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BIOSYNTHESIS OF IODININ: INCORPORATION OF [6-^{14}C]SHIKIMIC ACID

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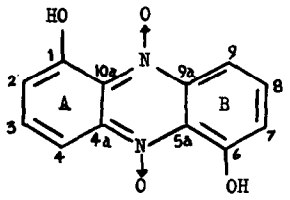
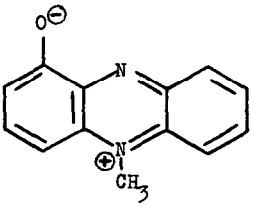
Iodinin (1), one of several pigments based on the phenazine ring system, is a product of *Brevibacterium iodinum* metabolism.[1] This and a related metabolite, pyocyanin (2), have been shown to derive from shikimic acid.[2,3,4,5]

Activity from [6-^{14}C]shikimic acid (as 3) has been found to be confined to C-4a, C-5a, C-9a and C-10a of phenazine-1-carboxylic acid (4),[3] a precursor of pyocyanin (2);[6] this result is supported by those obtained earlier for the incorporation of [1,6-^{14}C]shikimic acid (as 3) into pyocyanin (2) and phenazine-1-carboxylic acid (4).[4] We report here the results of a related study on iodinin (1).



(1)



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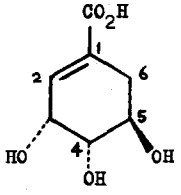
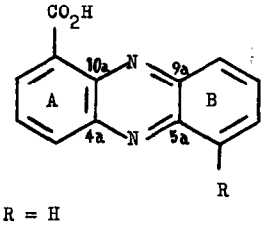


(3)



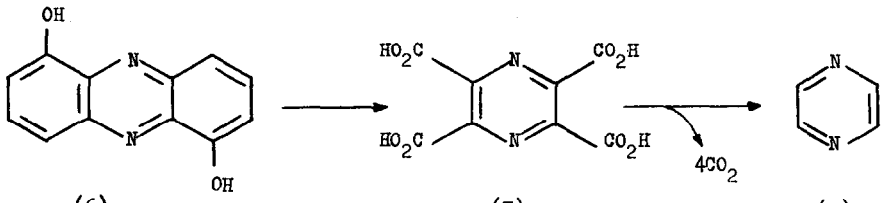
(4) R = H

(5) R = OH

Administration of D-[6-^{14}C]shikimic acid (as 3)[7] to *B. iodinum*, after the onset of pigment production, resulted in a quite remarkably efficient (54%) incorporation into iodinin whilst a 4% incorporation was recorded when the shikimic acid was fed just before

pigment production was apparent. Careful degradation of the iodinin (1 → 6 → 7 → 8) revealed a distribution of label unexpected of a singly labelled precursor: C-1, C-4, C-6 and C-9: 22%; C-4a, C-5a, C-9a and C-10a: 74% (see table).

Results differing from these have been reported[3] for iodinin with the same precursor: 12.4% and 103.6% respectively. The large experimental error in these results probably arises in the failure to purify the pyrazine obtained in the degradative sequence. We have converted our pyrazine (8) to N,N-diacetylpiperazine which is readily purified to constant activity. It should be noted that earlier results obtained for iodinin with [1,6-^{14}C]shikimic acid[5] are not compatible with any of the results obtained with [6-^{14}C]shikimic acid for either phenazine-1-carboxylic acid or iodinin.



(6) (7) (8)

Table. Distribution of [6-^{14}C] shikimic acid label in iodinin (1) [as % of phenazine-1,6-diol (6)]

	Experiment 1[a]	2[b]
Pyrazinetetracarboxylic acid (7)[c]	100	108
Pyrazine (8)[d]	78, 72	73, 74
Carbon dioxide	20, 19	24, 24

a. Shikimic acid fed over a period of 2 hours after onset of iodinin production; isolation of pigment after a further 12 hours. Incorporation: 54%.

b. Shikimic acid fed over a 9 hour period before iodinin production was apparent; isolation, as above. Incorporation: 4%.

c. Radioactivity determined on the tetramethyl ester.

d. Radioactivity determined on N,N-diacetylpiperazine, m.p. 137-138° (lit.[9] 138.5°).

In the efficient conversion of 6-hydroxyphenazine-1-carboxylic acid (5) into iodinin it has been shown that a specific hydroxylative decarboxylation occurs in which the carboxy-function is replaced by a hydroxy-group; other evidence showed that the hydroxy-group present in the acid (5) is introduced before phenazine-ring formation.[8] We have found further that radioactive (5) was isolated, following the addition of inactive material, from cultures to which [6-^{14}C]shikimic acid had been administered. The evidence strongly suggests that (5) is a normal intermediate in iodinin biosynthesis.

It follows then that since C-2, C-3, C-7 and C-8 in iodinin (1) are not labelled by [6-^{14}C]shikimic acid, ring A, i.e. the ring corresponding to the one bearing the carboxy-group in (5), is labelled at C-10a. Further, the distribution of radioactivity in iodinin requires that the two carbocyclic rings of the pigment be differently elaborated. The results may be accommodated by an unequal incorporation of radioactivity into rings A and B, which means a greater dilution of labelled shikimate by non-labelled pools leading to ring B than to ring A.

The alternative explanation is that the label in ring B is equally divided over two carbon atoms: C-5a and C-9, or C-6 and C-9a, i.e., C-6 of shikimic acid (3) becomes equivalent to C-4 or C-2 at some point in the elaboration of the phenazine ring system. This explanation is preferred since the observed distribution of label closely corresponds with the theoretical ratio of 25:75 and is unaffected by the time of precursor feeding.† The results[3] for phenazine-1-carboxylic acid indicate no symmetrization of label and the difference between the results for this metabolite and iodinin may be associated with the additional hydroxy-group present in the iodinin precursor (5).

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† Equilibration between C-6 and C-5 or C-2 of shikimic acid seems unlikely as this implies that ring B shikimate passes through a symmetrical intermediate before linking with ring A and distribution of label should again be subject to variation with time of feeding.

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