Tetrahedron Letters No. 8, pp 639 - 642, 1976. Pergamon Press. Printed in Great Britain.

BIOSYNTHESIS OF MICROBIAL PHENAZINES: INCORPORATION OF SHIKIMIC ACID

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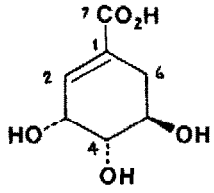
(Received in UK 1 January 1976; accepted for publication 15 January 1976)

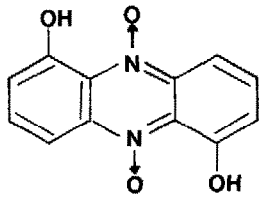
Shikimic acid (1) is clearly implicated as an intermediate in the biosynthesis of microbial phenazines, e.g. iodinin (2)[1,2] and phenazine-1-carboxylic acid (5)[2,3] and it can act as the sole carbon source in the formation of the phenazine skeleton.[4] The results of experiments with ^{14}C labelled shikimic acid[1-3] show that, if the genesis of (2) and (5) is from two molecules of (1), then they are formed according to pattern (3) or (4). Definitive evidence which shows that two shikimic acid units are involved, and which distinguishes between (3) and (4), is lacking however. This information is an essential preliminary in the further elucidation of the pathway between (1) and the phenazine metabolites. We report here the results of experiments with [2-^{2}H]- and [1,6,7-$^{14}C_3$]-shikimic acid which prove that the phenazine nucleus is generated as in (3) from two shikimic acid units.

DL-[2-^{2}H]shikimic acid (as 1) was prepared from [3-^{2}H]propiolic acid by modification of a published procedure.[5] We were unable to remove 11% of DL-[2-^{2}H]-4-epishikimic acid which contaminated the product but this did not affect the validity of subsequent experiments as a pure sample of this material was shown not to label iodinin (2) in vivo.

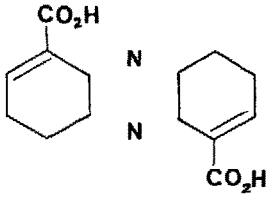
A mixture of DL-[2-^{2}H]- and DL-[1,6-$^{14}C_2$]-shikimic acid (as 1) was administered to cultures of Brevibacterium iodinum. The iodinin isolated (22% incorporation of both ^{14}C and ^{2}H) had a mass spectrum with molecular ions corresponding to dideuteriated (7.5%), monodeuteriated (32%) and unlabelled (60.5%) species. The presence of dideuteriated material proves that iodinin arises from two molecules of shikimic acid.



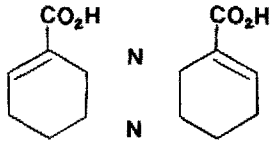
(1)



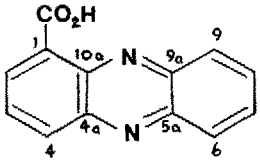
(2)



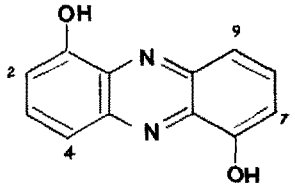
(3)



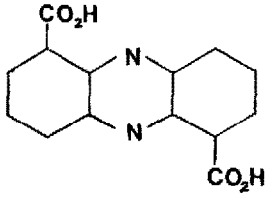
(4)



(5)



(6)



(7)

Catalytic reduction of the labelled iodinin gave (6) without loss of deuterium. Exchange in boiling aqueous 0.5M sodium hydroxide resulted in essentially complete loss of deuterium within 4 hr. (k approx 4.0×10^{3} sec^{-1}) Rates of base catalysed exchange at the positions ortho and para to the hydroxy-groups in (6) were found to be 4.7×10^{3} sec^{-1} and 1.8×10^{3} sec^{-1}. These were determined in [2,4,7,9-$^{2}H_{4}$]phenazine-1,6-diol* obtained by treating (4) with excess sodium deuteroxide in deuterium oxide for 16 hrs at 140 - 160°; even with the exchange reaction temperature raised to 200°, an insignificant amount of meta deuteriation took place. The rate of exchange of meta deuterons could therefore not be determined, but it must be very much slower than 1.8×10^{3} sec^{-1}. In the phenazine-1,6-diol derived from the biosynthetic experiment, the higher exchange rate is associated with all the deuterons present. They must therefore be located ortho to the hydroxy-groups since the alternative para location is inconsistent with ^{14}C labelling studies.† This shows that the biosynthesis of iodinin (2) follows pattern (3) and not the alternative (4) which requires half the deuterium label to be meta to the hydroxy-groups.

The incorporation[3] of [G-^{14}C]shikimic acid into phenazine-1-carboxylic acid (3) in Pseudomonas aureofaciens cultures gave material in which the carboxy-group was labelled to the extent of 10.9%. This result does not allow distinction to be made between biosynthesis from one or two units of shikimic acid which require respectively 14.3% and 7.7% of the radioactivity to be located in the carboxy-group. In support, however, of the biosynthesis of (5) involving two molecules of shikimic acid, we have found that D-[1,6,7-$^{14}C_{3}$]shikimic acid‡ is incorporated into phenazine-1-carboxylic acid (5) in Ps. aureofaciens cultures with 21% of the activity located in the carboxy-

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* Labelling pattern consistent with changes in the n.m.r. spectrum of the disodium salt.

† That exchange ortho to hydroxy-groups in (4) is faster than para is an interesting point, with some analogy.[6]

‡ This material was prepared from [U-^{14}C]pyruvic acid following a published method.[7]

group; from previous work[2,3] the remaining activity must be confined to C-1,-4,-4a,-5a,-6,-9,-9a, and -10a. Our results suggest that (1) is equally incorporated into both "halves" of (5), but it must be noted that this result would inevitably be obtained if a symmetrical intermediate of the type (7) was involved in the pathway, even if only one shikimic acid unit was utilized for the biosynthesis of (5).

Acknowledgements: We are indebted to Mr. T. Etherington for expert technical assistance and Mr. R. Robinson for mass spectra.

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