A ONE STEP SYNTHESIS OF RING LABELLED MELATONIN-^{3}H WITH HIGH SPECIFIC ACTIVITY

Yu-Ying Liu and Martha Minich
Chemical Research Department, Hoffmann-La Roche Inc.
Nutley, New Jersey 07110 (USA)

SUMMARY

A mixture of brominated melatonin derivatives has been synthesized for use as starting material for preparation of ring tritium labelled melatonin by catalytic hydrogenolysis. The high specific activity obtained makes this product useful in radioimmunoassay studies.

Key Words: N-Acetyl-5-methoxy-6-bromotryptamine, N-Acetyl-5-methoxy-4-bromotryptamine, (4,6-$^{3}H_{1}$)melatonin, Catalytic hydrogenolysis

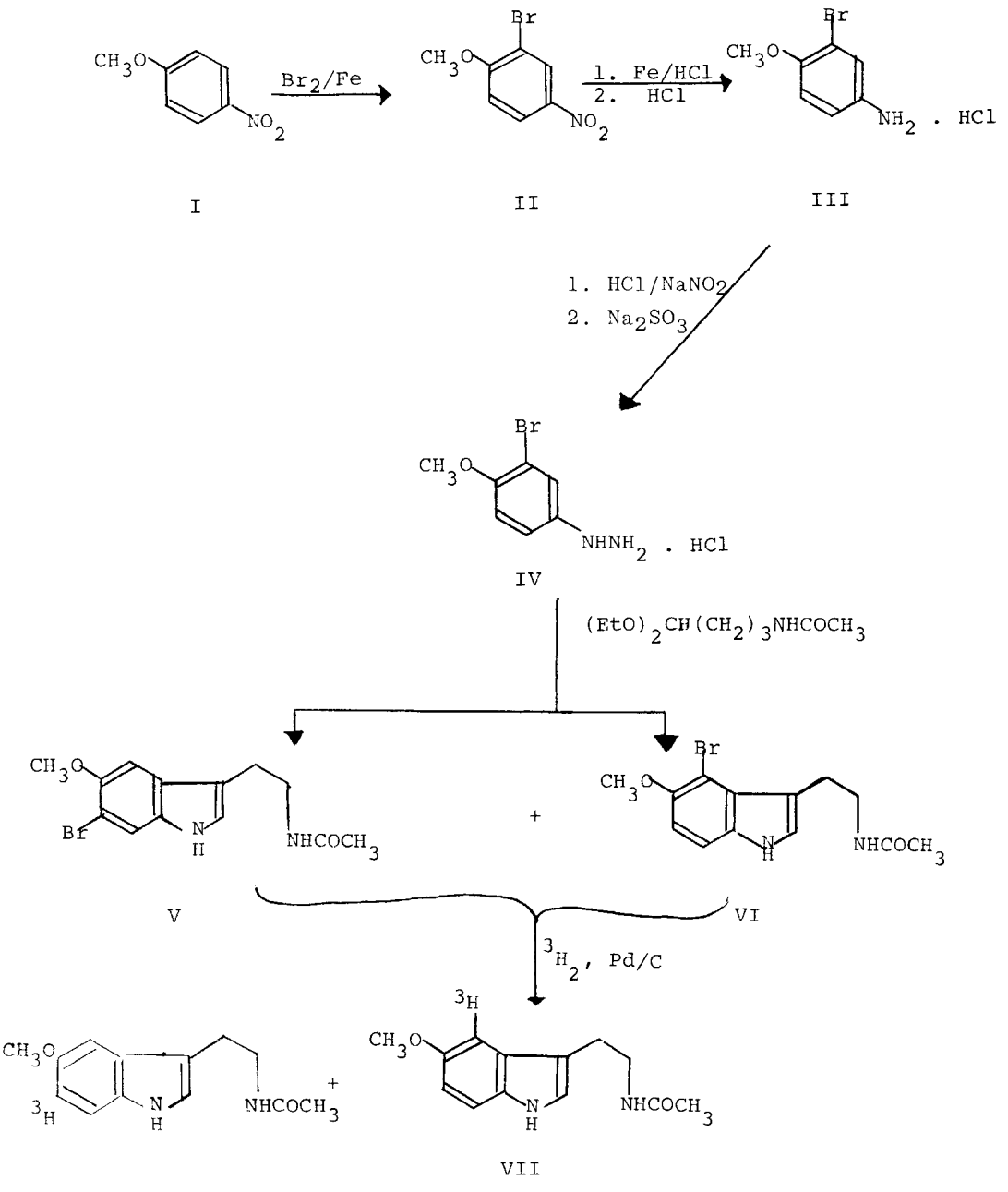
INTRODUCTION

We were interested in the preparation of tritium labelled melatonin (VII), N-acetyl-5-methoxytryptamine, at high specific activity for use in a radioimmunoassay procedure. The compound is commercially available with a tritium label in the acetyl group or in the ethyl side chain. To minimize any loss of the label by an exchange process, a ring labelled material is more desirable, and its preparation led us to develop the synthesis of a suitable halogenated precursor. Because of the high level of radioactivity required, a one step reductive hydrogenolysis of this precursor with tritium gas would be the most economical approach. Attempts to brominate melatonin directly in the aromatic ring were unsuccessful. Although several literature references[1-5] describe the synthesis of tryptamine, we found that these are not adaptable to the synthesis of brominated analogs.

0362-4803/81/060791-07$01.00

Received September 18, 1979
Revised November 6, 1979 and January 24, 1980

Scheme. Preparation of (4,6-$^{3}H_2$)melatonin.



Keglevic[6] and co-workers reported that 4-acetylamino butanal diethyl acetal and p-methoxyphenylhydrazine hydrochloride cyclized in 25% acetic acid to give melatonin in 26% yield. Based on this work, we were able to prepare a mixture of N-acetyl-5-methoxy-6-bromotryptamine (V) and N-acetyl-5-methoxy-4-bromotryptamine (VI) in an analogous fashion starting with 3-bromo-4-methoxyphenylhydrazine hydrochloride (IV), which was prepared from 4-nitroanisole (I) by bromination, reduction and hydrazine formation (Scheme). Since compounds V and VI have very similar chemical properties and are difficult to separate, they were used as a mixture for the hydrogenolysis reaction. Hydrogenolysis with deuterium gas in the presence of 10% Pd/C and triethylamine in dry THF gave deuterated melatonin with an incorporation of d_o 13.5%, d_1 83.1%, d_2 3.4% (mass spectrum). Therefore, melatonin prepared from these precursors is probably a mixture of mostly the 4 and the 6-labelled derivatives. Additional evidence to this point is the specific activity of 26.3 Ci/mmol which resulted from carrier free tritium gas reduction under the same conditions. Of course, the mixture of melatonin-4-^3H and of melatonin-6-^3H results in (4,6-$^3H_1$)melatonin.

Experimental

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Radiochemical purity was determined on thin-layer chromatograms (Brinkmann precoated silica gel F-254) with a Packard Model 7201 Radiochromatogram Scanner System and radioactivity was measured by the liquid scintillation technique with a Packard Tricarb Model 2010 spectrometer. Spectra were recorded on standard instruments.

2-Bromo-4-nitroanisole (II) - To a solution containing 71 g (0.46 mol) of p-nitroanisole and 0.7 g of iron in 200 ml of acetic acid, 24 ml of bromine was added dropwise. After the addition, the resulting solution was warmed at 70°C for 1.5 hr and then poured into ice water. This mixture was extracted with chloroform and the organic extracts were combined, washed with 2N sodium hydroxide solution and water, dried ($MgSO_4$), filtered and concentrated in vacuo to yield 93.8 g (0.4 mol, 87%) of II, m.p. 102° (lit. m.p. 106°).[7]

3-Bromo-4-methoxyaniline hydrochloride (III) - To a suspension of 68 g of iron powder in a solution of 93.8 g (0.4 mol) of II in 200 ml of 50% ethanol was added, in small portions at the beginning, 4 ml of concentrated hydrochloric acid in 20 ml of 50% ethanol. The reaction mixture became warm and the reaction was controlled by alternately cooling in an ice bath or heating to maintain gentle evolution of gases. After the addition, the reaction mixture was refluxed for 1.5 hr, poured into ice water and extracted with chloroform. The chloroform extracts was evaporated to give the crude product. This was dissolved in ethanol and treated with ethanolic hydrogen chloride to precipitate III. Recrystallization from methanol-ether afforded 64.9 g (0.27 mol, 68% of pure product (III), m.p. 247° dec. lit. m.p.254-255°[8]

3-Bromo-4-methoxyphenylhydrazine hydrochloride (IV) - To a solution of 857 mg (3.59 mmol) of III in 3 ml of water and 0.6 ml (7.2 mmol) of 12N hydrochloric acid at 0°C was added a solution (0°C) of 249 mg (3.61 mmol) of sodium nitrite in 1 ml of water.

This diazonium salt solution was added very rapidly to a solution (0°C) containing 2.28 g (18.05 mmol) of sodium sulfite in 6.5 ml of water. The resulting mixture was heated to 70°C. Sufficient hydrochloric acid was now added to make the solution acidic to litmus, and heat was continued at 75°C overnight. To the hot solution was added 8 ml of concentrated hydrochloric acid, and the mixture was cooled. The crude product which precipitated was filtered and purified by crystallization from hot water yielding 570 mg (2.25 mmol, 63%) of IV. Anal. Calcd. for $C_7H_{10}N_2OClBr$: C, 33.16; H, 3.97; N,11.05; Br, 31.52. Found: C, 32.98; H, 3.98; N, 10.89; Br, 31.31.

N-Acetyl-5-methoxy-6-bromotryptamine (V) and N-Acetyl-5-methoxy-4-bromotryptamine (VI) - A solution of IV (226 mg, 0.89 mmol) and 4-acetylaminobutanal diethyl acetal (182 mg, 0.9 mmol) in 10 ml of 25% acetic acid was heated at 80°C for 1.5 hr. The mixture was made basic with potassium carbonate and then extracted with chloroform. The combined organic extracts were concentrated *in vacuo* and the residue was purified by column chromatography on silica gel using ethyl acetate elution to yield 227 mg (0.73 mmol, 82%) of colorless solid, m.p. 147-148°, which consisted of 60% of VI and 40% of V based on nmr analysis. This mixture was used for the labelling synthesis with no further purification; uv $\lambda_{max}^{isopropanol}$ nm (E), 226 (30800), 286 (6450), 303 (6050), 316 (3140); ir $\nu_{max}^{CHCl_3}$, cm^{-1}: 3480, 1663, 1520, 1235; nmr ($CDCl_3$) δ: compound V 1.92 (s, 3H, $C\underline{H}_3CO$), 3.54 (m, 2H, $C\underline{H}_2NH$), 2.90 (t, 2H, $C\underline{H}_2CH_2NH$), 3.89 (s, 3H, $OC\underline{H}_3$), 5.6 (broad, 1H, $N\underline{H}Ac$), 7.05 and 7.54 (m, 2H, Ar); compound VI, 1.92 (s, 3H, $C\underline{H}_3CO$), 3.54 (m, 2H, $C\underline{H}_2NH$), 3.21 (t, 2H,

$C\underline{H}_2CH_2NH$), 3.88 (s,3H,$OC\underline{H}_3$), 5.6 (broad, 1H, $N\underline{H}Ac$), 6.89 and 7.24 (m, 2H, Ar); ms, 310, 312 (M+).

(4,6-$^3H_1$)melatonin (VII)-A mixture consisting of V and VI (44 mg, 0.14 mmol) and of 30 µl (0.22 mmol) triethylamine was dissolved in anhydrous THF and 10 mg of 10% Pd/C was added. After evacuation to a pressure of 1 micron, 10 Ci of tritium gas was admitted , and the reaction mixture was stirred at room temperature overnight. Hydrogen gas was added the next day to complete the hydrogenolysis. After two hours, unreacted hydrogen gas was removed from the system, the catalyst was filtered off and volatile components removed by high vacuum transfer at -190°. The residue was purified on a short silica gel column eluting with ethyl acetate to provide a total of 2.43 Ci (21 mg) of (4,6-$^3H_1$)melatonin having a specific activity of 26.3 Ci/mmol and a radiochemical purity of 99% [tlc: silica gel; EtOAc/$CHCl_3$/MeOH (10:10:1), R_f 0.36]. Evidence confirming this radiochemical purity has been obtained by the use of this product, at several dilutions, in a radioimmunoassay procedure for melatonin.[9]

Acknowledgement

We thank Dr. R. P. W. Scott and his staff in our Physical Chemistry Department, in particular, Dr. W. Benz for mass spectra, Dr.V. Toome for uv spectra, Mr. S. Traiman for ir spectra, Dr. T. Williams for nmr spectra, and Dr. F. Scheidl for microanalyses.

References

1. Abramovitch, R. A., and Shapiro, D.-J. Chem. Soc: 4589 (1956).
2. Speeter, M. E., and Anthony, W. C.-J. Amer. Chem. Soc. 76: 6208 (1954).

3. Shaw, G. J.-J. Labelled Compounds and Radiopharmaceuticals 14: 897 (1978).

4. Szmuszkovicz, J., Anthony, W. C., and Heinzelman, R. V.-J. Org. Chem. 25: 857 (1960).

5. Abramovitch, R. A.-J. Chem. Soc: 4593 (1956).

6. Keglevic, D., Stojanac, N., and Desaty, D.-Croat. Chem. Acta. 33: 83 (1961).

7. Staedel, A.-Ann. 217: 67 (1883).

8. Benevento, A.-Gaz. Chim. Ital. 28 II: 202 (1898).

9. Spector, S. and Wurzburger, R.-Personal Communication.