SYNTHESIS OF D/L-NOREPINEPHRINE-(PHENYL-U-^{13}C)

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ABSTRACT

The synthesis of phenyl-U-carbon-13 labelled D,L-norepinephrine from catechol-U-^{13}C in three steps is described. In the first step, the Friedel-Crafts acylation of the labelled part with N-phthaloylglycylchloride yielded the aromatic ketone adduct. Subsequent hydrolysis and catalytic hydrogenation produced the desired product in a 5% overall yield.

Key Words: D/L-norepinephrine, carbon-13, Friedel-Crafts acylation

INTRODUCTION

Norepinephrine (NE) is a hormone and neurotransmitter directly related to some of the most widely spread diseases of mankind: hypertension, heart disease, and mental illness[1]. To elucidate the connection between the kinetics of NE in humans and the diseases mentioned above, various techniques have been developed for the quantitative measurement of NE levels in biological fluids. The most common technique for studying NE kinetics in humans uses tritiated NE as a radiotracer[2]. Mass spectrometric studies on the metabolism and kinetics of NE require the use of a stable isotopomer of this compound. Synthesis of various deuterated (ring[3] and alkyl chain[3,4]) and ^{13}C-labelled (alkyl chain[5]) species have been reported. Our aim was to obtain a multiply ring labelled NE for studies in humans, one which would retain its isotopic content during metabolism to oxidized metabolites and during chemical manipulations. A ring labelled species would contain mass shifted fragment ions at high m/z, a valuable characteristic for quantitative analysis, and making it a useful internal standard for sensitive mass spectrometric assays.

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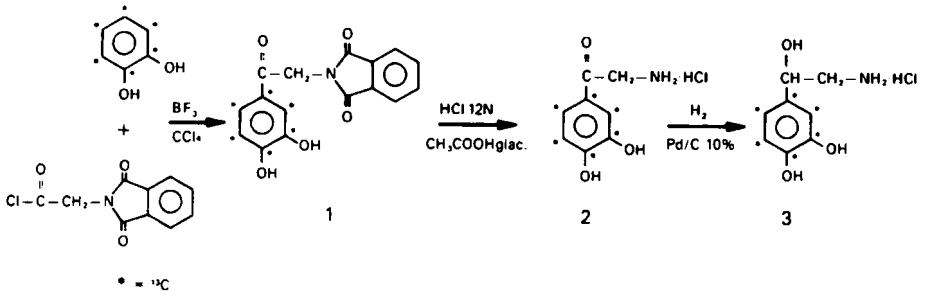
SCHEME



RESULTS AND DISCUSSION

Considering the starting material and its value, a short and efficient synthesis of NE was desirable. The simplest approach seemed a Friedel-Crafts acylation between the catechol and glycine parts of NE. To avoid polymerization and to enhance the reactivity of the glycine part, the phthaloyl group was chosen as the protecting group for the amine moiety and the acid was transformed into its chloride. As acylation catalysts, we tried boron trifluoride, aluminum chloride and iodine. Boron trifluoride was found to be most effective.

In an attempt to increase the yield of the acylation, one of the approaches was to protect the hydroxy groups of the catechol from oxidation or polymerization. When one of the hydroxyls was protected with a methyl group and the other one was free (i.e., guaiacol), the reaction resulted in two products in the ratio of three to one - the desired ring acylation and competitive O-acylation products. Blocking both hydroxyl groups (2x $-CH_3$, veratrol; $-OCH_3$, -OBz, benzylguaiacol; 2x -OBz, dibenzylcatechol) prevented the O-acylation reaction, but the ring acylation yields were even smaller. Direct acylation of catechol was therefore chosen.

After separation by chromatography, adduct 1 still contained some of the unreacted N-phthaloylglycine. This impurity was hydrolyzed and eliminated in the second step. The mass spectra of the derivatized unlabelled and ^{13}C-labelled condensation products are shown in Fig. 1.

In the search for the right conditions for the hydrogenation of 2 to 3, we observed that using absolute ethanol as the solvent for hydrogenation resulted in the ethyl ether of the benzylic hydroxy function as the final product. This

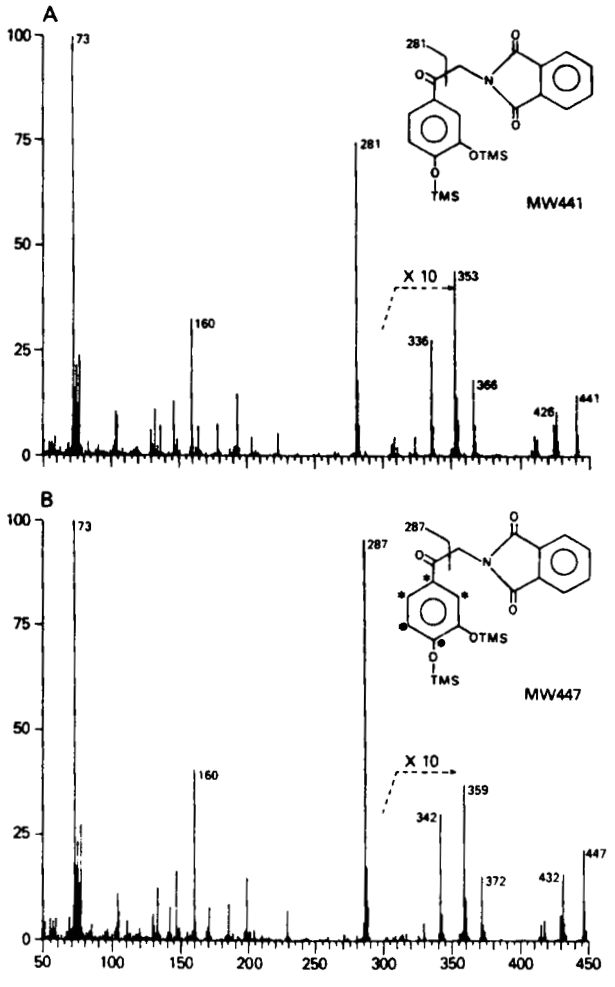


Figure 1: Mass spectra of trimethylsilylated (TMS) unlabelled (upper, A) and ^{13}C-labelled (lower, B) condensation product 1 (see scheme).

fact was reported[11] previously in the synthesis of 6-aminoisoproterenol. Also in one of our hydrogenation attempts the hydrogenation was too vigorous, causing hydrogenolysis of the benzylic hydroxy group, and a mixture of norepinephrine and dopamine was obtained. The products of hydrogenation were separated by counter current chromatography using the solvent system of n-butanol/water saturated with barium or sodium chloride as described elsewhere[10]. On small scale the procedure above produced D/L-norepinephrine as the hydrochloride (3) in 5% overall yield based on catechol. Previously reported

syntheses for various labelled NE cite 5-15% overall yield[9,12]. The mass spectra of the derivatized unlabelled and ^{13}C-labelled NE are shown in Fig. 2.

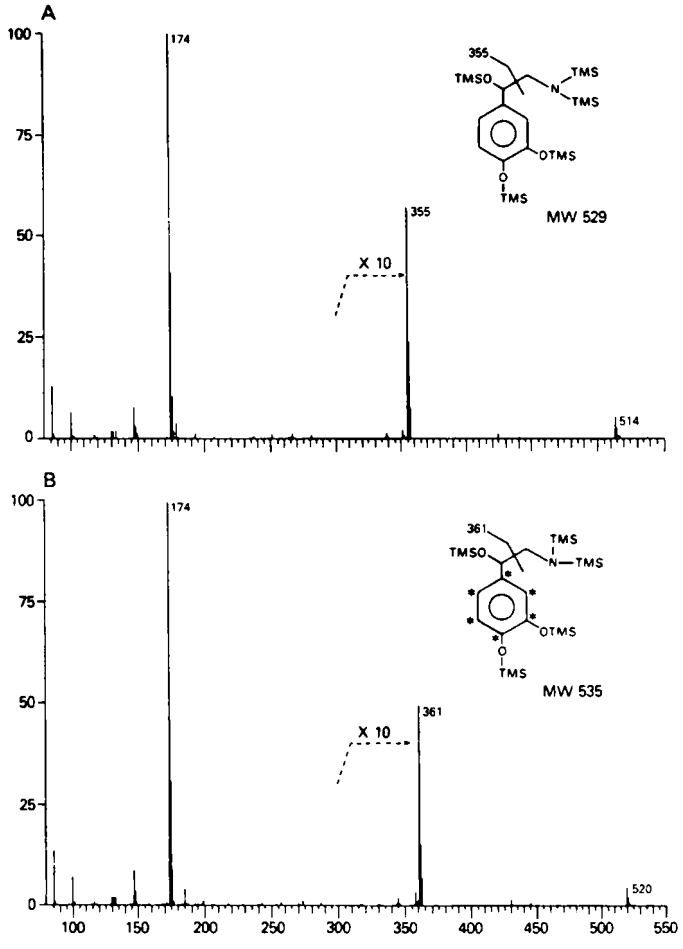


Figure 2: Mass spectra of the trimethylsilylated unlabelled (upper, A) and ^{13}C-labelled (lower, B) norepinephrine.

EXPERIMENTAL

Materials and Methods: Catechol-U-^{13}C was purchased from Cambridge Isotope Laboratories, Inc., Woburn, MA. N-phthaloylglycylchloride[6] was prepared from commercially available N-phthaloylglycine (Aldrich). Mass spectral analyses were performed with a Finnigan 3200 gas chromatograph-mass spectrometer (GC-MS)

operated under computer control (Teknivent). Typical analysis conditions were: GC column 1% OV-1, 80-100 mesh; column temperature, 120-250° C at 10° C/min; helium flow rate, 15 ml/min; electron impact ionization, 70 eV ionizing energy. For GC-MS analyses, the samples were derivatized for 15 min. in a solution of bis (trimethylsilyl) trifluoroacetamide (Alltech Ass.) in acetonitrile (1:1) at room temperature. High resolution MS measurements were performed with a VG 7070 mass spectrometer. High performance liquid chromatography (HPLC) analyses were performed with a Waters Radial-Pak C-18 reverse phase column (5 m, 10.0 cm x 5 mm ID)[7]. High-speed countercurrent chromatography (CCC) separations were performed with a commercially available (P.C. Inc., Potomac, MD) multilayer coil planet centrifuge described earlier[8]. The hydrogenation was performed in a vortex low pressure hydrogenator (J.B. Thompson Co., Inc., Cumberland, MD).

3,4-dihydroxy-α-phthalimidoaceto-$^{13}C_6$-phenone (1). A two necked 50 ml reaction flask fitted with a condenser, a gas inlet and a magnetic stirring bar was charged with catechol-U-^{13}C (1 g, 8.62 mmol) and carbon tetrachloride (10 ml). Under continuous stirring boron trifluoride was bubbled, at a slow rate, for 10 min. at room temperature. Freshly prepared N-phthaloylglycylchloride (1.96 g, 8.77 mmol) and carbon tetrachloride (7 ml) were added to the pinkish suspension. The reaction mixture was stirred along with continuous boron trifluoride bubbling, for 2 h at room temperature, 1 h at 40°C and 1 h at 55°C. At this point water (30 ml) was added with stirring. After 5 min the dark-red residue was isolated by decantation, then washed with water (2 x 10 ml). From the combined liquids, 162 mg of unreacted catechol-U-^{13}C were crystalized overnight. The red solid was dissolved in ethyl acetate, transferred to a clean flask, concentrated to dryness, and the product freeze-dryed. The red sticky solid was dissolved in diethyl ether and a large excess of hexane was added, causing red solid flakes to precipitate after a few minutes. Filtration and drying produced the crude adduct (1.276 g) as a red powder which was then purified by countercurrent chromatography. The solvent system was n-hexane/ethyl acetate/methanol/water (1:3:2:2). The lower aqueous phase was used as the mobile phase. After chromatography, the product obtained (410 mg) was a mixture of the adduct and N-phthaloylglycine (3:1), as evidenced by MS (M^+ measured 303.0839; M^+ calculated 303.0838), corresponding to 14% yield.

2-amino-1-(3,4-dihydroxyphenyl-$^{13}C_6$)ethanone (2). 1 was hydrolyzed to 2 by a previously published procedure[9]. In a glass tube (8 cm long and 2 cm in diameter), an aliquot of 1 (130 mg) and a mixture of 0.5 ml of each of 12 N HCl and glacial acetic acid were combined. After sonication for 2-3 min., the tube was sealed under vacuum and heated at 100° C for seven days. The dark content of the tube was then diluted with distilled water (10 ml), filtered from the black residue, and freeze dryed. This procedure was repeated twice with the remaining adduct 1, but purified 2 was not isolated (M^+ measured 173.0780; M^+ calculated 173.0783).

2-amino-1-(3,4-dihydroxyphenyl-$^{13}C_6$)ethanol (3). Noradrenalone 2 (30 mg) was dissolved in 50% ethanol (30 ml) and hydrogenated over 10% palladium on powdered charcoal (Matheson, Coleman & Bell) (13.4 mg) at room temperature and at 32 psi pressure for 45 minutes. Filtered free of the catalyst and freeze-dryed, the crude product (30 mg) contained 6 mg NE $^{13}C_6$ as determined by HPLC. This corresponds to 35% yield from adduct 1 or 5% from catechol. The crude product was purified by countercurrent chromatogarphy. The solvent system was n-butanol/NaCl saturated aqueous solution, adjusted to pH 3.4 with 0.1 N HCl (1:1). The lower aqueous phase was used as the mobile phase.

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