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# SYNTHESIS OF TRITIUM-LABELLED STOBADINE, A NEW CARDIOPROTECTIVE AGENT

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## SUMMARY

Synthesis of dihydrochloride of (-)-cis-2,8-dimethyl-6-[$^3$H]-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3b]indole by catalytic reductive dehalogenation of dihydrochloride of (-)-cis-2,8-dimethyl-6-bromo-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3b]indole is described together with the reaction conditions of the tritiation using 5% $Pd/BaSO_4$ (UVVVR) in 0.1 M phosphate buffer pH 7.4. Specific activity of the product was 0.9 TBq.mmol$^{-1}$, its radiochemical purity 96%.

Keywords: [$^3$H]stobadine, 6-bromostobadine, dehalogenation

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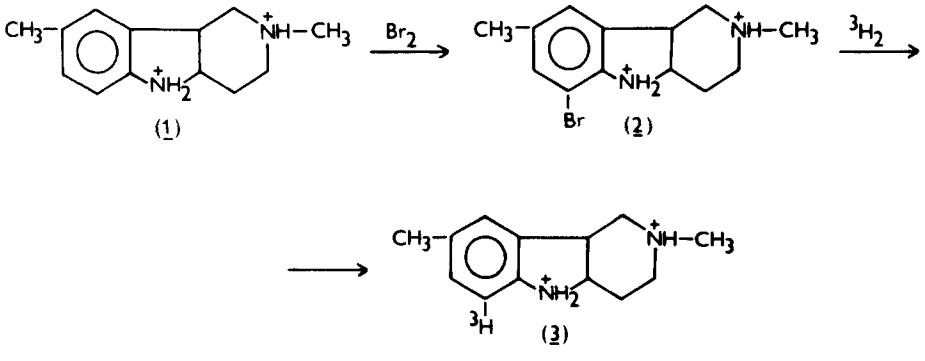
## INTRODUCTION

Stobadine, dihydrochloride of (-)-cis-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3b]indole (1), is a new cardioprotective agent from the gamma-carboline structural group (1).

Within a long-term toxicological investigation of stobadine in dogs, a simple spectrofluorometric analytical method was developed for the determination of serum concentrations of the drug (2). However, the method is of limited sensitivity and is inappropriate for full-range experimental pharmacokinetic studies. Therefore a labelled material was needed.

As attempts to prepare [$^3$H]stobadine by isotope exchange in the system gas-solution did not give a product of molar activity higher than 67 GBq.mmol$^{-1}$, it was necessary to try another way of preparation of the labelled drug.

This paper describes the synthesis of [$^3$H]stobadine of high molar activity based on catalytic reductive dehalogenation of the bromo-derivative of stobadine.




(1) (2) (3)

Figure 1: Scheme of [$^3$H]stobadine synthesis

## RESULTS AND DISCUSSION

The first step of the synthesis of [$^3$H]stobadine (3), i.e. bromation of stobadine (1), was based on a previous synthetic work done on racemic gamma-carbolines (3). The published procedure had to be modified due to the different salt of the starting compound used in our procedure. As the dihydrochloride is not sufficiently stable, the salt of the base with palmitic acid was used for the storage of the drug. This salt was used also in the reaction. As the addition of the dipalmitate to the acidic reaction medium resulted in a suspension, a comparatively higher volume of acetic acid and longer reaction time were used to improve suspending and to prolong the contact of reacting molecules. Drying of collected ethereal extracts can be considered the "bottle--neck" of this part of synthesis. In the case of incomplete drying the resulting precipitate, after acidification with hydrogen chloride, was unstable and it decomposed within a few minutes.

Analytical profile of 6-bromostobadine is in Table I. The data were compared with those published for stobadine (4).

The presence of bromine in the molecule of bromostobadine is well documented by mass spectrum of the substance. The approximately equal occurence of two stable isotopes of bromine, i.e. $^{79}$Br and $^{81}$Br, caused the appearance of double maxima differing in two units of m/e in the mass spectra. It is visible in the case of the maxima of the molecular ion $M^+$ (m/e equal to 280 and 282; the molecular weight of bromostobadine is 281.20), of the ion $M^+$- - $NH(CH_3)_2$ (m/e equal to 235 and 237), etc.

The exact position of bromine on the aromatic ring was specified by NMR spectroscopy using homodecoupling of protons of the aromatic methyl group and the NOE differential experiment (5). Both

methods confirmed the ortho-position of aromatic protons towards the methyl group.

Table I. Analytical profile of 6-bromostobadine

| | | | |
|---|---|---|---|
| m.p. | [°C] | 150-155 | (decomposition) |
| UV[a] | λ [nm] | 246 | $\varepsilon = 8.85.10^3$ |
| | | 305 | $\varepsilon = 3.64.10^3$ |
| IR[b] | $\nu$ [$cm^{-1}$] | 3418, 3300 | $\nu$(NH) |
| | | 2929, 2853 | $\nu$(CH) |
| | | 2691 | $\nu(NH_2^+)$ |
| | | 2448 | $\nu(NH^+)$ |
| | | 1615, 1592 | $\nu(C{=}C_{arom})$ |
| | | 1470 | $\nu(CH_2)$ |
| $^1$H NMR[c] | δ/ppm | 7.26 | 1H, d, H-7 |
| | | 7.15 | 1H, d, H-9 |
| | | 2.89 | 3H, s, $NCH_3$ |
| | | 2.29 | 3H, s, $CH_3$ |
| MS[d] | m/e | 280, 282 | $M^+$ |
| | | 235, 237 | $M^+ - NH(CH_3)_2$ |

[a] methanol

[b] KBr pellets

[c] methanol-$d_4$

[d] E.I., 75 ev

In trace experiments (Table II) we studied the influence of the type of catalyst and reaction medium on specific activity and radiochemical yield of the reaction. High molar activity can be achieved only if the rate of catalytic reductive dehalogenation

is substantially higher than the rate of isotope exchange (6). The highest specific activities were reached by using 5% $Pd/BaSO_4$ (UVVVR) as a catalyst, when requirements of both high reaction rate and low exchange were met. The specific activities obtained were close to theoretical values.

## EXPERIMENTAL

UV spectra were measured in methanol using a M40 spectrophotometer (Carl Zeiss, Jena, GDR). IR spectra were recorded on a Perkin-Elmer 983G apparatus using KBr technique. $^1H$ NMR spectra were obtained on a Bruker AM-300 apparatus in methanol-$d_4$ at 298K; TMS was used as an internal standard. Mass spectra were recorded on a Jeol JMS D 100 apparatus using electron impact (75 eV) technique.

For thin-layer chromatography of 6-bromostobadine, Silufol UV 254 plates (Kavalier, Votice, Czechoslovakia) were used. The two solvent systems used were benzene-isopropanol-methanol-conc. ammonia 70:30:20:5 (system A) and cyclohexane-benzene-methanol-triethylamine 70:20:10:5 (system B). $R_f$ values of stobadine and its bromoderivative, using systems A and B, are in Table III. Fluorescent spots were detected using a UV lamp 254 nm.

Activities were measured on a Packard-Tri-Carb apparatus in the liquid scintilator SLD (Lachema, Brno, Czechoslovakia). Radiochemical purity was determined by HPLC method on a model 8500 Varian liquid chromatograph. The separation was carried out on a CGC 3.3x150 mm column packed with Sepharon SGX C18 7 μm (Laboratórní přístroje, Prague, Czechoslovakia). The mobile phase, aqueous solution of $NaH_2PO_4$ (0.05 M), tributylamine (0.005 M) and methanol (8%), pH 2.6, was pumped through the column at a flow-rate of 1 $ml.min^{-1}$.

Table II. Reaction conditions of catalytic reductive dehalogenation

| Reaction medium | Catalyst | Reaction time [min] | Radioactivity of reaction mixture [MBq] | [$^3$H]stobadine | | |
|---|---|---|---|---|---|---|
| | | | | Radioactivity [MBq] | [GBq.mmol$^{-1}$] | Chemical yield [%] |
| 0.1 M $NH_4OH$ | A | 40 | 156 | 14.2 | 0.95 | 68 |
| 0.1 M NaOH | A | 36 | 146 | 22.6 | 1.32 | 78 |
| Methanol | A | 49 | 164 | 8.0 | 0.72 | 51 |
| 0.1 M Phosphate buffer, pH 7.4 | A | 20 | 142 | 36.2 | 1.77 | 93 |
| 0.1 M Phosphate buffer, pH 7.4 | B | 27 | 149 | 32.8 | 1.68 | 89 |
| 0.1 M NaOH | C | 25 | 178 | 18.6 | 1.18 | 72 |

Catalyst: A - 8 mg of 5% $Pd/BaSO_4$ (UVVVR)
B - 8 mg of 5% $PdO/BaSO_4$ (Merck)
C - 3 mg of 10% Pd/C (Fluka)

Table III. $R_f$ values of stobadine and its bromoderivative

| System[a] | Compound | $R_f \pm$ S.D.[b] |
|---|---|---|
| A | stobadine | 0.52 ± 0.01 |
| | bromostobadine | 0.64 ± 0.02 |
| B | stobadine | 0.16 ± 0.01 |
| | bromostobadine | 0.25 ± 0.01 |

[a] for description of systems see text

[b] standard deviations of five measurements

Tritium (without carrier) in a volume activity of 200 MBq.ml$^{-1}$ was purchased from Techsnabexport (USSR). Catalyst 5% Pd/$BaSO_4$ (UVVVR) was prepared at the Institute for Research, Production and Application of Radioisotopes, Prague, Czechoslovakia, 5% PdO/$BaSO_4$ was from Merck, Darmstadt, FRG, 10% Pd/C from Fluka, Buchs, Switzerland.

Other chemicals were from Lachema, Brno, Czechoslovakia.

For studies of hydrogenation reactions in cold and trace experiments, the apparatus described earlier (7) was used. For preparation of [$^3$H]stobadine in a higher molar activity, the modified apparatus of Wenzel (8) was used. Immediately after finishing the reaction tritium was returned to the container and by measuring pressure the consumption of tritium was determined.

Dihydrochloride of (-)-cis-2,8-dimethyl-6-bromo-2,3,4,4a,5,9b--hexahydro-1H-pyrido 4,3b indole (2)

To 3.3 g (4.6 mmol) of dipalmitate of stobadine a cold mixture of $H_2SO_4$ (0.75 ml) and $CH_3COOH$ (12 ml) was added and the resulting

suspension was well stirred. 0.8 g (5 mmol) of bromine was dropped in during 15 minutes and the orange-coloured suspension was mixed at room temperature for 2 hours. Then 40 ml of cold water were added and the suspension was made alkaline with the chilled solution of 20 g NaOH in 100 ml of water. The resulting precipitate (Na-palmitate) was filtered off and washed 3 times with diethyl ether. The clear filtrate was also extracted 3 times with diethyl ether. The yellow extracts were collected and dried overnight above a molecular sieve (Dusimo S-3A, Lachema, Brno, Czechoslovakia). Saturated diethyl ether solution of hydrogen chloride was dropped into the dry extracts and the white pracipitate was filtered off and vacuum dried. The yield of the product was 1.35 g (83%). The purity of the product was confirmed by TLC (one spot in both systems).

Catalytic reductive dehalogenation of 6-bromostobadine (2) (trace experiments)

Into a 1 ml reaction flask 7.8 mg (22 μmol) of 6-bromostobadine (2) was placed along with catalyst (8 mg of 5% $Pd/BaSO_4$, 8 mg of 5% $PdO/BaSO_4$, 3 mg of 10% Pd/C), as well as 0.5 ml of solvent (see Table II). The flask was connected to the apparatus for trace experiments and chilled to 210 K. After degasing tritium was introduced and the mixture was stirred at a tempereture of 295 K. After finishing the reaction the radioactivity of the reaction mixture was determined and labile radioactivity was removed by 3x2 ml of methanol. Catalysts were filtered off by 2 cm column of silicagel.

Dihydrochloride of (-)-cis-2,8-dimethyl-6-[$^3$H]-2,3,4,4a,5,9b--hexahydro-1H-pyrido 4,3b indole (3)

7.8 mg (22 μmol) of 2 was dissolved in 0.6 ml of 0.1 M phosphate

buffer, pH 7.4, and placed with a catalyst (8 mg of $Pd/BaSO_4$) into a 1 ml reaction flask. Catalytic reductive dehalogenation took place for 26 minutes at 295 K and at tritium pressure of 0.10 MPa. After finishing the reaction tritium from labile bindings was removed in a closed system by 3x2 ml of methanol and the catalyst was filtered off by a 2x1 cm column of silicagel. The yield of 3 was 18.8 GBq with a specific activity of 0.91 $TBq.mmol^{-1}$ and radiochemical purity of 96%.

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