High-Performance Liquid Chromatographic Assay of Isoquinoline Alkaloid Formation from Reaction of Biogenic Amines and Aldehydes

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Abstract □ To understand the role that tetrahydroisoquinoline formation may play in alcoholism and drug toxicology, high-performance liquid chromatography with electrochemical detection was used to monitor the overall rate of reaction, in pH 7.4 buffer, between the catecholamines (dopamine, α-methyldopamine, dihydroxyphenylpropanolamine, deoxyepinephrine, levodopa, α-methyldopa, epinephrine, levarterenol, and isoproterenol) and acetaldehyde. The observed overall rate of reaction varied from 0.38 to 0.0013 liter/mole sec. In addition, the reaction rate of the neurotransmitter dopamine was measured for various aldehydes (formaldehyde, acetaldehyde, glyoxylic acid, paraldehyde, malonaldehyde, glyceraldehyde, and chloral hydrate). The observed overall rate of reaction varied from 5.3 to 0.0011 liters/mole sec. Penicillamine prevented formation of the tetrahydroisoquinoline alkaloids when initially present in concentrations equal to or greater than the aldehyde concentration.

Keyphrases □ Catecholamines, various—reaction with acetaldehyde, formation rate of isoquinoline alkaloids □ Aldehydes, various—reaction with dopamine, formation rate of isoquinoline alkaloids □ Isoquinoline alkaloids—formation rate by reaction of various catecholamines and aldehydes □ Alkaloids, isoquinoline—formation rate by reaction of various catecholamines and aldehydes

An understanding of the mechanism and kinetics of the Pictet–Spengler condensation is significant. The reaction, involving the formation of tetrahydroisoquinoline alkaloids from biogenic amines and aldehydes, may be important in alcoholism and the toxicology of several pharmaceuticals adventitiously coadministered with alcohol (1–9). While some effort has been expended to evaluate the role of these alkaloids *in vivo*, few supporting chemical studies have been reported (10).

Recently, this laboratory developed a new approach to the assay of phenolic alkaloids involving high-performance liquid chromatography (HPLC) with electrochemical detection (11). The new method is sufficiently sensitive to be useful for monitoring isoquinoline alkaloids in biological material, and data have been obtained for the banana (12), cocoa (13), and human urine[1]. The present brief report describes the use of HPLC to determine the overall formation rate of tetrahydroisoquinoline alkaloids from the Pictet–Spengler reaction at physiological pH. The inhibition of alkaloid formation by competitive reaction of penicillamine with acetaldehyde also was examined. This investigation was undertaken as part of a long-term study on the role of *in vivo* isoquinoline formation in drug toxicology.

EXPERIMENTAL

A modular HPLC system[2] was used with an amperometric detector operated at +0.8 v *versus* the saturated calomel electrode. A 50-cm × 2-mm glass column was dry packed with a pellicular cation-exchange resin[3]. The aqueous mobile phase consisted of 0.018 *M* H_2SO_4 and either 0.1 or 0.2 *M* Na_2SO_4, depending upon the relative capacity factors of the alkaloids and parent amines.

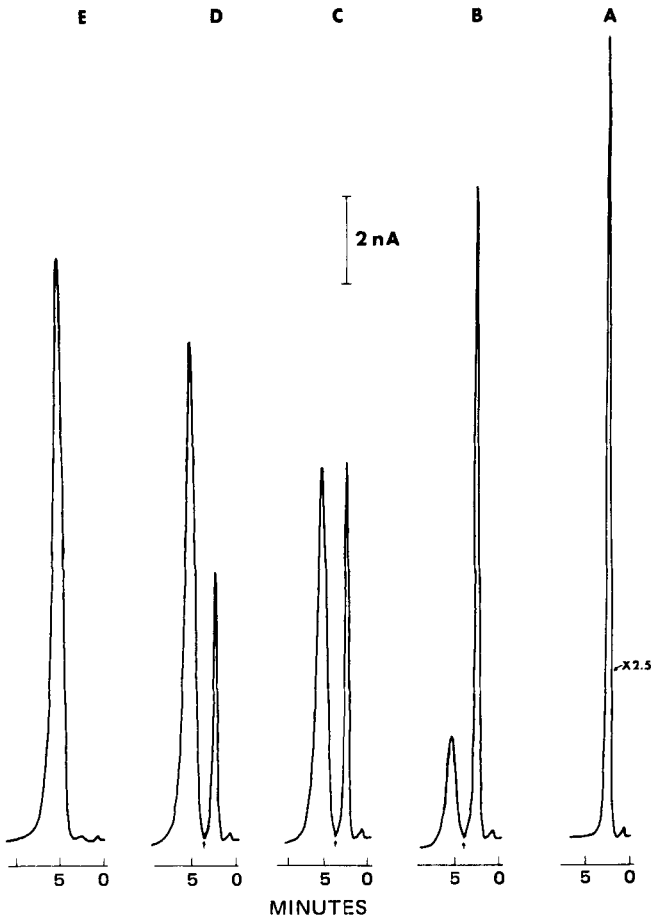


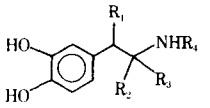
Figure 1—*Representative chromatograms illustrating conversion of dopamine to salsolinol at pH 7.4 monitored by HPLC with electrochemical detection. The initial concentration of dopamine was 1 m*M*, and that of acetaldehyde was 20 m*M*. Key: A, 0 sec; B, 25 sec; C, 101 sec; D, 149 sec; and E, 300 sec.*

Reactions were carried out at 37° in 0.1 *M* phosphate buffer, pH 7.4. The amines tested included dopamine, α-methyldopamine, 3,4-dihydroxyphenylpropanolamine, deoxyepinephrine, levodopa, α-methyldopa, epinephrine, levarterenol, isoproterenol, and serotonin. The compounds (as salts), except α-methyldopa and α-methyldopamine[4], were obtained from commercial suppliers[5].

Acetaldehyde, formaldehyde, malonaldehyde, glyceraldehyde, glyoxylic acid, paraldehyde, or chloral hydrate was added to a 1 m*M* amine solution so that the final analytical concentration of the aldehyde was 20 m*M*. Serial aliquots (25 μl) of the reaction mixture were transferred to test tubes containing 10 ml of the acidic mobile phase at 0°, thereby quenching the reaction both thermally and by dilution. Each tube was

[1] R. M. Riggin and P. T. Kissinger, unpublished results.
[2] Model LC-40, Bioanalytical Systems.
[3] Zipax SCX, du Pont.
[4] Donated by the Merck Institute, West Point, Pa.
[5] Sigma Chemical Co., Aldrich Chemical Co., and Regis Chemical Co.

Table I—Reaction of Catecholamines with Acetaldehyde at pH 7.4



Compound	R_1	R_2	R_3	R_4	k_{obs}, liter/mole sec[a]
Dopamine	H	H	H	H	0.38
α-Methyldopamine	H	CH_3	H	H	0.36
3,4-Dihydroxyphenyl-propanolamine	OH	CH_3	H	H	0.32
Deoxyepinephrine	H	H	H	CH_3	0.30
Levodopa	H	COOH	H	H	0.16
α-Methyldopa	H	COOH	CH_3	H	0.14
Epinephrine	OH	H	H	CH_3	0.10
Levarterenol	OH	H	H	H	0.075
Isoproterenol	OH	H	H	$CH(CH_3)_2$	0.0013

[a] Observed second-order rate constants for the disappearance of catecholamines in the presence of acetaldehyde at pH 7.4 and 37° determined under pseudo-first-order conditions *via* HPLC.

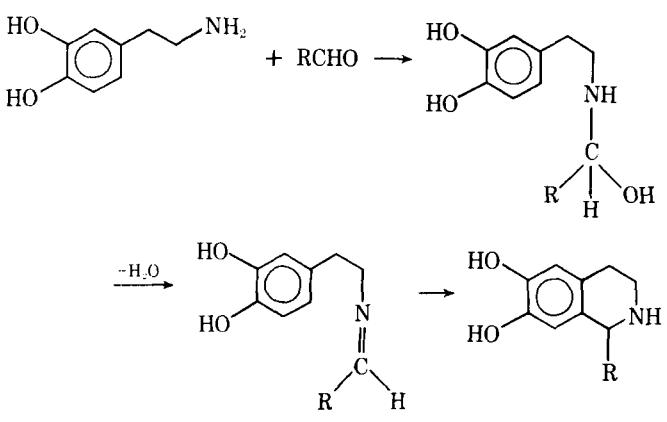
immediately capped, vortexed, and kept on ice prior to chromatographic analysis. A similar sequence was used during competitive inhibition studies, except that penicillamine was mixed with the deoxygenated amine solutions prior to aldehyde addition. All chromatographic data were analyzed by manual peak height measurement and fit, using a simple linear regression analysis assuming pseudo-first-order behavior.

RESULTS AND DISCUSSION

Neuroamines condense *in vivo* with aldehydes to form isoquinoline alkaloids of potential pharmacological importance. Since the reaction is apparently nonenzymatic, the chemistry in homogeneous solution probably has a direct bearing on the *in vivo* process. A thorough study of this chemistry (14) is complicated by the large number of reactions (protonations and hydrations) coupled to the major steps indicated in Scheme I, using dopamine as an example.

While all of the details are not well understood, a useful first step is to assess the influence of amine and aldehyde structure on the overall rate of alkaloid formation. HPLC is well suited to this objective since, in most cases, the irreversible reaction is sufficiently slow to permit discrete analysis of the reaction mixture quenched by acidification, dilution, and temperature. For example, the time course of the reaction between dopamine and acetaldehyde can be examined by HPLC as illustrated in Fig. 1. All alkaloids of interest are phenolic and can be readily oxidized at a graphite electrode following separation by HPLC. The current resulting from this electrochemical reaction is an excellent measure of the quantity eluted from the column.

Rate constants obtained for the condensation of acetaldehyde with several catecholamines are presented in Table I. Where the biogenic amine is β-hydroxylated (*e.g.*, levarterenol), the reaction is complicated by the stereochemistry of the final product(s). Serotonin (5-hydroxytryptamine), which is known to react with acetaldehyde (15, 16), has a k_{obs} of 0.0034 liter/mole sec for the formation of 1-methyl-6-hydroxytryptoline under the conditions described in Table I. Observed rate constants for the condensation of endogenous or exogenous aldehydes with dopamine are reported in Table II. The hypnotic compounds, chloral hydrate and paraldehyde, react, but slowly, as do aldehydes that result from lipid peroxidation (17).



Scheme I

Table II—Reaction of Dopamine with Various Aldehydes at pH 7.4

Compound	k_{obs}, liters/mole sec[a]
Formaldehyde	5.3
Acetaldehyde	0.38
Glyoxylic acid	0.33
Paraldehyde	0.069
Malonaldehyde	0.048
Glyceraldehyde	0.021
Chloral hydrate	0.0011

[a] Observed second-order rate constants for the disappearance of dopamine at pH 7.4, 37°, determined under pseudo-first-order conditions *via* HPLC.

An attempt was made to examine the role of sulfhydryl-containing compounds (*i.e.*, penicillamine and cysteine) in preventing tetrahydroisoquinoline alkaloid formation. With variable amounts of the sulfhydryl compounds present in the initial catecholamine reaction mixture, acetaldehyde combined with the sulfhydryl compound in a 1:1 ratio. With stoichiometric amounts of penicillamine and acetaldehyde, no measurable alkaloid was produced even at catecholamine concentrations 10-fold in excess. Penicillamine and cysteine protect animals from the detrimental effects of acetaldehyde by the formation of the corresponding thiazolidine compounds (18–20). This observation may eventually prove useful in alcohol detoxification therapy.

This HPLC approach is applicable to a number of related problems in alkaloid chemistry because of its excellent selectivity and sensitivity (detection limits below 100 pg are routine). Whether the Pictet–Spengler reaction is important in the pharmacology of amine or aldehyde drugs remains to be determined. *In vivo* experiments bearing on this question are planned. The methodology for brain tissue assay has already been developed (21).

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