THE ORIGIN OF THE BENZOQUINONE RING OF COENZYME Q_9 IN THE RAT

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It has been suggested (Olson et al., 1960, 1961) that the aromatic ring system of coenzyme Q_9, in the rat, is derived from phenylalanine. To test this hypothesis, uniformly C^{14} labeled L-phenylalanine was administered to rats previously maintained on a vitamin A deficient diet to obtain elevated levels of the coenzyme (Gloor and Wiss, 1959). The observed per cent incorporations of isotope into total body coenzyme Q_9 in two experiments, after 90 minutes, are shown in Table I.

TABLE I

Incorporation of C^{14} from Phenylalanine-U-C^{14} into CoQ_9

Expt.	Dose of C^{14}, mC	No. of rats	Period of A deficiency, weeks	Mg of crude CoQ[a]		% C^{14} in CoQ_9[b]
				Liver	Carcass	
A	0.5	10	6	14.0	32.0	0.0025
B	1.0	10	3	6.5	21.0	0.0031

[a] This yield is derived from spectrophotometric determination after the first alumina column.

[b] The recovery is based on the yield of crude CoQ and the specific radioactivity of the highly purified CoQ_9 dihydro-diacetate derivative.

The values are slightly higher than those previously reported from this laboratory with normal animals (Olson et al., 1961). The coenzyme Q_9 was isolated from the livers and carcasses of the animals, combined, and chromatographed five

times or more on alumina, and twice on paper to constant specific activity. After dilution with carrier coenzyme Q_9* to about 200 mg, the isotopic product was recrystallized twice from cold methanol. The dihydro-diacetate was then prepared by reductive acetylation and recrystallized twice from 95 per cent ethanol. The specific activity of the diacetate derivative did not change during the recrystallizations.

In a determination of the structure of coenzyme Q_{10}, the dihydro-diacetate was treated with ozone in acetic acid, followed by reductive cleavage of the ozonide, and oxidation of the obtained phenylacetaldehyde derivative to 3', 6'-diacetoxy-4', 5'-dimethoxy-2'-methyl-phenylacetic acid with potassium permanganate (Morton et al., 1958). Although Gloor, Schindler and Wiss (1961) reported the degradation of labeled samples of coenzyme Q (derived from 2- and 4-C^{14}-mevalonic acid) by this method, Lawson et al. (1961) failed to obtain a pure specimen of the phenylacetic acid derivative in a subsequent tracer experiment. Similarly, in our hands, the original ozonolytic procedure led to poor and variable yields. A new method was, therefore, devised in which the dihydro-diacetate of coenzyme Q_9 was treated with ozone in ethyl acetate at low temperature and in the presence of water. The ether soluble material obtained in this way was treated further with neutral potassium permanganate at room temperature, and the acidic fraction was sublimed in high vacuum. The desired 3', 6'-diacetoxy-4', 5'-dimethoxy-2'-methyl-phenylacetic acid, typically obtained in 39 per cent of the theoretical yield, quickly crystallized on solution in ether, followed by addition of light petroleum (b. p., 35-50°). The product recrystallized twice in the same way had m.p. 132.5-134° (micro-block).

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Anal. Calcd. for $C_{15}H_{18}O_8$: C, 55.21; H, 5.56. Found: C, 55.30, 55.03; H, 5.21, 5.40.

A further increase in m.p. to 136-138° (micro-block) was obtained by chromatography of this acid on silicic acid, using benzene-ethyl ether elution. The purity of this compound was further established by gas-liquid chromatography, both of the free acid and the methyl ester, on silicone coated Chromosorb W at 176°. Typical retention times were as follows; acid, 8 minutes; methyl ester, 11 1/2 minutes. A somewhat lower melting point (121-124°) for this acid was obtained by Morton et al. (1958).

The water soluble fraction from the ozonolysis contained levulinaldehyde, representative of the isoprenoid side chain. This material was isolated as the bis-dinitrophenylhydrazone. Results obtained on degradation of two labeled coenzyme Q_9 samples are shown in Table II.

TABLE II

C^{14} Distribution in Coenzyme Q_9 Biosynthesized from L-phenylalanine-U-C^{14}

Expt.	CoQ_9 dihydro-diacetate[a] Spec. activ. $\mu C/mM \times 100$	Phenylacetic acid[a] Spec. activ. $\mu C/mM \times 100$	Phenylacetic acid[a] % of original	Levulinaldehyde[b] Spec. activ. $\mu C/mM \times 1000$	Levulinaldehyde[b] % of original
A	2.99	2.64	88.3	0.0	
B	8.3	5.92	71.3	3.2	30.8

[a] Counted in a Tri-Carb liquid scintillation spectrometer

[b] The levulinaldehyde 2,4-dinitrophenylhydrazone samples were counted as infinitely thick discs of 1 cm^2. In Expt. A, about 12% of total activity is unaccounted for, and should have been found in the levulinaldehyde fraction. This amount of activity would have given a counting rate of 1-2 cpm above background; the sample was essentially inactive, under these counting conditions. To obtain the % of original activity, the specific activity has been multiplied by 8, since 8 moles of levulinaldehyde are obtained per mole of dihydro-diacetate.

These experiments provide the first unequivocal evidence that the aromatic ring of phenylalanine is used as a precursor of the benzoquinone nucleus of

coenzyme Q9 in the rat. The percentage incorporation of activity from phenylalanine into coenzyme Q although low (see Table I) is of the same order of magnitude as that observed with acetate, and the observed activity from phenylalanine-U-C^{14} is located largely in the benzoquinone portion. The total activity in the isoprenoid side chain is very much lower, consistent with partial degradation of the labeled phenylalanine, and reincorporation of the aceto-acetate into terpene derivatives via mevalonate. It seems probable that the phenylalanine is converted to homogentisic acid and thence to 2-methyl-hydroquinone as originally proposed by Olson et al. (1961).

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