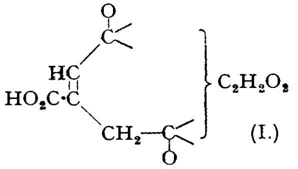
2. *Puberulic and Puberulonic Acids. Part II. Structure.*

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

Oxidation of puberulic acid with alkaline hydrogen peroxide yields aconitic acid. On the basis of this and other evidence, puberulic acid is formulated as 6 : 7-dihydroxytropolone-4-carboxylic acid (II), or one of its tautomeric forms. The properties of puberulonic acid, in particular its ready conversion into (II) in boiling aqueous solution, indicate that it has structure (VI), although a final decision between this and an isomeric structure containing a γ- rather than a δ-lactone grouping cannot yet be made.

Preliminary work on the structure of the mould products, puberulic and puberulonic acids (Birkinshaw and Raistrick, *Biochem. J.*, 1932, **26**, 441; Barger and Dorrer, *ibid.*, 1934, **28**, 11) suggested that they had the molecular formulæ $C_8H_6O_6$ and $C_8H_4O_6$ respectively, but in Part I of this series (preceding paper) we amended the formula of puberulonic acid to $C_9H_4O_7$. The properties of puberulic acid as outlined in the previous paper indicate that it contains a carboxyl and three enolic groupings, but no direct evidence has been obtained for the presence of a free carbonyl group. It is apparent that the puberulic acid molecule must contain therefore the equivalent of five double bonds if the remaining oxygen atom is present as an ether linkage, or four double bonds if it is present as a masked carbonyl group. The nature of the ultra-violet absorption spectrum (preceding paper) and the facts that puberulic acid neither absorbs bromine from an acetic acid solution nor forms an adduct with maleic anhydride eliminate possible open-chain structures. In any case, the accommodation of at least four double bonds in an open chain of seven carbon atoms (allowing for the carboxyl group) would require the presence of acetylenic or cumulative ethylenic linkages.

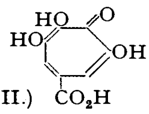
Important evidence on the nature of the cyclic system present in puberulic acid has been gained from oxidative degradation. Except for carbon dioxide, no identified products had been previously obtained from the oxidation of puberulic acid with, *e.g.*, permanganate, chromic acid, neutral hydrogen peroxide, alkaline hydrogen peroxide, or iodine and potassium hydroxide (Barger and Dorrer, *loc. cit.*). We have repeated the oxidation with alkaline hydrogen peroxide and have obtained aconitic acid in 40% yield; the identity of this product has been confirmed by direct comparison with an authentic specimen and by its hydrogenation to tricarballylic acid. On the other hand, oxidation with nitric acid gave only oxalic acid (1 mole) and carbon dioxide, and ozonolysis gave carbon dioxide (5·2 moles) with a small amount of an unidentified non-volatile acidic residue. This formation of aconitic acid establishes the relative positions of six of the eight carbon atoms of puberulic acid, and the molecule may now be represented as (I): A six-membered ring in puberulic acid would necessarily be benzenoid, and benzenoid structures have already been shown to be untenable (preceding paper). Thus, if the acid is monocyclic, there must be present either a five-membered or a seven-membered ring, and, since structures based on these systems could not accommodate the equivalent of four double bonds, the unplaced oxygen must be embodied in an inert carbonyl group rather than in an ether linkage. This argument also eliminates structures containing an oxygen-heterocyclic ring. A further theoretical possibility would be a formula based on the *bicyclo*[0 : 1 : 4]heptane system, but no reasonable structures can be devised which are derived either from this bicyclic system or from a monocyclic five-membered ring and at the same time account for the formation of aconitic acid by oxidation. Of seven-membered ring structures based on (I), the only possibility is (II) (or one of its tautomeric forms), according to which the acid is formulated as a derivative of *cyclo*heptatrienolone (tropolone) (III).



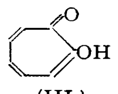
(I.)

The occurrence of tropolone derivatives in Nature is now well established, *e.g.*, the thujaplicins (Erdtman *et al.*, *Nature*, 1948, **161**, 719; *Acta Chem. Scand.*, 1948, **2**, 625, 639, 644), colchicine (Dewar, *Nature*, 1945, **155**, 141, 479; Arnstein *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 1669; Lettré, *Angew. Chem.*, 1947, **59**, *A*, 218), and purpurogallin (Barltrop and Nicholson, *J.*, 1948, 116; Haworth, Moore, and Pauson, *ibid.*, p. 1045) which occurs as its glucoside, eriophyesin. There is also evidence that stipitatic acid, a mould product of *Penicillium stipitatum* (Birkinshaw, Chambers, and Raistrick, *Biochem. J.*, 1942, **36**, 242), is a hydroxytropolonecarboxylic acid (Dewar, *Nature*, 1945, **155**, 50; Corbett, Johnson, and Todd, unpublished work), and indeed Dewar (*loc. cit.*) has suggested, although without any experimental evidence, that puberulic acid might be a hydroxystipitatic acid.

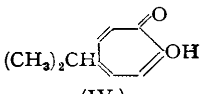
Characteristic features of the various tropolones hitherto examined have been (i) the inert carbonyl group which gives no characteristic reactions, (ii) the formation of substitution rather than addition products with bromine, and (iii) the formation of benzenoid derivatives containing one extra carboxy-group on fusion with potassium hydroxide. Reactions (i) and (ii) are shown



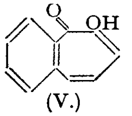
(II.)



(III.)



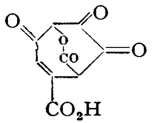
(IV.)

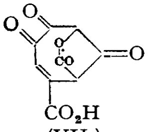


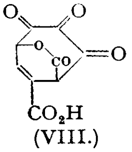
(V.)

by puberulic acid, but the potassium hydroxide fusion has so far failed to yield an aromatic product. The acid is unchanged by potassium hydroxide below 320°; above this temperature, gas is evolved and considerable charring occurs, but no recognisable product has been isolated. In this connection, it is interesting to note that, whereas γ-thujaplicin (IV) was converted with potassium hydroxide into *p*-*iso*propylbenzoic acid at 230° (Erdtman *et al.*, *loc. cit.*), and benztropolone (V) into α-naphthoic acid at 185° (Cook and Somerville, *Nature*, 1949, **163**, 410), stipitatic acid, a monohydroxytropolone derivative, required to be heated to 300° to give 5-hydroxy*iso*phthalic acid (Birkinshaw, Chambers, and Raistrick, *loc. cit.*), so that the relative stability of puberulic acid, a dihydroxytropolone derivative, towards potassium hydroxide is not unexpected.

Perhaps the most interesting aspect of the structure (II) for puberulic acid is the manner in which it explains the relationship between puberulic and puberulonic acids. We have shown (*loc. cit.*) that puberulonic acid, on being heated with dilute sulphuric acid under reflux, is converted into puberulic acid with evolution of carbon dioxide : $C_9H_4O_7 + H_2O \longrightarrow C_8H_6O_6 + CO_2$. It is now found that this change occurs even on heating the acid with water at 100° and thus there is a strong suggestion of the presence of a potential β-keto-acid structure in puberulonic acid. We have also previously demonstrated the existence of α-diketone and lactone groupings and we now propose structure (VI) for puberulonic acid in order to accommodate all these features :


(VI.)


(VII.)


(VIII.)

It will be evident that hydrolysis of the lactone group in (VI) gives a β-keto-acid which, on loss of carbon dioxide, passes into a ketonic form of puberulic acid. Structure (VI) also accounts for the α-diketonic properties of puberulonic acid and hence its yellow colour, as well as for the fact that it cannot be acetylated.

Other possible formulæ for puberulonic acid are (VII) and (VIII), if we exclude structures containing a double bond at a bridgehead. Structure (VII) contains a γ-lactone grouping and there is no decisive evidence available at present to distinguish between it and (VI). Whereas the ready opening of the lactone ring on heating the acid with water and the fact that the quinoxaline derivative of puberulonic acid does not evolve carbon dioxide when heated with dilute aqueous methanolic hydrochloric acid tend to favour the δ-lactone structure, the recovery of puberulonic acid on acidification of a solution of the sodium salt is perhaps more indicative of a γ-lactone formula.

The 1 : 2 : 3-triketone structure (VIII) is perhaps less likely, since such a compound would be expected to exist in a hydrated form and exhibit strong reducing properties. Further work is necessary, however, to decide between structures (VI) and (VII).

EXPERIMENTAL.

Conversion of Puberulonic Acid into Puberulic Acid.—A continuous stream of nitrogen, freed from carbon dioxide and oxygen, was passed through a boiling suspension of puberulonic acid (224 mg.) in water (25 c.c.). The carbon dioxide evolved in the reaction was collected quantitatively in barium hydroxide solution (98·8 c.c. of 0·1923N.), and at the end of 8 hours the excess of barium hydroxide was determined by titration (volume of barium hydroxide neutralised by carbon dioxide, 9·5 c.c.; calc. for 1 molar equivalent of carbon dioxide, 10·4 c.c.). The yellow crystalline solid (170 mg.) formed in the course of the reaction was separated and mixed with a further small quantity of solid obtained by continuous extraction of the filtrate for 8 hours. The crude mixture, m. p. 308°, was recrystallised from methanol to give a faintly yellow micro-crystalline powder, m. p. 311°, alone or mixed with an authentic specimen of puberulic acid.

Fusion of Puberulic Acid with Potassium Hydroxide.—Puberulic acid (200 mg.), water (0·5 c.c.), and potassium hydroxide (1 g.) were heated in a salt-bath at 240° and a further quantity of potassium hydroxide (1 g.) was added to increase the fluidity. The bath temperature was slowly raised to 300°, the melt remaining yellow and frothing considerably. After 10 minutes at 300—320°, the melt was cooled, dissolved in water (8 c.c.), and acidified with 3N-hydrochloric acid (12 c.c.) which caused vigorous evolution of gas. The copious precipitate was allowed to settle at 0° overnight, then separated, and dried (117 mg.), m. p. 300°. Mixed with an authentic specimen of puberulic acid, the product had m. p. 310°. The ferric chloride reaction of the material was identical with that of puberulic acid. The filtrate was evaporated to dryness at 50°/13 mm., and the solid residue remaining was continuously extracted with dry ether. Evaporation of the ether failed to yield any residue.

Repetition of the above experiment at a higher temperature, 320—340°, for 20 minutes caused extensive charring and yielded on acidification a deep reddish-brown mass that had m. p. >350° and was not identified.

Oxidation of Puberulic Acid with Hydrogen Peroxide.—Puberulic acid (560 mg.) was dissolved in N-sodium hydroxide (11 c.c.), hydrogen peroxide (4·9 c.c. of 31%) added, and the mixture set aside for 72 hours at room temperature, whereupon the solution changed from orange-red to colourless. The colourless solution was acidified with N-hydrochloric acid (12 c.c.) (evolution of carbon dioxide), and the mixture evaporated at 50°/13 mm. The colourless, rather gummy residue was continuously extracted with dry ether for 9 hours and gave, after removal of the ether *in vacuo*, a white solid contaminated with a trace of colourless resin. Purification was effected by repeated recrystallisation from dry ether-light petroleum (b. p. 40—60°) and finally by recrystallisation from 2N-hydrochloric acid. The purified product formed small white prisms, m. p. 187—189° (decomp.), which had m. p. 186—187° (decomp.) when mixed with authentic aconitic acid (m. p. 186—187°) (Found: C, 41·8; H, 3·5%; equiv. (titration), 59·4, 56. Calc. for $C_6H_6O_6$: C, 41·4; H, 3·5%; equiv., 58).

The above degradation product gave, under the conditions of the fluorescein reaction 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.

Reduction of the Hydrogen Peroxide Oxidation Product.—The hydrogen peroxide oxidation product (116 mg.) was dissolved in methanol (25 c.c.) and hydrogenated at atmospheric pressure using Adams's platinum oxide catalyst. The hydrogen uptake was slightly in excess of that calculated for one double bond and was complete in 1 hour. The methanol was removed *in vacuo*, leaving an oil which solidified when kept for 24 hours at 0°. Recrystallisation from acetone (charcoal) yielded tricarballylic acid (50 mg.), m. p. 157—158°; mixed with authentic tricarballylic acid (m. p. 162—163°) it had m. p. 158—160°. Confirmation of the identity was provided by the fluorescein test: the colour observed corresponded precisely with that shown by an authentic specimen.

Nitric Acid Oxidation of Puberulic Acid.—Puberulic acid (50 mg.) was heated with distilled nitric acid (2 c.c.) at 100° for 2 hours. Copious brown fumes were evolved and the mixture rapidly changed from red to pale brown. It was evaporated to dryness at 100°, and water (2 c.c.) added to the residue, which was again taken to dryness. This process was repeated three times, the last trace of volatile material being removed *in vacuo*, leaving a colourless solid residue (20 mg.). This residue gave no colour reaction with ferric chloride but a blue colour with diphenylamine, and decolorised potassium permanganate when gently warmed. Purification was effected by sublimation at 80—90°/2 × 10^{-2} mm. and yielded oxalic acid (16·7 mg.), m. p. 187—188°, showing no depression on admixture with an authentic specimen of oxalic acid.

Ozonolysis of Puberulic Acid.—Ozonized oxygen was passed through a suspension of puberulic acid (198 mg.) in water (50 c.c.) and then through standard barium hydroxide until dissolution of the acid was complete (3 days). Carbon dioxide equivalent to 5·2 mols. per mol. of puberulic acid was evolved. The titratable acidity of the clear solution was initially 20 c.c. of 0·1N-alkali and decreased to 7·15 c.c. of 0·1N-alkali after ozonization. Steam-distillation of the residue showed that none of the titratable acidity was due to volatile acids, nor did the distillate give any reaction with Brady's reagent. Evaporation of the ozonized solution gave a small amount of an acidic gum (10 mg.) which gave no characteristic colour reactions and was not further investigated.

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