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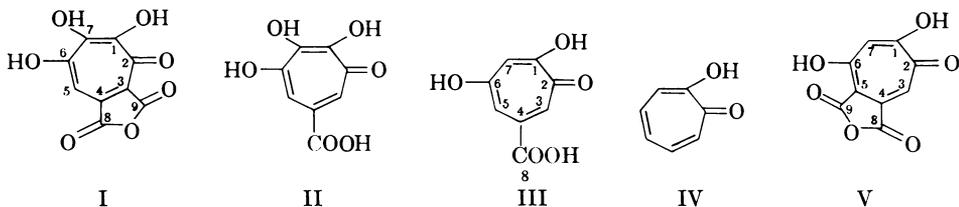
# THE BIOGENESIS OF THE MOLD TROPOLONES\*

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The discovery of the mold metabolites puberulonic acid (I) and puberulic acid (II)<sup>1</sup> and stipitatic acid (III)<sup>2</sup> presented a difficult structural problem to organic chemistry until, in 1945, Dewar<sup>3</sup> made the ingenious proposal of the existence of tropolone (IV) as a new aromatic system. Dewar<sup>3</sup> correctly assigned the structure III to stipitatic acid. The work of Todd and his collaborators<sup>4</sup> led to I for puberulic acid. Aulin-Erdtman<sup>5, 6</sup> and Todd *et al.*<sup>7</sup> showed puberulonic acid to be II. Recently, a fourth mold tropolone, stipitatic acid, has been characterized<sup>8-10</sup> as V.



No less perplexing than the structural problem is the question of the biogenesis of this group of natural substances. Several proposals have been put forward.<sup>11-15</sup> The currently popular schemes envision tropolone biogenesis not to be the result of any known mode of sugar or acetate metabolism<sup>14</sup> but postulate, for example, the cyclization of an octulonic acid phosphate derivative.<sup>15</sup>

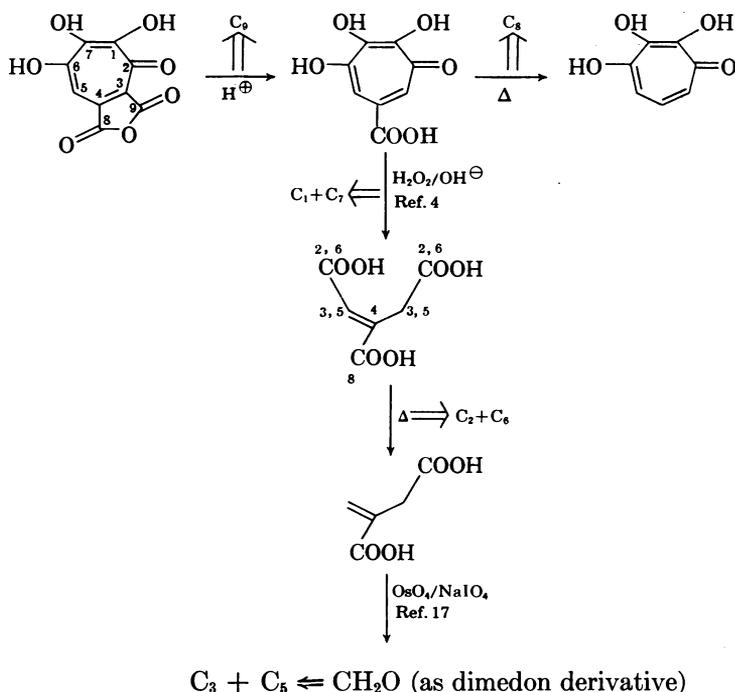
It is the purpose of this paper to present the results of a study on the incorporation of certain labeled substrates into puberulic acid and puberulonic acid by *Penicillium aurantio-virens*, and on the basis of these results to propose that the mold tropolones arise via a direct pathway from simple one and two carbon precursors. Moreover, we propose that they belong to the same general biosynthetic realm as such substances as orsellinic acid and cyclopaldic acid.<sup>§</sup>

*Experimental.*—To a standard Czapek-Dox nutrient medium was added a small amount of an appropriately labeled substrate. The medium was then inoculated with *Penicillium aurantio-virens* Biourge (77) CBS R2138.<sup>1</sup> Growth proceeded for 4-6 weeks, at which time puberulic acid and puberulonic acid were isolated and separated by the method of Barger and Dorrer.<sup>16</sup>

The acids were degraded by the following sequence, each step allowing the determination of the activities of certain carbon atoms as indicated.

The radioactivities of the samples were determined by combustion to carbon dioxide and counting in an ion chamber by means of a vibrating-reed electrometer. The results obtained are given in Table 1 and are expressed as the percentage activity in the indicated carbon atoms relative to puberulonic acid as 100 per cent.

The phenylenediamine derivative<sup>18</sup> and pyridine complex of puberulonic acid<sup>18</sup> and the diacetyl derivative of puberulic acid<sup>2</sup> were prepared and their activities compared with those of the parent compounds to insure that the observed activities



$C_4$  by difference

of puberulonic acid and puberulic acid were not inaccurate by reason of a highly active impurity. This precaution was taken in the degradation of tropolones derived from each substrate. In all cases, the activities of the derivatives were within 3 per cent of those of the parent compounds.

TABLE 1

Per cent activity in	PRECURSOR			
	1- $C^{14}$ acetate	2- $C^{14}$ acetate	1- $C^{14}$ glucose	$C^{14}$ formate
$C_1 + C_7$	...	$24 \pm 4$	$36 \pm 4$	100
$C_2 + C_6$	$76 \pm 12$	...	...	...
$C_3 + C_5$	...	$54 \pm 4$	$31 \pm 4$	...
$C_4$	$24 \pm 14$	...	...	...
$C_8$	...	$22 \pm 5$	$20 \pm 4$	...
$C_9$	...	...	$13 \pm 4$	...

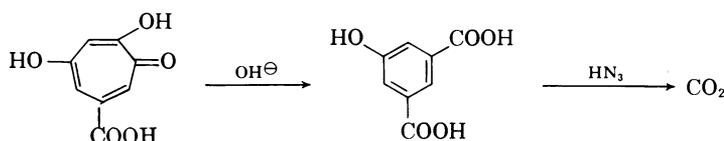
The incorporation of bicarbonate was also studied. It was very much less efficiently utilized than any of the other substrates investigated. Ten per cent of the activity was found in  $C_9$ . Further degradations have not been completed, but the very low level of incorporation suggests that carbon at the oxidation level of carbon dioxide is not a specific precursor of any of the carbon atoms of the mold tropolones.

*Discussion.*—The intermediacy of puberulic acid in the degradative sequence presents certain potential difficulties in the interpretation of the experimental results. Thus puberulic acid is, by virtue of enolization, a symmetrical molecule which may render ambiguous any distinctions between the origin of  $C_1$  and  $C_7$ , between  $C_2$  and  $C_6$ , and between  $C_3$  and  $C_5$ . In the case of  $C_2$ ,  $C_6$  and  $C_3$ ,  $C_5$  this

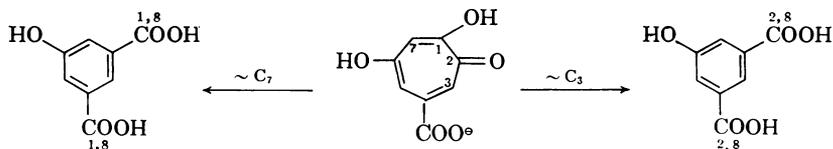
ambiguity is resolved as both members of each pair have a similar origin.

From the results with 2-C<sup>14</sup> acetate it can be seen that four of the carbon atoms in puberulonic acid are derived from the methyl carbon of acetate. These are C<sub>3</sub>, C<sub>5</sub>, C<sub>8</sub>, and either C<sub>1</sub> or C<sub>7</sub>. An assumption made in this and other subsequent similar interpretations is that the carbon atoms in the tropolone derived from a particular carbon of a precursor are labeled to at least approximately the same extent. Thus the fact that, with 2-C<sup>14</sup> acetate as precursor, 54 per cent of the activity is found in C<sub>3</sub> + C<sub>5</sub> implies that *both* C<sub>3</sub> and C<sub>5</sub> are derived from the methyl carbon of acetate and not that only one of these two carbons is so derived, with the activity in this position being twice that of other carbons (such as C<sub>8</sub>) also derived from the acetate methyl.

Bentley<sup>14</sup> has previously described a degradation of stipitatic acid derived from 1-C<sup>14</sup> acetate in which he concluded that C<sub>4</sub> and C<sub>6</sub> but *not* C<sub>2</sub> are derived from the acetate carboxyl carbon. The basis of the latter conclusion was the assumption that the base-catalyzed rearrangement of stipitatic acid would extrude, to a significant extent, both C<sub>1</sub> and C<sub>2</sub> into the new carboxyl carbon of the 5-hydroxybenzene-1,3-dicarboxylic acid product. When stipitatic acid biosynthesized from 1-C<sup>14</sup> acetate was subjected to this rearrangement and the isophthalic acid derivative decarboxylated by the Schmidt reaction, the carbon dioxide so obtained had only a small activity—leading to the conclusion that the carboxyl carbon of acetate is not incorporated into C<sub>2</sub> of the tropolone skeleton.



We do not believe the assumption made in this deduction is valid. Stipitatic acid is not a symmetrical molecule, and the migration of either C<sub>3</sub> or C<sub>7</sub> will proceed along pathways of different energies. This will almost surely lead to a predominance of rearrangement in one of the two possible senses. Under the highly alkaline conditions of the rearrangement, the C<sub>3</sub> carboxyl group will be ionized. There is evidence<sup>19</sup> in the case of the benzylic acid rearrangement—a reaction analogous to the ring contraction of tropolones—that the site of attack by base controls the direction of rearrangement. In the case of stipitatic acid, therefore, attack of base at C<sub>2</sub> followed by migration of C<sub>3</sub> will be hindered by the presence of the carboxylate anion relative to attack of base at C<sub>1</sub> followed by migration of C<sub>7</sub>. This will result in the preferential (not necessarily exclusive) occurrence of C<sub>1</sub> of stipitatic acid as the new carboxyl carbon in the 5-hydroxybenzene-1,3-dicarboxylic acid. Bentley's degradation does not, therefore, preclude the possibility that C<sub>2</sub> of the tropolone skeleton can originate in the carboxyl carbon of acetate.

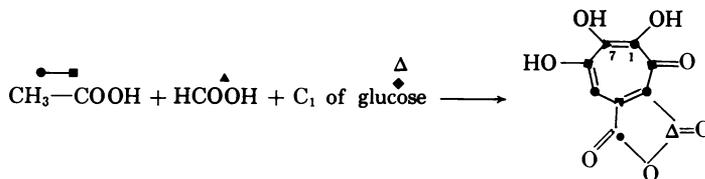


In fact, our results with 1-C<sup>14</sup> acetate are most convincingly interpreted as indicating that C<sub>2</sub>, C<sub>4</sub>, and C<sub>5</sub> of the tropolone nucleus are derived from the acetate carboxyl carbon.

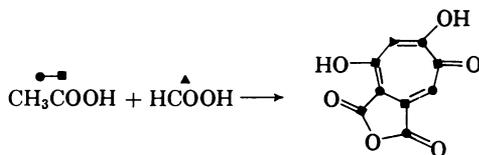
The results with C<sup>14</sup> formate show that either C<sub>1</sub> or C<sub>7</sub> of puberulonic acid can be supplied by formate.

The experiments with 1-C<sup>14</sup> glucose suggest the occurrence of six carbons in puberulonic acid, which can be derived from C<sub>1</sub> of glucose. These are: C<sub>1</sub>, C<sub>3</sub>, C<sub>6</sub>, C<sub>7</sub>, C<sub>8</sub>, and C<sub>9</sub>. We interpret this result as indicating that the glucose is first dissimilated to smaller fragments before its incorporation into the mold tropolones, and that the results with 1-C<sup>14</sup> glucose are, in the main, the sum of those with 2-C<sup>14</sup> acetate and C<sup>14</sup> formate. The unusual exception is C<sub>9</sub> of puberulonic acid, which is not derived from either of the two acetate carbons nor from formate but is supplied by C<sub>1</sub> of glucose. The simplest, though by no means unique, explanation of this result is that C<sub>9</sub> is derived from a one-carbon pool, which can, in turn, be derived from C-1 of glucose, but that this particular one-carbon pool is not in significant metabolic contact with added sodium formate in *P. aurantio-virens*.

These results lead to the following proposal for the origin of the various carbon atoms of puberulonic acid:



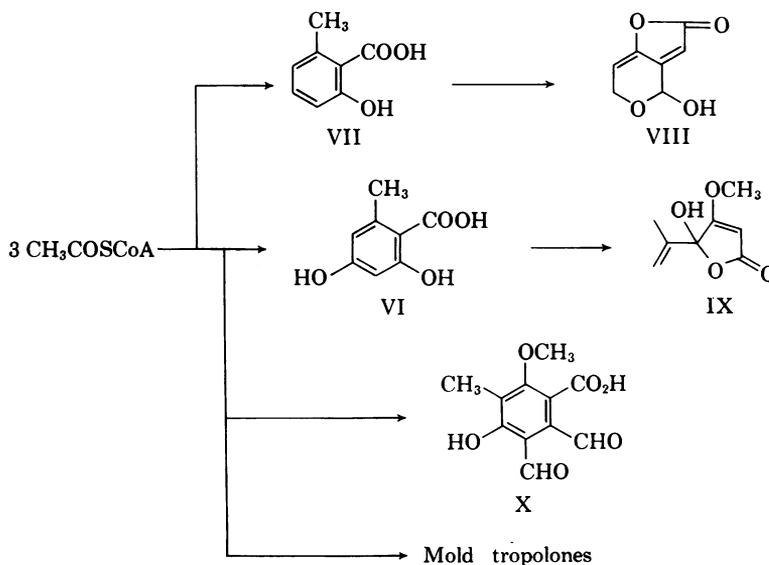
There remains, of course, the ambiguity about the origin of C<sub>1</sub> and C<sub>7</sub> mentioned earlier. Of the two alternatives, we prefer the one presented here because Bentley<sup>20</sup> has shown that C<sub>9</sub> of stipitatic acid is derived from the carboxyl carbon of acetate. Thus, stipitatic acid has the following distribution of acetate and formate carbons based on a reinterpretation of Bentley's earlier work:<sup>14</sup>



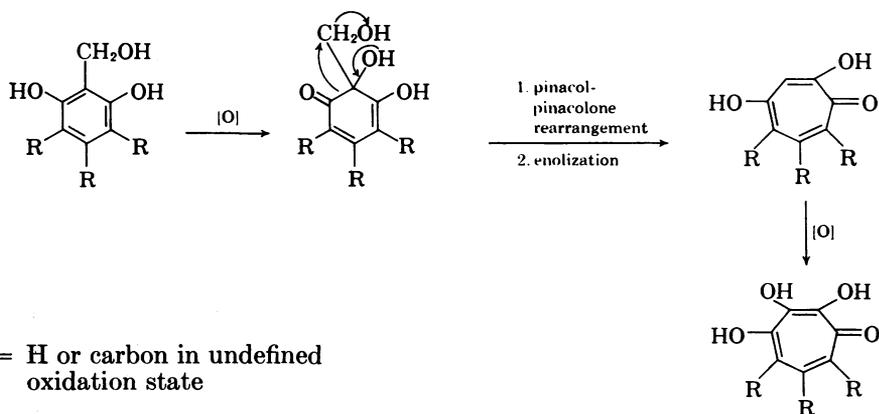
Since, in stipitatic acid, a carboxyl carbon of acetate is attached at C<sub>5</sub> we believe it more likely that the additional one carbon fragment present in puberulonic acid is attached at C<sub>3</sub> rather than replacing the acetate carboxyl carbon at C<sub>6</sub>. This raises the possibility of a ten-carbon intermediate in the biosynthesis of puberulonic acid. Tannenbaum has described<sup>15</sup> the isolation from *P. stipitatum* of what may prove to be such a ten-carbon tropolone.

The route by which the mold tropolones are formed from their simple precursors cannot be discussed in detail with only the present evidence at hand. However, an intriguing series of structural relationships can be deduced, which will provide a basis for further experimentation. The condensation of three acetyl CoA<sup>21</sup> (or malonyl CoA<sup>22</sup>) fragments to produce orsellinic acid (VI) is a process that finds experimental justification in the work of Birch, particularly his studies on the bio-

synthesis of 6-methylsalicylic acid (VII).<sup>23</sup> This latter substance has been shown by the beautiful work of Tannenbaum and Bassett<sup>24</sup> and of Bu'lock and Ryan<sup>25</sup> to undergo an oxidative ring fission and so to give rise to patulin (VIII). A similar ring fission reaction has been postulated<sup>26</sup> to explain the biosynthesis of penicillic acid (IX). Birch has suggested<sup>27</sup> that the acquisition of two one-carbon units by orsellinic acid or a derivative can lead to such ten-carbon substances as cyclopaldic acid (X).



We should like to suggest that an oxidative ring enlargement of a substance such as cyclopaldic acid is the biosynthetic pathway by which tropolones are produced. One of the many detailed mechanisms that can be envisioned to effect this change is the following:



For stipitonic acid a scheme of the above kind operating on the appropriate nine-

carbon precursor will produce an intermediate with the correct level and site of nuclear oxidation. Another attractive mechanism for oxidative ring enlargement would involve an oxidative ring fission followed by a subsequent recyclization of the acyclic intermediate. Indeed, such a scheme is in closer formal analogy to the mechanism of ring opening that produces patulin.

It is impossible to say whether or not the extra carbon is lost in puberulonic acid biosynthesis before or after ring enlargement. Many such questions as to sequence of transformations, precise structure (including appropriate biological activation) of intermediates, etc., must await further experimental developments for their solution.

The isolation by Bentley and Thiessen<sup>28</sup> of an enzyme system from *P. stipitatum* that catalyzes the decarboxylation of the nine-carbon tropolones to their eight-carbon analogues makes it virtually certain that the nine-carbon stipitatic acid and puberulonic acid are the immediate precursors of their eight-carbon analogues, stipitatic acid and puberulic acid.

An interesting possible confirmation of the suggestion that penicillic acid belongs in the same biosynthetic region with puberulonic acid and puberulic acid is the fact that all three substances have been isolated from the same strain of *P. puberulum*.<sup>1</sup>

*Summary.*—The mode of incorporation of certain labeled substrates into the mold tropolones, puberulonic acid, and puberulic acid has been studied. The origin of the various carbon atoms of the tropolones has been determined, showing that these substances are probably built up directly from simple one- and two-carbon precursors. It has been proposed that the mold tropolones are biogenetically related to benzenoid metabolites such as orsellinic acid and cyclopaldic acid.

\* Presented at the 137th meeting of the American Chemical Society, Cleveland, Ohio, 1960; cf. Abstracts of papers 39-0. A preliminary communication of some of these results has appeared in *Biochem. Biophys. Res. Comm.*, **2**, 107 (1960).

† Aided by a grant from the Brown Hazen Fund of the Research Corporation.

‡ Contribution No. 2630.

§ R. Bentley and J. Murray have both independently suggested in personal communications the possibility of such a relationship.

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## ON THE ELECTRON DONATING PROPERTIES OF CARCINOGENS\*

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A. and B. Pullman<sup>1</sup> have shown that polynuclear carcinogenic aromatic hydrocarbons could be characterized by the presence of a strong "K-region," provided that the action of this region was not compensated by the presence of an active "L-region." These authors also showed that the energies of the highest filled orbitals of these carcinogens were low. The energy of the highest filled orbital was characterized by the Pullmans by the "K-value" which is a linear function of the ionization potential. It is rather unfortunate that these authors should have chosen the same letter for the characterization of a region (two C-atoms) and an energy value, which, inevitably, has to lead to confusion. For this reason, in the present paper, we will use the letter P (in honor of the Pullmans) to characterize the energy of the highest filled orbital or the lowest empty orbital. The P for the highest filled will, according to the original denotation, have, as a rule, a plus; the P for the lowest empty orbital, a negative sign.† This theory was, for the time being, limited to polynuclear aromatic hydrocarbons. Kofahl and Lucas found a certain parallelism between carcinogenic activity and the affinity of the hydrocarbons for the Ag ion.<sup>2</sup> Weil-Malherbe<sup>3</sup> and others found, in certain cases, a tendency for complex formation with purines. Mason<sup>4</sup> pointed to certain relations of orbital energies. None of these theories could, till now, be extended to a wider variety of carcinogens, belonging to various chemical groups, and so it may not be superfluous to look for other connections between carcinogenicity and chemical properties.

It was suggested, in a previous paper,<sup>5</sup> that indoles owe their strong electron donor properties to the strong "local" donating property of certain C atoms, characterized by a high negative formal charge.<sup>6</sup> These C atoms, being part of a conjugated system, might then draw for the electrons, to be donated, on the  $\pi$  pool of