SPECIFICALLY LABELLED BETA-CARBOLINES FOR BIOMEDICAL INVESTIGATIONS

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SUMMARY

Five radioactive and three deuterium labelled 1,2,3,4-tetrahydro-beta-carbolines (THBCs) have been synthesized from tryptamine hydrochloride or 5-methoxytryptamine hydrochloride and formaldehyde, glyoxylic acid or pyruvic acid. Radioactive labelling was performed using [^{14}C] and [^{3}H]labelled compounds, while the deuterium labels were introduced using lithium aluminium deuteride. The specific activities obtained for [^{14}C] labelled THBCs varied between 1.0 and 5.0 mCi/mmol, while a value of 30.1 mCi/mmol was obtained for the tritiated compound. The isotopic purities of the deuterated THBCs were in the range of 76.3 - 96.2 %.

INTRODUCTION

1,2,3,4-tetrahydro-beta-carbolines (THBCs) are known to occur in human, animal and plant tissues (1-3). The synthesis of a number of THBCs takes place from aldehydes and tryptamines quite simply in vitro at room temperature in mildly acidic conditions. This is also the supposed biosynthetic principle for these compounds in tissues (4,5).

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Biosynthesis of THBCs from tryptamines and pyruvic or glyoxylic acid is predictable because in vitro the reactions in question occur at almost the same conditions as those yielding THBCs from tryptamines and aldehydes.

Eight labelled THBCs have been prepared (Fig. 1) for biosynthetic studies on these compounds and for quantitative studies on their occurrance in animal and human tissues by using gas chromatography-mass spectrometry (GLC-MS).

EXPERIMENTAL

Synthesis of 1-[^{14}C]-6-methoxy-1,2,3,4-tetrahydro-beta-carboline, 1-[^{14}C]-6-MeO-THBC (I)

27.0 mg (0.12 mmol) of 5-methoxytryptamine hydrochloride (Sigma) and 3.0 mg (0.1 mmol) of [^{14}C]-formaldehyde (New England Nuclear, specific activity 10.0 mCi/mmol) were incubated for 12 h in a water solution of pH 4. The solution was made alkaline by adding 10 % KOH and extracted with ethyl acetate. Most of the solvent was evaporated and the rest of it (about 0.5 ml) was applied on a TLC plate (Kieselgel 60 F_{254}, AC-Alufolien, art. 5554, Merck) as a narrow band. Using the solvent system n-butanol-acetic acid-water (4:1:1) 1-[^{14}C]-6-MeO-THBC had an Rf-value of 0.43. The band at this Rf-value was transfered to a chromatographic column packed with the same material as the adsorbent used in TLC (c.f. above).

1-[^{14}C]-6-MeO-THBC was eluted from the column using ethyl acetate, and the solvent was removed by a stream of nitrogen. The specific activity of (I) was 2.9 mCi/mmol.

1-[^{3}H]-6-methoxy-1,2,3,4-tetrahydro-beta-carboline, 1-[^{3}H]-6-MeO-THBC (II)

This compound was prepared from 16.3 mg (0.07 mmol) of 5-methoxytryptamine hydrochloride and 1.8 mg (0.06 mmol) of [^{3}H]-formaldehyde (New England Nuclear, specific activity 85 mCi/mmol) by a method analogous to that

used for 1-[^{14}C]-6-MeO-THBC. The specific activity of (II) was 30.1 mCi/mmol.

1-[^{14}C]-1,2,3,4-tetrahydro-beta-carboline, 1-[^{14}C]-THBC (III)
1-[^{14}C]-THBC was prepared from 23.5 mg (0.12 mmol) of tryptamine hydrochloride (Fluka) and 3.0 mg (0.1 mmol) of [^{14}C]-formaldehyde (New England Nuclear, specific activity 16.7 mCi/mmol) in the same way as compound (I). In TLC, an Rf-value of 0.47 was observed using the solvent system n-butanol-acetic acid-water (4:1:1). The specific activity of (III) was 5.0 mCi/mmol.

1,2,3,4-tetrahydro-beta-carboline-1-carboxylic-[^{14}C] acid, THBC-1-^{14}COOH (IV)
13.5 mg (6.8 µmol) of tryptamine hydrochloride and 0.51 mg (6.8 µmol) of 1-[^{14}C]-glyoxylic acid sodium salt (Amersham, specific activity 7.33 mCi/mmol) were incubated at 35°C for 20 h in a buffer solution of pH 3.5. The solution was applied as a narrow band on a TLC plate. After chromatography using the solvent system n-butanol-acetic acid-water (4:4:1) the band of THBC-1-^{14}COOH with an Rf-value of 0.50 was extracted with concentrated acetic acid. The specific activity of (IV) was 1.8 mCi/mmol.

1-methyl-1,2,3,4-tetrahydro-beta-carboline-1-carboxylic-[^{14}C] acid, 1-Me-THBC-1-^{14}COOH (V)
This compound was obtained from 0.43 mg (2.1 µmol) of tryptamine hydrochloride and 0.24 mg (2.1 µmol) of 1-[^{14}C]-pyruvic acid sodium salt (Amersham, specific activity 23 mCi/mmol) in the same way as compound (IV). The Rf-value in TLC was 0.48. The specific activity of (V) was 1.0 mCi/mmol.

Synthetic procedures analogous to those described above were applied for the THBCs (I-V) prepared from non-labelled reagents. Results from

mass spectrometric analysis of the compounds thus obtained are summarized in Table 1.

1-D_2-1,2,3,4-tetrahydro-beta-carboline, 1-D_2-THBC (VI)

100 mg (0.54 mmol) of 1-oxo-THBC, synthesized according to ref. (6), was suspended in 50 ml of dry tetrahydrofuran, and 100 mg (2.4 mmol) of lithium aluminium deuteride was carefully added. The mixture was refluxed for 12 h and unreacted lithium aluminium deuteride was eliminated by the addition of water. The solid mass from filtration was extracted with dichloromethane. After evaporation of the extraction solvent 1-D_2-THBC was recrystallized from benzene. The yield was 85 mg (90 %), m.p. 202 - 204°C, reported 201 - 203°C (6).

3,3,4,4-D_4-1,2,3,4-tetrahydro-beta-carboline, 3,3,4,4-D_4-THBC (VII)

200 mg (1.0 mmol) of tryptamine-α,α,β,β-D_4 hydrochloride (6) and 90 mg (1.0 mmol) of glyoxylic acid monohydrate (Sigma) were stirred in a buffer solution of pH 3.5 at room temperature for 5 h. The precipitate obtained, consisting of 3,3,4,4-D_4-THBC-1-carboxylic acid, was collected on a filter.

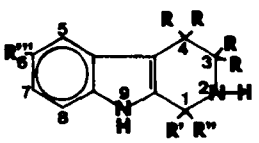
3,3,4,4-D_4-THBC-1-carboxylic acid was decarboxylated by incubation for 1 h in a strongly acidic solution at 70°C. 3,3,4,4-D_4-THBC was precipitated by adding 2 M KOH. After filtration and recrystallization from ethanol, the yield was 70 mg (40 %), m.p. 200 - 203°C, reported (6) 204 - 205°C (for non-labelled THBC).

3,3,4,4-D_4-1-methyl-1,2,3,4-tetrahydro-beta-carboline,
3,3,4,4-D_4-1-Me-THBC (VIII)

200 mg (1.0 mmol) of tryptamine-α,α,β,β-D_4 hydrochloride (6) and 110 mg (1.0 mmmol) of pyruvic acid (Boehringer Mannheim) were incubated in a phosphate buffer solution of pH 3.5 at 35°C for 20 h, during which time a precipitate was formed. The crystals obtained (3,3,4,4-D_4-1-Me-THBC-1-carboxylic acid) were dissolved in 15 % hydrochloric acid. A white precipitate was obtained when this solution was stirred at 60°C for

1/2 h, followed by an addition of 2 M KOH. After filtration and washings using cold water the yield was 82 mg (43 %), m.p. 174 - 177°C, reported (8) 175 - 177°C (for non-labelled 1-Me-THBC).

The radioactivities of the synthesized labelled THBCs were counted at 20°C on a Wallac 1215 Rackbeta liquid scintillation counter using preset counting windows for [^{14}C] and [^{3}H].
The mass spectra were recorded using a Jeol JMS D300 mass spectrometer with a JMA mass analysis system. The ionization electron beam energy was normally 23 eV but 15 eV was applied for the determinations of isotopic purity. The ionization current was 300 µA and the ion chamber temperature was 230°C. Samples were admitted using a solids insertion probe.



	R	R'	R''	R'''	C_1
I	H	H	H	OCH_3	^{14}C
II	H	H	^{3}H	OCH_3	^{12}C
III	H	H	H	H	^{14}C
IV	H	H	$^{14}COOH$	H	^{12}C
V	H	CH_3	$^{14}COOH$	H	^{12}C
VI	H	D	D	H	^{12}C
VII	D	H	H	H	^{12}C
VIII	D	CH_3	H	H	^{12}C

Fig. 1. Structures of the synthesized compounds.

RESULTS AND DISCUSSION

Performing the syntheses of [^{3}H] and [^{14}C] THBCs according to those of the corresponding unlabelled compounds, it was expectable that the Rf-values

in TLC were identical with those observed for the reference compounds. TLC-purification of the THBCs was successful due to the remarkably higher polarities of the compounds used as starting materials in the syntheses. Compounds (IV) and (V) as well as their non-labelled analogues could be extracted from TLC adsorbent only using concentrated acetic acid. Ethyl acetate was used for this purpose in the cases of the other THBCs. The mass spectra of the extracted THBCs showed closely similar peak patterns to those in the spectra obtained for the corresponding reference compounds (Table 1.).

Table 1. Relative intensities (%) of the main peaks in the mass spectra of the THBCs and their reference compounds.

compound	m/z (relative intensity %)
I	202(42), 173(100), 158(52)
I_{ref}	202(49), 173(100), 158(50)
II	202(40), 173(100), 158(38)
II_{ref}	202(49), 173(100), 158(50)
III	172(34), 143(100)
III_{ref}	172(30), 143(100)
IV	170(45), 169(92), 143(100)
IV_{ref}	170(90), 169(100), 143(30)
V	186(70), 185(100), 171(35)
V_{ref}	186(72), 185(100), 171(22)

The syntheses of deuterated THBCs yielded specifically labelled compounds (Fig. 1) with isotopic purities ranging from 76.3 to 96.2 % (Table 2).

In general, the isotopic purity was sufficient when reduction by lithium aluminium deuteride was used as the last step in the labelling procedure. In agreement with previously published data (9), the THBCs synthesized from deuterated tryptamine hydrochloride showed a lower isotopic purity. The yield of compound (VI) could be increased by

adding of dilute H_2SO_4 into the mixture containing 1-D_2-THBC and other reaction products of $LiAlD_4$. This presumably results from H^+ ions breaking down Al-complexes. The rather poor isotopic purities of the compounds (VII) and (VIII) are possibly due to proton exchange in the acidic reaction conditions used. The syntheses of THBCs from tryptamine hydrochloride and glyoxylic or pyruvic acid gave approximately 40 % yields.

In syntheses using tryptamine hydrochloride and formaldehyde (I-III) the yields generally were less than 25 %, while for the corresponding radioactive THBCs, a 20 % excess of tryptamine hydrochloride was used with increased yields. Unreacted tryptamine hydrochloride was easily separated by TLC.

Table 2. Isotopic purities of the deuterium labelled compounds. The results have been corrected for $(M-H)^+$ peaks.

compound	D_0	D_1	D_2	D_3	D_4
D_4-tryptamine	0	0	0	3.6	96.4
D_4-1-Me-THBC	4.7	0	9.1	2.6	83.6
D_4-THBC	7.3	0	0	16.4	76.3
D_2-THBC	3.8	0	96.2	-	-

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References

1. Airaksinen M.M. and Kari I. Med. Biol. 59: 21(1981)
2. Leino M. Kari I. Airaksinen M.M. and Gynther J. Exp. Eye Res. 36: 135(1983)
3. Rimon R. Airaksinen M.M. Kari I. Gynther J. Venäläinen E. Heikkilä L. Ryyppö J. and Palo J. Ann. Clin. Res. 16: 171(1984)
4. Rommelspacher H. Coper H. and Strauss S. Life Sci. 18: 81(1976)

5. Rosengarten H. Meller E. and Friedhoff A.J. Biochem. Pharmacol. 24: 1759(1975)

6. Peura P. Gynther J. and Ylinen M. Acta Pharm. Fenn. 91: 55(1982)

7. Ho T.B. and Walker K.E. Org. synth. 51: 136(1971)

8. Peura P. and Nousiainen E. Acta Pharm. Fenn. 90: 175(1981)

9. Gynther J. Peura P. and Salmi S. Acta Chem. Scand. B (in press)