TROPOLONE BIOSYNTHESIS: ORIGIN OF THE CARBOXYL GROUPS OF STIPITATONIC AND STIPITATIC ACIDS

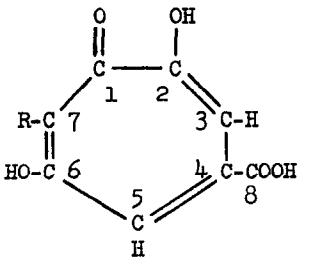
Ronald Bentley

Department of Biochemistry and Nutrition, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

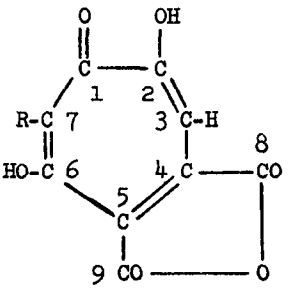
Received August 8, 1960

A study of the biosynthesis of stipitatic acid (I, R = H), by Penicillium stipitatum NRRL 2104, has shown that acetate has a major role while formate is the precursor of C_7 (Bentley, 1958). It has now been determined that stipitatonic acid (II, R = H; Segal, 1959) is also formed by strain NRRL 2104 but was destroyed under the strongly acid conditions used to isolate stipitatic acid. The enzymatic decarboxylation of stipitatonic acid has also been described (Bentley and Thiessen, 1959).

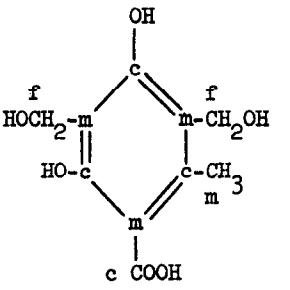
To investigate the origin of C_8 and C_9 in stipitatonic acid, P. stipitatum NRRL 2104 was grown on 1-C^{14}-glucose from the time of inoculation, or on ordinary glucose with subsequent addition of labeled acetates on the seventh day of growth. The percentage recoveries of added C^{14} in the combined tropolones were as follows; glucose-1-C^{14}, 5.1; acetate-1-C^{14}, 20.0; acetate-2-C^{14}, 34.6. The culture medium, originally 700 ml., was adjusted to pH 6.7 and concentrated in vacuum to about 50 ml. Stipitatic acid precipitated on addition of HCl to pH 3.5 and was completely removed by subsequent continuous ether extraction. On lowering the pH to 2.0, a crystalline, orange-yellow precipitate separated slowly on standing at 0°. This material recrystallized readily from water as beautiful orange-yellow plates and is a mono-sodium salt of the di-acid form of stipitatonic acid (Anal. Calcd. for $C_9H_5O_7Na$; Na, 9.3%. Found; Na, 9.6%). It was readily converted to stipitatonic acid either by trituration with 3N HCl or by treatment with Amberlite IR 120 (H) in aqueous solution.



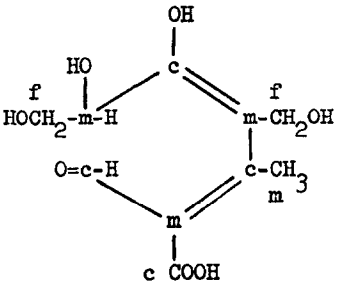
I



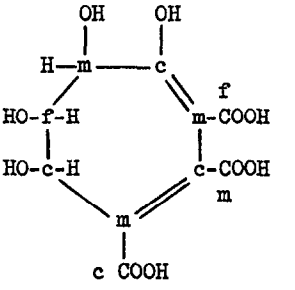
II



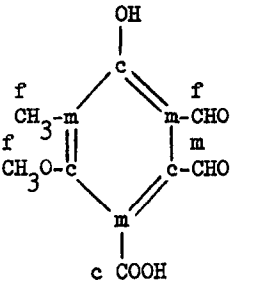
III



IV



V



VI

The labeled stipitatonic acid samples, purified by sublimation, were decarboxylated (Corbett et al., 1950) to yield C_9 as $BaCO_3$ (87 - 90% of theoretical) and crude stipitatic acid. After recrystallization, stipitatic acid was decarboxylated (Bentley, 1958) yielding C_8 as $BaCO_3$. The results of these experiments are summarized in Table I; it is clear that C_8 of stipitatonic acid is derived from the methyl carbon of acetate and C_9 from the carboxyl carbon.

TABLE I

	% Total radioactivity		
Carbon of stipitatonic acid	Glucose-1-C^{14}	Acetate-2-C^{14}	Acetate-1-C^{14}
C_8	19	25	4
C_9	5	1	26

Richards and Ferretti (1960) have described partial chemical degradations of labeled puberulic acid (I, R = OH) and puberulonic acid (considered to be II, R = OH) from P. aurantio-virens. They have concluded that C_1, C_3,

C_5 and C_8 are derived from the methyl carbon of acetate, as is the case in stipitatic acid, and C_2, C_4 and C_6 from acetate carboxyl. Our previous conclusion that only C_4 and C_6 of stipitatic acid are derived from acetate carboxyl rested on the assumption that the new carboxyl formed on benzenoid re-arrangement of stipitatic acid was derived equally from C_1 and C_2. Richards and Ferretti have now suggested that the electrostatic influence of the COOH group may result in the preferential elimination of C_1 as the new COOH group; in this event, C_2, C_4 and C_6 of stipitatic acid are derived from acetate carboxyl. Although this suggestion is without experimental support, considerations presented later relating tropolone and other aromatic syntheses indicate that the conclusion may be valid. Despite these general similarities in the two tropolone series, a major difference is apparent in the origin of the labile carboxyl group, C_9. In stipitatonic acid, this group is unambiguously derived from acetate carboxyl*; in puberulonic acid, neither acetate carbon serves as a precursor, leading Richards and Ferretti to suggest a C_1 precursor such as formate for C_9, and also by analogy with stipitatic acid, for C_7.

The different origin of the labile carboxyl groups of stipitatonic and puberulonic acids, at first puzzling, may be understood when the precise structures of these acids are considered. Although stipitatonic acid is unambiguously the 4,5-dicarboxylic acid, the precise location of the tropolone function on the four OH groups of puberulonic acid is less well understood. There are, in fact, marked differences between the two anhydrides. We have observed that whereas stipitatonic acid reacts readily with methanol, losing CO_2 and forming the methyl ester of stipitatic acid, puberulonic acid is much more stable; further, tropolone-3,4-dicarboxylic anhydride is unaffected by hot alcohol, alone or in the presence of acids (Crow et al., 1952). In tropolone 3,4-dicarboxylic acids, and in puberulonic acid, the separation of the carbonyl frequencies in the IR spectra is usually 60 cm^{-1} (Doi and Kitahara, 1958); in stipitatonic acid, this difference is 78 cm^{-1}. It is therefore most likely that puberulonic acid is to be regarded as the anhydride of 6,7-dihydroxy-tropolone-3,4-dicarboxylic acid and not, as indicated earlier, as II, R = OH. A different biogenetic origin for the carboxyl of puberulonic acid attached to C_3 and the carboxyl of stipitatonic acid attached to C_5 is therefore reasonable.

An interesting possibility is that orsellinic acid, or a biologically active derivative, may be the precursor of the tropolones as originally postulated by Seshadri (1955) but by a rather different mechanism. Addition of two C_1 units to orsellinic acid yields III; m and c indicate the methyl and

---

*In a personal communication, Professor J. H. Birkinshaw has notified me of a similar observation in his laboratories.

carboxyl atoms of acetate and f, a carbon derived from formate. Ring fission similar to that observed in patulin biosynthesis (Bu'Lock and Ryan, 1958; Tanenbaum and Bassett, 1959) would lead to IV and a re-cyclization to the seven membered ring compound, V. At some stage, appropriate oxidations of CH_3 and CH_2OH to COOH must be presumed. Stipitatonic acid would be formed from V by loss of H_2, H_2O and the formate derived COOH; puberulonic acid by loss of 2 H_2 and the acetate carboxyl derived COOH. Alternatively, a common precursor for puberulonic and stipitatonic acids is not mandatory and stipitatonic acid might be derived by a single C_1 addition to orsellinic acid. The labeling pattern in III is identical to that postulated for cyclopaldic acid (VI; Birch and Kocor, 1960) and III might be a common precursor for cyclopolic and cyclopaldic acids as well as the mold tropolones. An analogous case of a benzenoid compound carrying groups derived from formate (2) and methyl and carboxyl of acetate in the same sequence is found in citrinin (Schwenck et al., 1958) and with one less formate derived group in mycophenolic acid (Birch et al., 1958 a,b).

In an attempt to derive support for this hypothesis, carboxyl labeled orsellinic acid has been prepared and added to a P. stipitatum culture growing on non-labeled glucose. Most of the added radioactivity was recovered from the culture medium by ether extraction at pH 3.5 and there was only a very slight incorporation into stipitatonic acid. It is possible that this negative result is due to the fact that orsellinic acid itself does not penetrate the mold mycelium and the hypothesis cannot be abandoned without further investigation.

REFERENCES

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

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

Birch, A. J., English, R. J., Massy-Westropp, R. A., Slayton, M. and Smith, H., J. Chem. Soc., 365 (1958 a).

Birch, A. J., English, R. J., Massy-Westropp, R. A. and Smith, H., J. Chem. Soc., 369 (1958 b).

Birch, A. J. and Kocor, M., J. Chem. Soc., 866 (1960).

Bu'Lock, J. D. and Ryan, A. J., Proc. Chem. Soc., 222 (1958).

Corbett, R. E., Hassall, C. H., Johnson, A. W. and Todd, A. R., J. Chem. Soc., 1 (1950).

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

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

Richards, J. H. and Ferretti, L. D., Biochem. Biophys. Res. Comm., 2, 107 (1960).

Schwenck, E., Alexander, G. J., Gold, A. M. and Stevens, D. F., J. Biol. Chem., 233, 1211 (1958).

Segal, W., J. Chem. Soc., 2847 (1959).

Seshadri, T. R., J. Sci. Indus. Res., India, 14 B, 248 (1955).

Tanenbaum, S. W. and Bassett, E. W., J. Biol. Chem., 234, 1861 (1959).