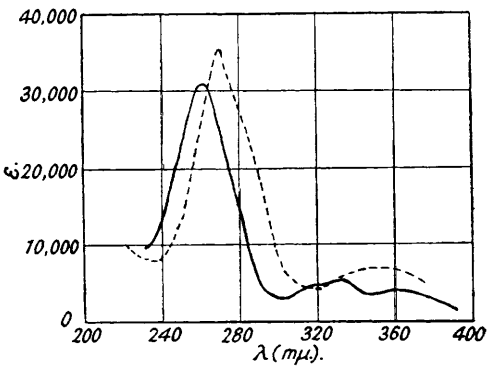
31. *The Structure of Stipitatic Acid.*

By R. E. Corbett, A. W. Johnson, and A. R. Todd.

Oxidation of stipitatic acid with alkaline hydrogen peroxide gives a mixture of aconitic and malonic acid, indicating that stipitatic acid is 6(4)-hydroxytropolone-4(6)-carboxylic acid (I or II).

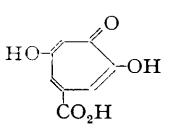
Stipitatic acid, $C_8H_6O_5$, was isolated from the culture media of *Penicillium stipitatum* Thom by Birkinshaw, Chambers, and Raistrick (*Biochem. J.*, 1942, **36**, 242), who carried out a number of degradative experiments but were unable to advance a satisfactory structural formula. The compound was optically inactive, contained no *C*-methyl groups but had three active hydrogen atoms, and it titrated as a dibasic acid. On acetylation, two isomeric diacetyl derivatives were obtained, both soluble in sodium carbonate solution, and on methylation two isomeric trimethyl derivatives were formed, both neutral and insoluble in cold dilute aqueous sodium hydroxide. Other derivatives of stipitatic acid which were described included a dimethyl compound, soluble in dilute sodium hydroxide but insoluble in sodium hydrogen carbonate, formed from it by the action of hot methanolic hydrogen chloride or from disodium stipitatate by methyl iodide; a dibasic monomethyl derivative obtained by treatment of the acid with methyl sulphate in the presence of methanolic potassium hydroxide; and finally a monobromo-substitution product prepared by bromination in aqueous acetic acid. This bromo-derivative titrated as a dibasic acid and gave a neutral compound, "bromotrimethylstipitatic acid," on methylation with diazomethane. These facts indicated the presence of two enolic (or phenolic) groupings of varying acid strengths and one carboxyl group, whose presence was confirmed by decarboxylation of stipitatic acid to give a compound, $C_7H_6O_3$, which was monobasic and gave a blood-red precipitate with ferric chloride. Although no evidence was obtained for the presence of a free carbonyl group, hydrogenation in the presence of platinum, or reduction with zinc and acetic acid, gave products which gave positive carbonyl reactions, and this suggested the presence of a masked carbonyl group in stipitatic acid. The five oxygen atoms were thus accounted for in two enolic hydroxyl groups, one carboxyl and one masked carbonyl group. The titratable acidity of reduced stipitatic acid was one-half that of the original acid, presumably owing to the reduction of the acidic enolic group. A most important observation was made on fusing stipitatic acid or its monomethyl derivative with potassium hydroxide at 300°, when 5-hydroxy*iso*phthalic acid was obtained in good yield. No satisfactory structure was advanced to explain all of these findings and, in particular, a dihydroxyformylbenzoic acid structure was shown to be untenable. The similarity in the general behaviour of stipitatic acid and puberulic acid, noted by Raistrick and his colleagues, is further emphasised by the similarity of their ultra-violet absorption spectra (see figure).

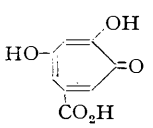


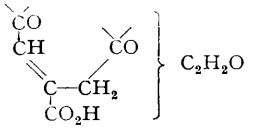
Ultra-violet absorption spectra of puberulic (-----) *and stipitatic acid* (——) *in aqueous solutions.*

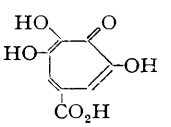
In 1945, Dewar (*Nature*, **155**, 50) re-examined the above evidence and suggested that stipitatic acid was probably the hydroxytropolonecarboxylic acid (I), or the tautomeric form (II), although no further experimental evidence was provided. Nevertheless, Dewar showed that such a structure would offer a satisfactory explanation of the various reactions described by Birkinshaw, Chambers, and Raistrick (*loc. cit.*). Thus the isomeric trimethyl compounds are derived from (I) and (II) and the dimethyl derivative is probably methylated on the

carboxyl and ketol groups, the acidity being due to the remaining enolic grouping. The formation of 5-hydroxy*iso*phthalic acid was ascribed to a benzilic acid type of rearrangement


(I.)


(II.)


(III.)


(IV.)

of the tropolone ring and this view is supported by more recent work on tropolone derivatives (*e.g.*, Barltrop and Nicholson, *J.*, 1948, 116; Erdtman and Gripenberg, *Acta Chem. Scand.*, 1948, **2**, 625; Cook and Somerville, *Nature*, 1949, **163**, 410).

We have now obtained degradative proof that structure (I) correctly represents stipitatic acid, for oxidation with alkaline hydrogen peroxide at room temperature gives a mixture of aconitic and malonic acid. The isolation of aconitic acid indicates that stipitatic acid can be represented by (III), and as a benzenoid structure has been shown to be untenable, the only feasible structure for the molecule is (I) or the tautomer (II). Since it has been shown (Corbett, Johnson, and Todd, *J.*, 1950, 6) that puberulic acid is represented by (IV) or one of its tautomeric forms, this acid can be regarded as a hydroxy-stipitatic acid; that this might prove to be the case was originally suggested by Dewar (*Nature*, 1945, **155**, 479).

Further evidence in favour of structure (I) is obtained by the periodate oxidation of hydrogenated stipitatic acid, 0·85 mole of reagent being consumed, indicating the presence of a 1 : 2-diol structure; in the course of this experiment we confirmed that the titratable acidity of stipitatic acid is halved after reduction.

Experimental.

Stipitatic acid was prepared from cultures of *P. stipitatum* Thom as described by Birkinshaw, Chambers, and Raistrick (*loc. cit.*).

Oxidation of Stipitatic Acid with Hydrogen Peroxide.—Stipitatic acid (1·5 g.) was dissolved in aqueous sodium hydroxide (30 c.c. of N.), hydrogen peroxide (14 c.c. of 31%) added, and the mixture left for 72 hours at room temperature, the solution changing from orange-red to pale golden-yellow. This solution was acidified with hydrochloric acid (32 c.c. of N.) (evolution of carbon dioxide), and the mixture evaporated at 50°/13 mm. and dried *in vacuo* over phosphoric oxide. The colourless, rather gummy residue was continuously extracted with dry ether for 9 hours, and the extract was then evaporated giving a white solid contaminated with some colourless resin (100 mg.). The white solid was dissolved in dry ether (5 c.c.), and light petroleum (b. p. 40—60°) was added at the b. p. until a faint cloudiness was produced. The solution, set aside at 0° for 24 hours, deposited small colourless prisms (30 mg.), m. p. 186—187°. The m. p. was not raised by recrystallisation from ether–light petroleum and was unchanged on admixture with authentic aconitic acid (m. p. 186—187°) (Found : C, 41·5; H, 3·5. Calc. for $C_6H_6O_6$: C, 41·4; H, 3·5%).

Under the conditions of the fluorescein reaction, the oxidation product gave, with resorcinol–sulphuric acid, a red solution with a pale greenish-yellow fluorescence in daylight, and a beautiful sky-blue fluorescence in ultra-violet light, identical with that observed using authentic aconitic acid. With pyridine and acetic anhydride it showed the same series of colour changes as authentic aconitic acid.

The mother-liquors, after removal of the aconitic acid, were again brought to the b. p. and a further quantity of light petroleum added to induce further crystallisation. The solution was set aside at 0° for 24 hours, and a further crop of somewhat less pure aconitic acid (20 mg.), m. p. 160—165°, collected. The mother-liquors were evaporated to dryness, and the white residue (50 mg.) sublimed at 80—85°/10^{-5} mm. giving a colourless micro-crystalline sublimate (21 mg.), m. p. 120—125°, and leaving behind a residue of slightly impure aconitic acid, m. p. 178—179°. The sublimate was purified by resublimation at 80°/10^{-4} mm. and formed small colourless prisms, m. p. 131—133°, which had m. p. 134—135° when mixed with malonic acid (m. p. 135—136°) (Found : C, 34·7; H, 4·4. Calc. for $C_3H_4O_4$: C, 34·6; H, 3·8%). The identity of this product with malonic acid was confirmed by the identity of their colour changes with acetic anhydride and by treatment with resorcinol–sulphuric acid under the conditions of the fluorescein reaction, both giving orange-red solutions with a yellow fluorescence in daylight and a pale greenish-blue fluorescence in ultra-violet light.

Periodate Oxidation of Reduced Stipitatic Acid.—Stipitatic acid (616 mg.) was dissolved in dry methanol (75 c.c.) and hydrogenated at atmospheric pressure using Adams's platinum catalyst, 4·6 moles of hydrogen being absorbed. Aliquots of the methanolic solution, titrated before and after reduction, required 13·3 c.c. and 6·37 c.c. of N/100-alkali, respectively. Removal of the methanol under reduced pressure left a colourless gum, which was exactly neutralised with N-sodium hydroxide and extracted continuously with ether for 9 hours. Evaporation of the ethereal extract yielded only a trace of a neutral gum which gave no reaction with Brady's reagent. Reduced stipitatic acid was regenerated from its sodium salt by passage through a pre-treated resin column and, after removal of the water by evaporation under reduced pressure, was obtained as a colourless gum (376 mg.). A solution of this reduced stipitatic acid in water was oxidised with sodium periodate; it consumed 0·85 mole of oxidising agent.

Light Absorption.—An aqueous solution of puberulic acid showed maxima at 2700 and 3500 A.; $\varepsilon_{max.}$ 35,200 and 7160. An aqueous solution of stipitatic acid showed maxima at 2620, 3320, and 3600 A.; $\varepsilon_{max.}$ 30,800, 5150, and 4050 respectively.

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