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BIOSYNTHESIS OF IODININ: INCORPORATION OF D-[1-^{14}C]-, D-[6-^{14}C]- AND D-[1,6,7-$^{14}C_3$]-SHIKIMIC ACID

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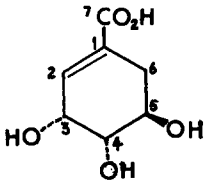
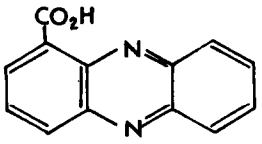
Earlier results from this laboratory[1] gave the distribution of D-[6-^{14}C]shikimic acid (as 1) label in iodinin (2) as 22% in C-1, C-4, C-6, and C-9 and 74% in C-4a, C-5a, C-9a, and C-10a. These results were at variance with those reported for iodinin (12% and 104%) and phenazine-1-carboxylic acid (3) (1% and 102%) from the same precursor;[2] both sets were incompatible with earlier results on iodinin derived from DL-[1,6-$^{14}C_2$]shikimic acid (50% and 25%, the remaining 25% being in C-2, C-3, C-7, and C-8).[3]



(1)



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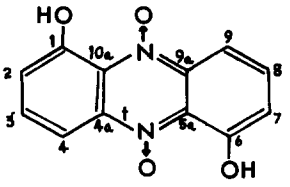
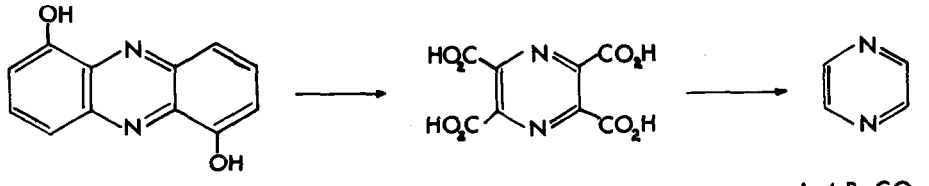


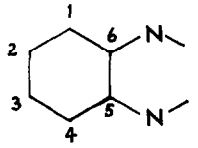
(2)

Our distribution was interpreted as the label in ring A being confined to C-10a, and that in ring B being equally divided over C-5a and C-9 (or C-6 and C-9a) which would give a distribution of 25 and 75%. To resolve the two possible modes of symmetrisation of ring B (shikimic acid C-6 becoming equivalent to C-2 or C-4) we have now fed D-[1-^{14}C]shikimic

acid;[4] results are given in the table. Using our previous degradation route (2) ⟶ (4) ⟶ (5) ⟶ (6) and methods, up to 48% of the activity of (5) was unaccounted for in the pyrazine and $BaCO_3$. The explanation of this apparent loss probably lies in the copper chromite reagent used in the last decarboxylative step (5) ⟶ (6) causing degradation of some of the pyrazine ring to fragments which were collected and counted as $BaCO_3$, in this case active $BaCO_3$ being diluted with inactive material. Using copper-bipyridyl-quinoline[5] (CuQ) as the decarboxylation reagent 100% of this label was found in the carbon dioxide of step (5) ⟶ (6) corresponding to C-1, C-4, C-6 and C-9 of iodinin (2).



(4) (5) (6)



(7)

Degradation of the pyrazine ring, now labelled, by the copper chromite reagent also accounts for the appreciable activity found in C-1, C-4, C-6 and C-9 of iodinin using D-[6-^{14}C]shikimic acid[1,2] (although the same degradation applied to phenazine-1-carboxylic acid had given only 1% of the label in these positions[2]). Repetition of our earlier work[1] but with CuQ decarboxylation now gave consistent results with only a small activity in the $BaCO_3$; careful drying of the hygroscopic N,N-diacetylpiperazine used for the determination of the activity of the pyrazine gave 100% of the activity in C-4a, C-5a, C-9a and C-10a of the phenazine system. These results, together with the label distribution in iodinin

derived from D-[1,6,7-$^{14}C_3$]shikimic acid[4] (see table) are not only self consistent but also substantiate Hollstein and McCamey's conclusion that shikimic acid (numbering) relates to the phenazine nucleus as in (7),[2] in iodinin as well as in phenazine-1-carboxylic acid[2] and pyocyanin.[2,6]

TABLE

^{14}C-Labelled Shikimate precursor	Incorporation[a] %	Distribution of activity as % of phenazine-1,6-diol: Pyrazinetetracarboxylic acid[b]	Pyrazine	Carbon Dioxide[c]
1	42	100	0	52, 67[d]
		102	0	100, 101[e]
6	31	102	113[f] 100[g]	1.2[e]
1,6,7	34[h]	99	55[f] 47[g]	49[e]
			56[f] 50[g]	50[e]

a. Based on phenazine-1,6-diol.

b. Radioactivity determined on tetramethyl ester.

c. Radioactivity determined on $BaCO_3$ in scintillator.

d. Decarboxylation by heating with copper chromite.

e. Decarboxylation by heating with Cu, bipyridyl and quinoline.

f. Radioactivity measured on pyrazine assayed by u.v.

g. Radioactivity determined on N,N-diacetylpiperazine.

h. Allowing for loss of $\frac{1}{3}$ of the activity.

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3. M. Podojil and N.N. Gerber, Biochemistry, 9, 4616 (1970).

4. K.H. Scharf and M.H. Zenk, J. Labelled Compounds, 7, 525 (1972).

5. Copper powder (20 mg), 2,2'-bipyridyl (5 mg), and pyrazinetetracarboxylic acid (14 mg) in CO_2-free quinoline (1.5 ml) heated in a bath at 210° for 2 hr in a stream of CO_2-free nitrogen, which then passed through, successively, saturated aqueous mercuric chloride (2 traps) and aqueous barium hydroxide (38 g/l; 2 traps).

6. M.E. Flood, R.B. Herbert and F.G. Holliman, J. Chem. Soc., Perkin I, 622 (1972).