# SYNTHESIS AND CHARACTERISATION OF DEUTERATED CLENBUTEROL AND TWO EQUINE METABOLITES

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## ABSTRACT

The synthesis of deuterated clenbuterol (1) and 2 major equine metabolites of clenbuterol 1-(4-amino-3,5-dichlorophenyl)ethane-1,2-diol (2) and 2-[2-(4-amino-3,5-dichlorophenyl)-2-hydroxyethylamino]-2-methylpropan-1-ol (3) is described. Clenbuterol-$d_9$ (1) was obtained from 1-(4-amino-3,5-dichlorophenyl)-2-bromoethan-1-one (6) after reaction with *tert*-butylamine-$d_9$ in tetrahydrofuran (THF) and a subsequent reduction of the obtained aminoketone with sodium borohydride in methanol. Clenbuterol diol metabolite 2 was prepared from 1-(4-amino-3,5-dichlorophenyl)-2-bromoethan-1-one (6) by reaction with sodium acetate followed by reduction of the keto group with sodium borohydride. Analogously, the hydroxy metabolite 3 was obtained after reaction of 1-(4-amino-3,5-dichlorophenyl)-2-bromoethan-1-one (6) with 2-amino-2-methylpropanol in THF and subsequent reduction of the obtained product with sodium borohydride in methanol. All products were characterised by mass spectrometry and nuclear magnetic resonance.

## INTRODUCTION

Clenbuterol is a ß-agonist/antagonist bronchodilator and the only member of this group approved by the US Food and Drug Administration for use in horses. Because clenbuterol may be used as a therapeutic medication, in racehorses, particularly for bronchospasm, it is classified by the Association of Racing Commissioners International (ARCI) as a Class 3 agent, and its detection in post performance samples may lead to sanctions against trainers.

To develop a highly sensitive and specific analytical method for clenbuterol (Lehner *et al.* 2001) the synthesis of deuterated clenbuterol (clenbuterol-$d_9$) and its 2 major equine metabolites 2 and 3 (J. F. Quirke, Boeringer Ingelheim Vetmedica GmBH, personal communication; Schmid *et al.* 1990) was required.

## MATERIALS AND METHODS

### *2-[2-(4-Amino-3,5-dichlorophenyl)-2-hydroxyethylamino]-2-methyl-$d_3$-propane-$d_6$ (clenbuterol-$d_9$)* (1)

The synthesis of deuterated clenbuterol was performed following the procedure described by Keck *et al.* (1972) and using deuterated *tert*-butyl-$d_9$-amine (Isotec Inc) instead of *tert*-butylamine. The product was purified upon column chromatography on silica gel and crystallised from ethyl ether.

***Characterisation data:*** Colourless crystals from ethyl ether, Mp. 182-185°C; $^1$H-NMR (300 MHz, $CDCl_3$: δ (ppm) 2.91 (dd, 1 H, J 10.5 Hz, J 12.0 Hz, C$H_2$N), 3.18 (dd, 1H, J 2.4 Hz, J 12.0 Hz, C$H_2$N), 4.48 (s, 2H, N$H_2$), 5.30 (dd, 1H, J 2.4 Hz, J 10.5 Hz, C$H$OH), 7.29 (s, 2 H, 2 x $H_{AR}$).

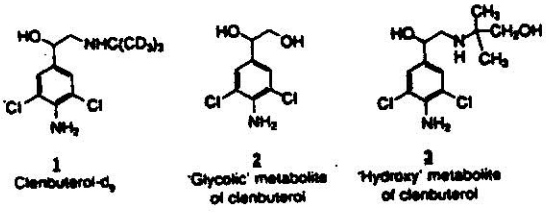


*Fig 1: Structures of deuterated clenbuterol-$d_9$ and 2 major equine metabolites of clenbuterol.*

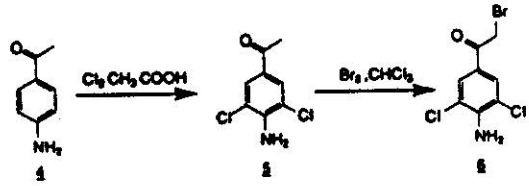


*Fig 2: Synthesis of 1-(4-amino-3,5-dichlorophenyl)-2-bromoethan-1-one (6).*

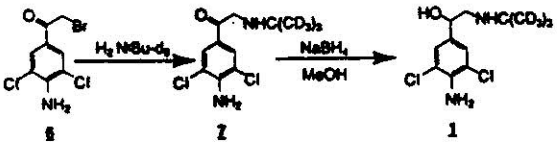

*Fig 3: Synthesis of clenbuterol-$d_9$.*

### *1-(4-Amino-3,5-dichlorophenyl)ethane-1,2-diol* (2)

A chloroform solution of 1-(4-amino-3,5-dichlorophenyl)-2-bromoethan-1-one (6) was added to a concentrated aqueous sodium acetate solution containing *tetra*-n-butylammonium bromide as phase transfer catalyst. The mixture was stirred vigorously for 3 days at room temperature. The chloroform phase was then separated, washed with water and dried with $MgSO_4$. After evaporation of the solvent, satisfactorily pure 2-(4-amino-3,5-dichlorophenyl)-2-oxoethyl acetate (8) was isolated (yield 88%) which, without any further purification, was reduced with $NaBH_4$ in methanol over a period of 24 h. Methanol was evaporated from the reaction mixture and clenbuterol metabolite 2 was purified upon column chromatography.

***Characterisation data:*** Colourless crystals from acetone-hexane, Mp. 90-93°C; $^1$H-NMR (300 MHz, $CDCl_3$): δ (ppm) 1.99 (broad triplet, 1 H, $CH_2OH$), 2.50 (d, 1 H, $CHOH$), 3.55-3.75 (m, 2 H, $CH_2OH$), 4.46 (bs, 2 H, $NH_2$), 4.65-4.73 (m, 1 H, $CHOH$), 7.21 (s, 2 H, 2 x $H_{AR}$); $^1$H-NMR (300 MHz, $CDCl_3$ with a drop of $D_2O$): δ (ppm) 3.60 (dd, 1 H, J 8.1 Hz, J 11.4 Hz, $CH_2OH$), 3.71 (dd, 1H, J 3.6 Hz, J 11.4 Hz, $CH_2OH$), 4.68 (dd, 1 H, J 3.6 Hz, J 8.1 Hz, $CHOH$), 7.21 (s, 2 H, 2 x $H_{AR}$).

### *2-[2-(4-amino-3,5-dichlorophenyl)-2-hydroxyethylamino]-2-methylpropan-1-ol* (3)

The synthesis was performed by reaction of 1-(4-amino-3,5-dichlorophenyl)-2-bromoethan-1-one (6) with 2-amino-2-methylpropanol in THF and

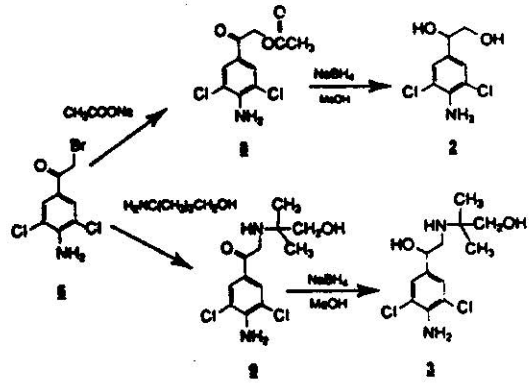

*Fig 4: Synthesis of 2 major equine metabolites of clenbuterol 2 and 3.*

subsequent reduction with NaBH4 following the procedure described by Keck *et al.* (1972).

***Characterisation data:*** Colourless crystals from acetone-hexane Mp. 149-150°C; $^1$H-NMR (300 MHz, DMSO-$d_6$): δ (ppm) 0.91, 0.92 (2 s, 6 H, 2 x $CH_2$), 2.57 (m, 2 H, $CH_2N$), 3.17 (qAB, 2 H, $CH_2OH$), 4.31 (dd, 1H, J 5.1 Hz, J 7.5 Hz, $CHOH$), 5.36 (s, 2H, $NH_2$), 7.19 (s, 2 H, 2 x $H_{AR}$).

## RESULTS AND DISCUSSION

The crucial substrate for the synthesis of all 3 compounds was 1-(4-amino-3,5-dichlorophenyl)-2-bromoethan-1-one (6), which was obtained as shown in Figure 2. Chlorination of the commercially available 4-aminoacetophenone (4u) in acetic acid gave the 3,5-dichloroderivative 5 (Lutz *et al.* 1947) which, after bromination in chloroform according to Keck's procedure (Keck *et al.* 1972), provided bromoketone 6. This compound was the initial substrate for the synthesis of all 3 target compounds: both metabolites of clenbuterol and deuterated clenbuterol-$d_9$.

Clenbuterol-$d_9$ was synthesised according to the procedure described by Keck *et al.* (1972). The reaction of 1-(4-amino-3,5-dichlorophenyl)-2-bromoethan-1-one (6) with *tert*-butyl-$d_9$-amine in

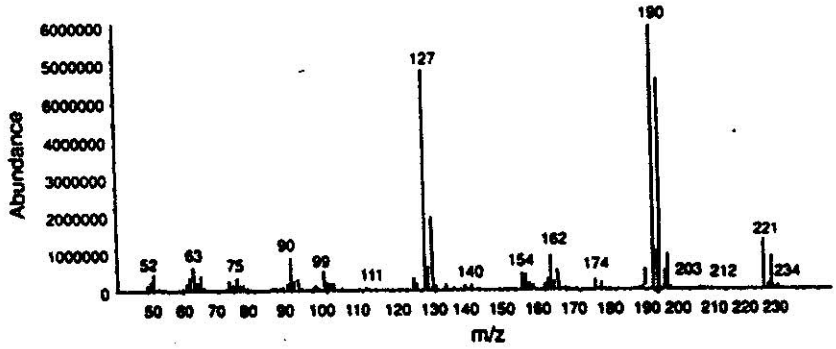


*Fig 5: Mass spectrum (EI mode) of 1-(4-amino-3,5-dichlorophenyl)ethane-1,2-diol (2).*

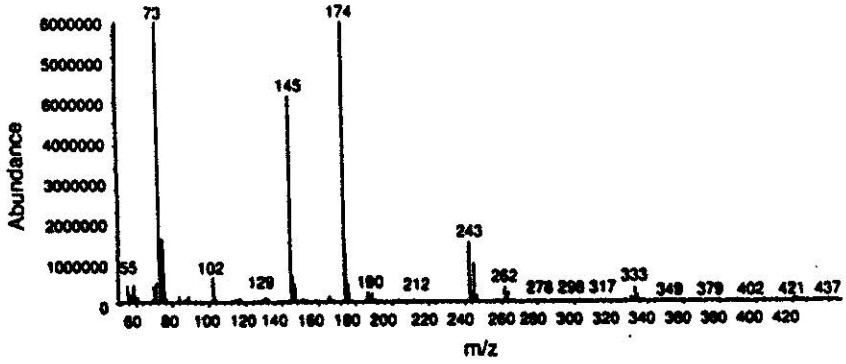


*Fig 6: Mass spectrum (EI mode) of 2-[2-(4-amino-3,5-dichloropbenyl)-2-bydroxyetbylamino]-2-methylpropan-1-ol (3) derivatised with BSTFA-1%TMCS.*

THF afforded 2-[2-(4-amino-3,5-dichlorophenyl)-2-oxoethylaminol-2-methyl-$d_3$-propane-$d_6$ (7) (Fig 3). The reduction of the carbonyl to hydroxy group in 7 was performed using sodium borohydride in methanol. Clenbuterol-$d_9$ (1) was characterised by electron impact MS and $^1$H-NMR. The mass spectrum of the TMS derivative of clenbuterol-$d_9$ revealed the expected isotope shifts showing the molecular ion at m/e 357 of very low abundance and being 9 mass units higher than that of clenbuterol (data not shown).

The glycolic metabolite of clenbuterol was obtained after reaction of 6 (Fig 4) with sodium acetate in a 2 phase solvent system water/chloroform in the presence of phase transfer catalyst *tetra*-n-butylammonium bromide. The substitution product - acetate 8 was obtained in high yield.

The joint reduction of the ketone and the ester group was made by sodium borohydride in methanol. The $^1$H-NMR spectrum of 2 registered in deuterated chloroform is consistent with the assigned structure and significantly simplifies when registering the spectrum after addition of a drop of deuterium water. The glycolic metabolite of clenbuterol was also characterised by mass spectrometry. The electron impact mass spectrum of 2 (Fig 5) contains 2 abundant and characteristic fragment ions at m/z 190 and 127 corresponding to $C_7H_6Cl_2NO^+$ (loss of $CH_2OH$) and $C_6H_6ClN^+$ respectively. The molecular ion is represented by m/z 221.

The second clenbuterol metabolite 3 was obtained after treating 1-(4-amino-3,5-dichloro-phenyl)-2-bromoethan-1-one (6) with 2-amino-2-methylpropanol in THF and reducing the obtained product with sodium borohydride in methanol (Keck *et al.* 1972, Fig 6).

The EI mass spectrum of bis-TMS derivative of 3 contains 3 abundant fragment ions at m/z 145, 174 and 243, where the first 2 correspond to the ions $^t$Bu-OTMS$^+$ and $CH_2NH^t$Bu-OTMS$^+$ respectively.

## Conclusions

The synthesis of clenbuterol-$d_9$ and 2 major equine metabolites of clenbuterol was accomplished. These compounds were designed for analytical methods for clenbuterol. Particularly the deuterium labelled clenbuterol has enabled us to develop a highly sensitive serum test for clenbuterol (Lehner *et al.* 2001). Use of clenbuterol-$d_9$ as an internal standard allowed detection of clenbuterol in serum samples at concentrations down to 10 pg/ml.

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