Evidence for a Mono-oxygenase Mechanism in the Biosynthesis of the Fungal Tropolones

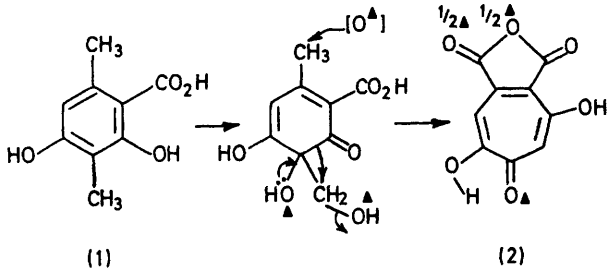
By A. I. SCOTT* and K. J. WIESNER

(*Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut* 06520)

Summary Interpretation of the incorporation data for $^{18}O_2$ and $CH_3{}^{14}C^{18}O_2H$ into stipitatonic acid in *Penicillium stipitatum* leads to the postulate of a mono-oxygenase mechanism for the biosynthesis of fungal tropolones.

IT has been demonstrated recently[1] that 3-methylorsellinic acid (**1**) serves as a precursor of stipitatonic acid (**2**) in *Penicillium stipitatum*. Two principal mechanisms can be suggested for the oxidative transformation of the benzenoid

to the troponoid ring system. In the first of these (Scheme 1) ring enlargement of a hydroxymethyl precursor by a mono-oxygenase enzyme affords the seven-membered ring by a 1,2-shift.[2] Alternatively, ring cleavage by a typical dioxygenase process allows reclosure of an acyclic keto-ester (Scheme 2).[3]



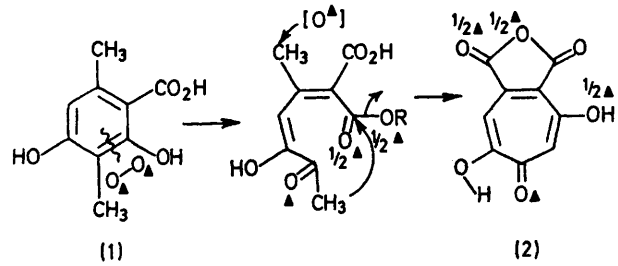
(1) (2)

SCHEME 1

In order to distinguish between these possibilities (and their several minor sequential variants) the fermentation was conducted in an atmosphere of $^{18}O_2$. If no exchange with ^{16}O from the aqueous medium occurs, the intermediates and mechanism implicit in Scheme 1 lead to a tropolone ring having O(1) enriched in ^{18}O and O(3) independently enriched by oxidation of the 6-methyl group of 3-methylorsellinic acid.†

On the other hand, the dioxygenase mechanism (Scheme 2) would afford a species containing two oxygen atoms, at O(1) and O(6), incorporated from the same molecular species of $^{18}O_2$.‡

After incubation of *P. stipitatum* in an enriched oxygen atmosphere (containing 20% ^{18}O–^{18}O) for 4 days the resultant stipitatonic acid was isolated, purified, and a



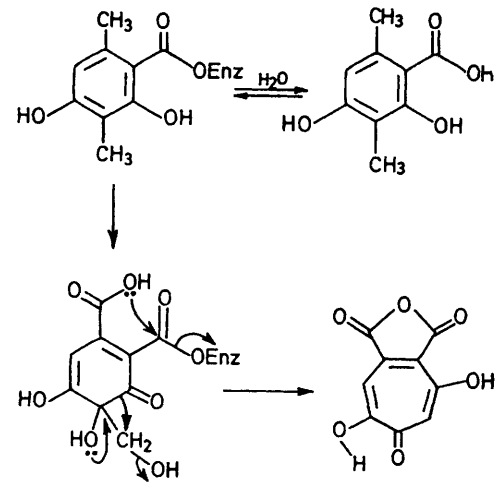
(1) (2)

SCHEME 2

portion converted into ethyl stipitatate (**3**) and 6-hydroxytropolone (**4**). (**3**) lacks the original oxygen atoms O(4) and O(5) whilst (**4**) has lost oxygen atoms O(3)—O(5).

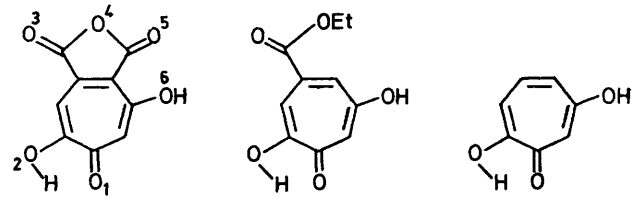
The ^{18}O enrichments for these compounds were determined by high resolution mass spectrometry and the results for $M + 2$ species are summarised in the Table.

The most important aspect of the data is the absence of any $M + 4$ species in stipitatonic acid (**2**) and its derivatives (**3**, **4**). These results strongly indicate the operation of the mono-oxygenase pathway for both areas of oxidation, *i.e.* one oxygen atom is independently introduced into both the tropolone nucleus and the anhydride function with the same



SCHEME 3

specific incorporation. The latter oxygen is then randomised between O(3) and O(4). If the oxidation had involved rupture of the aromatic ring and recyclisation of the acyclic species (Scheme 2) then in order to account for the complete absence of the $M + 4$ species, it would be



(2) (3) (4)

necessary to invoke complete exchange† of one of the introduced oxygens and complete retention of the other. This explanation is shown to be unlikely by the following experiment. When [1-^{14}C,^{18}O (93%)]sodium acetate was administered to the organism, the specific incorporation (^{14}C) into stipitatonic acid (**2**) was 4·0%. On the basis of previous work with aromatic systems[4] the intermediate 3-methylorsellinic acid must have carried 3·7% ^{18}O enrichment in the phenolic hydroxy-groups. As can be seen from the Table, the ^{18}O analysis of stipitatonic acid indi-

† The further complication arises that the O(3) enrichment can also be randomised to O(4) *via* the symmetry of the carboxy-function. This argument applies equally to the dioxygenase mechanism which also requires distinct generation of the anhydride grouping by a process which again introduces ^{18}O at O(3) [O(4)] in a manner which can lead to $M + 2$ but not $M + 4$ species. Analysis of the derivatives (**3**) and (**4**) is used to distinguish between the two oxidation sites.

‡ Apart from the anhydride enrichment mentioned above, the equilibration of label in the acyclic intermediate of Scheme 2 could result in up to 50% loss of one of the labelled O atoms, O(6). However, excluding complete exchange of all the carboxyl (or latent carbonyl) oxygen in the keto-ester, operation of the dioxygenase mechanism would be detected by the appearance of an $M + 4$ peak in the mass spectrum of (**2**).

Fermentation	% *Enrichment of* M + 2 (2)	(3)	(4)
No. 1 ($^{18}O_2$)	8·4 ± 0·2	5·6 ± 0·6	4·0 ± 0·5
No. 2 ($^{18}O_2$)	16·5 ± 0·4	13·0 ± 0·6	—
No. 3 (^{18}O acetate)	1·73 ± 0·03	1·55 ± 0·03	—
No. 4 (^{18}O acetate)	1·54 ± 0·03	1·38 ± 0·03	—

cates an exchange with H_2O from the medium of 80% at O(6) and O(2) and 95% at O(5). From the $^{18}O_2$ experiment it was found that O(1) retained ^{18}O and we regard this as good evidence for operation of the hydroxy-dienone–tropolone rearrangement of Scheme 1. In this process O(1) is transferred from tertiary alcohol to tropolone carbonyl, neither function being exchangeable under the conditions of the experiment. On the other hand, the acyclic dioxygenase intermediate (Scheme 2) would be expected to show massive exchange of all the carbonyl functions. In fact the unique retention at O(1) is most logically interpreted as indirect proof for the mono-oxygenase mechanism of Scheme 1 leading to the hypothesis shown in Scheme 3 for the overall biosynthetic transformation.

We thank the N.I.H. for support of this work and Dr. W. J. McMurray for m.s. measurements.

(*Received, 19th June* 1972; *Com.* 1062.)

[1] A. I. Scott, H. Guilford, and E. Lee, *J. Amer. Chem. Soc.*, 1971, **93**, 3534.
[2] J. H. Richards and L. D. Ferretti, *Proc. Nat. Acad. Sci. U.S.A.*, 1960, **46**, 1438.
[3] T. A. Geissman and D. H. G. Crout in 'Organic Chemistry of Secondary Plant Metabolism,' Freeman, Cooper and Co., New York, 1969, p. 134.
[4] S. Gatenbeck and K. Mosbach, *Acta Chem. Scand.*, 1959, **13**, 1561.