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PREPARATION OF TRITIUM-LABELED BIOGENIC AMINES AND THEIR ANALOGS

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The possibility has been shown of introducing a tritium label into biogenic amines and their analogs in nonaqueous systems. Labeled compounds possessing a specific activity sufficient for performing many biological investigations have been obtained in good yields.

Investigations of recent years have shown the exceptionally important role of biogenic amines in the performance of adaptive reactions of the organism. At the present time the biosynthetic pathways of these biologically active compounds have been largely elucidated and determined with accuracy, and the main enzyme systems participating in the multistage processes have also been studied. For subsequent biochemical, physiological, and chemical investigation a continuous improvement and the introduction of modern methods of determining both the biogenic amines themselves and their transformation products in the organism are necessary. One of such methods is the use of radioactive preparations [1, 2].

In the present paper we consider the introduction of a tritium label into dopamine, norepinephrine, phenylephrine, β-phenylethylamine, tyramine, and metanephrine by the method of heterogeneous catalytic isotope exchange in solution with gaseous tritium.

Since, when this type of reaction is carried out in aqueous media, intensive isotope exchange takes place between the gaseous tritium and the solvent, and also in view of the fact that many biogenic amines are labile in aqueous solutions, we used methanol as the solvent. We first studied the stability of the biogenic amines and the distribution of the isotopic label in the molecules of these compounds under the conditions that we proposed to use for the catalytic reactions with gaseous tritium. In order to determine the optimum reaction time of isotope exchange we studied the kinetics of the inclusion of 0.1% tritium into the tyramine molecule (Fig. 1). We simultaneously determined the tyramine content by spectral methods (UV). As can be seen from Fig. 1, after about 3 h the rate of inclusion of the label decreases substantially and the concentration of tritium has fallen by only 5%, and therefore it was decided to limit the reaction time to three hours.

In studying the stability of the structures investigated, we treated norepinephrine and metanephrine with gaseous deuterium (pressure 250 mm Hg) in methanol for 3 h and, without additional purification, analyzed the compounds obtained by the method of PMR, UV, and mass spectroscopy.

The mass spectra of the deuterated and initial norepinephrine and metanephrine are shown in Fig. 2. As can be seen from a comparison of spectra a and b and of spectra c and d, the molecular peaks and the main peaks of the fragments coincide and, therefore, the treatment of norepinephrine and metanephrine under the selected conditions is not accompanied by the decomposition of the substance.

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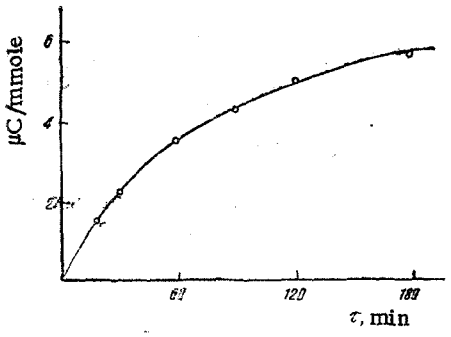


Fig. 1. Change in the specific activity of tyramine during the isotope exchange reaction with 0.1% tritium (23°C, pressure 250 mm Hg).

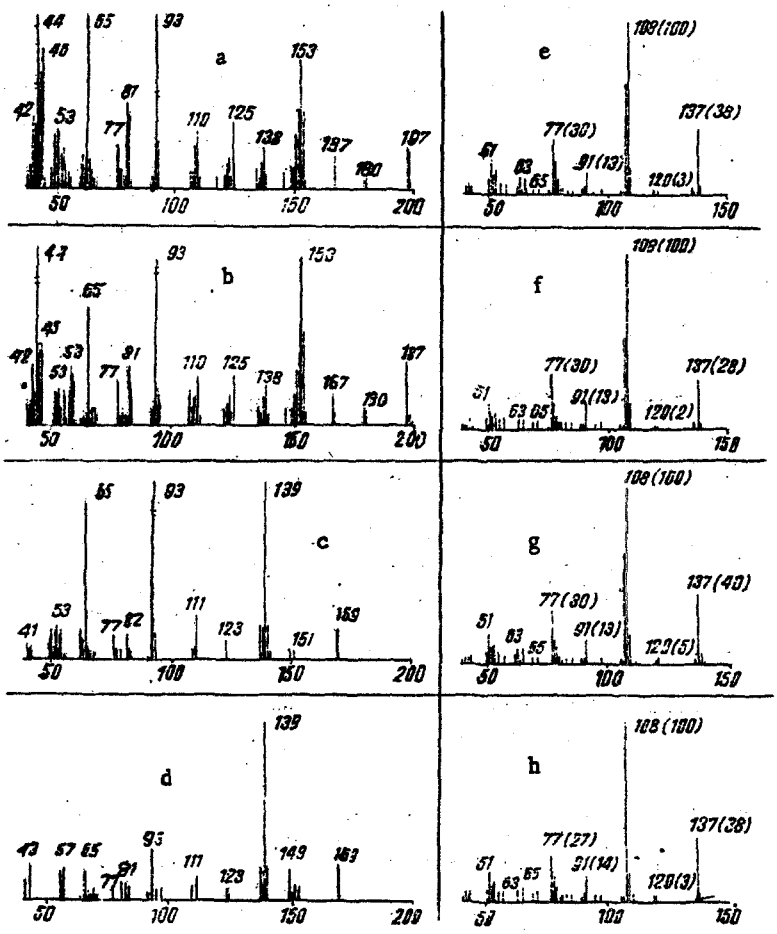


Fig. 2. Mass spectra of biogenic amines before (a, c, e) and after (b, d, f, g, h) their treatment with 100% deuterium. The relative intensities of the peaks of the main ions (m/e) are shown in parentheses: a, b) methanephrine; c, d) norepinephrine; e, f, g, h) tyramine; f) solvent methanol; g) methanol containing 7% of conc. HCl; h) methanol containing 7% of conc. NH_4OH.

The PMR spectra of these compounds also proved to be identical: in the aromatic region, a multiplet, δ 6.72-7.08; in the region of methyl groups, a quartet, δ 4.72-4.96, hydrogen at C-2; in the region of ethylene groups, a multiplet, δ 2.93-3.22, hydrogen at C-1; and in the case of metanephrine, in addition, in the region of methyl groups a singlet, δ 3.893, OCH_3, and a singlet, δ 2.758, NCH_3.

The UV spectra of the deuterium-treated and the initial metanephrines [λ_{max}279 nm (in methanol), λ 3330] and norepinephrines [λ_{max} 282 nm (in methanol) λ 2869] were identical, but the concentrations of metanephrine and norepinephrine (the comparison was made from the adsorption at λ_{max}) isolated after treatment with deuterium had decreased by approximately 5% and 20%, respectively.

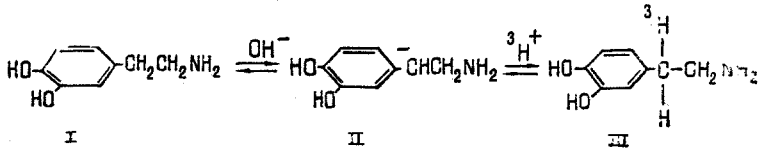
Thus, the results obtained indicate a fair stability of the labile biogenic amines on their treatment with the hydrogen isotope under the conditions that we have proposed.

In order to determine the distribution of the isotopic label it was necessary to increase the concentration of deuterium. With this aim, tyramine — a relatively stable and well-crystallizing compound — was subjected to treatment with deuterium under more severe conditions. The reaction was performed at a pressure of 650 mm Hg, for 24 h in methanol, in methanol containing 7% of concentrated HCl, and in methanol containing 7% of concentrated NH_4OH. The deuterated tyramine was purified by crystallization from a mixture of hexane, benzene, and methanol and then its UV, PMR, and mass spectra were studied.

As can be seen from the facts given, the structures of the compounds isolated were identical with the initial one. Their mass spectra were given in Fig. 2. The mass spectra (f, g, h) of tyramine treated with deuterium under different conditions are practically identical. They show the absence of any degradation of the substance whatever. The PMR spectra are similar to the spectra of the initial tyramine, which is given in Fig. 3. The UV spectra of all the samples had λ_{max} 283 nm (in methanol), ε 2031. The PMR spectra of tyramine contained four groups of two-proton signals (Fig. 3). In these compounds, the hydrogen atoms attached to oxygen and nitrogen are rapidly replaced in deuteromethanol and therefore give no signals. On comparing the integral values of the proton signals of the PMR spectra of the initial and deuterium-treated tyramines, it was found that the largest amount of deuterium was included when the deuterium-exchange reaction was carried out in pure methanol.

On the basis of the results of a comparison of the integral values of the proton signals, it may be assumed that isotope exchange is most probable in the adsorption of the molecules of biogenic amines by the C-2,4,8 section on the active centers of the catalyst. The ratios of the amounts of deuterium included at C-2 and C-2,4,8 in pure methanol, acidified methanol, and ammonia-containing methanol were 1:1.5; 1:2.03; and 1:2.47, respectively.

Thus, the distribution of deuterium in tyramine differed from the distribution of a tritium label in dopamine (5-hydroxytyramine) when the isotope exchange reaction was performed in phosphate buffer (pH 8-9) with gaseous tritium [3]. Evans [3] explains the inclusion of almost 90% of the radioactivity at the C-2 position by the formation of the intermediate carbanion II from dopamine (I) in its conversion into [^{3}H]dopamine (III).



The conditions for performing the reaction in methanolic solution apparently do not promote the stabilization of the carbanion (II). This leads to a situation in which the exchange of deuterium with the protons of the ring and with the benzyl protons becomes more probable than the formation of the carbanion (II) with the subsequent inclusion of the hydrogen isotope.

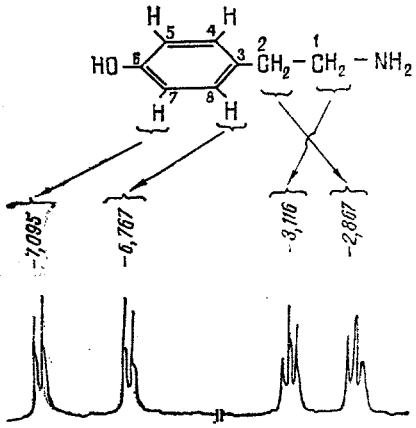


Fig. 3. PMR spectrum of tyramine.

The probabilities of adsorption on the active center of the catalyst of the protons at C-2, C-4, and C-8 are close, and therefore the amount of isotopic label at C-4,8 should exceed the amount of deuterium included at C-2, as is observed experimentally.

As a result of the investigations performed, the following conditions have been selected for isotopic exchange between the compounds mentioned above and 100% tritium; solvent, methanol; reaction time, 3 h; temperature, 23°C. After the treatment of the biogenic amines and their analogs with gaseous tritium [4], they were freed from degradation products by crystallization. The radiochemical purity of the compounds was analyzed by a method described previously [5] (Fig. 4). It can be seen from Fig. 1 that the introduction of a tritium label is greatly affected by the physical properties of the compound (the mobility of the compound on TLC is one of the manifestations of such influence). It was found that the following relationship exists: the greater the Rf value of the compound when it is chromatographed on cellulose F in the butanol—acetic acid—water (4:1:5) system, the greater is the specific activity. It is also interesting to note that two compounds — phenylephrine and tyramine — although they have close chromographic mobilities, differ strongly in their specific activities. This is probably connected with the presence of a hydroxy group at C-2 which creates additional hindrances to the adsorption of the molecules by the C-2,4,8 section. It is known that the heterogeneous isotope exchange reaction with gaseous tritium in solution is very sensitive to steric factors. It has been shown [3] that position 9 of methotrexate (there is a methyl substituent in position 10) contains 30% of the total radioactivity, while in folic acid, which has a structure close to it (with hydrogen in position 10), there is 60% of the total radioactivity in position 9.

EXPERIMENTAL

The hydrochlorides of dopamine, norepinephrine, phenylephrine, β-phenylamine, tyramine, and metanephrine were commercial preparations. The catalyst (10% $Pd/BaSO_4$) and the solvents were prepared and purified by standard methods. Radioactivities were measured in a scintilla-

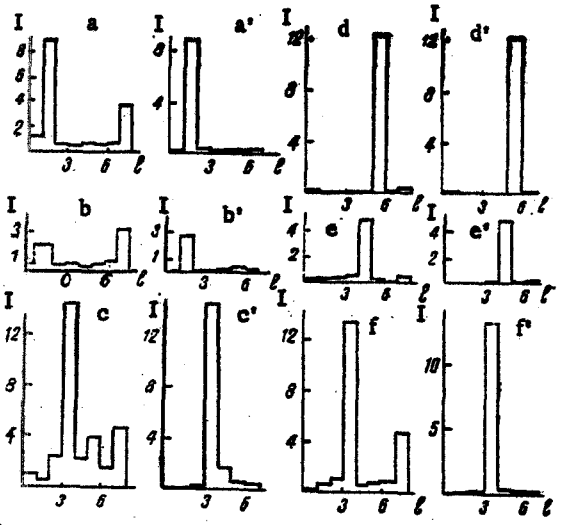


Fig. 4. Radiochemical purities of the biogenic amines and their analogs before (a, b, c, d, e, f) and after (a', b', c', d', e', f') the purification of the labeled preparation (I, counts/sec·10^{-4}, l, cm); a, a') dopamine; b, b') norepinephrine; c, c') phenylephrine; d, d') β-phenylethylamine; e, e') tyramine; f, f') metanephrine.

TABLE 1. Heterogeneous Isotopic Exchange of Hydrogen in Biogenic Amines and Their Analogs with Pure Tritium (methanol, 23°C, 3 h)

Compound	Mol. wt.	Wt., mg	Radioactivity of the preparation, mCi		Yield, %	Sp. act., mCi/mmole	R_f in the butanol—acetic acid—water (4:1:5) system
			unpurified	after purification			
Dopamine·HCl	189,63	30,1	38,8	15,0	82	115	0,26
Norepinephrine·HCl	205,65	30,7	21,6	2,1	67	22	0,18
Phenylephrine·HCl	203,67	30,4	66,2	33,1	86	258	0,45
β-Phenylamine·HCl	157,63	31,1	277,9	120,0	99	609	0,64
Tyramine·HCl	173,63	30,4	141,8	73,1	92	454	0,46
Metanephrine·HCl	233,7	30,5	47,1	20,1	85	181	0,38

tion counter with a recording efficiency for the preparations of ~12% in dioxane scintillator [6]. Analytical thin-layer chromatography was performed in system (1), butanol—acetic acid—water (4:1:5), on Cellulose F. The radiochemical purities of the products were monitored as described previously [5] (see Fig. 4) in system (1). Solutions in methanol of the labeled amines obtained were stored at 0°C. The concentrations of the labeled compounds were determined by spectral methods: dopamine at λ_{max} 282 nm (in methanol, ε 3175); phenylephrine at λ_{max} 275 nm (in methanol, ε 2291); and β-phenylethylamine at λ_{max} 257 nm (in methanol, ε 189).

UV spectra were recorded on a Specord UV-VIS spectrometer, mass spectra on an LKB 9000 instrument with direct introduction of the sample into the source at an ionization energy of 70 eV, and PMR spectra on a Varian SC-300 spectrometer with a working frequency of 300 MHz combined with a 620 L = 100 computer in the pulsed regime with a Fourier transformer at 22°C (except for metanephrine, the spectrum of which was measured at 50°C), at a concentration of 25 mg/ml in deuteromethanol.

All the preparative experiments connected with the kinetic investigations (0.1% tritium) and with the study of the distribution of the isotopic label (100% deuterium) were performed by the method and with the instruments and apparatus described elsewhere [7] at a molar ratio of catalyst to substance of 2:1, at room temperature.

The isotope exchange reaction with 100% tritium was carried out as described previously [4]. A tube was charged with 1 ml of absolute methanol containing approximately 30 mg of the substrate, 10% $Pd/BaSO_4$ was added (in a molar ratio of catalyst to compound of 2:1), the tube was frozen in liquid nitrogen and was evacuated to a pressure of $1 \cdot 10^{-3}$ mm Hg, and it was filled with gaseous tritium to a pressure of 250 mm Hg and was then heated to room temperature and was kept for 3 h with stirring by a magnetic stirrer. The catalyst was filtered off and was washed with methanol (3 × 10 ml). The filtrate was diluted to 50 ml with methanol and was evaporated, and the residue was again dissolved in 50 ml of methanol and evaporated to eliminate labile tritium.

The labeled compounds obtained were purified in the following way: the residue after the evaporation of the solvent was taken up in the minimum amount of methanol and, in the case of dopamine, tyramine, β-phenylethylamine, and metanephrine, benzene—hexane (1:1) or, in the case of phenylephrine and norepinephrine, benzene—hexane (1:10) was added until a precipitate appeared. Then the reaction mixture was kept at 0°C overnight, and the precipitate was filtered off and was washed with hexane. Information on the specific activities and yields are given in Table 1.

SUMMARY

The possibility has been shown of introducing a tritium label into biogenic amines and their analogs in nonaqueous systems. Labeled compounds have been obtained with yields and with specific activities sufficient for performing many biological investigations.

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