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SYNTHESIS OF NONADEUTERO-CLENBUTEROL

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The synthesis of nonadeutero-clenbuterol, a useful tool for the quantitation of clenbuterol by gas chromatography-mass spectrometry, is described.

Key words : Clenbuterol - Deuterium labelling

Clenbuterol, 1-(4-amino-3,5-dichlorophenyl) 2-tertiobutyl-aminoethanol 1, is a very potent beta-2 sympathomimetic amine. Compounds of this pharmacological class are essentially useful for their bronchodilator and mucolytic properties but clenbuterol is often used in a quite different way: to dope horses for races and also, illegally to increase the muscular mass of calves. But, as it is active at very low doses (5 μg/kg/day for calves, 10-40 μg/day for humans) and as its volume of distribution is high (about 6 l/kg), detection and quantification of clenbuterol in plasma require sensitive and specific analytical methods, like GCMS (1). Quantification by GCMS necessitates the use of an internal standard; we describe in this paper the synthesis of nonadeutero-clenbuterol 2.

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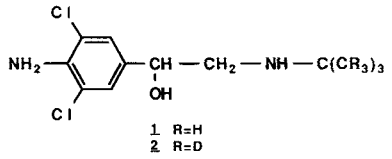
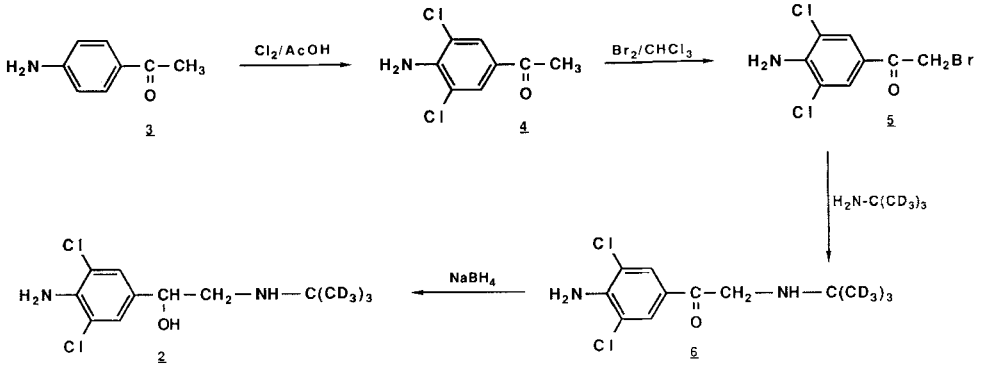


Figure 1

4-amino-3,5-dichloro-acetophenone 4 synthesised according to Lutz et al. (2) was brominated with bromine in chloroform; the bromo derivative 5 was treated with nonadeuterated tertiobutylamine (98% d9, Merck Sharp Dohme) and the resulting aminoketone 6 reduced to the aminoalcool 2 with sodium borohydride (method adapted from (3)).



The structure of d9-clenbuterol was verified by comparing its electron impact mass spectrum, obtained by solid probe introduction into the source of an LKB 2091 mass spectrometer, with that of d0-clenbuterol (Table 1).

1	2	
276/278/280 (1)	285/287/289 (1)	$M^{+\bullet}$
243/245/247 (5)	249/251/253 (3)	$(M - H_2O - CR_3)^+$
190/192/194 (5)	190/192/194 (6)	$(M - CH_2\text{-}NH\text{-}C(CR_3)_3)^+$
86 (100)	95 (100)	$(CH_2\text{=}NH\text{-}C(CR_3)_3)^+$
57 (30)	66 (31)	$C(CR_3)_3^+$

Table 1. Electron impact mass spectra of d0- and d9-clenbuterol (m/z (intensity %))

To confirm that d9-clenbuterol can be used in a GCMS assay, it was derivatised with bis-silyl trifluoroacetamide; the trimethylsilyl ether thus formed was chromatographed on a 25 m OV-1 fused silica capillary column, showing a unique chromatographic peak (fig 2): no ion at m/z 86 (the base peak of d0-clenbuterol) could be detected in d9-clenbuterol, so that in the GCMS assay of clenbuterol with d9-clenbuterol as internal standard, no interference will occur between the two compounds. The mass spectra of the two derivatives are explained in table 2.

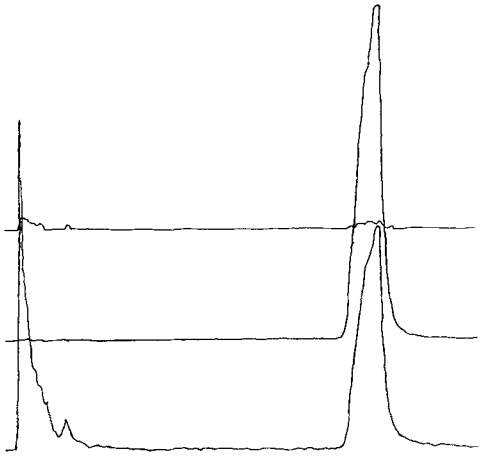


Fig 2. Mass fragmentogram of d9-clenbuterol trimethylsilyl ether; upper trace : m/z 86 (x10); middle trace : m/z 95 (x1); lower trace : total ion current (x1)

1	2	
333/335/337 (1)	342/344/346 (1)	$(M - CH_3)^+$
277/279/281 (1)	278/280/282 (3)	$(M - NH\text{-}C(CR_3)_3 + R)^{+\bullet}$
262/264/266 (5)	262/264/266 (5)	$(M - CH_2\text{-}NH\text{-}C(CR_3)_3)^+$
243/245/247 (2)	249/251/253 (2)	$(M - TMSOH - C(CR_3)_3)^+$
86 (100)	95 (100)	$(CH_2\text{=}NH\text{-}C(CR_3)_3)^+$
73 (18)	73 (18)	TMS^+
57 (30)	66 (31)	$C(CR_3)_3^+$

Table 2. Electron impact mass spectra of the trimethylsilyl ether of d0- and d9-clenbuterol (m/z (intensity %))

Experimental

The purity of all compounds was checked by TLC; the mass spectra were in accordance with the proposed stuctures.

4-amino-3,5-dichloroacetophenone 3.

A solution of 10 g of chlorine in 125 ml of concentrated acetic acid was added rapidly to a solution of 10 g of 4-aminoacetophenone in 125 ml of acetic acid at a temperature below 5°C. Immediately after the mixing, 2 l of ice-water were added and the resulting precipitate filtered off and recristallised from ethanol: 5 g (34%).

m/z 203/205/207 $M^{+\bullet}$, 188/190/192 $(M - \bullet CH_3)^+$, 160/162/164 $(M - \bullet CO\text{-}CH_3)^+$

4-amino-alpha-bromo-3,5-dichloroacetophenone 4.

3.8 g (18.7 mmole) of 3 were refluxed in 50 ml of chloroform. 2.98 g (18.7 mmole) of bromine were added over 15 minutes; reflux was continued for 20 minutes and some ethanol added to dissolve a light precipitate which was formed. The solution was concentrated and left overnight at 0°C. The precipitate was further purified by chromatography on silica.

4.36 g (82%) monobrominated product are obtained containing traces of non- and di-brominated derivatives.

m/z 281/283/285/287 $M^{+\bullet}$, 188/190/192 $(M - \bullet CH_2\text{-}Br)^+$

Nonadeutero-4-amino-alpha-tertiobutylamino-3,5-dichloroacetophenone 5.

1.4 g (5 mmole) of 4 were dissolved in 20 ml of chloroform under argon. To this solution was added 1.4 g of nonadeuterated tertiobutylamine (20 mmol) in 5 ml of chloroform. After heating for 2 h at 60°C in a sealed tube (to avoid loss of tertiobutylamine), the solution was cooled, washed with water and brine and dried over sodium sulfate. 300 mg of HCl in 20 ml of ethanol were added and after one night at 0°C the chlorhydrate was filtered off and dried in vacuum; 0.71 g (44%).

m/z 265/267/269 (M -•CD_3)$^+$, 188/190/192 (M -•CH_2-NH-C$(CD_3)_3$)$^+$,
160/162/164 (M -•CO-CH_2-NH-C$(CD_3)_3$)$^+$, 95 (CH_2=NH-C$(CD_3)_3$)$^+$

Nonadeutero-1-(4-amino-3,5-dichlorophenyl)-2-tertiobutylaminoethanol 2

540 mg (1.7 mmole) of 5 were dissolved at 0°C in 5 ml water and 5 ml methanol. 170 mg (5 mmole) of sodium borohydride in 0.5 ml of water were added dropwise at a pH maintained with hydrochloric acid between 3 and 7. After adjustment of the pH to 12 with NaOH N, the compound was extracted with ethyl acetate, which was dried and evaporated. The residue was purified by column chromatography on silica ($CHCl_3$-MeOH 95-5). The chlorhydrate formed by action of HCl in ethanol on the compound was recristallised from isopropanol-diisopropyloxide; 200 mg (35%).

(mass spectrum : table 1).

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