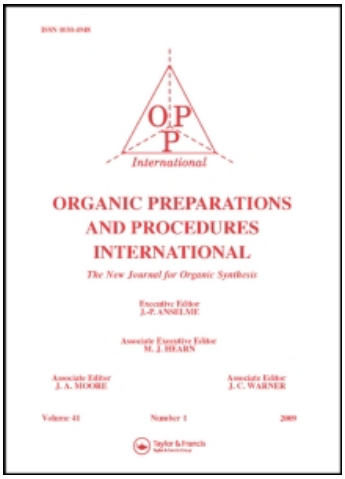

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A FACILE SYNTHESIS OF DOPAMINE-4-SULFATE

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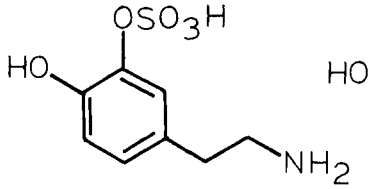
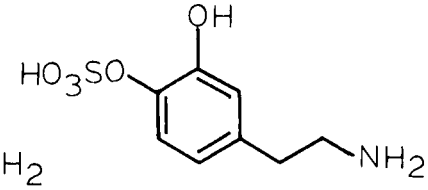
The catecholamine neurotransmitters norepinephrine (NE) and epinephrine (EPI) as well as the putative neurotransmitters dopamine (DA) and 5-hydroxytryptamine (5-HT) are known to be rapidly conjugated in general circulation as well as in a variety of peripheral tissues.[1] Sulfoconjugation predominates in man[2] and has been the subject of a number of recent investigations.[3] Of particular interest to us was the finding from several of these studies that sulfoconjugation may have a biological role beyond that of biodegradation of phenolic substances. Specifically, the indications that dopamine sulfates I and II might be involved in plasma transport of dopamine[4] and/or act as biosynthetic precursors of epinephrine[5] were intriguing. In order to further investigate the pharmacological properties of the dopamine-3- and -4-sulfates (I and II respectively), we required gram quantities of each in pure form. Jenner and Rose[6] had



I

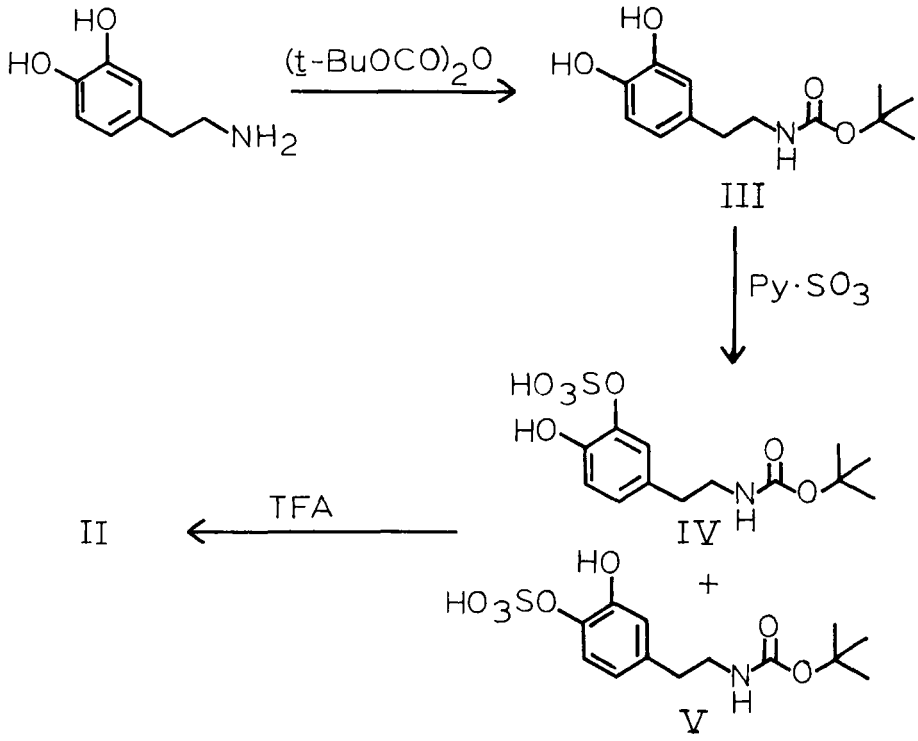


II

previously reported the synthesis of the dopamine sulfates I and II. Their procedure, however, required the purification and separation of I

and II by ion exchange chromatography, and provided only milligram quantities of each.

Our regioselective synthesis of dopamine-4-sulfate (II) (Scheme 1) employed the pyridine-sulfur trioxide complex[7] as a mild reagent for the production of the sulfate conjugate IV (although only a single spot was

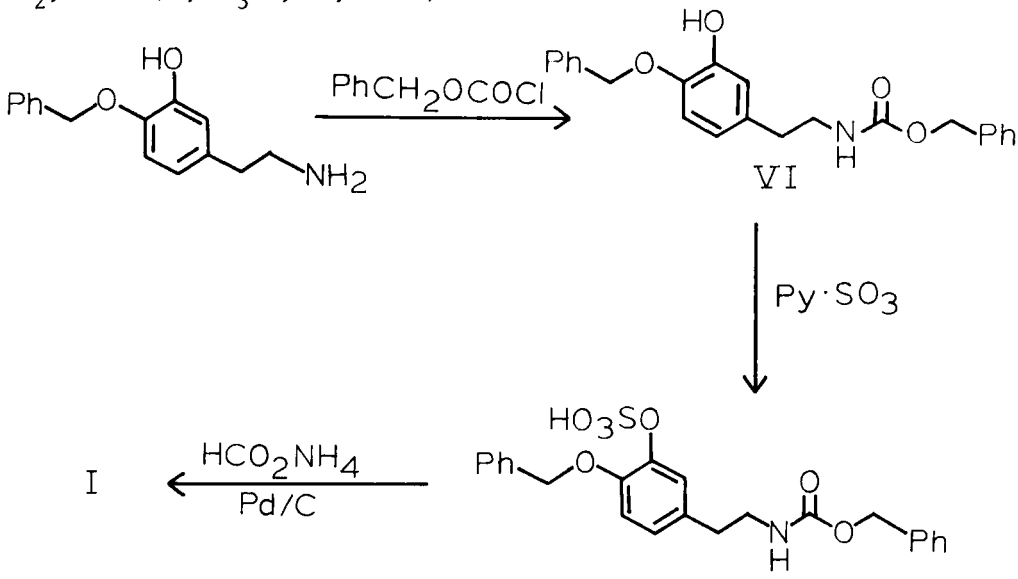


Scheme 1

detected on thin layer chromatography, a mixture of IV and V was probably produced). Deprotection of the crude product was accomplished using trifluoroacetic acid; the solid resulting from evaporation was washed with water to remove any of the highly aqueous soluble 3-sulfate I. This afforded II upon filtration.

The preparation of the dopamine-3-sulfate (I) was accomplished using a modification of procedure reported by Hegedüs.[8] Starting with 4-benzyl

dopamine[8] (Scheme 2) the amine was protected as the carbobenzoxy urethane VI, and the sulfation accomplished using the pyridine-sulfur trioxide complex.[7] Removal of the nitrogen and phenolic protecting groups was performed in one step by catalytic transfer hydrogenation[9] (monitoring for the disappearance of starting material by tlc) due to the instability of the 3-sulfate I under standard catalytic hydrogenation conditions (1 atm H_2, 10% Pd/C, CH_3OH, RT, 2 hrs).



Scheme 2

Examination of the ^1H NMR spectra of the 3- and 4-sulfates I and II readily provided confirmation of the position of the sulfate ester. In the case of the 3-sulfate I, the proton in the 2 position on the aromatic ring (identified by its meta coupling constant J = 1.5 Hz) was shifted downfield ($\Delta\delta$ approximately 0.25 ppm) relative to the remaining aromatic protons due to its proximity to the sulfate ester in the 3-position. Similarly, for the 4-sulfate II, the proton in the 5-position of the aromatic ring (ortho coupled, J = 8 Hz) was deshielded by the 4-sulfate ester ($\Delta\delta$ approximately 0.40 ppm).

As far as we are aware, there is no mention in the literature of the decomposition of the dopamine sulfates on storage at ambient temperatures. However, we have observed that the shelf life of II is somewhat limited. When analyzed by HPLC (see Experimental), a sample of the 4-sulfate II (prepared 11 months earlier and originally homogeneous by tlc) contained only about 50% of unchanged II. The other components consisted of dopamine and an unidentified material (tlc provided a similar result). Although we have not analyzed an eleven month-old sample of the 3-sulfate I, a two month-old sample (HPLC analysis) showed no detectable decomposition, and a four month-old sample (HPLC analysis) showed only a trace amount of dopamine.

To avoid possible dopamine contamination due to decomposition of the dopamine sulfates during storage, we recommend that samples be authenticated prior to use.

EXPERIMENTAL SECTION

Melting points were obtained using a Thomas-Hoover "Uni-Melt" melting point apparatus and are uncorrected. ^{1}H NMR were recorded on either a Varian T-60 (60 MHz), or a Varian XL-100 (100 MHz) equipped with a Nicolet 1080 computer. All chemical shifts are reported in δ units (ppm) downfield of internal tetramethylsilane. Infrared (IR) spectra were recorded on either a Perkin Elmer 521 or 283B spectrophotometer. Mass spectra were obtained with either an Associated Electrical Industries, Ltd., MS-902 or a Varian CH7 spectrometer at an ionizing voltage of 70 eV. Combustion analyses were performed on either a Perkin Elmer 240 or a Control Equipment 240X instrument. Thin layer chromatography was performed on Brinkman pre-coated plates (Silica Gel 60F-254). The tlc plates were visualized by one of the following: UV, ninhydrin spray (0.3 g in 100 mL n-butanol and 3 mL glacial acetic acid), or cerric sulfate spray. The following solvent systems were utilized: (1) chloroform-methanol (9:1); and (2) toluene-ethyl acetate (3:1). All solvents and reagents were "reagent grade" and were not purified prior to use. High performance liquid chromatography (HPLC) was performed on a Waters HPLC equipped with a Waters U6K injector, a Waters 6000A solvent delivery system, a Schoeffel UV detector, and a Waters C_{18} μ bondapak column (4mm x 30 cm).

N-t-butoxycarbonyldopamine (III).- Dopamine hydrochloride (Cordova Chemical Co.) (10 g, 52.7 mmol) was added to a solution of triethylamine (7.3 mL,

5.3 g, 52.7 mmol) in 100 mL DMF. A solution of di-t-butyldicarbonate (Aldrich) (12.6 g, 58 mmol) in 20 mL DMF was then added. There was an initial evolution of gas. The mixture was allowed to stir at room temperature for 12 hrs. The reaction mixture was filtered to remove triethylamine hydrochloride and concentrated under reduced pressure. Ethyl acetate (150 mL) was added and the resulting mixture was washed with cold 1N HCl (3 x 40 mL). The organic phase was dried ($MgSO_4$), suction filtered and concentrated under reduced pressure to give a colorless oil. This oil was crystallized from ethyl acetate-hexane to give 10.5 g (79%) of III as a white solid, mp. 135-137°; R_f (1): 0.35; IR (KBr): 3485, 3380, 1679, 1610, 1515 cm^{-1}; ^1H NMR (60 MHz) (DMSO-d_6): δ 8.1 (br s, 2H), 6.8-6.37 (br m, 3H), 3.42-2.83 (br m, 3H), 2.73-2.33 (br m, 2H), 1.42 (s, 9H); mass spectrum, m/e: 253 (M^+), 197, 180, 136, 123, 77, 57.

Dopamine-4-sulfate (II).- To a solution of III (4 g, 158 mmol) in 32 mL DMF was added pyridine-sulfur trioxide complex (Aldrich) (3.6 g, 22.6 mmol) and the reaction was allowed to stir 12 hrs at room temperature. Tlc (1) indicated that no starting material remained. The solvent was first removed under reduced pressure and then under high vacuum. Removal of the t-butoxycarbonyl group was accomplished by treatment of the oil with 7:3 trifluoroacetic acid-glacial acetic acid (20 mL) at room temperature for 30 min. Excess reagent was removed under reduced pressure followed by high vacuum. The brown solid was suspended in 20 mL water and then chilled in an ice bath. The white solid was isolated by suction filtration, washed with 5 mL cold water, 5 mL ethanol and finally 10 mL diethyl ether. Air drying gave 2 g (54.4%) of II as a white solid, mp. 257-259°; R_f (2): 0.51; IR (KBr): 3400, 3200, 1595, 1525, 1505, 1280-1250, 1235-1210, 1050, 838 cm^{-1}; ^1H NMR (60 MHz) (DMSO-d_6): δ 8.33-6.33 (br m, 4H absent with D_2O), 7.12 (d,1H, J = 8 Hz), 6.73 (m, 2H), 3.33-2.50 (br m, 4H); mass spectrum, m/e: 153 (M^+-SO_3), 124, 123, 106, 80.

Anal. Calcd for $C_8H_{11}NO_5S$: C, 41.20; H, 4.75; N, 6.01
Found: C, 41.04; H, 4.75; N, 6.01

N-Carbobenzyloxy-4-benzyldopamine (VI).- Benzyldopamine hydrochloride[8] (5 g, 17.9 mmol) was suspended in 25 mL water with sodium bicarbonate (3.75g, 44.6 mmol) and 10 mL dioxane. To this mixture was added benzylchloroformate (Aldrich) (5 x 0.56 mL) over a period of 30 min. The

mixture was allowed to stir 2 hrs at room temperature. The reaction mixture was diluted into 100 mL water and acidified with 1N HCl to pH 1. This mixture was extracted with ethyl acetate (3 x 50 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated to a brown solid under reduced pressure. Recrystallization from ethyl acetate-hexane yielded 4.58 g (68%) of VI as a grey solid, mp. 124-126°; R_f (2): 0.50; IR (KBr): 3520, 3337, 1687, 1543, 1521, cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.58-7.20 (m, 10H), 7.0-6.52 (m, 3H), 5.68 (s, 1H), 5.12 (s, 4H), 4.90-4.57 (br m, 1H), 3.42 (q, 2H, J = 6 Hz), 2.68 (t, 2H, J = 6 Hz); mass spectrum, m/e: 377 (M^+), 316, 225, 181, 120, 91.

Anal. Calcd for $C_{23}H_{23}NO_4$: C, 73.19; H, 6.14; N, 3.71
Found: C, 72.73; H, 6.15; N, 3.60

Dopamine-3-sulfate (I).- To a solution of VI (3.55 g, 9.4 mmol) in 21 mL dry DMF was added pyridine-sulfur trioxide complex (2.9 g, 18.2 mmol), and the resulting solution was stirred for 12 hrs. Tlc (2) indicated that all starting material had been consumed. The solvent was removed first under reduced pressure and then under high vacuum. Deprotection was accomplished by dissolving the oil in 11 mL methanol and stirring with ammonium formate (2.0 g, 31.7 mmol), 10% palladium on carbon (1.8 g) and 0.5 mL acetic acid. Gas evolution began after 40 min and at this point tlc indicated complete reaction. The mixture was suction filtered to remove solids, and the filtrate was concentrated to an oil, first under reduced pressure and then under high vacuum for 12 hrs. The product was crystallized from water-ethanol and recrystallized from the same solvent system to yield 1.1 g (50%) of I as a grey solid, mp. >250° (Lit.[8] 275-277°); R_f (2): 0.58; IR (KBr): 3400, 3120, 1620, 1595, 1510, 1285-1235, 1060, 1050, 805, 795 cm^{-1}; ^1H NMR (100 MHz) (DMSO-d_6): δ 8.1-7.2 (br m, 4H, absent with D_2O), 7.06 (d, 1H, J = 1.5 Hz), 6.80 (m, 2H), 3.09-2.56 (br m, 4H); mass spectrum, m/e: 153 ($M^+ - SO_3$), 124, 123, 106.

Anal. Calcd for $C_8H_{11}NO_5$: C, 41.20; H, 4.75; N, 6.01
Found: C, 41.35; H, 4.95; N, 6.09

HPLC of the Dopamine-sulfates I and II.- A 10 μg/mL DMSO solution of the 4-sulfate II (synthesized eleven months earlier and stored at ambient temperature) was prepared and 3 μL of this solution was analyzed by HPLC using as an eluant an aqueous solution which was 10% in methanol, 0.5% in acetic acid, and 0.005 M in sodium hexamate. The flow rate was 1 mL per

min, and the pressure was 1000 psi. UV detection was at 254 nm. Approximately 50% of the material was eluted in a peak at 5 min retention time (the 4-sulfate II). The remainder of the material was divided equally between two peaks, one at 7.2 min retention time (unidentified) and the other at 9.0 min retention time (dopamine).

On HPLC analysis of the 3-sulfate I (10 μg/mL in DMSO, conditions the same as for II above) after 2 months of storage, there was found a single peak (I) eluting at 5.0 min retention time (9 μL injection). Analysis of I after 4 months of storage (9 μL injection) showed a trace (< 1%) of an additional peak at 9 min retention time (dopamine).

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