Incorporation of 6-Hydroxyphenazine-1-carboxylic Acid into Iodinin

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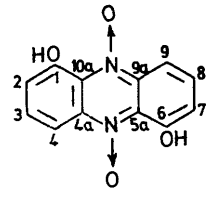
Summary 6-Hydroxyphenazine-1-carboxylic acid was shown to be an efficient precursor for iodinin in *Brevibacterium iodinum*, the hydroxy-group in the precursor being present before formation of the phenazine ring system; the result has important implications in respect of the biosynthesis of this ring system.

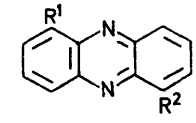
IODININ (**1**), a metabolite of *Brevibacterium iodinum* (*Pseudomonas iodina*),[1] is known to be derived from shikimic acid.[2] Apart, however, from the finding that phenazine-1,6-diol (**2**) and its 5-oxide, which are produced by *B. iodinum* in small amounts,[3,4] are efficiently incorporated into iodinin,[5] the pathway which leads from shikimic acid has not been established.

We have recently shown that (**3**) and (**8**) are specifically converted into pyocyanin (**9**) by *Ps. aeruginosa* by a hydroxylative-decarboxylation.[6] It seemed likely that a similar process might lead to iodinin from (**4**) or related compounds, but when 7,9-dideuteriophenazine-1,6-dicarboxylic acid [as (**4**)] or 7,9-dideuterio-6-hydroxymethylphenazine-1-carboxylic acid [as (**5**)] were fed to *B. iodinum*, either over the period of pigment production or over the period of growth preceding it, no deuteriated iodinin was obtained. Similarly, 1-carboxy-5-methyl-6,7,8,9-tetradeuteriophenazinium chloride [as (**8**) HCl], 6,7,8,9-tetradeuteriophenazine-1-carboxylic acid [as (**3**)] or a mixture of equal quantities of 1,2,3-trideuterio-4- and 1,2,3,4-tetradeuterio-6-hydroxyphenazine [as (**6**)] were not incorporated. On the other hand, both the phenazin-1-ol and 1-carboxylic acid were incorporated into (**10**), previously observed as a minor co-metabolite only in another micro-organism, although produced from phenazin-1-ol by disrupted cells of *B. iodinum*.[7] The conversion of the tetradeuterio-carboxylic acid occurred without loss of deuterium and is thus analogous to the conversion into pyocyanin.[6]

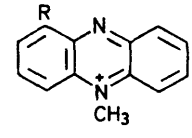
From the above evidence, it follows that one of the hydroxy-groups in iodinin is present before formation of the phenazine ring system. Accordingly, 7,9-dideuterio-6-hydroxyphenazine-1-carboxylic acid [as (**7**)] was fed to



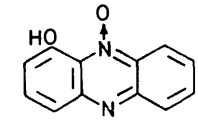
(1)



(2) $R^1 = R^2 = OH$
(3) $R^1 = CO_2H, R^2 = H$
(4) $R^1 = R^2 = CO_2H$
(5) $R^1 = CO_2H, R^2 = CH_2OH$
(6) $R^1 = H, R^2 = OH$
(7) $R^1 = CO_2H, R^2 = OH$



(8) $R = CO_2^-$
(9) $R = O^-$



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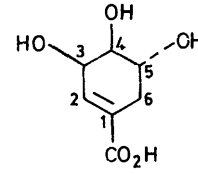

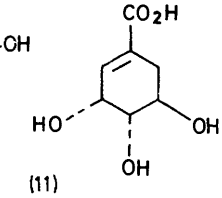

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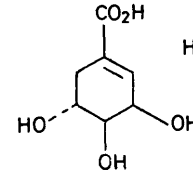

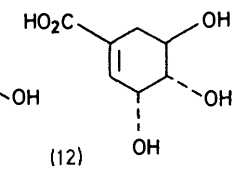

(12)

B. iodinum. The iodinin isolated showed an incorporation (16%) without deuterium loss; this high level of incorporation of the hydroxy-acid, greater than that observed for phenazine-1,6-diol, is in keeping with it being a true precursor for iodinin. It thus appears probable that the micro-organism produces (**3**) and 6-hydroxyphenazine-1-carboxylic acid (**7**) which are then converted by the same, or similar enzyme systems, into (**10**) and iodinin (**1**) respectively, *N*-oxide formation occurring either before or after hydroxylative decarboxylation.

[1,6-^{14}C]Shikimic acid† has been found to be incorporated into iodinin (**1**)[2] with high efficiency, thus demonstrating that the immediate precursor of the phenazine ring system lies close to shikimic acid. The label from the [1,6-^{14}C]-shikimic acid was distributed as follows in iodinin: C-2, -3, -7, and -8: 25%; C-1, -4, -6, and -9: 50%; and C-4a, -5a, -9a, and -10a: 25%. The arrangement of shikimic acid molecules shown as (**11**) was proposed to account for this result; this arrangement, however, is inconsistent with our results, but there is an alternative arrangement (**12**) which will fit both sets of findings. Further, the observed labelling pattern requires that both units from which the phenazine system is formed should be identical or so close in the biosynthetic pathway that no significant dilution occurs between the one and the other. It is significant that (**12**) is the only arrangement allowing the formation of 6-hydroxyphenazine-1-carboxylic acid (and hence iodinin) from two units with *identical* substitution patterns. This pattern would be derived by entry of a nitrogen function at C-2 of shikimic acid (or a derivative thereof) rather than at C-6 as in the formation of anthranilic acid[8] which together with chorismic acid is unlikely as an intermediate in phenazine biosynthesis.[9] Although such a reaction has no precedent, carboxylation at C-2 of shikimic acid has been noted in naphthaquinone biosynthesis.[10]

We are currently experimenting with shikimic acid labelled in such a way that the arrangements (**11**) and (**12**) can be distinguished.

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† I.U.P.A.C. numbering.

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