Synthesis of Puberulonic Acid[1]

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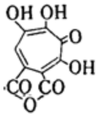
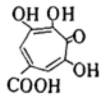
Puberulonic acid (I) was first isolated, together with puberulic acid (II), by Birkinshaw and Raistrick[2] from cultures of the moulds *Penicillium puberulum* Bainer and *P. aurentio-virens* Biourge. These mould tropolones (I and II) have been reported to have a significant antibiotic activity against various Gram-positive organisms[3]. The structure I for puberulonic acid has been accepted reasonably by the presence of a dicarboxylic anhydride grouping evidenced from the data of the infrared spectrum[4], ultraviolet spectrum[5] and titration[6], and by the decarboxylation of it to puberulic acid[6,7] (II). As other mould tropolones, stipitatonic acid[8] (III) and stipitatic acid[9] (IV) have been isolated from culture of *P. stipitatum* Thom. Of the above four mould tropolones, puberulic acid[10] (II) and stipitatic acid[11,12] (IV) have already been synthesized by Johnson and his collaborators from 1, 2, 4-trimethoxybenzene and diazoacetate.



I



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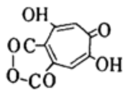

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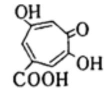

IV

The present authors have been able to synthesize puberulonic acid (I), the details of which will be reported in this communication.

The starting material used was tropolone-3, 4-dicarboxylic anhydride[13] (V), which had been obtained in a poor yield by alkaline hydrogen peroxide oxidation[14] of purpurogallin, of which synthesis[15] had been completed by Haworth and his co-workers. The structure of the anhydride V had been proved by the conversion of it to benzene-1, 2, 3-tricarboxylic acid with alkali-fusion.

As an approach to the synthesis of puberulonic acid (I), it was adopted, that an introduction of bromine, followed by replacing of the bromine with the hydroxyl group, into the anhydride V, was carried out and that a further brominationhydroxylation process were repeated in the resulted hydroxytropolone-dicarboxylic anhydride (XIII). To the reaction for replacing bromine atom with the hydroxyl group, the Rosenmund method[16], in which *o*-bromobenzoic acid was converted into salicylic acid, was satisfactorily applied.

When 3-bromotropolone (VI) was heated with sodium β-naphthalenesulfonate in dilute alkaline solution, 3-hydroxytropolone (VII) was obtained in a 40% yield. This fact proves that a normal nucleophilic substitution of the bromine into the hydroxyl group took place in the above reaction, contrary to formation of 4-hydroxytropolone, a *ciné* (abnormal) substitution product, in high temperature hydrolysis of 3-bromotropolone (VI) with 75% potassium hydroxide[17]. Application of similar reaction to 3, 7-dibromotropolone (VIII) afforded in less yield 3, 4-dihydroxytropolone (IX), which was proved to be identical with the compound known as "decarboxylated puberulic acid"[6,18].

Bromination of tropolone-3, 4-dicarboxylic anhydride (V) in acetic acid gave easily 7-bromotropolone-3, 4-dicarboxylic anhydride (X) in a good yield. Heating of the anhydride X in

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1) This article was read at the 11th Annual Meeting of the Chemical Society of Japan, April, 1957; partly read by T. Nozoe at the 16th International Congress of IUPAC in Paris, July 18, 1957.

2) J. H. Birkinshaw and H. Raistrick, *Biochem. J.*, **26**, 441 (1932).

3) A. E. Oxford, H. Raistrick and G. Smith, *Chem. & Ind.*, **61**, 485 (1942).

4) A. W. Johnson, N. Sheppard and A. R. Todd, *J. Chem. Soc.*, **1951**, 1139.

5) G. Aulin-Erdtman, *Acta Chem. Scand.*, **4**, 1325 (1950); **5**, 301 (1951).

6) R. E. Corbett, C. Hassal, A. W. Johnson and A. R. Todd, *J. Chem. Soc.*, **1950**, 1.

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8) a) W. Segal, *Chem. & Ind.*, **1957**, 1040. b) K. Doi and Y. Kitahara have corrected the earlier formulation of stipitatonic acid by W. Segal to that of III. This Bulletin, **31**, 788 (1958).

9) J. H. Birkinshaw, A. R. Chambers and H. Raistrick, *Biochem. J.*, **36**, 242 (1942).

10) R. B. Johns, A. W. Johnson and J. Murray, *J. Chem. Soc.*, **1954**, 198.

11) J. R. Bartels-Keith, A. W. Johnson and W. I. Taylor, ibid., **1951**, 2352.

12) R. B. Johns, A. W. Johnson and M. Tisler, ibid., **1954**, 4605.

13) T. Nozoe et al., *Science Repts. Tohoku Univ., Ser.* I, **38**, 257 (1954).

14) W. D. Crow, R. D. Haworth and P. R. Jefferies, *J. Chem. Soc.*, **1952**, 3705.

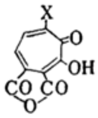
15) D. Caunt, W. D. Crow, R. D. Haworth and C. A. Vodoz, ibid., **1950**, 1631.

16) K. W. Rosenmund and H. Harms, *Ber.*, **53**, 2226 (1920).

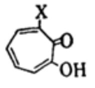
17) Y. Kitahara, *Science Repts. Tohoku Univ., Ser.* **I**, **39**, 258 (1956).

18) G. Barger and O. Dorrer, *Biochem. J.*, **28**, 11 (1934).

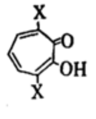
water at 180°C afforded 3-bromo-6-carboxytropolone[19] (XI), a bromination product of 4-carboxytropolone (XII). This fact, together with derivation of 3-hydroxytropolone (VII) from the compound XIII described below, appears to determine the position of the bromine-atom in the molecule X.



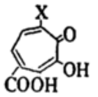
V : X=H
X : X=Br
XIII : X=OH



VI : X=Br
VII : X=OH



VIII : X=Br
IX : X=OH

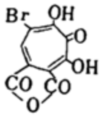


XI : X=Br
XII : X=H
XIV : X=OH

Reaction of the bromo derivative X with sodium β-naphthalenesulfonate in weak alkaline solution for four hours gave 3-hydroxytropolone-4, 5-dicarboxylic anhydride (XIII) in a good yield. The replacement of the bromine-atom in the compound X with hydroxyl group appeared to be more facile than in the cases of 3-bromotropolone (VI) and 3, 7-dibromotropolone (IX), the fact of which indicates probably, that the bromine-atom in the former X is activated by the dicarboxylic anhydride grouping situated at the *p*-position.

It was found, that stipitatonic acid[8a] (III) gives stipitatic acid (IV) under the same condition as that[6] of formation of puberulic acid (II) from puberulonic acid (I). On the contrary, 3-hydroxytropolone derivative XIII did not decarboxylate under the same condition, but caused decarboxylation under heating with water in a sealed tube at 180°C to 5-carboxy-3-hydroxytropolone (XIV), which corresponds to "isostipitatic acid"[10,12], synthesized already by Johnson and others. Subsequent heating of the compound (XIV) at its melting point gave 3-hydroxytropolone (VII). 3-Hydroxytropolone-4, 5-dicarboxylic anhydride (XIII) was also obtained by treatment of the corresponding bromo derivative X with 75% potassium hydroxide or by persulfate oxidation of the anhydride V, the details of which will be reported elsewhere.

It was found, that the monobromination of the hydroxy compound XIII was unexpectedly difficult. Addition of bromine to XIII in acetic acid appeared to result in consumption of about one-half of the calculated amount of bromine, and the successive isolation procedure of the product left only charred matter. This fact recalls an observation of Johnson and others on bromination of isostipitatic acid[10] (XIV). In this series of experiments, however, 7-bromo-3-hydroxytropolone-4, 5-dicarboxylic anhydride (XV) could be obtained by the bromination of XIII in the presence of sodium hydrogen sulfite in a 50% yield.



XV

Fig. 1 shows the ultraviolet absorption spectra of the foregoing four intermediates (V, X, XIII and XV). The absorption maximum near 350 mμ in curves (1) and (2) is respectively split into three absorption peaks in curves (3) and (4), the fact of which is probably characteristic of those of 3-hydroxytropolones[20].

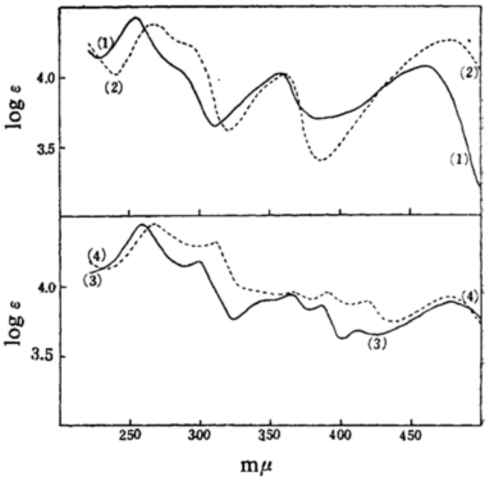


Fig. 1. Ultraviolet absorption spectra in methanol.

(1) : Tropolone-3, 4-dicarboxylic anhydride (V)
(2) : 7-Bromotropolone-3, 4-dicarboxylic anhydride (X)
(3) : 3-Hydroxytropolone-4, 5-dicarboxylic anhydride (XIII)
(4) : 7-Bromo-3-hydroxytropolone-4, 5-dicarboxylic anhydride (XV)

Treatment of the bromo derivative XV with sodium β-naphthalenesulfonate in slightly alkaline solution resulted in formation of puberulonic acid (I) in a 35% yield. The nucleophilic reactivity of the bromine atom in XV seemed to be distinctly smaller than that of the bromine in X. Puberulonic acid (I) synthesized as above did not show any depression of the melting point on admixture with a natural one[21].

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[19] Y. Kitahara, *Science Repts. Tohoku Univ., Ser.* I, **40**, 74 (1956).

[20] T. Nozoe et al., ibid., **37**, 191 (1953); **40**, 121 (1957).

[21] It was separated from a mixture of puberulonic and puberulic acids, m. p. 275°C (decomp.), which Professor J. H. Birkinshaw had kindly sent to us.

Puberulic acid (II), obtained by decarboxylation of the synthesized puberulonic acid, also showed no depression of the melting point on admixture with a natural one[21].

In Fig. 2 are shown the comparisons of the ultraviolet absorption spectra of synthetic puberulonic and puberulic acids with natural ones, and in Table I is shown, that of the infrared absorptions (6 μ bands) of synthetic and natural puberulonic acid.

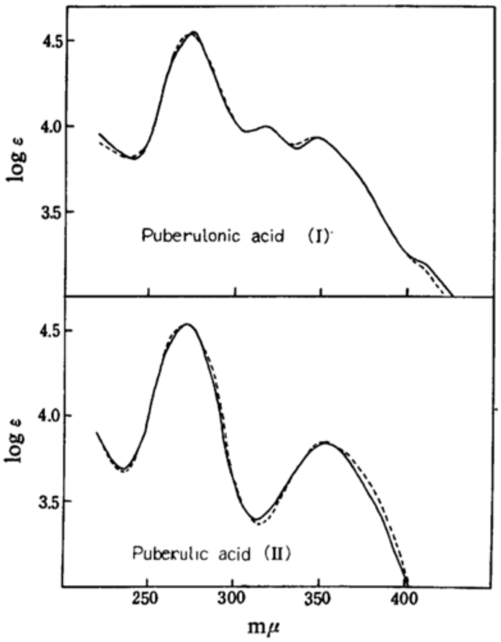

Fig. 2. Ultraviolet absorption spectra in water.
(——) : Synthetic products
(------) : Natural products

TABLE I. COMPARISON OF THE INFRARED ABSORPTION OF SYNTHETIC AND NATURAL PUBERULONIC ACID

Puberulonic acid	M.p.	Main strong bands cm^{-1}				
Synthetic[22]	293°C	1830	1765	1618	1545	KBr
Natural[22]	293°C	1830	1765	1618	1545	KBr
Johnson et al.[4]	298°C	1830	1770	1615	1544	Nujol

Experimental[23]

3-Hydroxytropolone (VII).—A mixture of 3-bromotropolone (VI, 0.60 g.), sodium β-naphthalenesulfonate (0.76 g.), sodium hydroxide (0.4 g.), copper bronze (60 mg.) and water (45 cc.) was heated under reflux for 4 hr. The filtrate, from which copper was removed, was acidified with dilute sulfuric acid to Congo-red and extracted with ethyl acetate (100 cc.). The evaporated residue of the extract, after sublimation in vacuo followed by crystallization from cyclohexane, gave 3-hydroxytropolone (VII, 0.14 g., 44%), m. p. 136°C, undepressed on admixture with an authentic specimen[20].

3, 4-Dihydroxytropolone (IX).—A mixture of 3, 7-dibromotropolone (VIII, 0.28 g.), sodium β-naphthalenesulfonate (0.7 g.), sodium hydroxide (0.2 g.), copper powder (10 mg.) and water (30 cc.) was heated under reflux for 8 hr. The similar procedure as above described, after sublimation in vacuo and subsequent crystallization from ethyl acetate, yielded 3, 4-dihydroxytropolone (IX, 35 mg., 22%), colorless prisms, m. p. 237°C (reported 233∼235°C[18], 237∼238°C[6]).

Found: C, 54.58; H, 4.00. Calcd. for $C_7H_6O_4$: C, 54.55; H, 3.92%.

The ultraviolet absorption, having maxima at 272 mμ (log ε 4.64) and 355 (3.95) in water, was identical with that of the reported decarboxylated puberulic acid[6].

7-Bromotropolone-3, 4-dicarboxylic Anhydride (X).—To a stirred mixture of the dicarboxylic anhydride (V, 2.5 g.), sodium acetate trihydrate (1.8 g.) and acetic acid (40 cc.) was added, dropwise, bromine (2.1 g.) in acetic acid (5 cc.). After being stirred for 2 hr. at room temperature, the mixture was diluted with water, acidified with dilute sulfuric acid to Congo-red, and then extracted with methyl ethyl ketone. The concentrated residue of the extract was crystallized from acetic acid to form 7-bromotropolone-3, 4-dicarboxylic anhydride (X, 3.0 g., 86%), yellow needles, m. p. 197∼198°C. The analytical sample, m. p. 198°C, was obtained after sublimation in vacuo following recrystallization from acetic acid.

Found: C, 39.73; H, 1.42. Calcd. for $C_9H_3O_5Br$: C, 39.85; H, 1.11%.

$\lambda_{\max}^{\text{MeOH}}$ mμ (log ε): 265 (4.38), 362 (4.03), 480 (4.27).

Decarboxylation of X.—A mixture of the bromotropolonedicarboxylic anhydride (X, 70 mg.) and water (3 cc.) was heated in a sealed tube at 180°C for 2 hr. The evaporated residue, after sublimation in vacuo and successive crystallization from methanol, afforded 3-bromo-6-carboxytropolone (XI, 20 mg.), pale yellow prisms, m. p. 233∼234°C, alone or mixed on admixture with an authentic specimen[17].

3-Hydroxytropolone-4, 5-dicarboxylic Anhydride (XIII).—A mixture of the bromo derivative (X, 0.5 g.), sodium β-naphthalenesulfonate (0.42 g.), sodium hydroxide (0.3 g.), copper powder (10 mg.) and water (6 cc.) was heated on a boiling water bath for 4 hr. The filtrate, after copper was removed from the mixture, was acidified with dilute sulfuric acid to Congo-red and extracted with ether for 48 hr. continuously. The evaporated residue of the extract, after being washed with a small volume of methanol, gave 3-hydroxytropolone-4, 5-dicarboxylic anhydride (XIII), yellow prisms, m. p. 254∼255°C. Yield, 0.32 g. (83%). The analytical sample melted at 258°C was obtained by sublimation in vacuo and by successive recrystallization from acetic acid.

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[22] The infrared spectra were measured by a Perkin-Elmer Model 21 Infrared Spectrometer with a rock salt prism.

[23] All melting points are uncorrected.

Found: C, 51.74; H, 2.02. Calcd. for $C_9H_6O_6$: C, 51.93; H, 1.94%.

$\lambda_{\max}^{\text{MeOH}}$ mμ (log ε): 258 (4.44), 300 (4.14), 365 (3.94), 385 (3.88), 410 (3.69), 475 (3.90).

Decarboxylation of XIII. — A mixture of XIII (100 mg.) and water (4 cc.) was heated in a sealed tube at 180°C for 2 hr. The reaction mixture was evaporated and the residual solid was washed with ethyl acetate. The soluble part in the same solvent afforded 3-hydroxytropolone (VII, 15 mg.), undepressed on admixture with an authentic specimen[20]. The less soluble part, after crystallization from water, gave 5-carboxy-3-hydroxytropolone (XIV, 40 mg.), m. p. 279°C (decomp.), of which further heating at its decomposition point yielded 3-hydroxytropolone. The product XIV is assumed to correspond to impure "isostipitatic acid"[10,12], having m. p. of 282°C.

7-Bromo-3-hydroxytropolone-4, 5-dicarboxylic Anhydride (XV).—To astirred mixture of the hydroxytropolone-dicarboxylic anhydride (XIII, 0.21 g.), sodium acetate trihydrate (0.14 g.) and acetic acid (4 cc.), was added dropwise a solution of bromine (0.16 g.) in acetic acid (1 cc.) at room temperature. At the point of dropping of about one-half volume of bromine, sodium hydrogen sulfite (20 mg.) was added, further bromination being followed. Then, the reaction proceeded smoothly and the reaction mixture became a transparent solution, which was allowed to evaporate in vacuo over an alkali-desiccator. The residue was diluted with water, acidified with dilute sulfuric acid to Congo-red, and extracted with methyl ethyl ketone. The residue obtained after removal of the solvent crystallized from acetic acid to give the bromo derivative (XV, 0.16 g.), yellow needles, m. p. 236~237°C (decomp). The analytical sample melted at 240°C (decomp.) was obtained by sublimation in vacuo followed by recrystallization from acetic acid.

Found: C, 38.00; H, 1.27. Calcd. for $C_9H_3O_6Br$: C, 37.66; H, 1.05%.

$\lambda_{\max}^{\text{MeOH}}$ mμ (log ε): 266 (4.44), 310 (4.31), 365 (3.96), 390 (3.96), 420 (3.89), 480 (3.92).

Puberulonic Acid (I).—A mixture of the above bromo compound (XV, 0.57 g.), sodium β-naphthalenesulfonate (0.7 g.), sodium hydroxide (0.56 g.), copper powder (20 mg.) and water (7 cc.) was heated on a boiling water-bath for 8 hr. The solution obtained by removal of copper was acidified with dilute sulfuric acid to Congo-red and extracted wih ether for 48 hr. continuously. The evaporated residue of the extract crystallized to give yellow solids, m. p. 265~270°C (decomp.), of which washing with a small volume of methanol, sublimation in vacuo and subsequent recrystallization from methanol gave puberulonic acid (I, 15 mg., 35%), yellow crystals, m. p. 293°C (decomp.), undepressed on admixture with natural puberulonic acid[21].

Found: C, 48.04; H, 1.62. Calcd. for $C_9H_4O_7$: C, 48.23; H, 1.80%.

The ultraviolet absorption (in water) of the synthesized puberulonic acid (I) has maxima at 275 (4.54), 317 (4.00) and 447 mμ (log ε 3.94), identical with those of the authentic specimen[6].

Puberulic Acid (II).—A mixture of the above synthesized puberulonic acid (I, 80 mg.) and water (2 cc.) was heated under reflux for one hour. The evaporated residue of the mixture was dissolved in sodium hydrogen carbonate solution and treated with active charcoal. The solution obtained by removal of the charcoal crystallized by acidification with dilute sulfuric acid to give puberulic acid (II, 45 mg.), colorless crystals, m. p. 317~318°C, not depressed on admixture with natural puberulic acid[21].

Found: C, 48.37; H, 2.96. Calcd. for $C_8H_6O_6$: C, 48.49; H, 3.05%.

The ultraviolet absorption spectrum (in water), having maxima at 274 (4.54) and 352 mμ (log ε 3.84), was identical with that of the authentic specimen[6]. Heating of the synthesized puberulic acid (II) at 350°C and subsequent sublimation in vacuo afforded 3,4-dihydroxytropolone (IX), undepressed on admixture with an authentic specimen.

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