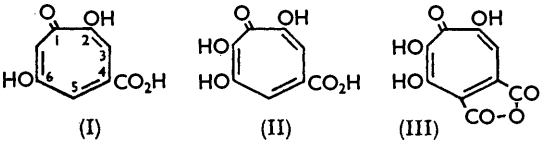
571. *Stipitatonic Acid. A New Mould Tropolone from* Penicillium stipitatum *Thom.**

By W. SEGAL.

Stipitatonic acid (6-hydroxytropolone-4,5-dicarboxylic acid anhydride) has been isolated from the Czapek–Dox culture fluid of *Penicillium stipitatum*. Its properties and structure are analogous to those of puberulonic acid. Partial decarboxylation of stipitatonic acid produces stipitatic acid. This accumulates in the culture fluid at the expense of the stipitatonic acid.

As a preliminary to the study of the biosynthesis of stipitatic acid, the mould tropolone elaborated by *P. stipitatum*,[1] a method of assaying the metabolite was investigated. As a result a new mould tropolone, stipitatonic acid, has been isolated from the culture fluid.

Reviews [2] have described the chemistry of the three known mould tropolones, namely, stipitatic (I), puberulic (II), and puberulonic acid (III). To date the mould tropolones have been reported as metabolites of the genus *Penicillium* only: puberulic and puberulonic acid from *P. puberulum* Bainier,[3] *P. aurantio-virens* Biourge,[3,4] *P. Johannioli* Zaleski, and *P. cyclopium-viridicatum* series; [5] stipitatic acid from *P. stipitatum* Thom [1] only.



(I) (II) (III)

Stipitatonic acid, $C_9H_4O_6$, was obtained as bright yellow prisms from the culture fluid by concentration under reduced pressure, acidification, and ether-extraction. Stipitatic acid is extracted simultaneously but the new metabolite can be separated by selective sublimation. The composition, colour, and colour reactions of stipitatonic acid suggested its relation to stipitatic acid (I) in the manner [6] of puberulonic (III) to puberulic acid (II). This was confirmed by the quantitative conversion of stipitatonic into stipitatic acid under the conditions reported [7] for converting puberulonic acid into puberulic acid, namely, refluxing in aqueous solution.

Puberulonic acid condenses with *o*-phenylenediamine,[4b] giving a compound, $C_{15}H_8O_5N_2$, which was assigned structure (IV; R = OH) when the anhydride nature of puberulonic acid was established spectroscopically.[6b,8,9] By similar treatment stipitatonic acid gives a refractory vermilion-coloured compound, $C_{15}H_8O_4N_2$, analogous to the puberulonic acid condensation compound. Crow, Haworth, and Jefferies [10] obtained from tropolone-3,4-dicarboxylic anhydride and *o*-phenylenediamine a brown crystalline product which could not be recrystallized; they suggested that it was probably an *N*-*o*-aminophenylimide (V). Stipitatonic acid also produces an intermediate condensation compound (probably $C_{15}H_{10}O_5N_2$) analogous to the compound described by Crow *et al.*[10] Attempts to recrystallize it were unsuccessful and heating it under reflux in a large volume of ethanol

* Briefly reported in part in *Chem. and Ind.*, 1957, 1040; 1958, 1726.

[1] Birkinshaw, Chambers, and Raistrick, *Biochem. J.*, 1942, **36**, 242.

[2] Nozoe, *Fortschr. Chem. org. Naturstoffe*, 1956, **13**, 232; Pauson, *Chem. Rev.*, 1955, **55**, 9; Cook and Loudon, *Quart. Rev.*, 1951, **5**, 99.

[3] Birkinshaw and Raistrick, *Biochem. J.*, 1932, **26**, 441.

[4] (*a*) Barger and Dorrer, *ibid.*, 1934, **28**, 11; (*b*) Corbett, Hassall, Johnson, and Todd, *J.*, 1950, 1.

[5] Oxford, Raistrick, and Smith, *Chem. and Ind.*, 1942, **41**, 485.

[6] (*a*) Aulin-Erdtman and Theorell, *Acta Chem. Scand.*, 1950, **4**, 1490; (*b*) Johnson, Sheppard, and Todd, *J.*, 1951, 1139.

[7] Corbett, Johnson, and Todd, *J.*, 1950, 6.

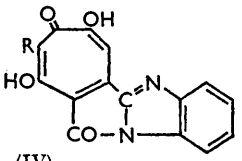
[8] Aulin-Erdtman, *Acta Chem. Scand.*, 1951, **5**, 301.

[9] *Idem, ibid.*, 1950, **4**, 1325.

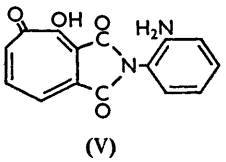
[10] Crow, Haworth, and Jefferies, *J.*, 1952, 3705.

gave the vermilion-coloured product. This conversion supports the structure (V) suggested by Crow *et al.*

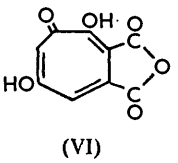
In dioxan the ultraviolet absorption of stipitatonic acid is highly comparable with that of puberulonic acid, the bands for the latter being at somewhat longer wavelengths. Aulin-Erdtman [8] has drawn attention to the unsuitability of water of undefined pH as solvent for measuring the detailed absorption spectra of easily ionizable solutes. It appears that



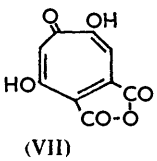
(IV)



(V)



(VI)

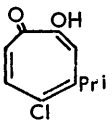


(VII)

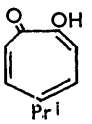
stipitatonic acid is more highly dissociated in water than in dioxan. The spectrum of an aqueous solution (yellow) has a relatively feeble band at 432 mμ. The spectrum of a dioxan solution of comparable concentration (colourless) lacks this band. Addition of water to a colourless dioxan solution produces a marked yellow colour.

The infrared absorption spectrum of stipitatonic acid in " Nujol " mull shows a doublet at 1642 and 1621 cm.$^{-1}$ and a band at 1599 cm.$^{-1}$ [assigned to the tropolone nucleus (cf. doublet at 1639 and 1626 and band at 1577 cm.$^{-1}$ for tropolone-3,4-dicarboxylic anhydride [10])], and intense bands at 1746 and 1824 cm.$^{-1}$ (anhydride); the separations of the anhydride peaks for spectra of puberulonic acid [6b,9] and tropolone-3,4-dicarboxylic anhydride [10] (60 and 45 cm.$^{-1}$ respectively) are somewhat less than for stipitatonic acid.

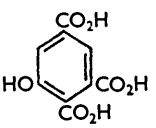
These reactions and properties of stipitatonic acid may be explained by the structures (VI) or (VII). Coupling of tropolones with diazonium salts has been claimed by Pauson [2] as a test for a vacant 5-position in tropolones. An alkaline solution of stipitatonic acid with diazotized *p*-toluidine gave (on acidification) a reddish-brown amorphous solid which, however, was not an azo-compound; attempts to crystallize it produced tars or gums. Stipitatic acid, which possesses a vacant 5-position, gave a similar but paler amorphous solid. In this respect stipitatic and stipitatonic acid are similar to 5-chloro-hinokitiol (VIII) which gives reddish-brown amorphous products with diazonium salts.[11] In addition γ-thujaplicin (IX) affords [12] " red products " on treatment with diazotized amines.



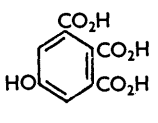
(VIII)



(IX)



(X)



(XI)

The isomerization of tropolones to benzenoid compounds by alkali [2] has on occasions been carried out under very drastic conditions (potassium hydroxide at 300°); though the possibility of migration of groups should not be overlooked, this has not been reported for tropolonoid compounds and potash fusion affords unambiguous preparations of phenol-polycarboxylic acids from the corresponding sulphonated acids. Crow *et al.*[10] reported the isomerization of tropolone-3,4-dicarboxylic anhydride to hemimellitic acid by fusion with sodium hydroxide. Fusion of stipitatonic acid with potassium hydroxide yielded a phenolic acid which on methylation afforded trimethyl 6-methoxybenzene-1,2,4-tricarboxylate.[13] Thus the phenolic acid has structure (X), and stipitatonic acid has structure (VII). The isomeric structure (VI) would be expected to produce the acid (XI), the fully methylated derivative [13] of which melts at 85°, 60° lower than that of the fully methylated acid (X).

[11] Nozoe, Mukai, and Takase, *Proc. Japan Acad.* 1951, **27**, 236.
[12] Erdtman and Gripenberg, *Acta Chem. Scand.*, 1948, **2**, 625.
[13] Gardner, Grove, and Ismay, *J.*, 1954, 1817.

On this basis the *o*-phenylenediamine condensation compound of stipitatonic acid has structure (IV; R = H) or the isomeric structure in which the 5-substituent is bonded to both nitrogen atoms. The intermediate compound is probably the corresponding *N*-*o*-aminophenylimide analogous to (V).

Rate of Production of Stipitatonic Acid.—The concentration of stipitatonic acid in the culture medium reaches a maximum after approximately 25 days; thereafter that of stipitatic acid increases at the expense of the stipitatonic acid. In spite of the instability of stipitatonic acid its conversion into stipitatic acid could be avoided by isolating the metabolites from the samples of acidified culture fluid maintained at room temperature throughout the determination. The quantitative partial decarboxylation and determination of the carbon dioxide produced served as a means of assaying the stipitatonic acid present in the ether-extracted metabolites. Examination of the extracts * by sublimation, fractional crystallization, and paper chromatography failed to detect the presence of more than traces of other components. The results of the determination are summarized in the Table.

Rate of production of stipitatonic acid by P. stipitatum *Thom. growing on Czapek–Dox medium at* 24°.

Incubation period (days)	pH	Residual glucose (%)	Yield of ether-soluble extract (g./l.)	Stipitatonic acid in medium (g./l.)	Stipitatonic acid in extract (%)	Stipitatic acid in extract (by diff.) (%)
8	3·97	4·0	0·108	0·036	33·4	—
13	4·10	3·5	0·308	0·166	54·1	—
25	3·90	2·2	1·138	0·882	77·5	22·5
33	3·81	1·5	1·309	0·535	40·8	59·2
35	3·81	1·5	1·023	0·370	36·1	63·9
39	3·74	1·1	1·233	0·259	21·0	79·0

Experimental

The strain of *Penicillium stipitatum* Thom used (L.S.H.T.M. Cat. No. P 199) has been described by Birkinshaw *et al.*[1] The cultural conditions were those described by them.

Isolation and Purification of Stipitatonic Acid.—After 25 days' growth the filtered Czapek–Dox metabolism solution (35 l.) was concentrated under reduced pressure at 45—50° to about 2·5 l., after which it was cooled to 0° and acidified with sulphuric acid (30% v/v) until the pH was 2·0. It was then exhaustively extracted with ether in a continuous liquid–liquid extractor until the extract issuing became colourless (5—6 days). During the extraction a mixture of stipitatic and stipitatonic acid separated, and after removal of this and drying of the filtrate (Na_2SO_4), the remaining solutes were recovered by removal of the ether at reduced pressure (total yield of crude solids, 15·5 g.). Hot acetone removed most of the stipitatonic acid from the powdered extract. The stipitatic acid simultaneously dissolved was partially precipitated by concentrating the acetone extract. Excessive concentration to remove stipitatic acid caused co-precipitation of stipitatonic acid. The solids in the acetone solution were recovered by evaporation at reduced pressure on the water-bath. Sublimation of the residue in a high vacuum at $<130°$ separated stipitatonic (1·2 g.) from stipitatic acid which sublimed only at 190—200° at the same pressure. The yield of stipitatonic acid here is lower than indicated in the Table, apparently owing to decomposition during the concentration of the metabolite solution. The yellow sublimate was crystallized from acetone and *stipitatonic acid* obtained as bright yellow prisms, m. p. 237—237·5° (decomp.) (Found: C, 52·1; H, 2·2. $C_9H_4O_6$ requires C, 51·9; H, 1·9%), $\lambda_{max.}$ in distilled water 253, 333, 369, 432 mμ (log ε 4·45, 3·81, 3·94, 3·36 respectively), in dioxan 258, 355, 382 mμ (log ε 4·39, 3·98, 3·97 respectively). Stipitatonic acid is readily soluble in cold water producing a deep yellow solution, stable for several days at room temperature. It is insoluble in light petroleum, sparingly soluble in hot chloroform, carbon tetrachloride, and benzene, and slightly soluble in ether and ethyl acetate. Addition

* The extracts after 8 and 13 days' inoculation were gummy and contained intractable components. Subsequent extracts possessed the properties of a mixture of stipitatic and stipitatonic acid.

of sodium hydrogen carbonate solution to an aqueous solution of the acid produces a deepening of the colour which fades slowly. Acidification of the colourless solution partially restores the original yellow colour. Aqueous neutral ferric chloride produces a light-brown stable colour with aqueous solutions of the acid. The solid acid is very slowly soluble in concentrated sulphuric acid with no marked colour change.

Conversion into Stipitatic Acid.—Stipitatonic acid (71·0 mg.) in air-free distilled water (10 ml.) was heated for 8 hr. under reflux while nitrogen (free from carbon dioxide and oxygen) was passed through the solution and bubbled through two cells of standard barium hydroxide solution (10·00 and 5·00 ml.). Carbon dioxide was evolved after a few minutes and the solution became progressively paler during the heating. On cooling, cream-coloured needles separated and the hydrolysis product was collected at 0° and dried to constant weight (59·3 mg.). Evaporation of the filtrate on the water-bath at reduced pressure afforded a cream-coloured solid (3·5 mg.) (total yield, 62·8 mg.; the process $C_9H_4O_6 + H_2O \longrightarrow C_8H_6O_5 + CO_2$ requires 62·1 mg.). Back-titration of the barium hydroxide solution with standard hydrochloric acid indicated that $3{\cdot}45 \times 10^{-4}$ mole of carbon dioxide was produced (theory. $3{\cdot}41 \times 10^{-4}$ mole). After sublimation *in vacuo* at 190°, the hydrolysis product had m. p. 302—304° (decomp.), undepressed on admixture with stipitatic acid. Colour reactions with ferric chloride, sodium hydroxide, and concentrated sulphuric acid were identical with those of stipitatic acid, and the ultraviolet absorption spectrum was identical with that of this acid. Acetylation (sodium acetate as catalyst) gave diacetylstipitatic acid of m. p. 172° (decomp.), identical (mixed m. p.) with a specimen prepared as described by Birkinshaw *et al.*[1]

Condensation Compounds of Stipitatonic Acid with o-*Phenylenediamine.*—o-Phenylenediamine (60 mg.) and stipitatonic acid (107 mg.) in ethanol (15 ml.) were heated on the water-bath for 3·5 hr. Brown plates soon separated and were collected (103 mg.); they included a few small reddish needles. Attempts to recrystallize the product from a large volume of ethanol converted it into vermilion-coloured needles which had no definite m. p. but charred slowly above 300° and were not molten at 350°. The sample partially sublimed in the m. p. tube at about 300°, giving dark red needles. The vermilion-coloured condensation product {7,10-*dihydroxy*-8,11 *dioxobenzo*[d]*cyclohepta*[3,4]*pyrrolo*[1,2-a]*imidazole* (IV) or the 6,9-*dihydroxy-isomer*} was difficult to purify and was virtually insoluble in the common organic solvents (Found: C, 63·6; H, 2·8; N, 9·6. $C_{15}H_8O_4N_2$ requires C, 64·2; H, 2·9; N, 10·0%). The brown crystalline intermediate condensation product (N-o-*aminophenyl*-3,6-*dihydroxy*-3-*oxocyclohepta*-1,5,6-*triene*-1,2-*dicarboxyimide*), decomp. >320°, was also difficult to purify and sparingly soluble in the common organic solvents (Found: N, 9·6. $C_{15}H_{10}O_5N_2$ requires N, 9·4%). It could not be recrystallized.

Fusion of Stipitatonic Acid with Potassium Hydroxide and Methylation of the Product.—Stipitatonic acid (80 mg.) was dissolved in water (1 ml.) in a nickel crucible, and potassium hydroxide (2 g.) added. The crucible was heated in a Woods-metal bath slowly and kept at a bath-temperature of 310° for 15 min., during which the melt remained clear, showing no tendency to char. After cooling, distilled water was added and the ice-cold solution acidified with hydrochloric acid. Continuous extraction in a liquid–liquid extractor with ether, drying of the extract (Na_2SO_4), and removal of the solvent afforded a white solid acid (70 mg.). Sublimation in a high vacuum gave first oxalic acid, m. p. and mixed m. p. 188°, then at 180° a pale yellow compact mass. This was probably an anhydride as it readily dissolved in water to a yellow, highly fluorescent solution which rapidly became colourless on gentle warming. The corresponding acid was obtained as white crystals, m. p. 278—281°, by removal of the water at room temperature. It gave an intense cherry-red colour with aqueous ferric chloride and a positive fluorescein reaction on fusion with resorcinol, but not by the wet reaction involving concentrated sulphuric acid. With ethereal diazomethane in the presence of methanol it afforded white crystals which sublimed in a high vacuum at 100°. Crystallization from ethanol gave trimethyl 6-methoxybenzene-1,2,4-tricarboxylate as prisms, m. p. 145°, identical in m. p., mixed m. p., and infrared absorption spectrum with a specimen prepared by Gardner *et al.*[13] (Berner[14] reports m. p. 144°) (Found: C, 55·6; H, 4·8. Calc. for $C_{13}H_{14}O_7$: C, 55·3; H, 5·0%).

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[14] Berner, *J.*, 1946, 1052.

for determining the infrared absorption spectrum of stipitatonic acid, and to Mr. J. F. Grove (Imperial Chemical Industries Limited, Akers Research Laboratories) for an authentic specimen of trimethyl 6-methoxybenzene-1,2,4-tricarboxylate and for determining the infrared absorption spectrum of this compound derived from stipitatonic acid. This work has been done during the tenure of an Imperial Chemical Industries Research Fellowship.

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