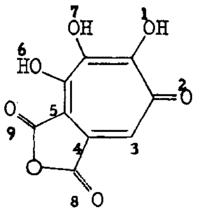
TROPOLONE BIOGENESIS

John H. Richards and Louis D. Ferretti

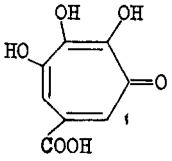
Gates and Crellin Laboratories of Chemistry*
California Institute of Technology, Pasadena, California

Received February 2, 1960

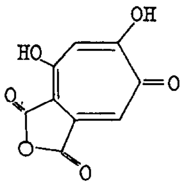
The discovery of the tropolones puberulonic acid (I) and puberulic acid (II) (Birkinshaw and Raistrick, 1932) and of stipitatic acid (IV) (Birkinshaw _et al._, 1942) opened an area in chemistry which presented a difficult structural problem until the tropolone ring system was postulated (Dewar, 1945) as a structural feature of these mold metabolites. Recently a fourth member, stipitatonic acid (III), has been added (Segal, 1957, 1958; Doi and Kitahara, 1958) to this list.



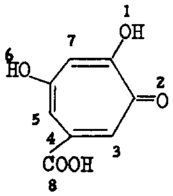
I



II



III



IV

The occurrence of these curious structures in nature has prompted a great deal of speculation on the biogenetic origin of the mold tropolones (Robinson, 1955; Seshadri, 1955; Birch, 1957; Bentley, 1958; Tannenbaum, _et al._, 1959). The currently popular schemes envision tropolone biogenesis not to be the result of any known mode of sugar or acetate metabolism (Bentley, 1958) but postulate, for example, the cyclization of an octulonic acid phosphate derivative (Tannenbaum _et al._, 1959). It is the purpose of this note to

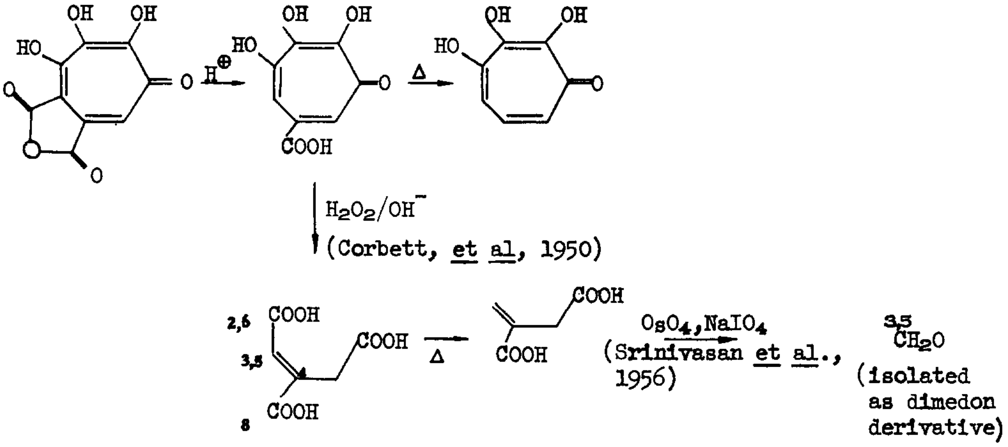
---

[*] Contribution No. 2546

present the results of a study on the incorporation of certain labeled substrates into puberulic acid and puberulonic acid by P. aurantio-virens Biourge and, on the basis of these results, to propose that the tropolones arise via a direct pathway from acetate and formate.

A Czapek-Dox medium containing 1-C^{14} acetate or 2-C^{14} acetate or 1-C^{14} glucose was inoculated with P. aurantio-virens Biourge (77) CBS R2138 (Birkinshaw and Raistrick, 1932). Puberulic acid and puberulonic acid were isolated and separated (Barger and Dorrer, 1934).

The acids were degraded according to the following sequence.



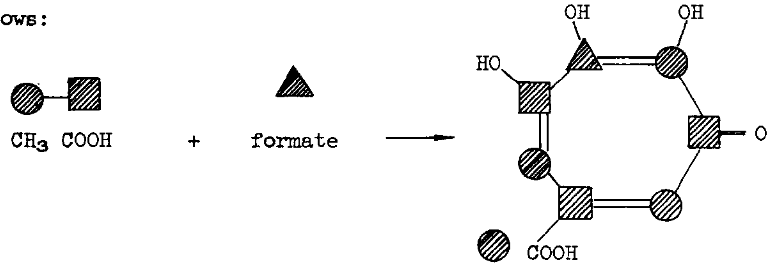
The activities of the various degradation products were determined by combustion of the sample to carbon dioxide and counting in an ion chamber by means of a vibrating reed electrometer. The results obtained (expressed as % activity in the designated carbon atoms relative to that of puberulic acid) are: from 1-C^{14} glucose $C_1 + C_7 = 41 \pm 4\%$, $C_3 + C_5 = 36 \pm 4\%$, $C_8 = 23 \pm 5\%$; from 2-C^{14} acetate $C_1 + C_7 = 24 \pm 4\%$; $C_3 + C_5 = 54 \pm 4\%$, $C_8 = 22 \pm 5\%$; from 1-C^{14} acetate $C_2 + C_6 = 76 \pm 12\%$; $C_4 = 24 \pm 14\%$. In those cases where the same precursors were used, these results are in agreement, within the limits of experimental error, with those published for stipitatic acid (Bentley, 1958).

That C_7 of stipitatic acid originates in formate has been demonstrated (Bentley, 1958). We interpret our results with 2-C^{14} acetate as indicating that C_1, C_3, C_5 and C_8 are derived from the methyl carbon of acetate and propose that the results with 1-C^{14} glucose are a combination of those obtained with 1-C^{14} acetate and C^{14} formate, reflecting a dissimilation of glucose to the level of one and two carbon intermediates before incorporation into the tropolone. This proposal accounts for the finding (Tannenbaum et al., 1959) that the stipitatic acid has an activity almost five times as great as that of the phenylalanine or the tyrosine isolated from P. stipitatum grown on a substrate of 1-C^{14} glucose.

In the interpretation of the earlier results it was assumed (Bentley, 1958) that the base catalyzed rearrangement of stipitatic acid will extrude, to a significant extent, both C_1 and C_2 into the carboxyl carbons of the 5-hydroxybenzene-1,3-dicarboxylic acid product. However, under the conditions of the rearrangement, the carboxyl group will probably be ionized, rendering more facile the migration of C_7, which is removed from electrostatic influence of the carboxylate anion, than of C_3, which is close to this anionic center. This will result in the preferential (not necessarily exclusive) occurrence' of C_1 as the new carboxyl carbon in the isophthalic acid derivative.

These remarks remove the implication (Bentley, 1958) that C_2 cannot originate in the carboxyl carbon of acetate. In fact, we suggest that the correct interpretation of the experimental data is that C_2, C_4 and C_6 are all derived from the acetate carboxyl carbon.

We therefore propose that puberulic acid biogenesis may be represented as follows:



Stipitatic acid would have an analogous origin. The presence of a decarboxylase in P. stipitatum has been demonstrated (Bentley and Thiessen, 1959) and it has been proposed that the nine carbon metabolites are intermediates preceding the eight carbon tropolone carboxylic acids. The only remaining question is the origin of C_9. It is not derived from either carbon of acetate and we suggest that it represents the incorporation of an additional one carbon fragment, e.g. formate. The question of whether the seven membered tropolone ring is formed by an expansion of some cyclic six membered precursor or by a direct cyclization of a seven carbon chain is not resolved by these experiments.

REFERENCES

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

Bentley, R., Biochem. Biophys. Acta, 29, 666 (1958).

Bentley, R. and Thiessen, Nature, 184, 552 (1959).

Birch, A. J., Fortschritte der Chemie Organischer Naturstoffe, 14, 186 (1957).

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

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

Corbett, R. E., Johnson, A. W. and Todd, A. R., J. Chem. Soc. 1950, 6.

Dewar, M. J. S., Nature, 155, 50 (1945).

Doi, K. and Kitahara, Y., Bull. Chem. Soc., Japan, 31, 788 (1958).

Robinson, R., "Structural Relationships of Natural Products," Clarendon Press, Oxford, 1955, pg. 57.

Segal, W., Chem. and Ind., 1957, 1040.

Segal, W., Chem. and Ind., 1958, 1726.

Seshadri, T. R., J. Sci. Indus. Res. (India) B14, 248 (1955). Cf. Stickings, C. E. and Raistrick, H., Ann. Rev. of Biochem. 25, 236 (1956).

Srinivasan, P. R., Shigeura, H. T., Sprecher, M., Sprinson, D. B. and Davis, B. D., J. Biol. Chem., 220, 477 (1956).

Tannenbaum, S. W., Bassett, E. W., and Kaplan, M., Arch. Biochem. Biophys., 81, 169 (1959).