Tetrahedron Letters No. 33, pp 2987 - 2990.

0040-4039/78/0808-2987$02.00/0

# INCORPORATION OF SHIKIMIC ACID INTO IODININ

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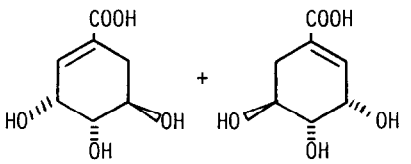
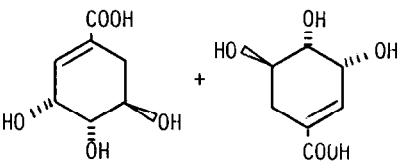
The role of shikimic acid (1) as precursor for a number of antibiotic phenazines[1] has been established among others for iodinin (2). Our previous results[10] have led to the conclusion that for iodinin the possible pairing schemes of two shikimic acid molecules can be narrowed down to schemes A and B. We have been able to eliminate pairing scheme A by the direct $^{13}C$-NMR method for elucidating the biosynthetic pathway.

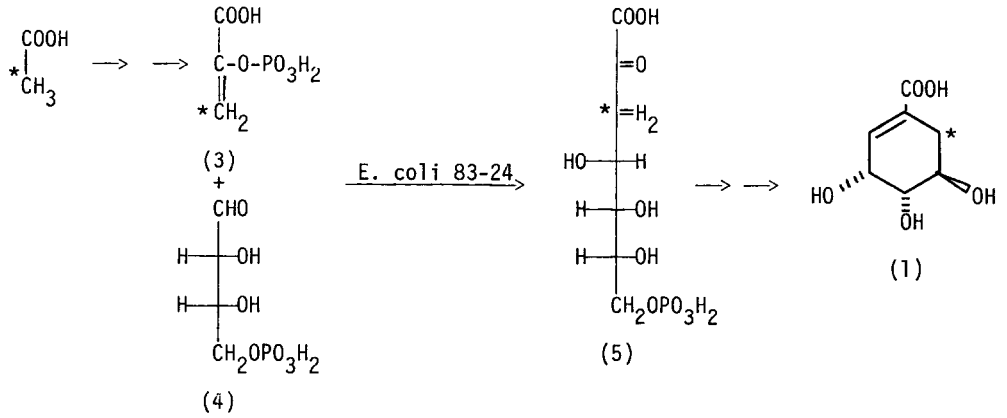



(1) A

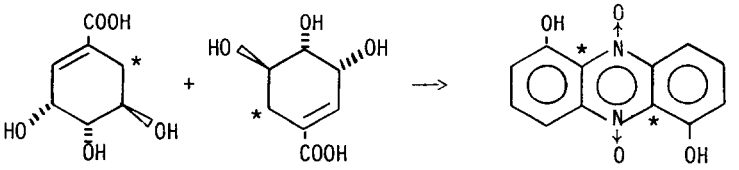



B

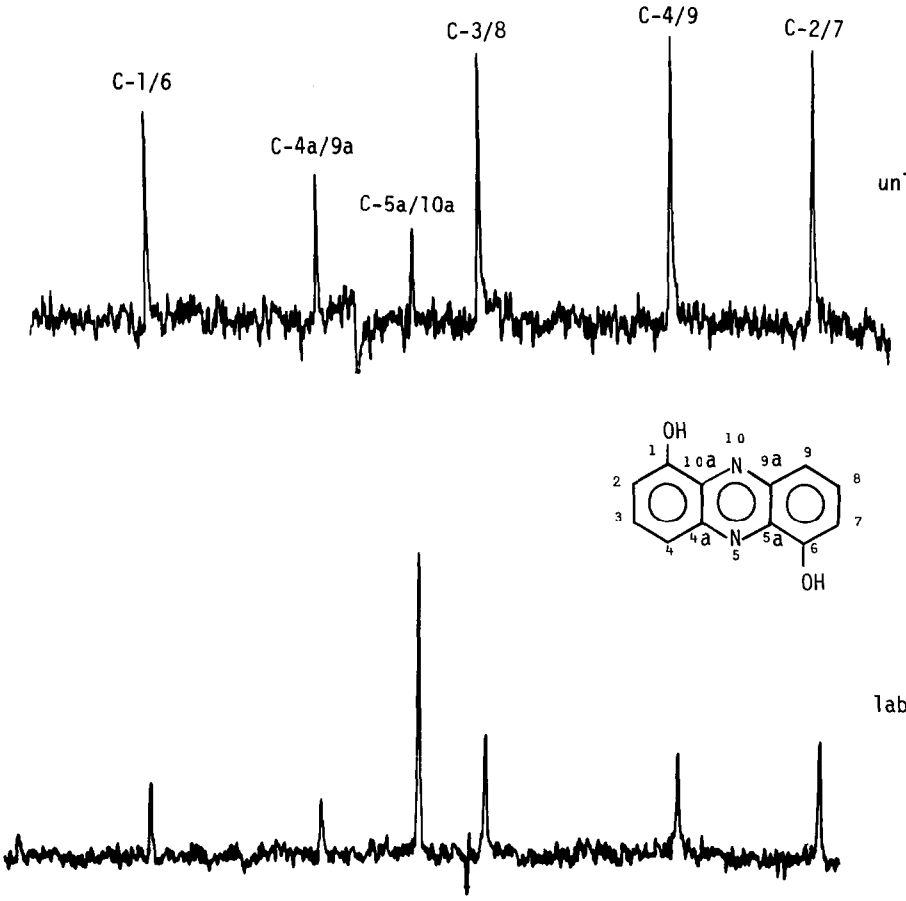
Acetic acid-2-$^{13}C$, 91.92% enriched, was converted via its potassium salt with benzoyl bromide into acetyl bromide[3]. The acid bromide was treated with cuprous cyanide to give pyruvonitrile[3] which was hydrolyzed with aqueous hydrochloric acid to pyruvamide[3] and then to pyruvic acid-$^{13}C$.[3] The pyruvic acid was brominated to bromopyruvic acid[4] and phosphorylated with trimethylphosphite to yield phosphoenol pyruvic acid-3-$^{13}C$ [5] (3), which was converted to its analytically pure monocyclohexylammonium salt (dec. 130-142°) in 27% overall yield from AcOH. Its $^{13}C$-NMR spectrum confirmed this structure and the compound showed 91.9% enrichment. The phosphoenol pyruvic acid was coupled with D-erythrose-4-phosphate (4) via 3-deoxy-D-arabino-2-heptulosonic acid-7-phosphate-3-$^{13}C$ (5) to shikimic acid-6-$^{13}C$ (1) by a cellfree extract of E. coli 83-24. The method was a 45-fold scaled up adaptation of a known procedure.[6] The overall yield from phosphoenol pyruvic acid was 40-60%. The $^{13}C$ enrichment in (1) had dropped to 40% as evidenced by mass spectrometry.




After it had been ascertained that a concentration of shikimic acid of 2 g/ℓ, as required for a reliable $^{13}$C-NMR analysis, fed to Brevibacterium iodinum (strain 26) did not upset the metabolism as to prevent production of iodinin, two 250 ml erlenmeyers with 50 ml production medium each[2] were inoculated with B. iodinum, agitated on a rotary shaker at 60 rpm at 29° and fed each with 25 mg shikimic acid-6-$^{13}$C, 40% enriched, on the 3rd, 4th, 5th and 6th day after inoculation. Thus, a total of 200 mg shikimic acid was fed. The total yield of crude iodinin was 179 mg. Because of its poor solubility in suitable NMR-solvents, it was reduced with $H_2$/Pt in dioxane to 1,6-dihydroxyphenazine[10] and recrystallized from dioxane, mp 268-269°. The $^{13}$C-NMR spectrum of a saturated dioxane solution of this 1,6-dihydroxyphenazine was measured (Varian XL-100, $D_2O$ lock, external TMS, pulse width 25 μs ∿ 45°, 7s delay, 6940 scans (unlabeled), 6956 scans (labeled), $^1$H frequency 100 MHz, decoupling with 2000 Hz band width, FT mode, coupled to a Nicolet TT-100 data system). The spectra of unlabeled 1,6-dihydroxyohenazine and 1,6-dihydroxyphenazine resulting from the feeding experiment are shown below and a comparison of chemical shifts and relative peak intensities is made in the subsequent table. Thus, the labeled spectrum shows a 5.8-fold enrichment of the equivalent positions C-5a and C-10a with a calculated 8.4% incorporation of the label[8] in agreement with a biosynthetic pathway




Moreover, our results show that the hydroxyls in iodinin must have arisen by decarboxylative hydroxylation. If those hydroxyls had been the original 4-hydroxyls in shikimic acid, the $^{13}$C-NMR spectrum would have shown enrichment of the equivalent positions 4a/9a. This direct evidence complements and confirms Holliman's recent conclusions arrived at by D-exchange rate measurements.[9]




| Peak (from left to right) | Assigned position[7] | ppm Relative to TMS in $CDCl_3$[7] | ppm in dioxane (this work) unlabeled | labeled | Rel. intensity labeled / Rel. intensity unlabeled |
|---|---|---|---|---|---|
| 1 | 1/6 | 153.4 | 153.5 | 153.6 | 1.1 |
| 2 | 4a/9a | 142.2 | 142.5 | 142.7 | 1.0 |
| 3 | 5a/10a | 135.9 | 136.4 | 136.4 | 5.8 |
| 4 | 3/8 | 131.3 | 132.0 | 132.1 | 1.0 |
| 5 | 4/9 | 119.3 | 119.5 | 119.6 | 0.9 |
| 6 | 2/7 | 110.6 | 110.1 | 110.3 | 1.0 |

Acknowledgements:

This work was supported by NIH grant No. A109598. The Nicolet FT data system was obtained with partial support from the NSF, grant No. MPS75-06111, under the departmental instrument grant program.

We are grateful for the generous gift of acetic-2-$^{13}$C acid from and consultation with Dr. T. W. Whaley, Los Alamos Scientific Laboratory.

The technical assistance of Miss S. Weiss is gratefully acknowledged.

References:

1. U. Hollstein, G. E. Krisov and D. L. Mock, Tetrahedron Letters, 326 (1976).
2. U. Hollstein and L. G. Marshall, J. Org. Chem., 7, 3510 (1972). The production medium for Ps. aureofaciens was used.
3. H. S. Anker, J. Biol. Chem., 176, 1334 (1948).
4. D. B. Sprinson and E. Chargaff, J. Biol. Chem., 164, 424 (1946).
5. V. M. Clark and A. J. Kirby, Biochim. Biophys. Acta, 78, 732 (1963).
6. K. H. Scharf and M. H. Zenk, J. Label. Compounds, 7, 525 (1971).
7. E. Breitmaier and U. Hollstein, J. Org. Chem., 41, 2104 (1976).
8. A. G. McInnes, J. A. Walters, J. L. C. Wright and L. C. Vining, in Topics in Carbon-13 NMR Spectroscopy, G. C. Levy, Ed., Vol. II, Wiley, New York, 1976, p. 125.
9. R. B. Herbert, F. G. Holliman and J. B. Sheridan; Tetrahedron Letters, 639 (1976).
10. U. Hollstein and D. A. McCamey, J. Org. Chem., 38, 3415 (1973).

**(Received in USA 22 May 1978; received in UK for publication 20 June 1978)**