Communications

Facile Pyridoxal-Catalyzed Racemization of Nornicotine and Related Compounds

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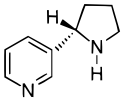
Received December 19, 1995

Despite recent advances in asymmetric synthesis, in many cases classical resolution is the method of choice for preparing pure enantiomers. Indeed, "65% of non-natural enantiomeric drugs are made by resolution of racemic drugs or intermediates and not by asymmetric synthesis".[1] In such cases, racemization of the unneeded isomer and cycling through the resolution process might be desirable.

Our studies of the metabolic disposition of tobacco alkaloids in humans[2−5] require gram quantities of deuterium-labeled nicotine, its metabolites, and other alkaloids. The syntheses utilized for preparing these labeled compounds involve a resolution step in order to obtain the desired (*S*)-enantiomers.[5−7] Consequently, it would be advantageous if unneeded deuterated intermediates with the (*R*)-configuration could be racemized and recycled through the resolution step. Since the syntheses involve resolution of racemic deuterium-labeled nornicotine derivatives, a method for racemization of nornicotine [3-(2-pyrrolidinyl)pyridine, **1**] and related compounds (e.g., 5-bromonornicotine-*2,4,6-d*$_3$, **2**) was sought.



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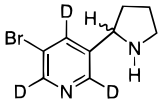

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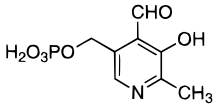

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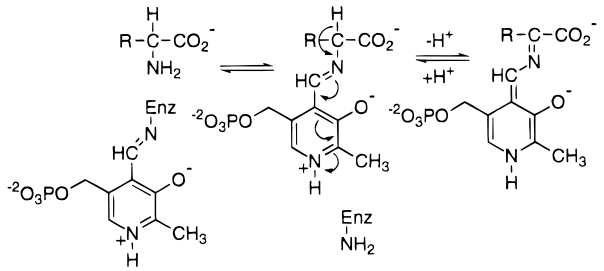
Pyridoxal phosphate **3** is a cofactor in enzyme-catalyzed racemization reactions of amino acids.[8] Mechanistic studies have established that these reactions involve the intermediacy of a Schiff's base formed between the aldehyde group of pyridoxal and the amino group of the amino acid.[9,10] Rearrangement of the Schiff's base occurs, resulting in loss of the proton attached to the chiral center (Scheme 1). The rearrangement is reversible, and hydrolysis releases the racemized amino acid. In model systems, pyridoxal will catalyze racemization of amino acids and amino acid derivatives. The rates of nonenzymatic racemization reactions are increased by cations such as Cu^{2+}, Fe^{3+}, and Al^{3+}, presumably due to stabilization of the Schiff's base intermediates.[11]

Scheme 1



Racemization of simple α-chiral amines catalyzed by pyridoxal or other aldehydes was considered possible. With secondary amines such as nornicotine **1**, iminium ions rather than Schiff's base intermediates were envisioned. Attempts were made to racemize nornicotine using various simple aldehydes and pyridoxal under conditions that would maximize formation of iminium ions.[12] Heating optically pure (*S*)-nornicotine (**1**[6]) for 3 h at 100 °C in pH 4.5 aqueous buffer with formaldehyde, butyraldehyde, or benzaldehyde resulted in no detectable racemization. Under these conditions, pyridoxal or pyridoxal phosphate, however, catalyzed nearly complete racemization (ee = 2.3%[13] using pyridoxal phosphate), as determined[7] by GC−MS analysis of the (−)-camphanic acid amide derivatives. Salicylaldehyde and 4-pyridinecarboxaldehyde did not effect any detectable racemization under these conditions, indicating that both the pyridine nitrogen and a hydroxyl adjacent to the aldehyde group of the catalyst are necessary.[14]

The effect of pH on the rate of racemization of nornicotine was studied (Table 1). The rate was maximal between pH 4 and 6, with virtually no racemization occurring above pH 9. Unlike the nonenzymatic pyridoxal-catalyzed racemization of amino acids, metal ions (Al^{3+} or Cu^{2+}), did not enhance the rate of racemization of nornicotine. This was not unexpected, since it is believed that metal ions stabilize a pyridoxal-amino acid Schiff's base intermediate,[11] but in the case of nornicotine it is difficult to envision the intermediacy of a Schiff's base. Metal ions would not be expected to have much effect on the formation or stability of iminium ions presumed to be intermediates in the racemization of nornicotine.

A mechanistic study was beyond the scope of the present work, but the steps depicted in Scheme 2 are consistent with the above-mentioned observations and

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(1) Stinson, S. C. *Chem. Eng. News* **1995**, *73*, 44.
(2) Benowitz, N. L.; Jacob, P., III; Denaro, C.; Jenkins, R. *Clin. Pharmacol. Ther.* **1991**, *49*, 270−277.
(3) Benowitz, N. L.; Jacob, P., III. *Clin. Pharmacol. Ther.* **1993**, *53*, 316−323.
(4) Benowitz, N. L.; Jacob, P., III; Fong, I.; Gupta, S. *J. Pharmacol. Exp. Ther.* **1994**, *268*, 296−303.
(5) Benowitz, N. L.; Jacob, P., III. *Clin. Pharmacol. Ther.* **1994**, *56*, 483−493.
(6) Jacob, P., III. *J. Org. Chem.* **1982**, *47*, 4165−4167.
(7) Jacob, P., III; Benowitz, N. L.; Shulgin, A. T. *J. Labelled Comp. Radiopharm.* **1988**, *25*, 1117−1128.
(8) Walsh, C. *Enzymatic Reaction Mechanisms*; W. H. Freeman and Company: San Francisco, 1977; p 798.
(9) Bruice, T. C.; Benkovic, S. J. *Bioorganic Mechanisms*; W. A. Benjamin, Inc.: New York, 1966; p 285.
(10) Dugas, H.; Penney, C. *Bioorganic Chemistry. A Chemical Approach to Enzyme Action*; Springer-Verlag: New York, 1981; p 420.
(11) Olivard, J.; Metzler, D. E.; Snell, E. E. *J. Biol. Chem.* **1952**, *199*, 669.
(12) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.
(13) Since there is some diastereoselection in the assay, favoring the (*R*)-isomer by a few percent, the ee values should not be considered exact.
(14) Although we cannot rigorously exclude the possibility that the hydroxymethyl group is involved with the catalysts, studies modeling pyridoxal-catalyzed reactions of amino acids make this possibility unlikely: ref 9, p 253.

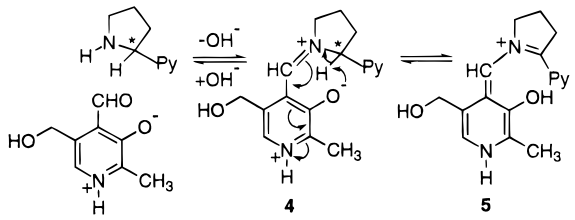
S0022-3263(95)02233-X CCC: $12.00

Table 1. Effect of pH on the Racemization of (*S*)-Nornicotine Catalyzed by Pyridoxal[a]

pH	4	5	6	7	8	9	10
ee of (*S*)-isomer,[b] (%)	72	60	69	81	92	97	99

[a] Reactions were carried out by mixing phosphate buffer (100 μL of 1 M) with 100 μL of 1 mg/mL of (*S*)-nornicotine[6] and 100 μL of 1 mg/mL of pyridoxal hydrochloride and then heating at 100 °C for 1 h. [b] See ref 13 in text.

Scheme 2



previous studies of pyridoxal-catalyzed reactions of amino acids. Reaction of nornicotine and the zwitterionic form of pyridoxal (the predominant form at pH 4.5[15]) would be expected to produce iminium ion **4**. The ionized hydroxyl could then serve as an intramolecular base catalyst, abstracting the proton attached to the chiral center, resulting in double bond migration to give **5**. The protonated pyridine nitrogen could serve as an "electron sink"[16] to facilitate the process, as in pyridoxal-requiring reactions of amino acids. Reversibility of this process would result in racemization. This scheme accounts for the requirement of both a pyridine nitrogen atom and hydroxyl adjacent to the aldehyde group in the catalyst.[14]

The reaction is preparatively useful. Ten grams of 5-bromonornicotine-*2,4,6*-d_3 (**2**[5]) (58% ee of (*R*)-isomer) was added to 250 mL of water, and glacial acetic acid was added to bring the pH to 5. This resulted in some precipitation of the acetate salt. Pyridoxal hydrochloride (0.5 g) was added, and the mixture was heated under reflux. The reaction was monitored by GC−MS analysis of the (−)-camphanic acid amide derivatives.[7] After 1 h, the enantiomeric purity was 33% ee, and after 18 h it was 15% ee. An additional 0.5 g of pyridoxal hydrochloride was added, and reflux was continued for an additional 5 h. After this, the racemization was complete or nearly complete, ee = 7%.[13] The reaction mixture was cooled, made basic with sodium hydroxide, extracted with methylene chloride, and distilled (Kugelrohr, 125−130 °C at 0.1 mmHg) to give an 83% yield of product. In another run, 2 g of pyridoxal hydrochloride was added in four portions during 48 h to a reaction mixture containing 17.5 g of **2** (initially 58% ee of (*R*)-isomer). An 87% yield was obtained, ee = 4%.[13]

The high isolated yields were gratifying, since iminium ions, which are seemingly obligatory intermediates, may be in equilibrium with enamines, which could lead to side reactions. For example, nicotine-$\Delta^{1',5'}$-iminium ion **6** undergoes dimerization in aqueous solution at room temperature, presumably via reaction with the $\Delta^{4',5'}$-enamine **7**.[17] In the case of pyridoxal-catalyzed racemization of nornicotine **1**, evidence that enamines are formed was obtained. Carrying out the reaction in D_2O resulted in deuterium exchange with all seven hydrogens on the pyrrolidine ring, as well as racemization, presumably through the intermediacy of equilibrating iminium ions and enamines. Consequently, pyridoxal-catalyzed exchange is potentially useful for preparing highly deuterated or tritiated amines.[18] These results also indicate that racemization of amines with a chiral center in the β-position could be catalyzed by pyridoxal.

6 **7**

Studies are in progress with other amines to evaluate the synthetic potential of pyridoxal-catalyzed racemization and deuterium exchange reactions.

Acknowledgment. I thank Dr. Alexander T. Shulgin (UCSF) and Dr. Dagmar Ringe (Brandeis University) for helpful discussions and Kaye Welch for editorial assistance. Research supported in part by grants DA02277 and DA01696 from the National Institute on Drug Abuse and by funds provided by the Cigarette and Tobacco Surtax of the State of California through the Tobacco-Related Disease Research Program of the University of California.

Supporting Information Available: Experimental procedures for racemization of and deuterium incorporation in **1** and GC−MS data documenting racemization and extent of deuterium incorporation (6 pages).

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(15) Reference 9, p 248.
(16) Reference 10, p 421.
(17) Brandange, S.; Lindblom, L. *Acta Chem. Scand.* **1979**, *B33*, 187−191.
(18) Pyridoxal-catalyzed exchange reactions have been used to synthesize deuterium-labeled amino acids: Thomas, A. F. *Deuterium Labeling in Organic Chemistry*; Appleton-Century-Crofts: New York, 1971.