253. *The Structure of Anhydrotetronic Acid.*

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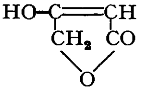
Anhydrotetronic acid, obtained by refluxing tetronic acid in aqueous solution, is shown by degradative studies to have structure (II).

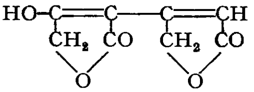
In the course of investigations in this laboratory on the hatching factor for the potato eelworm (*Heterodera rostochiensis* Wollenweber) produced by certain host species of *Solanaceæ* it was observed that anhydrotetronic acid possessed the property of hatching eelworm cysts (Calam, Todd, and Waring, to be published elsewhere). Since this acid was at that time the only active synthetic substance known, it was a matter of considerable importance for further work that its precise structure should be ascertained, and the investigation described in this paper was accordingly carried out.

Anhydrotetronic acid was first described by Wolff and Schwabe (*Annalen*, 1896, **291**, 251), who obtained it as colourless crystals containing one molecule of water of crystallisation by heat, ing an aqueous solution of tetronic acid (I). The anhydrous acid, $C_8H_6O_5$, was monobasic and had m. p. 263° (decomp.). Later Wolff (*Annalen*, 1901, **315**, 162) showed that it contained in all probability a reactive methylene group, since with aliphatic and aromatic aldehydes it gave alkylidene- or arylidene-bisanhydrotetronic acids; analogous compounds are obtained from tetronic acid on similar treatment. No further examination of anhydrotetronic acid seems to have been made.

Anhydrotetronic acid, like tetronic acid, owes its acidic properties to an enolic hydroxyl group; it gives a maroon colour with ferric chloride and couples readily with diazonium compounds. On treatment with diazomethane it gives a neutral *methyl ether* giving no ferric reaction, and on bromination it yields a *mono-bromo*-derivative. It shows strong selective light absorption in the ultra-violet region, and hydrolytic titration at 100° with N/100-sodium hydroxide shows only 20% of the uptake expected for one lactone group. Under optimum conditions anhydrotetronic acid is formed in a yield of only *ca.* 33% by heating aqueous solutions of tetronic acid, but when the water is replaced by aqueous morpholine reaction proceeds rapidly and the yield is almost quantitative. These facts suggest that anhydrotetronic acid has structure (II), but, especially since no analogous compound is obtained under similar conditions from either α- or γ-substituted tetronic acids, it was considered desirable to establish the validity of this structure by degradative methods.

(I.)

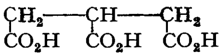




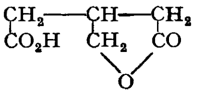
(II.)

Oxidation of anhydrotetronic acid with potassium permanganate gave oxalic acid, while fusion with potassium hydroxide yielded both oxalic and acetic acids. Refluxed with dilute sulphuric acid, anhydrotetronic acid evolved 1 mol. of carbon dioxide during 15 hours; the product, a resinous acid, gave a crystalline p-*phenylphenacyl* ester, but was not identified. Hydrogenation of anhydrotetronic acid in methanolic solution with a platinum catalyst proceeded rather slowly and ceased when about 2·5 mols. of hydrogen had been taken up. The product was not homogeneous and could be readily separated into neutral and acidic fractions. The acidic fraction was semi-solid, and no crystalline derivative could be prepared from it. It clearly contained an enolic hydroxyl group, since it gave a maroon ferric reaction and showed selective light absorption at 2330 A. (ε, 5000). Titration of the crude fraction gave an equivalent of 221, and hydrolytic titration caused a further uptake of alkali equivalent to 93% of that required for a second acid grouping. The failure to isolate any crystalline derivative from the material suggested that it was probably a mixture of acids; it was accordingly oxidised with potassium permanganate in the hope of obtaining identifiable fragments which would give some clue to its nature. Oxidation was at first rapid but slowed down later and was stopped when the equivalent of 6 atoms of oxygen had been used up. From the product, tricarballylic acid (propane-1 : 2 : 3-tricarboxylic acid) (III) and homoparaconic acid (homopilosinic acid, butanolide-2-acetic acid) (IV) were isolated. The isolation of (IV) is significant, since it is clear that in its formation the portion of the anhydrotetronic acid molecule (and of the enolic reduced acid) containing the enolic hydroxyl group has been destroyed. The lactone ring of (IV) is derived from one of the tetronic acid residues present in the starting material, and it is evident that the β-carbon atom in this residue must have been joined to the α-position of the second (enolic) residue in anhydrotetronic acid.

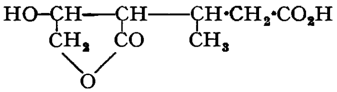
The distilled neutral fraction from the hydrogenation of anhydrotetronic acid was stable to permanganate and showed no selective light absorption in the ultra-violet region. It was evidently a mixture, since it gave analytical values lying between those corresponding to $C_8H_{10}O_4$ and $C_9H_{14}O_5$ and hydrolytic titration gave an equivalent of 101. Treatment with hydrazine gave a mixture of isomeric dihydrazides, $C_8H_{18}O_4N_4$, which was separated into one pure *dihydrazide*, m. p. 155°, which was sparingly soluble in methanol, and a mixture of more soluble dihydrazides, m. p. 125—140°. Both the dihydrazide, m. p. 155°, and the isomer mixture were oxidised by periodate, 3 mols. of reagent being consumed in each case. Since model experiments showed that hydrazides are oxidised by periodate according to the equation $2R\cdot CO\cdot NH\cdot NH_2 + 2O = R\cdot CO\cdot NH\cdot NH\cdot COR + N_2 + 2H_2O$, it can be deduced that the dihydrazides obtained above contain one α-glycol grouping. From these facts it is concluded that the neutral fraction from the hydrogenation contained the methyl ester of a lactonic hydroxy-acid, $C_8H_{12}O_4$, and the corresponding dilactone in their various stereoisomeric modifications; treatment of such a mixture with hydrazine would obviously yield a mixture of stereoisomeric dihydrazides. It may further be inferred that the potential carboxyl groups of the two tetronic acid molecules involved in the formation of anhydrotetronic acid are still present in the latter compound, and that the ring which has been opened by hydrogenolysis to form the acid $C_8H_{12}O_4$ is not the one bearing the enolic hydroxyl of anhydrotetronic acid. The acid $C_8H_{12}O_4$ is therefore regarded as a β-(2-hydroxybutanolide-1)-butyric acid (V).


$$\underset{CO_2H}{CH_2}-\underset{CO_2H}{CH}-\underset{CO_2H}{CH_2}$$

(III.)



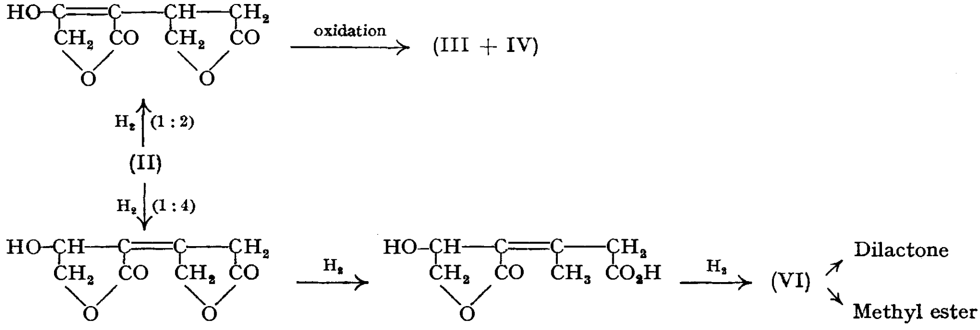
(IV.)



(V.)

These results suffice to establish beyond reasonable doubt structure (II) for anhydrotetronic acid. The course of the hydrogenation described above is explained on the basis of this structure according to the scheme given below. Addition of hydrogen in the 1 : 2-position of the conjugated system would give (VI), probably the major component of the acidic fraction, since as a substituted tetronic acid its further hydrogenation would be slow (Kumler, *J. Amer. Chem. Soc.*, 1938, **60**, 859); oxidation of (VI) would yield tricarballylic and homoparaconic acids. Addition of hydrogen in the 1 : 4-position would yield (VII) as initial product in which ready hydrogenolysis of one lactone ring would occur giving (VIII). Further hydrogenation of (VIII) would yield the lactonic acid (V), which either lactonises or is esterified by the solvent under the conditions of hydrogenation (cf. Waser, *Helv. Chim. Acta*, 1925, **8**, 117) giving the neutral fraction isolated.

(VI.)



The fact that α-substituted tetronic acids are recovered unchanged after treatment under conditions leading to the formation of anhydrotetronic acid (II) from tetronic acid is readily understood, but failure to prepare corresponding anhydro-acids from γ-substituted tetronic acids is rather surprising. On heating an aqueous solution of tetronic acid, the formation of anhydrotetronic acid can be followed by the rise in equivalent. Under similar conditions a definite, although smaller, rise in equivalent was observed with γ-methyltetronic acid (54% increase in 90 minutes) or γγ-dimethyltetronic acid (7% increase in 90 minutes), indicating that some condensation had occurred; no anhydro-acids could be isolated from the resulting solutions, nor could the starting material be recovered. Like α-acetyltetronic acid (Baker, Grice, and Jansen, *J.*, 1943, 241), anhydrotetronic acid ($pK = 1{\cdot}99$) is a much stronger acid than tetronic acid which has $pK = 3{\cdot}76$ (Kumler, *loc. cit.*), and as in the case of α-acetyltetronic acid this increase can be attributed to the increased resonance possibilities in the ionised form. Structural models show that substitution on the γ-carbon atom of tetronic acid would prevent the derived anhydro-acid from assuming a planar configuration, with consequent loss of resonance possibilities. As a result the derived anhydro-acid would be less stable and might decompose rapidly after formation or the condensation might take a different course after the initial aldol stage; either possibility would explain the experimental results.

EXPERIMENTAL.

Anhydrotetronic Acid.—(1) *In water.* Tetronic acid (5 g.) in water (15 c.c.) was refluxed for 10 minutes. On cooling, anhydrotetronic acid separated in small crystals (1·6 g.), m. p. 261°. Recrystallised from water it had m. p. 263° (decomp.).

(2) *In aqueous morpholine.* Tetronic acid (1·75 g.) in water (10 c.c.) and morpholine (1 c.c.) were refluxed for 10 minutes; the solution was cooled and acidified with sulphuric acid, giving 1·6 g. of

anhydrotetronic acid, m. p. 260° (decomp.). Recrystallised from water it had m. p. 263° (decomp.). Light absorption in alcohol: maxima at 3100 A. (ε, 14,200) and 2560 A. (ε, 9600).

Methyl Ether of Anhydrotetronic Acid.—Anhydrotetronic acid (200 mg.) in cold methanol (25 c.c.) was treated with excess of ethereal diazomethane. The solution was left overnight and then evaporated under reduced pressure, the residual oil was diluted with ether, and the brownish needles were collected and recrystallised from methanol (charcoal). The *ether* formed colourless plates, m. p. 185° (colours on heating) [Found: C, 55·0; H, 4·1; OMe, 16·1. $C_9H_8O_5$ requires C, 55·1; H, 4·1; OMe (1), 15·8%].

Bromination of Anhydrotetronic Acid.—Anhydrotetronic acid (100 mg.) in a few drops of water was covered with chloroform (2 c.c.), and a solution of bromine in chloroform (1 g. of bromine in 10 c.c. of chloroform) added slowly with shaking. Reaction appeared to be complete when 0·7 c.c. of solution had been added. The solution was filtered and allowed to evaporate at room temperature; a glass resulted which gradually solidified on standing to a hard white powder, m. p. 144—146°. Attempts to recrystallise the *bromo*-derivative failed (Found: C, 34·5; H, 2·7. $C_8H_5O_5Br,H_2O$ requires C, 34·4; H, 2·5%).

Oxidation of Anhydrotetronic Acid with Potassium Permanganate.—Anhydrotetronic acid (404 mg.) in water (20 c.c.) was treated with potassium permanaganate solution (1·5 g. in 100 c.c. of solution), of which 10 c.c. were taken up (*ca.* 5 atoms of oxygen). The solution was filtered and acidified with dilute sulphuric acid. Volatile acid corresponding to 8·5 c.c. of N/10-sodium hydroxide was removed by steam distillation. The residual solution was exhaustively extracted with ether, evaporation of which gave a yellow solid (340 mg.). Recrystallised from ether it gave colourless prisms, m. p. 102°; these were concluded to be oxalic acid from equivalent determination (Found: equiv., 64. Calc. for $C_2H_2O_4,2H_2O$: equiv., 63) and from the fact that they gave an insoluble calcium salt.

Potassium Hydroxide Fusion of Anhydrotetronic Acid.—Anhydrotetronic acid (250 mg.), potassium hydroxide (1 g.), and water (1 c.c.) were heated in a nickel crucible until fused. The melt was deep red at first but became yellow as the temperature was raised. When the temperature reached 250°, a further quantity (1 g.) of potassium hydroxide was added. The temperature was then raised to 300° and maintained for 10 minutes. The cooled melt was dissolved in water, acidified, and extracted with ether, evaporation of which left a brownish solid smelling of acetic acid. The residue was dissolved in water and the volatile acid removed by steam distillation. The steam distillate required 7·85 c.c. of N/10-sodium hydroxide (equivalent to 47·1 mg. of acetic acid; 0·7 mol.) for neutralisation. After neutralisation the distillate was evaporated and the sodium salt refluxed with *p*-phenylphenacyl bromide in alcohol. On cooling a *p*-phenylphenacyl ester separated. Recrystallised from alcohol it had m. p. 108—109°, undepressed by the *p*-phenylphenacyl ester of acetic acid.

Extraction of the steam distillation residue gave 126 mg. of oxalic acid which, recrystallised from ether, had m. p. 98—100° (Found: equiv., 62). The amount of oxalic acid obtained corresponded to 0·7 mol.

Treatment of Anhydrotetronic Acid with Sulphuric Acid.—Anhydrotetronic acid (508 mg.) was refluxed with N-sulphuric acid in a steam of nitrogen, the carbon dioxide evolved being collected in barium hydroxide solution.

Time (hrs.)	0·5	2·5	6·5	14·5	16·5
CO_2 (% mol.)	5·6	27·9	55·0	108·0	110·0

The reaction mixture was made alkaline and extracted with ether; no neutral fraction was obtained. On reacidification of the solution and extraction with ether it gave a brown resinous oil (400 mg.) (equiv., 135). The resin reduced Tollens's reagent and gave a positive Legal reaction; with ferric chloride it gave a weak green colour. When the acid (50 mg.) was treated with *p*-phenylphenacyl bromide in the usual manner it gave a colourless crystalline *ester*, m. p. 156—158° after recrystallisation from ethanol (Found: C, 75·3; H, 5·5. $C_{21}H_{18}O_4$ requires C, 75·4; H, 5·4%). Anhydrotetronic acid was recovered unchanged after being heated on the steam-bath for 2 hours with 2% oleum.

Hydrogenation of Anhydrotetronic Acid.—Anhydrotetronic acid (3 g.) in methanol (75 c.c.) was shaken with hydrogen in presence of platinum (from 0·25 g. of platinum oxide), 859 c.c. being absorbed in 4 hours. The catalyst was then removed and the methanol evaporated; water was added and the solution neutralised with N/10-sodium hydroxide and extracted continuously with ether for 24 hours. The ether was dried and removed, giving a neutral residue (0·9 g.), which distilled as a pale yellow viscous oil, b. p. 180—190° (bath temp.)/10^{-5} mm., stable to potassium permanganate in the cold [Found: C, 54·5; H, 6·6; OMe, 4·5; active H_2, 0·22; *M* (Rast), 210; equiv. by hydrolytic titration, 101, 98. Calc. for $C_9H_{14}O_5$: C, 53·5; H, 6·9; OMe (1), 15·3; active H_2 (1), 0·50%; *M*, 202; equiv. (2 potential CO_2H), 101. Calc. for $C_8H_{10}O_4$: C, 56·4; H, 5·9%; *M*, 170; equiv., 85]. Acidification of the alkaline solution followed by extraction with ether gave a semi-solid acid fraction (2 g.) (1st equiv., 221 in cold; 2nd equiv., 198 in warm. Calc. for $C_8H_8O_5$: 1st equiv., 184; 2nd equiv., 184). The substance was acid to Congo-red and was rapidly oxidised by permanganate in the cold. It was soluble in cold alkali and gave a maroon colour with ferric chloride. Light absorption in alcohol: maximum at 2330 A. (ε, 5000).

Hydrazides from Neutral Fraction from Hydrogenation.—The above neutral fraction (0·2 g.) was heated with hydrazine (1 g.) on a steam-bath for 3½ hours. Excess of hydrazine was removed under reduced pressure leaving a crystalline residue. Extraction with boiling methanol gave a crystalline almost insoluble *dihydrazide*, m. p. 155° (Found: C, 40·9; H, 7·5; N, 23·5. $C_8H_{18}O_4N_4$ requires C, 41·1; H, 7·7; N, 23·9%). The methanol extract was diluted with ethyl acetate and yielded a mixture of isomeric dihydrazides, m. p. 125—140° (Found: N, 23·9. Calc. for $C_8H_{18}O_4N_4$: N, 23·9%). The dihydrazide, m. p. 155° (13·4 mg.; 0·0573 × 10^{-3} mol.), in water was treated with 1 c.c. of 0·235M-sodium periodate; 0·173 × 10^{-3} mol. of periodate was absorbed (*i.e.*, 3·02 mols. of periodate per mol. of dihydrazide). The mixed dihydrazides (21·0 mg.) were treated with 2 c.c. of 0·235M-sodium periodate and used up 1·095 c.c. (*i.e.*, 2·89 mols. per mol.).

Oxidation of Benzhydrazide with Periodate.—Benzhydrazide (131·5 mg.; 0·968 × 10^{-3} mol.) treated with 5 c.c. of 0·235M-periodate used up 4·05 c.c., *i.e.*, 0·982 mols. per mol. The solid which separated, recrystallised from aqueous methanol, had m. p. 238—239° alone or mixed with dibenzoylhydrazine, m. p. 239°.

Oxidation of the Acid Fraction from Hydrogenation.—The acid fraction (1·17 g.) from the above hydrogenation was dissolved in acetone (20 c.c.) and water (20 c.c.), and 2% potassium permanganate was added dropwise with stirring at room temperature. When 3 atoms of oxygen had been taken up the rate of oxidation slowed considerably; in all 6 atoms of oxygen were added. The solution was filtered, acidified, and extracted with ether. On standing, crystalline material separated from the ether, m. p. 157—159°, undepressed in admixture with authentic tricarballylic acid (m. p. 158—159°). The solution was evaporated, giving an oil which subsequently crystallised and then had m. p. 70—73°. Recrystallised from chloroform-carbon disulphide it had m. p. 78°. Sublimation in a vacuum ($70°/10^{-5}$ mm.) gave colourless prisms, m. p. 80—81·5° (Found: C, 50·2; H, 5·9. Calc. for $C_6H_8O_4$: C, 50·0; H, 5·6%). The m. p. was not depressed on admixture with authentic homoparaconic acid (m. p. 82—82·5°; Polyakova, Preobrazhenskii, and Preobrazhenskii, *J. Gen. Chem. Russia*, 1939, **9**, 1402).

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