SYNTHESIS OF STABLE ISOTOPE LABELED NOREPINEPHRINE

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SUMMARY

Racemic norepinephrine was synthesized with three deuterium atoms on the alkyl chain. The deuteration was accomplished by D/H exchange on the intermediate, dibenzylaminodihydroxyacetophenone, followed by reduction of the keto moiety and cleavage of the benzyl-protecting groups with deuterium gas. Noradrenalone was also shown to be a possible intermediate for the incorporation of ^{18}O into norepinephrine.

Mass spectrometry and the technique of selective ion monitoring [1] for the quantitative measurement of minute quantitites of biologically-significant molecules have enjoyed widespread acceptance. One area for which it has been used extensively has been in the measurement of catecholamines and catecholamine metabolites in tissues and body fluids [2]. Stable isotope labeled molecules have been suggested as virtually ideal internal standards in such studies [3], and various such labeled catecholamines and metabolites have been synthesized [4,5,6]. However, the synthesis of stable isotope labeled norepinephrine (NE) has not been reported, even though this is the major neurotransmitter in the adrenergic nervous system [7]. Costa and co-workers [2,8] have reported a method for the quantitation of this amine using selective ion monitoring, however a homolog, α-methyl norepinephrine, served as the internal standard.

Trideutero-norepinephrine (NE-d_3) was synthesized by modification of the method outlined by Zenitz and Hartung [9]. Chloroacetocatechol [2-chloro-1-

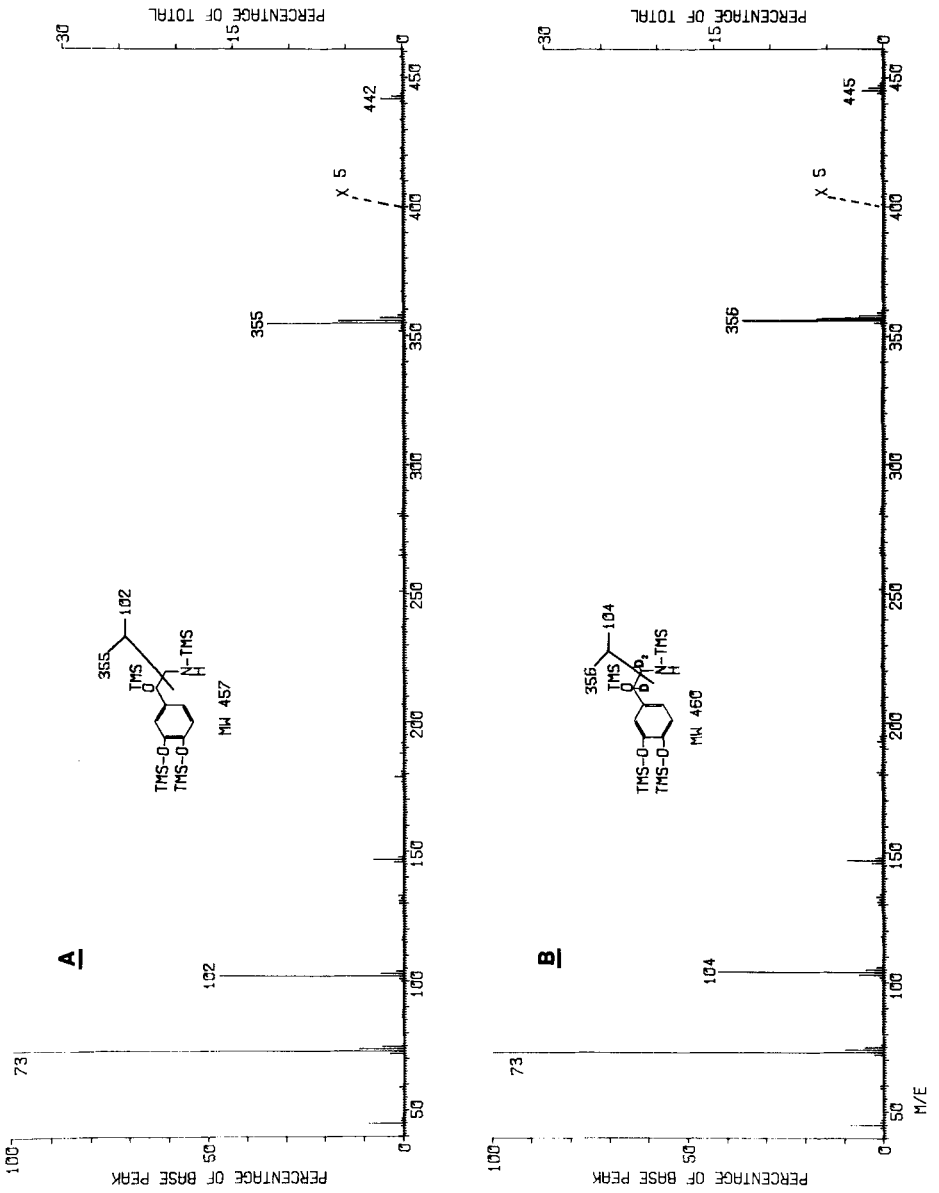
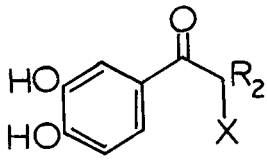


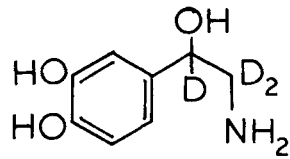
Figure: The mass spectra of tris(trimethylsilyl)norepinephrine (A) and tris(trimethylsilyl)trideuteronorepinephrine (B). Spectra were recorded under gas chromatography-mass spectrometry conditions with 70 eV electron-impact ionization.

(3,4 dihydroxyphenyl)ethanone, Ia] was condensed with dibenzylamine to yield 2-dibenzylamino-1-(3,4-dihydroxyphenyl)ethanone hydrochloride (Ib).



I

a. R=H, X=Cl
b. R=H, $X=\overset{+}{N}H(CH_2C_6H_5)_2Cl^-$
c. R=D, $X=\overset{+}{N}H(CH_2C_6H_5)_2Cl^-$
d. R=H, $X=\overset{+}{N}H_3Cl^-$



II

Deuterium incorporation into Ib was accomplished by the well-known acid catalyzed exchange of the protons alpha to the keto moiety. The first-order reaction proceeded with a rate constant of 0.23 hr^{-1} to yield product Ic. This deuterated ketone was then reduced under a deuterium atmosphere using a palladium on carbon catalyst [9] to simultaneously reduce the keto function and remove the benzyl protecting groups and yield the corresponding deuterated norepinephrine with an additional deuterium atom on the β-carbon atom (II).

The mass spectra of the tris(trimethylsilyl) derivative of authentic NE and synthetic NE-d_3 are shown in the Figure. The fragment ions at m/e 102 (A) and 104 (B) represent cleavage of the molecule between the α- and β-carbons with charge retention on the α-carbon. These ions indicate incorporation of deuterium onto the α-carbon as being 85.5 atom%-d_2, 12.7 atom%-d_1, and 1.8 atom%-d_0. The ions at m/e 355 (A) and 356 (B) arise from charge retention on the remaining portion of the molecule and isotope analysis indicated 95 atom%-d_1 and 5 atom%-d_0.

The commercial synthesis of norepinephrine proceeds by amination of chloroacetocatechol (Ia) with ammonia to give noradrenalone hydrochloride (Id) [10]. This is then reduced to norepinephrine by hydrogen using a palladium on carbon catalyst. The exchange of the protons alpha to the carbonyl group in noradrenalone hydrochloride (Id) was unsuccessful in spite of many attempts and precautions, the major reason being competing reactions which appeared to proceed at a rate faster than D/H exchange. The sensitivity of this amino-ketone

points out an advantage of the above synthesis which has benzyl groups protecting the amino group until the last step when the ketone is reduced and the benzyl groups are removed.

Noradrenalone was converted to a stable isotope labeled molecule after finding suitably mild conditions which allowed exchange of the keto-oxygen atom for ^{18}O. As shown in the Table, equilibrium exchange was attained in 24 hours using a trace of hydrochloric acid and 20%-^{18}O. Due to this rapid exchange with water, the conversion of ^{18}O-noradrenalone to ^{18}O-norepinephrine would have to proceed in an water-^{18}O environment or could be reduced by metal hydrides under anhydrous conditions. This conversion was not attempted. The high cost of this isotope of oxygen precluded the synthesis of higher isotopic purity ^{18}O-noradrenalone. However, recent reductions in the cost of this isotope may make this an attractive route to isotopically labeled norepinephrine especially suited for in vivo biochemical studies since, an unmeasurable isotope effect would be expected even in the worst case, in contrast to the corresponding deuterium-labeled norepinephrine.

TABLE

Exchange of the ketone-oxygen of Id with water - ^{18}O (20 atom%)

Time (Hrs)	Relative abundance m/e 137*	m/e 138	m/e 139
0	100	8.9	1.0
1.5	100	9.0	11.5
7	100	9.9	16.0
24	100	9.1	27.0+

*Id was isolated and inserted into the mass spectrometer ion source. The ion at m/e 137 represents cleavage between the α- and β-carbon with charge retention on the β-carbon atom.

+This abundance represents 21 atom%-^{18}O-incorporation.

EXPERIMENTAL

Mass spectra were recorded using a Finnigan Model 3100 mass spectrometer and a Model 6000 data system and Model 9500 gas chromatograph. A 1% OV-17 column was used for the gas chromatography. Melting points are uncorrected. The trimethylsilyl derivatives were prepared by heating the dried crystals of NE and NE-d_3 (1 mg) with bis(trimethylsilyl)trifluoroacetamide (100 µl) for 60 min at 60°.

2-dibenzylamino-1-(3,4-dihydroxyphenyl)ethanone hydrochloride (Ib).

This material was synthesized by the method of Zenitz and Hartung [9] in a 13.3 g yield (38%) by refluxing chloroacetocatechol with dibenzylamine in anhydrous ethanol. M.p. 163-165°. Mass spectrum, M^+ 347, m/e 210 (base peak), and m/e 137.

2-dibenzylamino-1-(3,4-dihydroxyphenyl)ethanone-2,2-d_2 hydrochloride (Ic).

One and six-tenths grams (4.6 mmole) of Ib was dissolved in 40 ml deuterium oxide (99 atom%), 20 ml dioxane, and 4 ml 20% deuterium chloride (99 atom%). The solution was degased under vacuum, sealed, and then heated at 80° for 4 days. After 4 days the sample was lyophilized to dryness and the same procedure repeated for a further 7 days after addition of fresh solvent. The product was isolated after evaporation of the solvent and recrystallized from cold dioxane/ether as white crystals. Yield 1.1 g (69%). M.p. 163-165°. Mass spectral analysis indicated 87.5 atom%-d_2, 11.8 atom%-d_1, and 0.7 atom%-d_0.

4-(2-amino-1-hydroxyethyl-1,2,2-d_3)benzene-1,2-diol(II, NE-d_3).

The deuterated product Ic was dissolved in 25 ml deuterium oxide with 10 ml acetic acid-d (99 atom%) and 0.8 g palladium on carbon (10%) was added. The mixture was put in a Parr hydrogenation apparatus and shaken with deuterium gas (30 psi, 99 atom%) for 4 hours, followed by standing an additional 12 hours under the deuterium atomsphere. The reaction mixture was filtered (cold) and a trace of sodium bisulfite added to the filtrate. The free base of norepinephrine was crystallized from this filtrate after evaporating to dryness and dissolving in 5 ml water to back exchange the labile hydrogens. The solution was cooled to 4° and 2 ml of concentrated ammonium hydroxide added according to the method of Tullar [11]. The mixture was frozen and, after 4 days, warmed to 4° and a light

yellow precipitate was collected by filtration. Yield 176 mg (24%). Anal. Calcd for $C_8H_8D_3NO_3$; C, 55.80; H†, 6.63; N, 8.13. Found‡: C, 55.7; H-D, 7.00; N,7.91. Mass spectral analysis (Figure) indicated 85.5 atom%-d_2. 12.7 atom%-d_1, and 1.1 atom%-d_0 on the α-carbon and 95 atom%-d_1 and 5 atom%-d_0 on the β-carbon which calculates to 81.2 atom%-d_3, 16.4 atom%-d_2, 2.3 atom%-d_1 and 0.1 atom%-d_0.

2-amino-1-(3,4-dihydroxyphenyl)ethanone-^{18}O (Id).

Two hundred milligrams of noradrenalone hydrochloride (Id) was dissolved in a mixture of 8 ml methanol, 1 ml acetonitrile, and 0.5 g water-^{18}O (20 atom%). Two to three milliliters of anydrous HCl gas was bubbled into the solution. An aliquot (10 µl) was taken for analysis before the addition of water - ^{18}O for the zero time measurement. Aliquots (10 µl) were taken after 1.5,7, and 24 hrs. The aliquots were evaporated to dryness in a glass capillary tube which was placed in a direct insertion probe for mass spectral analysis. The ion m/e 137 corresponds to cleavage adjacent to the carbonyl carbon with charge retention on the β-carbon, thus indicating the ^{18}O content. Results are given in the Table.

ACKNOWLEDGEMENT

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†Since hydrogen was to be measured gravimetrically as water, correction for the theoretical hydrogen value is made since 72.73% would be normal water and 27.27% would be deuterium oxide.

‡Analysis performed by Huffman Laboratories Inc., Wheatridge, Colorado.

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