer. Chem. Soc.*, 1957, **79**, 179; (*b*) *Experientia*, 1952, **8**, 377.

[10] (*a*) Corey and Haefele, *J. Amer. Chem. Soc.*, 1959, **81**, 2225; (*b*) Schmir, Cohen, and Witkop, *ibid.*, 1959, **81**, 2228.

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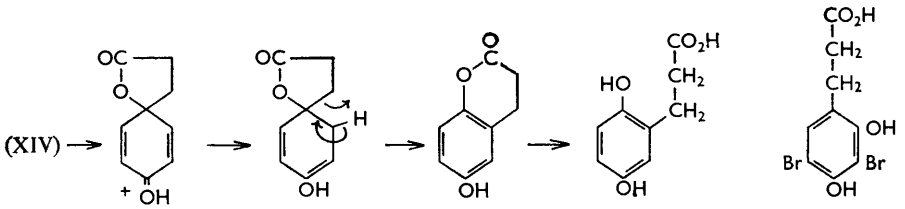
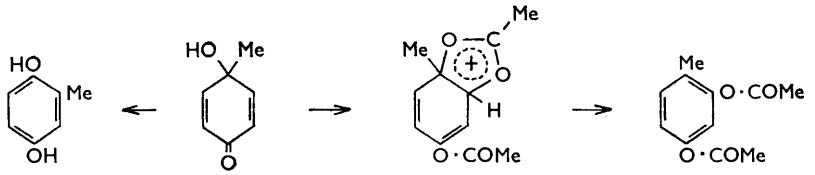
the mixture of products. Evidently, carbon migration, as we have envisaged for the formation of homogentisic acid, occurred with both spiro-lactones (XIV) and (XV) but was accompanied by oxygen migration in the latter case. Other examples of the influence of substituents on alternative pathways of dienone–phenol rearrangement are known.[11]



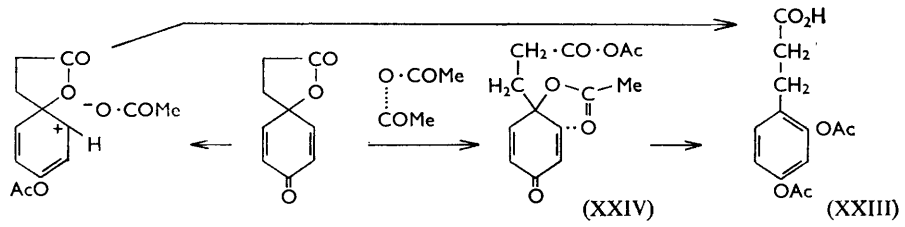
(XIV) (XVI) (XVII) (XVIII)

Rearrangement of the hydroxy-ketone (XIX) in dilute sulphuric acid gave 2-methylquinol (XX) but, in acetic anhydride–sulphuric acid, 2,4-diacetoxytoluene (XXII) was formed.[9a,12] When the spiro-dienone (XIV) was treated with acetic anhydride–sulphuric acid, 6-acetoxy-3,4-dihydrocoumarin (15%) and β-(2,4-diacetoxyphenyl)propionic acid (75%) were obtained. The formation of the coumarin may be attributed to a migration similar to that which occurs in dilute sulphuric acid. The reaction leading to the substituted propionic acid could be inter- or intra-molecular. In the case of the hydroxy-ketone and related compounds, the process has been interpreted [9a,12] as an intramolecular oxygen migration involving the intermediate (XXI).



(XX) (XIX) (XXI) (XXII)

We investigated the mechanism of formation of β-(2,4-diacetoxyphenyl)propionic acid using ^{18}O-labelled acetic anhydride. As the β-(2,4-dihydroxyphenyl)propionic acid obtained when the product of this reaction was hydrolysed could be shown by mass spectra measurements to be ^{18}O-labelled, an intramolecular process was excluded. Two alternative intermolecular processes can be envisaged. In one case, attack by " acetylium ion " on the carbonyl group of the dienone system would lead to attachment of an acetoxy-group at position 2 and formation of the diacetate (XXIII). In the other case, initial attack by the acetic anhydride on the lactone ring would lead to an intermediate (XXIV) which would undergo intramolecular rearrangement to the same product.



(XXIV) (XXIII)

The behaviour of diacetyl sulphide in reactions with quinones and phenols is similar to that of acetic anhydride.[13] It reacted with the spiro-lactone (XIV) in the presence either

[11] Loudon, *Progr. Org. Chem.*, 1961, **5**, 52.
[12] Metlesics, Wessely, and Budzikiewicz, *Tetrahedron*, 1959, **6**, 345.
[13] Metlesics, *Monatsh.*, 1957, **88**, 804.

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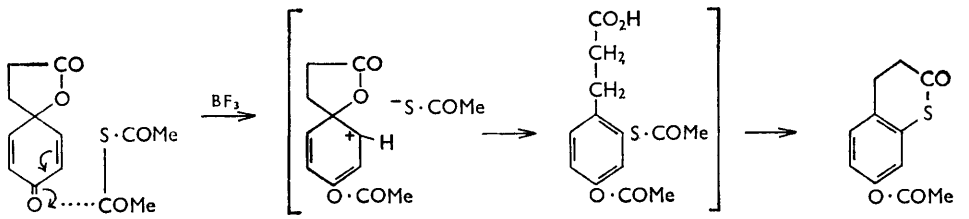
of concentrated sulphuric acid or of boron trifluoride to give a neutral product, $C_{11}H_{10}O_3S$, containing one acetyl group. The infrared absorption spectrum of this compound had a strong band at 1680 cm.$^{-1}$ characteristic of a thiolactone group. Hydrolysis with dilute acid and treatment with Raney nickel converted it into phloretic acid. This left no doubt that the neutral product was 7-acetoxy-3,4-dihydrothiocoumarin (XXV) and favoured a reaction sequence in which initial attack by the acetylium ion on the carbonyl group of the dienone system led to attachment of the thio-acetate group at position 2 of the benzene



(XIV) (XXVI) (XXV)

ring. The formation of the thiocoumarin (XXV) from the acetylated intermediate (XXVI) is similar to the conversion of β-(2,4-diacetoxyphenyl)propionic acid into 7-acetoxy-3,4-dihydrocoumarin, in low yield, by prolonged heating with acetic anhydride–boron trifluoride. The more facile reaction in the case of the sulphur analogue is attributed to the increase in nucleophilic character when sulphur replaces oxygen in such a compound.

These studies indicate that dienone–phenol rearrangement of a spiro-lactone such as (XIV) favours the carbon migration that has been envisaged in the pathway of biosynthesis leading from tyrosine to homogentisic acid. No evidence of the rearrangement involving oxygen migration has been obtained in this study, although in the case of some more highly substituted spiro-dienone lactones it is well authenticated.[14] This leaves open the possibility that suitable spiro-dienone lactones could serve as intermediates in the biosynthesis of coumarins. Clearly, these investigations must be extended to include *in vivo* studies using postulated intermediates before it can be said, with certainty, that the spiro-lactones are involved in biosynthetic pathways. The observation that spiro-dienones are produced enzymically,[1,15] and that dienone–phenol rearrangements are involved in several plausible pathways of biogenesis [16] are additional reasons for considering these spiro-lactones as possible intermediates in natural processes.

Experimental

Melting points were determined on a Kofler hot-stage apparatus. Ultraviolet spectra were measured on a Unicam S.P. 500 spectrophotometer and an Optica CF4 recording spectrophotometer. All spectra were obtained for ethanol solutions unless otherwise stated. Infrared spectra were determined on a Perkin-Elmer Infracord instrument model 137 in KBr discs or in chloroform solutions. Thin-layer chromatography was on Kieselgel G (Merck) using the solvent system benzene–methanol–acetic acid (45 : 8 : 4 v/v). Detection of spots on chromatograms was by the following spray reagents: (1) iodine in chloroform; (2) diazotised *o*-dianisidine; (3) ammoniacal silver nitrate. " Silica gel for chromatographic adsorption " (B.D.H. Ltd.) was used for column chromatography.

1-*Oxaspiro*[5,4]*deca*-6,9-*diene*-2,8-*dione* (XIV).—(*a*) Phloretic acid (8 g.) [10b] in glacial acetic acid (40 ml.) at 90° was treated with 30% hydrogen peroxide (6 ml.). Further aliquots of hydrogen peroxide were added every 0·5 hr. for 2·5 hr. The excess of hydrogen peroxide was decomposed by palladium and the solvent removed under reduced pressure to give a yellow gum (8·8 g.). Elution from a silica gel column (4 × 56 cm.) with chloroform (1·4 l.) and removal

[14] Asahina and Momose, *Ber.*, 1938, **71**, 1421; Leonard, Ph.D. Thesis, London, 1962.
[15] Hassall and McMorris, *J.*, 1959, 2831; Wachtmeister, *Acta Chem. Scand.*, 1958, **12**, 147.
[16] Barton and Cohen, " Festschrift Prof. Dr. A. Stoll," Birkhauser, Basel, 1957, p. 117.

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of the solvent gave the *spiro-lactone* (930 mg.) as plates, m. p. 108—109° (from chloroform–ether) (Found: C, 66·1; H, 4·9. $C_9H_8O_3$ requires C, 65·9; H, 4·9%), $\lambda_{max.}$ (in 20% acetonitrile–water), 232 mμ (ε 11,700), $\nu_{max.}$ 1779 (lactone CO), 1689 (conjugated CO), and 1639 cm.$^{-1}$ (conjugated C=C).

(*b*) Phloretic acid (1 g.) in peracetic acid (10 ml.) was kept at 65° for 2 hr. Evaporation of the solvent under reduced pressure gave a dark brown oil (650 mg.). The neutral component (18 mg.) in this oil was identified as the spiro-lactone.

(*c*) Lead tetra-acetate (40 g.) and phloretic acid (8 g.) in anhydrous methanol (150 ml.) were shaken mechanically for 4 hr. Removal of lead ion with 2N-sulphuric acid and chromatography of the product in chloroform on a silica gel column (66 × 4 cm.), gave a fraction which, on evaporation to dryness and addition of ether, gave the spiro-lactone (550 mg.).

(*d*) Phloretic acid (500 mg.) in 10% acetic acid (40 ml.) was electrolysed for 3 hr. at 0° in a cell with platinum electrodes (3 × 2 cm.) placed 1 cm. apart. Trial runs indicated that a current of 0·5 amp. at 50 v d.c. gave best results. The residue, obtained by evaporating the mixture to dryness, was dissolved in ethyl acetate, washed with saturated sodium hydrogen carbonate solution, and evaporated, to give the spiro-lactone (65 mg.), plates, m. p. and mixed m. p. 108—110° (from chloroform–ether).

Hydrogenation of the Spiro-lactone (XIV).—The spiro-lactone (120 mg.) was hydrogenated in ethanol (75 ml.) using 5% palladium–charcoal (1·8 g.). Uptake of hydrogen (1 mol.) was complete in 0·5 hr. The catalyst was filtered off and the ethanol solution evaporated, to give a colourless oil (110 mg.) which recrystallised from ether–chloroform to give phloretic acid as plates, m. p. and mixed m. p. 128—130°.

β-(2,5-*Dihydroxyphenyl*)*propionic Acid.*—3,4-Dihydro-6-hydroxycoumarin (400 mg.),[17] prepared from the dihydrocoumarin by oxidation with potassium persulphate in alkali, was heated with 10% sodium hydroxide (8 ml.) on a water-bath for 0·5 hr. The ether extract of the acidified solution was extracted with sodium hydrogen carbonate solution and the extract was acidified, extracted with ether, and evaporated, to give a pale yellow solid (160 mg.) which yielded the *dihydroxy-acid* as plates, m. p. 129—131° (from ether–chloroform) (Found: C, 59·3; H, 5·8. $C_9H_{10}O_4$ requires C, 59·3; H, 5·5%), $\lambda_{max.}$ 297 mμ (ε 4020) (addition of alkali changed this to $\lambda_{infl.}$ 262 mμ), $\nu_{max.}$ 1715 cm.$^{-1}$ (carboxyl CO). Thin-layer chromatography showed a single spot (R_F 0·25) giving a black colour with spray (3) and a rejection spot with spray (2).

β-(2,4-*Diacetoxyphenyl*)*propionic Acid.*—The crude product (95 mg.) obtained by acetylating β-(2,4-dihydroxyphenyl)propionic acid (200 mg.) [18] with acetic anhydride (14 ml.) and concentrated sulphuric acid (0·05 ml.) for 12 hr. at room temperature was chromatographed in chloroform on a column (20 × 1 cm.) of silica gel. Crystallisation of the main fraction from acetone–light petroleum (b. p. 40—60°) gave the *diacetoxy-acid* (40 mg.), m. p. 81—83° (Found: C, 58·2; H, 5·2. $C_{13}H_{14}O_6$ requires C, 58·6; H, 5·3%), $\lambda_{max.}$ 273 mμ (ε 2330), $\nu_{max.}$ 1760 (ester CO) and 1710 cm.$^{-1}$ (carboxyl CO). Thin-layer chromatography showed a single spot (R_F 0·55).

7-*Acetoxy*-3,4-*dihydrocoumarin.*—β-(2,4-Diacetoxyphenyl)propionic acid (250 mg.), acetic anhydride (10 ml.), and boron trifluoride etherate (4 drops) were left overnight at room temperature. After the addition of water (30 ml.), the mixture was worked up in the usual way for neutral material, to give an oil (12 mg.) which yielded the coumarin, m. p. 110—111° (from aqueous ethanol), identical (mixed m. p. and infrared spectrum) with an authentic sample.[18] Thin-layer chromatography of the product gave a single spot (R_F 0·85) which, on spraying with ammonia and spray (2), gave a characteristic red colour.

When the reaction was carried out at 90° a 17% yield of the dihydrocoumarin was obtained.

Reactions of the Spiro-lactone (XIV).—(*a*) *With dilute sulphuric acid at* 90°. The spiro-lactone (130 mg.) in acetonitrile (0·5 ml.) was treated with N-sulphuric acid (10 ml.) for 1 hr. at 90° and the solution extracted with ether. Thin-layer chromatography showed the presence of β-(2,5-dihydroxyphenyl)propionic acid and 3,4-dihydro-6-hydroxycoumarin, and the absence of β-(2,4-dihydroxyphenyl)propionic acid and 3,4-dihydro-7-hydroxycoumarin. The ether solution was extracted with aqueous sodium hydrogen carbonate and the dried ether solution evaporated, to give a solid (24 mg.) which sublimed at 120°/5 mm. to give 3,4-dihydro-6-hydroxycoumarin, m. p. and mixed m. p. 162—163°. The infrared spectrum was identical with that of an authentic sample. Thin-layer chromatography showed a single spot (R_F 0·63) which gave a positive reaction with spray (3).

[17] Fichter and Schlager, *Helv. Chim. Acta*, 1927, **10**, 409.
[18] Langley and Adams, *J. Amer. Chem. Soc.*, 1922, **44**, 2320.

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The alkaline extract was acidified, and extracted with ether. The dried extract was evaporated, to give an oil (46 mg.) which yielded β-(2,5-dihydroxyphenyl)propionic acid, m. p. 128—130° (from ether–chloroform), identical (mixed m. p. and infrared spectrum) with an authentic sample.

3,4-Dihydro-6- [17] and -7-hydroxycoumarin [18] were shown by thin-layer chromatography to be converted into β-(2,5-dihydroxyphenyl)- and β-(2,4-dihydroxyphenyl)propionic acid,[18] respectively, under the conditions described above. The acids were stable under these conditions.

(*b*) *With acetic anhydride–concentrated sulphuric acid.* The spiro-lactone (120 mg.) was set aside for 3 hr. at room temperature in acetic anhydride (10 ml.) containing concentrated sulphuric acid (0·04 ml.). After addition of water (20 ml.), the solution was extracted with ether and the dried ether solution evaporated to dryness, to give an oil (145 mg.). This was chromatographed on a silica gel column (30 × 1 cm.). Elution with chloroform gave first 6-acetoxy-3,4-dihydrocoumarin, m. p. and mixed m. p. 85—86° [from acetone–light petroleum (b. p. 40—60°)]. The infrared spectrum was identical with that of an authentic sample.[17] Thin-layer chromatography showed a single spot (R_F 0·8) and indicated that hydrolysis with N-sulphuric acid at 90° gave β-(2,5-dihydroxyphenyl)propionic acid.

Further elution of the column with chloroform gave β-(2,4-diacetoxyphenyl)propionic acid, white needles (80 m.g), m. p. and mixed m. p. [from acetone–light petroleum (b. p. 40—60°)] 82—83°. The infrared spectra of the product and of an authentic sample were identical Thin-layer chromatography showed that hydrolysis with N-sulphuric acid gave β-(2,4-dihydroxyphenyl)propionic acid.

(*c*) *With acetic anhydride–boron trifluoride.* The spiro-lactone (180 mg.) in acetic anhydride (4 ml.) was treated with boron trifluoride etherate (2 drops) overnight at room temperature. The mixture was worked up as in the previous experiment, to give 6-acetoxy-3,4-dihydrocoumarin (43 mg.) and β-(2,4-diacetoxyphenyl)propionic acid (85 mg.).

(*d*) *With* ^{18}O*-labelled acetic anhydride.* The ^{18}O-labelled acetic anhydride was prepared as follows. A mixture of glacial acetic acid (25 ml.) and ^{18}O-labelled water (40 ml.; approx. 2% ^{18}O-enriched) was refluxed for 24 hr. and treated with sodium hydroxide pellets until the solution was just alkaline to phenolphthalein. The sodium acetate obtained on evaporation was fused and powdered. Addition of concentrated sulphuric acid (15 ml.) and distillation gave ^{18}O-labelled acetic acid (22 ml.), b. p. 115—118°. The acid was dissolved in ether (250 ml.), *NN'*-dicyclohexylcarbodi-imide (36 g.) was added, and the mixture stirred for 4 hr. at room temperature. The dicyclohexylurea (35 g.) was filtered off, and fractional distillation then gave ^{18}O-labelled acetic anhydride (5 ml.), b. p. 135—138°.

The reaction was carried out as in (*b*), using the ^{18}O-labelled acetic anhydride. The crude β-(2,4-diacetoxyphenyl)propionic acid (350 mg.) was hydrolysed with N-sulphuric acid (25 ml.) for 2 hr. at 90°, to give β-(2,4-dihydroxyphenyl)propionic acid,[18] m. p. 165—166°. A sample (20 mg.) of this acid was fused for 1 hr. at 170° and then sublimed at 120°/5 mm. to give 3,4-dihydro-7-hydroxycoumarin,[18] m. p. 132—134°.

Analysis by mass spectroscopy of the acid and lactone from this experiment, together with samples of these compounds prepared by synthesis, gave the following results:

3,4-Dihydro-7-hydroxycoumarin			β-(2,4-Dihydroxyphenyl)propionic acid		
	Relative peak height			Relative peak height	
m/e	^{18}O-Enriched	Synthetic	*m/e*	^{18}O-Enriched	Synthetic
164	100	100	164	100	100
165	10·3	11·2	165	10·2	11·2
166	2·0	1·1	166	1·7	1·3

These results show ^{18}O-enrichment of the acid and the lactone by 0·5 and 0·9%, respectively.

(*e*) *With diacetyl sulphide–boron trifluoride.* The spiro-lactone (500 mg.), in diacetyl sulphide (10 ml.) and boron trifluoride etherate (4 drops), was left at room temperature overnight. The solution was diluted with ether, washed with water, dried, and evaporated to dryness under reduced pressure, to give an oil (830 mg.). Chromatography of a portion (500 mg.) of the oil on silica gel and elution with chloroform gave one main fraction. Evaporation to dryness and crystallisation of the residue (447 mg.) from ether–light petroleum (b. p. 40—60°) gave *7-acetoxy-3,4-dihydrothiocoumarin*, m. p. 69—70° (Found: C, 59·8; H, 4·8; S, 14·2; Ac, 20·6. $C_{11}H_{10}O_3S$ requires C, 59·5; H, 4·5; S, 14·4; Ac, 19·4%), $\lambda_{max.}$ 260 mμ (ε 7670), $\nu_{max.}$ 1680 cm.$^{-1}$ (thiolactone CO). Thin-layer chromatography in chloroform gave a single spot (R_F 0·8).

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(*f*) *With diacetyl sulphide-concentrated sulphuric acid.* The spiro-lactone (500 mg.) in diacetyl sulphide (10 ml.) was treated with concentrated sulphuric acid (0·1 ml.). Purification of the product, as in the previous experiment, gave 7-acetoxy-3,4-dihydrothiocoumarin (622 mg.), m. p. 68—69°.

Hydrolysis and Desulphurisation of 7-Acetoxy-3,4-dihydrothiocoumarin.—The thiocoumarin (220 mg.) in N-sulphuric acid (10 ml.) was heated overnight on a water-bath. The ether extract of the reaction mixture was evaporated to dryness, to give an oil (150 mg.) which was dissolved in ethanol (15 ml.) and refluxed for 4 hr. with Raney nickel (500 mg.). The catalyst was filtered off and the filtrate evaporated, to give an oil. Chromatography in chloroform on a silica gel column gave a major fraction (30 mg.), m. p. 127—129°, identified as phloretic acid by mixed m. p. and infrared spectrum.

Reaction of 3,5-Dibromo-1-oxaspiro[5,4]deca-6,9-diene-2,8-dione (XV) *with Dilute Sulphuric Acid at* 90°.—The dibromo-spiro-lactone [10b] (50 mg.) in acetonitrile (1 ml.) was refluxed with 4N-sulphuric acid (10 ml.) for 3 hr. After removal of acetonitrile by distillation the aqueous solution was extracted with ether. Thin-layer chromatography showed three spots: (a) R_F 0·5, identical with an authentic sample [10b] of β-(3,5-dibromo-2,4-dihydroxyphenyl)propionic acid, giving a red colour with spray (2), (b) R_F 0·45, identical with authentic β-(2,4-dibromo-3,6-dihydroxyphenyl)propionic acid [10b] giving a positive test with spray (3) and a rejection spot with spray (2), and (c) R_F 0·7 (trace), identical with 6,8-dibromo-3,4-dihydro-7-hydroxy coumarin [10b] giving a red colour with spray (2).

Identical results were obtained on treatment of the dibromo-spiro-lactone with N-sulphuric acid at 90°.

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