The Biosynthesis of Terreic Acid†

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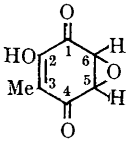
STRUCTURAL CONSIDERATIONS suggest that the acetate–malonate pathway is probably involved in the biosynthesis of terreic acid (I), a metabolite of *Aspergillus terreus*,[1] but the nature of the intermediates and the origin of the epoxide oxygen are less apparent, and have been the subject of speculation.[2] We report experiments which confirm the acetate origin of terreic acid (TA) and demonstrate that the carbon skeleton is derived from 6-methylsalicylic acid (6MS) and the epoxide oxygen from atmospheric oxygen.

A close metabolic relationship between 6MS and TA seemed probable when, in addition to TA, 6MS was isolated from the nutrient medium of *Aspergillus terreus* ATCC 12238. This view was reinforced when it was found that the maximum concentration of extracellular 6MS was reached about 12 hr. before that of extracellular TA.

[1-^{14}C]Acetate and [^{14}C]6MS (derived in the same organism from [1-^{14}C]acetate) were incorporated into TA with 1·1 and 15·5% efficiencies respectively. [2-^{14}C]Orsellinic acid was not incorporated. The carbon atoms C(3) and C(3′) were isolated from labelled TA by Kuhn–Roth oxidation followed by Schmidt degradation. Similar treatment of the labelled 6MS isolated carbon atoms C(6) and C(6′) from the metabolite. The specific activities (Table 1) of these carbons showed that in *A. terreus*, as in *P. griseofulvum*[3] and *P. patulum*,[4] 6MS was derived by the acetate–malonate pathway. They also clearly indicated that TA was formed by the same pathway *via* 6MS.



(Ia) ^{18}O at C(1) only
(Ib) ^{18}O at C(4) only
(Ic) ^{18}O at C(2) only
(Id) ^{18}O at epoxide only

On this basis the epoxide oxygen may have originated from malonate, water, or atmospheric oxygen. The origin was established by first determining the overall rate of exchange between oxygens in TA and water, using [95·0 atoms % ^{18}O]water–10% ethanol at 25°. No exchange of

TABLE 1

The degradation of TA and 6MS, showing specific activities in mμc/mmole

Sample	Total	Per acetate*	AcOH (Kuhn–Roth)	$BaCO_3$ (Schmidt)	$MeNH_2$ (Schmidt)
TA from [1-^{14}C]acetate	267	88·4	87·6	87·2	0·28
6MS from [1-^{14}C]acetate	179	44·8	44·4	44·1	0·08
TA from [^{14}C]6MS	46·0	15·3	15·5	15·1	0·13

* Based on 3 and 4 acetate units in TA and 6MS respectively.

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epoxide oxygen was anticipated and a mass spectrum of TA after 113 hr. exchange showed that equilibration was taking place at only three of the oxygens. Theoretically, if secondary isotopes can be neglected, the percentage of exchange ($O^{16} \rightarrow O^{18}$) at each of the three oxygens, at time t, is given by the three roots of the cubic equation:

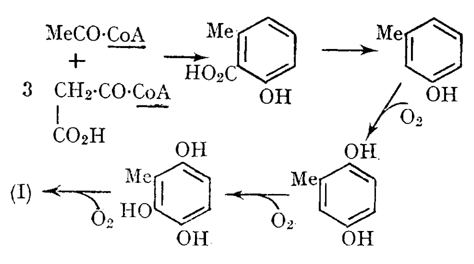
$$y^3 - (b + 2c + 3d)y^2 + 100(c + 3d)y - 10{,}000d = 0$$

where b, c, and d are percentages of the molecules containing 1,2, and 3 oxygen-18 atoms, respectively, at time t. These values were determined experimentally by quantitative examination of the M, $M + 2$, $M + 4$, and $M + 6$ ions in the mass spectrum of terreic acid isolated after 17 and 113 hr. exchange, and are shown in Table 2*a*. On substitution into the equation, they gave percentages of exchange of 8·5, 81·2, and 81·2[‡] at 17 hr. and 44·8, 95·25, and 95·25[‡] at 113 hr. The pseudo-first-order rate constants ([^{18}O]water is in large excess) calculated from these percentages are 0·113 and 0·0056 hr.$^{-1}$.[§] The slower rate is associated with the reaction at the enolic hydroxy-group, probably exchanging in a ketonic form, and the faster rate with the average of the rates at the two carbonyl groups.

TABLE 2

Change in isotope composition (%) *of TA molecular ion complex during exchange studies*

	(*a*) [^{16}O]TA *during exchange with* [^{18}O]*water*		(*b*) [^{18}O]TA *during exchange with* [^{16}O]*water*		
Ion	17 hr.	113 hr.	0 hr.	23 hr.	117 hr.
M	5·90	1·69	83·36	87·30	89·32
$M + 2$	23·21	3·16	15·66	12·39	10·53
$M + 4$	65·04	53·22	0·98	0·31	0·15
$M + 6$	5·86	41·93	—	—	—



When *A. terreus* was grown in an atmosphere enriched with oxygen-18, the isotope was incorporated into the terreic acid produced. The incorporated oxygen was allowed to exchange with [^{16}O]water under the conditions used previously and the molecular ion complex was quantitatively examined. The figures for the percentage of molecules containing 0, 1, and 2 oxygen-18 atoms, after 0, 23, and 117 hr. of exchange are given in Table 2*b*.

On the justifiable assumption that the $M + 4$ ion consists mainly of species containing one non-exchangeable oxygen (see below), the decrease in percentage of $M + 2$ ion with time is given by the increase in percentage of M ion. With the rates of exchange for (Ia) to (Id), the species in the $M + 2$ ion, established,[¶] and the total decrease in the $M + 2$ ion at $t = 23$ and 117 hr. known, the percentages of (Ia) + (Ib), (Ic), and I(d) at $t = 0$ can be calculated and are found to be 3·62, 4·93, and 7·11% respectively. These indicate that 77% of the $M + 4$ ion, *i.e.* 0·76% of the total molecular ion complex, contains oxygen-18 in the epoxide grouping and the total incorporation of oxygen-18 into this position is therefore 7·87%. Since the oxygen-18 enrichment in the experiment was 8·50%, the incorporation of oxygen-18 into the epoxide oxygen was 92·6% of the theoretical maximum and this oxygen is clearly derived from atmospheric oxygen.

From these results, and those reported recently

[‡] As the percentages of exchange at two oxygens is approximately equal, small experimental errors, probably due mainly to primary isotope effects in the fragmentation of the TA, give a small imaginary component to the two larger roots. The value of the two equal roots obtained when this component is ignored represents the average of the percentage of exchange at the two oxygens.

[§] For the slower exchange, rate constants of 0·0055 and 0·0056 hr.$^{-1}$ were obtained after 17 and 113 hr. exchange respectively. Only the results at 17 hr. could be used for the faster rate as after 113 hr. the rapid exchange was virtually complete.

[¶] A negligible primary isotope effect is assumed. If the exchange rates are 10% smaller, the value obtained for the total incorporation of oxygen-18 in the epoxide group is reduced by about 6%.

by Scott and Yalpani[5] which implicate 6MS as a precursor for *m*-cresol and toluquinol, it appears probable that the biosynthesis of terreic acid follows the pathway depicted.

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