Tetrahedron, 1960, Vol. 10, pp. 148 to 152. Pergamon Press Ltd. Printed in Northern Ireland

DIQUINONES—I

THE OOSPOREIN-TOMICHAEDIN DEGRADATION

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(*Received* 26 *March* 1960)

Abstract—It is established, by synthesis, that tomichaedin, the degradation product obtained from the dibenzoquinone, oosporein, by fusion with potash, has the structure 3-hydroxy-2-methyl-1,4-naphthaquinone-6-carboxylic acid. A mechanism for the reaction is proposed which accounts for all the products which have been isolated.

Iso-oosporein is identical with oosporein.

THE diquinone oosporein(I) has been encountered more frequently than most fungal quinones. Originally isolated from laboratory cultures of *Oospora colorans* van Beyma by Kögl and van Wessem[1], it was later obtained from *Chaetomium aureum* Chivers[2] (and other unidentified *Chaetomium* spp.)[3,4] *Verticillium psalliotae* Treschow,[5] the culture filtrate of a sterile mycelium,[4] and very recently, from an *Acremonium* sp.[6] The pigment chaetomidin, which Nishikawa[3] obtained from a *Chaetomidium* (= *Chaetomium*) sp. proved[7] to be identical with oosporein, and we have now shown that iso-oosporein,[8] the metabolic product of another unidentified mould, is also identical with oosporein. An isomeric structure was proposed by Shigematsu[8] as his material, and its derivatives, appeared to differ in several respects from oosporein, but the faults must be attributed to errors in the original work.[1] (At the time Shigematsu[9] was unaware of the later paper by Robertson *et al.*[2]) That oosporein and iso-oosporein are one and the same compound is now established by their infra-red spectra, which are identical having bands (KBr disk) at 3·03, 6·16, 7·24, 7·51, 7·71, 8·05, 9·35, 10·13, 10·48, 10·87, 13·06, 13·50, and 14·01μ.

Benzoquinones are reactive compounds and greater reactivity would be anticipated for dibenzoquinones, especially when the quinone rings are close enough to allow interaction. It has already been shown[10] that internuclear reactions can take place under acid conditions, with surprising results, and in the case of oosporein a remarkable transformation occurs under alkaline conditions. In their examination of the structure of oosporein, both Nishikawa[3] and Shigematsu[8] subjected it to fusion with potassium hydroxide and so obtained a yellow compound, tomichaedin, $C_{12}H_8O_5$, which gave a red solution in alkali. On zinc-dust distillation it yielded naphthalene, and from the

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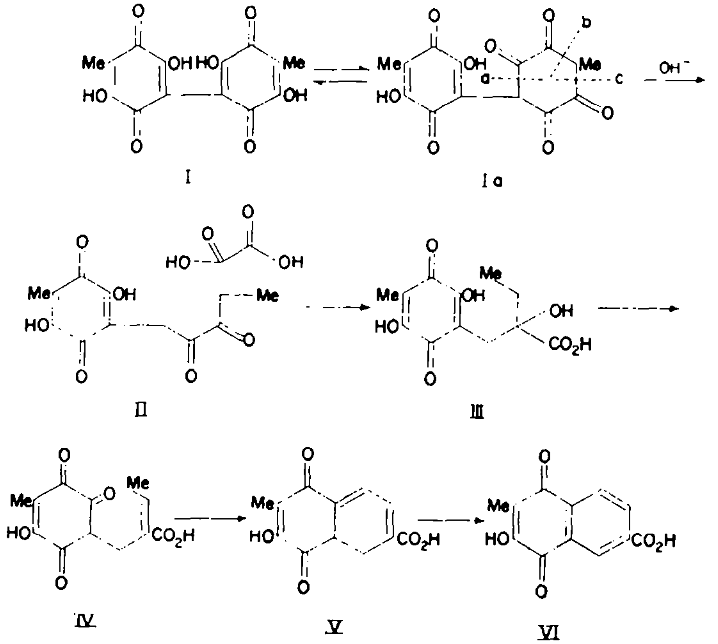
[1] F. Kögl and G. C. van Wessem, *Rec. Trav. Chim.* **63**, 5 (1944).
[2] G. Lloyd, A. Robertson, G. B. Sankey and W. B. Whalley, *J. Chem. Soc.* 2163 (1955).
[3] H. Nishikawa, *J. Fac. Agric. Tottori Univ.* **1**, 71 (1952).
[4] T. Rosett, Thesis, London University (1955).
[5] H. W. Reading, Thesis, London University (1955).
[6] P. V. Divekar, R. H. Haskins and L. C. Vining, *Canad. J. Chem.* **37**, 2097 1959).
[7] M. Itahashi, Y. Murakami and H. Nishikawa, *Tohoku J. Agric. Res.* **5**, 281 (1955).
[8] N. Shigematsu, *J. Inst. Polytechnics, Osaka City Univ.* Ser. C. **5**, 100 (1956).
[9] N. Shigematsu, personal communication.
[10] H. Erdtman, *Proc. Roy. Soc.* A **143**, 223 (1934); T. Posternak, *Helv. Chim. Acta* **39**, 1564 (1956).

formation of numerous derivatives[3,7] it appeared to be a hydroxynaphthaquinone-carboxylic acid. The presence of a methyl group was inferred from the analyses, and its location in the quinone ring adjacent to the hydroxyl group was indicated by the negative Kesting[11] tests given by tomichaedin and its derivatives, and by the formation of trimellitic acid on oxidation of tomichaedin with alkaline permanganate[7] or hydrogen peroxide.[8] The Japanese authors were thus driven to the conclusion that tomichaedin is 3-hydroxy-2-methylnaphthaquinone-6-or-7-carboxylic acid.



To account for the formation of such a compound we have suggested, in a preliminary communication,[12] the following mechanism, which leads to structure VI for tomichaedin. Structure Ia is a tetraketo tautomer of oosporein;* β-keto fission of bonds *a* and *b* would lead to the formation of the α-diketone II, and so to the intermediate III by a benzilic acid transformation. Dehydration of III would be expected under the reaction conditions, and if this produced the substituted crotonic acid IV (written in a tautomeric keto form), the bicyclic structure V could then be formed by aldol condensation. The final dehydrogenation to the naphthaquinone VI is probably effected by aerial oxidation as Shigematsu found that tomichaedin is not produced when oosporein is fused with potash in an atmosphere of hydrogen. In view of the many possible reactions, the low yields (10–15 %) are not surprising. If the diketone II is an intermediate then oxalic acid should be formed simultaneously, and this was in fact obtained by Nishikawa[3], but the origin of n-butyric acid, which Shigematsu isolated as its *p*-bromophenacyl ester, is less obvious. We have confirmed the formation of n-butyric acid and shown, by paper chromatography, that the volatile acids produced in the fusion reaction comprise n-butyric, propionic and acetic acids (in

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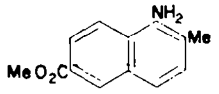
* This is written for convenience. Under the reaction conditions oosporein would be converted initially into its mesomeric anion.

[11] W. Kesting, *Ber. Dtsch. Chem. Ges.* **62**, 1422 (1929).

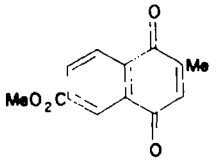
[12] J. Smith and R. H. Thomson, *Proc. Chem. Soc.* 95 (1959).

order of increasing concentration). If the initial degradation proceeds by fission of bonds *a* and *c* in Ia, it would give rise to α-oxobutyric acid; the same compound could also be formed from III by a reversed aldol condensation. It has not been possible to detect this product in the reaction mixture, but we have shown, in separate experiments, that on fusion with potassium hydroxide α-oxobutyric acid is destroyed and yields a mixture of n-butyric, propionic and acetic acids (in order of increasing concentration).

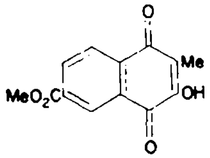
The structure of tomichaedin has been established by synthesis. 6-Methyl-5-nitro-2-naphthoic acid, derived from 2,6-dimethylnaphthalene,[13,14] was esterified and then reduced to the amine VII. This was coupled in aqueous acetone with diazotized aniline-2,5-disulphonic acid, and the resulting azo dye was reduced to the *p*-diamine from which the quinone VIII was obtained by oxidation with ferric chloride. Conversion to the epoxide and hydrolysis with cold sulphuric acid then gave the hydroxyquinone IX (tomichaedin methyl ester), whence hydrolysis with hot acid gave 3-hydroxy-2-methyl-1,4-naphthaquinone-6-carboxylic acid, identical with tomichaedin.



VII



VIII



IX

EXPERIMENTAL

6-Methoxycarbonyl-2-methyl-1-nitronaphthalene

A mixture of 6-methyl-5-nitro-2-naphthoic acid[13,14] (2·25 g), anhydrous potassium carbonate (24 g), and dimethyl sulphate (11·5 ml) was boiled in acetone (70 ml) for 1½ hr. The product, obtained on dilution, crystallized from methanol in straw-coloured needles, m.p. 140–141°; yield 2·35 g. (Found: C, 63·4; H, 4·5; N, 5·7. $C_{13}H_{11}O_4N$ requires: C, 63·7; H, 4·5; N, 5·7%).

6-Methoxycarbonyl-2-methyl-1-naphthylamine (VII)

6-Methoxycarbonyl-2-methyl-1-nitronaphthalene (4 g) in AnalaR ethyl acetate (100 ml) was hydrogenated over Adams' catalyst (200 mg) until 3 moles of hydrogen were absorbed. The catalyst was separated by filtration and the solvent removed under reduced pressure. The *amino-ester* crystallized from methanol in yellow micro needles, m.p. 143°; yield 2·56 g. (Found: C, 72·3; H, 6·0; N, 6·4. $C_{13}H_{13}O_2N$ requires: C, 72·5; H, 6·1; N, 6·5%).

The *acetyl derivative* crystallized from methanol in white needles, m.p. 216–217°. (Found: C, 69·8; H, 5·9; N, 5·3. $C_{15}H_{15}O_3N$ requires: C, 70·0; H, 5·9; N, 5·4%).

6-Methoxycarbonyl-2-methyl-1,4-naphthaquinone (VIII)

Aniline-2,5-disulphonic acid (5.2 g; 58·5% aqueous paste) in water (35 ml) containing sodium carbonate (0·63 g) was diazotized by the addition of conc HCl (1·7 ml) and a solution of sodium nitrite (0·8 g) in water (3·5 ml). An equal volume of acetone was added to the cold diazo mixture which was then stirred mechanically and treated dropwise (30 min) with a solution of 6-methoxycarbonyl-2-methyl-1-naphthylamine (2·3 g) in acetone (40 ml). After being stirred for 2–3 hr in the ice-bath and then for a further 2 hr at room temp, the mixture was set aside overnight. The bluish-red azo dye was collected, washed, and dried, and then crushed and suspended in hot water (85 ml). After addition of conc HCl (22·5 ml) the mixture was heated to 60° on a steam-bath and treated

[13] F. Mayer and E. Alken, *Ber. Dtsch. Chem. Ges.* **55**, 2278 (1922).

[14] C. C. Price, *J. Amer. Chem. Soc.* **62**, 2245 (1940).

gradually with zinc dust (11·5 g). Reduction was complete in 30 min. Residual zinc was removed by filtration and washed well with hot water. To the combined filtrate and washings (at 60°) there was added a filtered solution of anhydrous ferric chloride (8·4 g) in water (16·8 ml) containing conc HCl (1·4 ml). The solution immediately became green and the *quinone* which separated was collected, on cooling, and crystallized from methanol (charcoal) in pale-yellow needles, m.p. 157–158°; yield 1·24 g. (Found: C, 67·9; H, 4·4. $C_{13}H_{10}O_4$ requires: C, 67·8; H, 4·4%).

The *leuco-diacetate* formed long white needles (from light petroleum, b.p. 100–120°), m.p. 162°. (Found: C, 64·6; H, 5·1. $C_{17}H_{16}O_6$ requires: C, 64·55; H, 5·1%).

6-Methoxycarbonyl-2-methyl-1,4-naphthaquinone-2,3-epoxide

A hot solution of 6-methoxycarbonyl-2-methyl-1,4-naphthaquinone (0·62 g) in methanol (70 ml) was cooled to 45° and treated with hydrogen peroxide (1·3 ml; 30%) and a solution of sodium carbonate (0·25 g) in water (6·4 ml). The mixture was stirred at 45° for 5 min, diluted with water (300 ml), and cooled overnight on ice. The *epoxide* which separated, crystallized from aqueous methanol (charcoal) in cream-coloured needles, m.p. 136°; yield 0·40 g. (Found: C, 63·2; H, 4·1. $C_{13}H_{10}O_5$ requires: C, 63·4; H, 4·1%).

3-Hydroxy-6-methoxycarbonyl-2-methyl-1,4-naphthaquinone (IX)

6-Methoxycarbonyl-2-methyl-1,4-naphthaquinone-2,3-epoxide (0·42 g) was dissolved in ice-cold conc H_2SO_4 (4·5 ml) and, after 15 min at 0°, the solution was diluted with water (17 ml). The precipitated *hydroxyquinone* formed deep-yellow crystals, m.p. 213°, when crystallized from benzene-light petroleum (b.p. 100–120°); yield 0·29 g. (Found: C, 63·2; H, 4·0. $C_{13}H_{10}O_5$ requires: C, 63·4; H, 4·1%). The *acetate* crystallized from methanol in yellow plates, m.p. 144–145°. (Found: C, 62·2; H, 4·1. $C_{15}H_{12}O_6$ requires: C, 62·5; H, 4·2%).

3-Hydroxy-2-methyl-1,4-naphthaquinone-6-carboxylic acid (VI)

(a) A solution of the hydroxyquinone-ester (0·27 g) in conc H_2SO_4 (2·7 ml) was diluted with water (5·4 ml) and glacial acetic acid (2·7 ml) and boiled under reflux for 1 hr. On cooling, the solution was poured into water and the solid which separated was crystallized from methanol forming thin yellow needles, m.p. and mixed m.p. with tomichaedin, 282–283° (decomp) [lit[3,8] 284° (decomp); 279–280°]; yield 0·16 g. (Found: C, 62·2; H, 3·4. $C_{12}H_8O_5$ requires: C, 62·1; H, 3·5%). The infra-red and ultra-violet spectra of the natural and synthetic products were identical. Principal bands: (KBr disk) 3·1, 5·805, 5·97, 6·09, 6·23, 6·91, 7·18, 7·33, 7·59, 7·84, 8·28, 9·27, 12·70, 12·90, 13·63, 14·34, 14·86, 15·41 μ; λ max 260, 335, 382 mμ (log ε 4·41, 3·49, 3·24 respectively) λ inf 283 mμ (log ε 4·13) in ethanol; λ max 240, 286, 488 mμ (log ε 4·40, 4·38, 3·50 respectively) λ inf 345 mμ (log ε 3·13) in ethanolic $KHCO_3$.

(b) A solution of 6-methoxycarbonyl-2-methyl-1,4-naphthaquinone-2,3-epoxide (0·5 g) in conc H_2SO_4 (5 ml) was diluted with water (10 ml) and glacial acetic acid (5 ml) and boiled under reflux for 1 hr. The product (0·26 g) obtained on dilution of the solution was identical with that obtained in (a).

The quinone formed an acetate which crystallized from methanol in yellow prisms, m.p. 205° (lit[3,8] 205°). (Found: C, 61·1; H, 3·7. Calc. for $C_{14}H_{10}O_6$: C, 61·3; H, 3·7%).

Fusion of oosporein with potassium hydroxide

Oosporein (0·5 g) was fused with potassium hydroxide (5 g) in the way described by Nishikawa[3]. By acidification of the potassium salt which separated there was obtained a small amount of material which crystallized from methanol in yellow needles, m.p. 282° (decomp) (3–4 mg). The ultra-violet absorption of this product was the same as that of authentic tomichaedin. Acidification of the fusion liquor produced a strong smell of fatty acids and caused the separation of more yellowish-brown material. The mixture was steam-distilled and the distillate (A) made immediately alkaline to phenol red with aqueous barium hydroxide. The barium salts were converted to the ammonium salts for chromatography. The liquor which remained after steam-distillation was saturated with sodium chloride and extracted with ether. The material (B) obtained in this way was then examined chromatographically.

Chromatography

The volatile acids were identified by the method of Duncan and Porteous[15]. Four components were detected in (A). One component did not move from its starting position; the others corresponded in position to samples of acetic, propionic, and n-butyric acids co-chromatographed with the unknown mixture. The relative concentrations of the acids were acetic $\gg$ propionic $>$ n-butyric. The non-volatile material (B) contained several components and the best results were obtained by using the irrigants[16] (1) n-butanol/water/formic acid (10:4:3) or (2) s-butanol saturated with water/propionic acid (95:5). Four non-acidic components were detected on the unsprayed chromatograms. [R_f values in irrigant (2) were 0·84 (pink), 0·74, 0·53, 0·40 (all yellowish-brown)]. Two acidic components were detected when the chromatograms were sprayed with bromophenol blue[17] or similar indicators. One corresponded to oxalic acid but the other [R_f 0·65 in irrigant (2)] was not identified. No trace of an α-keto-acid could be found in either (A) or (B) although chromatograms of both fractions were treated with sprays specific for the detection of α-keto-acids.[18]

A sample of α-oxobutyric acid was fused with potassium hydroxide (same conditions as for oosporein). The fractions obtained by steam-distillation of the acidified mixture were examined chromatographically as before. The distillate was found to contain acetic, propionic and n-butyric acids and another component having R_f 0·0. No trace of α-oxo butyric acid was found, and examination of the residual liquor showed that the keto-acid had been completely destroyed.

Acknowledgements—One of us (J. S.) is grateful to the Executive Committee of the Shirley Institute for the award of a Fellowship. We are indebted to Dr. H. Nishikawa for a specimen of tomichaedin, to Drs. N. Shigematsu and W. B. Whalley for samples of oosporein, and to Dr. V. C. Farmer for the infra-red spectra.

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[15] R. E. B. Duncan and J. W. Porteous, *Analyst* **78**, 641 (1953).

[16] *Modern Methods of Plant Analysis* Vol. II, pp. 545 and 573. Springer-Verlag, Berlin (1955).

[17] A. R. Jones, E. J. Dowling and W. J. Skraba, *Analyt. Chem.* **25**, 395 (1953).

[18] Ref. 16, p. 552.