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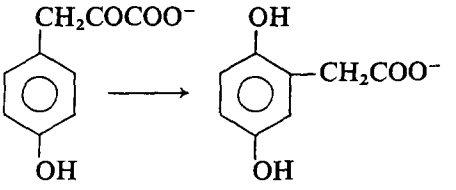
COMMUNICATIONS

Lack of Exchange of the Phenolic Function in the Enzymatic Conversion of *p*-Hydroxyphenylpyruvate to Homogentisate

The mechanism by which molecular oxygen becomes incorporated into aromatic substrates is one of continuing interest. Epoxides have been shown to be intermediates in certain instances (*1*). The purpose of this investigation in part has been to demonstrate whether an endoperoxide mechanism may be involved (*2*) in the enzymatic conversion of *p*-hydroxyphenylpyruvate (**1**) to homogentisate (**2**). Yasunobu *et al.* (*3*) reported



1 **2**

that 1 atom of atmospheric oxygen and 2 atoms of oxygen from water were incorporated during the conversion. More recent work by Linblad *et al.* (*4*) indicates that atmospheric oxygen is incorporated into both the new hydroxyl moiety and the carboxyl group as well.

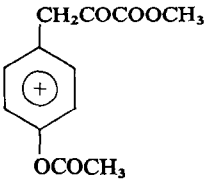
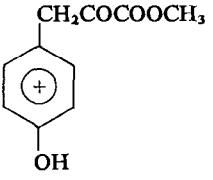
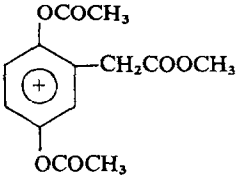
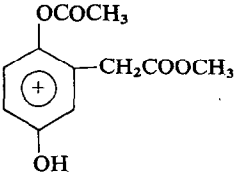
In order to gain new insight into this biochemical transformation and evaluate the various proposals (*1*, *2*, *4*), attention has been focused on the possible lability of the phenolic function in *p*-hydroxyphenylpyruvate during the transformation of this compound to homogentisate. The stability of this moiety was revealed by synthesizing *p*-hydroxyphenylpyruvic-4-^{18}O acid and examining the product of its metabolism.

The substrate, *p*-hydroxyphenylpyruvic-4-^{18}O acid, was synthesized from phenol-^{18}O (66.6% atom excess) in the following manner. The phenol was allowed to react with α,α-dichloromethyl methyl ether in the presence of aluminum chloride (*5*), yielding *p*-hydroxybenzaldehyde. This aldehyde was condensed with hydantoin (*6*) to form the substrate labeled unambiguously in the *para* position. After purification, the labeled *p*-hydroxyphenylpyruvate was enzymatically converted to homogentisate according to the procedure of LaDu and Zannoni (*7*). Both substrate and product were converted to the volatile derivatives, methyl *p*-acetoxyphenylpyruvate and methyl 3,5-diacetoxyphenylacetate, respectively, by methylation with diazomethane and acetylation with acetic anhydride. The results of the mass spectrometric analyses based upon two individual experiments are presented in Table 1. The percentage of retention of the oxygen-18 label in the *para* position is 96–98%.

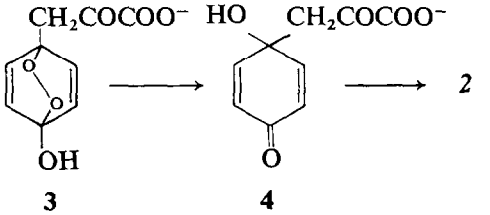
These results indicate that for all practical purposes the phenolic moiety in *p*-hydroxyphenylpyruvate is biologically stable, and consequently any endoperoxides of type (**3**)

TABLE 1

MASS SPECTROMETRIC ANALYSIS

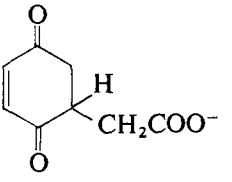
Structure	*m/e*	Experiment 1 (% excess ^{18}O)	Experiment 2 (% excess ^{18}O)
	M (236–238)	65.5 ± 0.2	65.1 ± 0.2
	M–42 (194–196)	65.7 ± 0.2	
	M (266-268)	63.1 ± 0.2	63.6 ± 0.3
	M–42 (224–226)	62.7 ± 0.2	
% Retention of ^{18}O		95.9 ± 0.8	97.7 ± 0.8

are unlikely intermediates. The basis for this supposition is that structure **3** may be expected to form the transient quinoid, **4**, in its conversion to **2**; and since quinones have been reported (*8*) to exchange oxygen in aqueous systems, structure **4** is ruled out on



3 **4**

these grounds. However, a hydrated quinoid form may result in the selective enzymatic removal of one of the two hydroxyl groups at the 4 position and, therefore, the endoperoxide mechanism cannot be totally eliminated. Likewise, these results raise serious questions about the proposed intermediacy (*4*) of a quinonoid structure **5** since, the original phenolic function in such a structure would be expected to be labile (*8*), and these results have shown unequivocally that this is not the case.



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