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OOSPOLIDE: A TEN-MEMBERED RING LACTONE COMPOUND ISOLATED FROM *OOSPORA ASTRINGENES*

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FROM a fungus, *Oospora astringenes*, eburicoic acid and several isocoumarin compounds (VII-IX) were isolated and their chemistry was described (1). Further investigation of the cultural medium by ion exchange chromatography has now effected a separation of a new water-soluble compound, oospolide, the structure (Ia) of which provides the first example of a ten-membered lactone occourring as a natural product.

Oospolide, colorless prisms, m.p. 111°, optically inactive, is an acidic substance, pKa'= 3.5, and its 0.1% solution showed pH ∿3.0. It gave poistive test to ferric chloride as well as bromine test for enol (2) and Legal test for active methylene. Its formula, $C_8H_{10}O_5$, suggested by elementary analysis was confirmed by titration with N/100 NaOH (found MW=186) and by mass analysis (M^+ 186). Oospolide showed strong UV absorption at 261-264 mμ (λmax 261 mμ, ε=12,600 in EtOH, and λmax 264 mμ, ε=12,600 in H_2O or 0.1-1N HCl) which shifted to longer wave-length about 30 mμ by basification of the solution (λmax 296 mμ, ε=14,100 in 0.1-1N NaOH) indicating that the compound has an enolized β-diketone chromophor, although attempts to obtain an isooxazole derivative failed. On standing the compound in an alkline solution the absorption gradually disappeared and the solution showed no absorption after several hours suggesting that the compound decomposes under basic conditions. The IR spectrum (in KBr) of oospolide exhibited weak bands at 2600-2800 and strong bands at 1735, 1660 and 1570 cm^{-1}, the latter two being consistent with an enolized β-diketone chromophor.

Oospolide reacts with diazomethane, though the resulting product was too unstable to characterize, the only crystalline derivative we have prepared being a p-bromophenacyl derivative (II), m.p. 110-112°, $C_{16}H_{15}O_6Br$, IR(KBr): 1735, 1665, 1583 cm^{-1}.

On permanganate oxidation, oospolide gave a crystalline dicarboxylic acid, m.p. 90-92°, $C_5H_8O_5$, which in NMR spectrum (in D_2O, 60 Mc, internal standard DSS) showed two triplets (each correspond to 2H, J=6 cps.) at δ 2.70 and 3.85, and a sharp singlet at δ 4.21 (2H). The structure (III) therefore assigned to this acid was proved by the direct comparisons (mixed m.p. and IR) with the specimen ([illegible]) obtained by oxidation of tetrahydro-γ-pyrone.

Much information of the structure was obtained from its NMR spectrum (in $CDCl_3$, 60 Mc, internal standard TMS) which accounted for all ten protons in the molecule. Two triplets (each corresponds to 2H, J=6 cps.) centered at δ 2.86 and 3.86, singlets at δ 4.13 (2H), 4.54(2H), 5.72 (1H), and a broad peak at δ 6.94 (disappeared on addition of D_2O) are attributed to CO-C$\underline{H}_2$-C$\underline{H}_2$-O, COO-C$\underline{H}_2$-CO, =C(OH)-C$\underline{H}_2$-O, -C$\underline{H}$=C- and -O$\underline{H}$, respectively. Since the proton at δ 6.94 must be attributed to the enol hydrogen and the compound showed no peak due to other terminal group, oospolide was concluded as being a ten-membered ring compound of either the structures I or VI. β-Diketone grouping in oospolide should be fixed in an enol-form by following reasons: i) it behaves as an acid stronger than acetic acid (pKa=4.76), ii) the UV absorptions in water and in ethanol have the same intensity (see above) [cf. acetylacetone (4) shows the change of intensity depending on the solvent; $\varepsilon^{277m\mu}$=1,900, (15% enol) in H_2O, and $\varepsilon^{272.5m\mu}$=10,500, (84% enol) in EtOH], and iii) the NMR signal due to olefinic proton does not exchange with deuterium by addition of D_2O while the hydroxyl proton exchanged smoothly. The UV absorption of oospolide was rapidly diminished by addition of sodium botohydride in methanol; a fact which favors the structure I than VI, and this was confirmed by ozonolysis of oospolide. The reaction yielded the acid (III) and glycolic acid (V) (identified as S-benzylpseudothiuronium salt, m.p. 140°) by decomposition of the ozonide with water, or the acid (III) and glycolaldehyde (IV) (identified as 2,4-dinitrophenylhydrazone, m.p. 152°) when the ozonide was decomposed by sulfinic acid, neither volatile acid, formaldehyde, glyoxilic acid, nor oxalic acid, which are possible products from VI, being detected in the mixture.

Of the two possible tautomers (Ia and Ib) we prefer Ia particularly in $CDCl_3$ solution, since the methylene group adjacent to the ether oxygen apparently showed long-range coupling (half-height width, 5 cps.), whereas the methylene adjacent to the lactone oxygen appeared as a sharp signal (half-height width, 2 cps.).

The mass spectrum, m/e 186 (0.5), 127(3), 111(15), 110(100), 109(9), etc., is another support of the structure. The fragmentation can be explained by Fig. II.

Oospolide may be synthesized in the fungus from an acetate, hydroxypyruvate, and β-hydroxy-

FIG. I

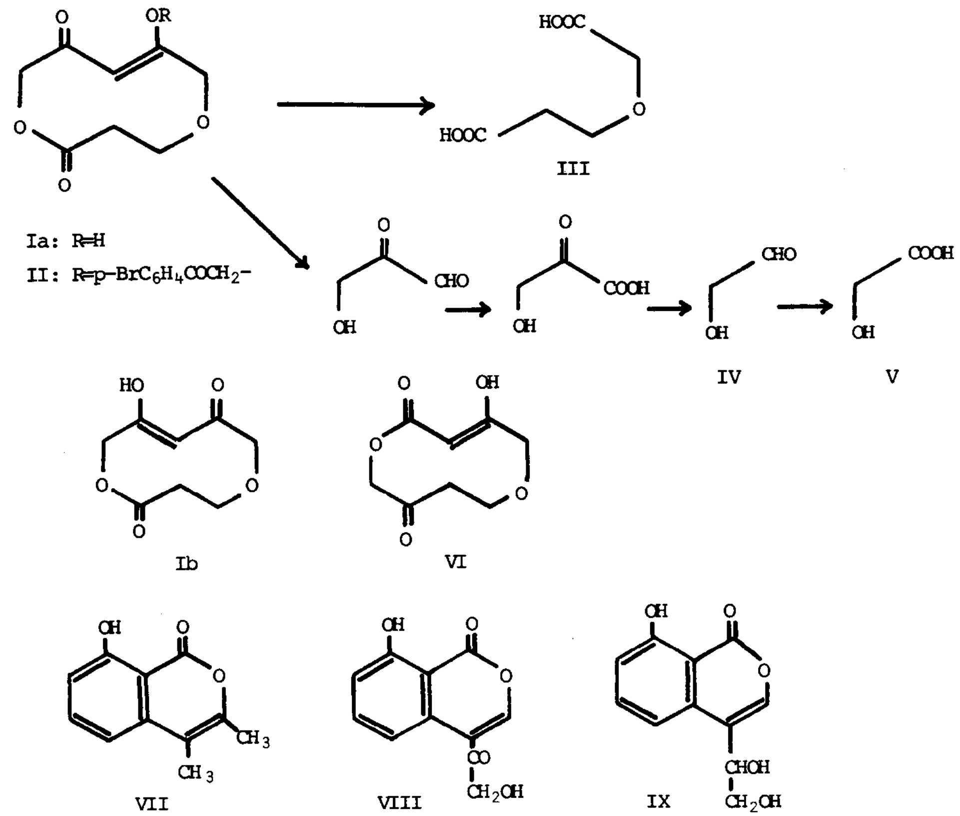
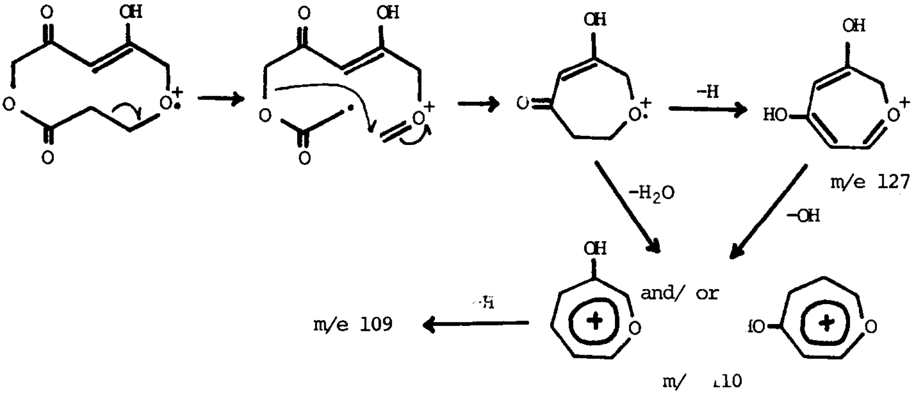

Ia: R=H
II: R=p-BrC6H4COCH2-
III
IV
V
Ib
VI
VII
VIII
IX

FIG. II


-H
-H2O
-OH
m/e 127
and/ or
-H
m/e 109
m/ 110

propionate. Biosynthetic investigation under this view is in progress.

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