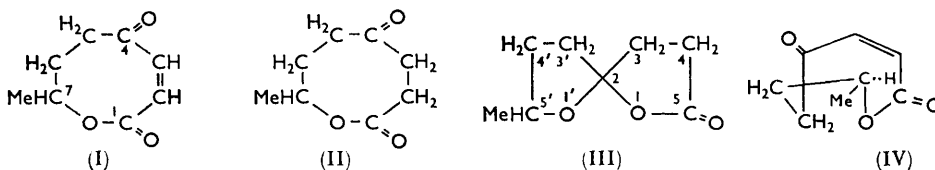


616. Metabolic Products of *Stemphylium radicinum*. Part II.¹
(-)-7-Hydroxy-4-oxo-oct-2-enoic Acid Lactone.

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The antifungal $C_8H_{10}O_3$ metabolic product of *Stemphylium radicinum* is shown to have structure (I).

THE antifungal keto-lactone, $C_8H_{10}O_3$, isolated from culture filtrates of *S. radicinum*¹ is shown to be (-)-7-hydroxy-4-oxo-oct-2-enoic acid lactone (I) by the following evidence. Catalytic reduction revealed the presence of one ethylenic double bond, and the three oxygen atoms in the dihydro-derivative (II) were shown to reside in lactone and ketone groups by titration and by formation of a semicarbazone, respectively. The keto-lactone (I) also reacted with semicarbazide acetate, but the ultraviolet (u.v.) absorption of the derivative was anomalous, suggesting that a simple semicarbazone was not formed. The keto-lactone (I) also reacted with semicarbazide acetate, but the ultraviolet (u.v.) absorption of the derivative was anomalous, suggesting that a simple semicarbazone was not formed. The u.v. absorption peak of (I), λ_{max} 220 m μ (log ϵ 4.05), absent in the dihydro-derivative (II), showed that the ketone and ethylenic link were conjugated: that the ethylenic bond was also conjugated with the lactone group followed from the spectrum, λ_{max} 207 m μ (log 3.87), of the alcohol obtained by reduction of (I) with sodium borohydride. Analyses of this transformation product were consistently poor but oxidation with chromic oxide quantitatively regenerated the ketone (I). Lithium aluminium hydride reduction of the keto-lactone (I) gave a triol, $C_8H_{16}O_3$, which had no carbonyl groups (infrared spectrum) and only weak u.v. end-absorption.



The nuclear magnetic resonance (n.m.r.) spectrum of (I) confirmed that the ethylenic bond was disubstituted (olefinic protons in AB double doublet at τ 2.81, 3.10, 3.33, 3.61, $J = 15.5$ c./sec.) and indicated a linear arrangement of the cross-conjugated system. It

¹ Part I, preceding Paper.

also showed the presence of a methyl group (3-proton doublet at τ 8.65, 8.75, $J = 6.5$ c./sec.) attached to a methine group (1-proton multiplet at τ 4.9) bearing the oxygen atom of the lactone bridge. Only four hydrogen atoms remained unaccounted for and these were contained in two methylene groups (A_2B_2 part of an A_2B_2X system at τ 7.2—8.1), one of which (τ 7.2) must be attached to the keto-group.

The eight-membered-ring lactone structure (I) was confirmed by hydrolysis and oxidation of the dihydro-derivative (II) to 4,7-dioxo-octanoic acid.² Although ring-opening of (II) with 0.1N-sodium hydroxide and distillation of the oily product gave a mixture, epimeric at position 2, of 5'-methyl-2,2'-spirobitetrahydrofuran-5-ones (III), rather than the expected 7-hydroxy-4-oxo-octanoic acid, oxidation of the spiro-lactone (III) with chromic oxide in the presence of sulphuric acid gave the desired diketo-acid. The expected small variation (0.08 τ) in the shielding of the protons of the 5'-methyl group, depending on whether this was *cis* or *trans* to the oxygen of the lactone ring, was observed in the n.m.r. spectrum of the mixed 2-epimeric spiro-lactones (III): the attempted isolation of the pure epimers by gas chromatography was unsuccessful.

The intensity of the u.v. peak of the lactone (I) indicates that the conjugated system is essentially planar and that the lactone exists in the rigid ring conformation (IV) (or its enantiomer). It is of interest that the coupling constant, $J_{2,3} = 15.5$ c./sec., is rather greater than those previously recorded (12—13 c./sec.) for olefinic protons in 8-membered rings,³ presumably due to the presence of two electron-withdrawing groups.⁴ The ring strain associated with (IV) is released in the dihydro-derivative (II) which can adopt a number of ring conformations, and the absence of any large change in the chemical shift of the C-methyl protons on reduction indicates that this group is *exo* in the preferred conformation (IV). In the *endo*-configuration the C-methyl group falls within the shielding cone of the lactone carbonyl group and a large downfield shift would have been expected on reduction.

EXPERIMENTAL

For general procedure see Part I.¹

(-)-7-Hydroxy-4-oxo-oct-2-enoic Acid Lactone (I).—The lactone¹ had λ_{\max} 220 μ ($\log \epsilon$ 4.05), ν_{\max} 3070 (=CH), 1719, 1697 (C=O), 1635 cm^{-1} (C=C), (in CCl_4) 3060, 1732, 1703 cm^{-1} . The unit cell was orthorhombic, $a = 10.03$, $b = 15.87$, $c = 5.11$ Å, $d = 1.253$. On microhydrogenation in acetic acid (Pd catalyst) the lactone took up 1.28 mol. of hydrogen. The lactone was very soluble in benzene and ethyl acetate: it was insoluble in light petroleum of b. p. 40—60° but was sparingly soluble in the fraction, b. p. 60—80°. It sublimed readily at 170°. The lactone was neutral but dissolved on warming in 0.1N-sodium hydroxide to give a solution from which it was not recovered on acidification. It gave no colour with ferric chloride and, although it did not reduce Fehling's solution, ammoniacal silver nitrate was slowly reduced on warming. It was unsaturated to potassium permanganate in acetone. Carbon dioxide was not liberated on acid hydrolysis. The amorphous orange precipitate obtained with Brady's reagent was intractable.

The derivative obtained by reaction with semicarbazide acetate in ethanol formed prisms, m. p. 301° (decomp.) (from aqueous acetic acid) (Found: N, 19.1. $\text{C}_9\text{H}_{13}\text{N}_3\text{O}_3$ requires N, 19.9%), ν_{\max} 1711 (C=O), 1694 (C=N), 1634 (C=C), 1579 cm^{-1} (CONH_2), λ_{\max} 302 μ ($\log \epsilon$ 3.78).

Catalytic Reduction of the Lactone (I).—The lactone (500 mg.) in ethyl acetate (40 ml.) was hydrogenated (uptake 1.00 mol.) in the presence of 10% palladium-carbon (50 mg.). The product, after filtration and recovery, crystallised from ethanol in needles (375 mg.), m. p. 155—156°, or from ethyl acetate-light petroleum as a mixture of needles and hexagonal plates, m. p. 155—156°, of 7-hydroxy-4-oxo-octanoic acid lactone (II), $[\alpha]_D -24^\circ$ (c 0.20, in EtOH) (Found: C, 61.2; H, 7.7%; Equiv. (lactone titration), 152. $\text{C}_8\text{H}_{12}\text{O}_3$ requires C, 61.5; H, 7.75%; M , 156), λ_{\max} 275 μ (ϵ 63), ν_{\max} 1734, 1712 (C=O), (in CCl_4) 1742, 1726 cm^{-1} , τ (number

² Kehler and Iglar, *Ber.*, 1899, **32**, 1176.

³ Chapman, *J. Amer. Chem. Soc.*, 1963, **85**, 2014.

⁴ Barfield and Grant, *J. Amer. Chem. Soc.*, 1961, **83**, 4726.

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of protons in parentheses) 8.72, 8.82 (3, doublet, $J = 6$ c./sec.), 5.1 (1, quartet, Me·CHO-), 7.3—8.4 (4, multiplet, CH₂·CH₂·CO), 7.4 (4, singlet, OOC·CH₂·CH₂·CO).

The two crystalline modifications had characteristic infrared spectra in the solid state but the solution spectra were identical. The compound was saturated to potassium permanganate in acetone. The *semicarbazone* formed prisms, m. p. 213—215° (from ethanol) (Found: N, 19.2; C₈H₁₅N₃O₃ requires N, 19.7%), λ_{max} . 228 m μ ($\log \epsilon$ 4.10), ν_{max} . 3470, 3220br (NH), 1726 (C=O), 1687 (C=N), 1581 cm.⁻¹ (CONH₂).

Reduction of the Lactone (I) with Alkali-metal Hydrides.—(a) *Lithium aluminium hydride.* The lactone (50 mg.) in tetrahydrofuran was added to lithium aluminium hydride (100 mg.) in tetrahydrofuran. After the initial reaction had subsided, the mixture was refluxed for 1.5 hr. The excess of hydride was destroyed by the addition of ethyl acetate followed by ice-water (10 ml.). Continuous extraction of the mixture with ethyl acetate gave a neutral yellow oil (21 mg.) which was distilled at 80°/10⁻³ mm. giving the oily *oct-2-ene-1,4,7-triol* (Found: C, 59.9; H, 10.45. C₈H₁₆O₃ requires C, 60.0; H, 10.1%). The infrared spectrum indicated the absence of carbonyl groups.

(b) *Sodium borohydride.* The lactone (25 mg.) in ethanol (2 ml.) was treated with sodium borohydride (20 mg.) in ethanol (1 ml.) for 1 hr. at 0° and then for 0.5 hr. at room temperature. After the excess of borohydride had been decomposed with acetic acid, the solvent was removed *in vacuo*, water (3 ml.) was added, and the product, recovered in ethyl acetate, was separated into neutral (15 mg.) and acidic (discarded) fractions by extraction with sodium hydrogen carbonate.

The oily neutral fraction was distilled at 130—150° (bath temp.)/0.15 mm., giving *4,7-dihydroxyoct-2-enoic acid 7-lactone* (Found: C, 60.6; H, 8.3. C₈H₁₂O₃ requires C, 61.5; H, 7.75%), ν_{max} . (film), 3400br (OH), 1700br (C=O), 1640 cm.⁻¹ (C=C), λ_{max} . 207 m μ ($\log \epsilon$ 3.87).

Oxidation of the oily lactone (5 mg.) in acetone with chromic oxide-sulphuric acid⁵ regenerated the lactone (I) (4 mg.), identified by comparison of the infrared spectra.

Hydrolysis of the Lactone (II).—The lactone (100 mg.) slowly dissolved in 0.1N-sodium hydroxide (13 ml.) during 18 hr. at room temperature. The solution was acidified with hydrochloric acid and extracted with ether. The recovered product, a neutral mobile oil, was distilled at 90—100° (bath temp.)/1.0 mm., giving *5'-methyl-2,2'-spirobitetrahydrofuran-5-one* (III) (mixture of 2-epimers) (Found: C, 61.1; H, 7.7. C₈H₁₂O₃ requires C, 61.5; H, 7.75%), ν_{max} . (liquid film or in CHBr₃) 1780sh, 1764 cm.⁻¹ (5-membered-ring lactone). The n.m.r. spectrum showed a pair of CH-Me doublets at τ 8.62, 8.73 and 8.70, 8.81, a multiplet at τ 5.7, and a complex system at τ 7.0—8.5.

Oxidation of the Spiro-lactone (III).—The spiro-lactone (17 mg.) in acetone (1 ml.) was treated with an excess of the chromic oxide reagent⁵ at 0° for 2 hr. The mixture was diluted with water and extracted with chloroform. Recovery yielded a solid product (7 mg.) which gave *4,7-dioxo-octanoic acid*, plates (3 mg.), m. p. 71—72° (from ethyl acetate-light petroleum) identical (mixed m. p. and infrared spectra) with a synthetic specimen.

4,7-Dioxo-octanoic Acid.—2-(3-Oxobut-1-enyl)furan (2.5 g.), concentrated hydrochloric acid (3 ml.), and ethanol (6 ml.) were heated under reflux for 2 hr. The black tarry product was extracted with chloroform and the organic layer was extracted with sodium hydrogen carbonate. The acidic fraction was recovered and crystallised from ether (charcoal) affording *4,7-dioxo-octanoic acid*, hexagonal plates (72 mg.), m. p. 72—73° (from ether-light petroleum) (lit.,² 75—76°) (Found: C, 55.8; H, 7.1. Calc. for C₈H₁₂O₄: C, 55.8; H, 7.0%).

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⁵ Curtis, Heilbron, Jones, and Woods, *J.*, 1953, 457.