Synthesis of D-threo-2,2-[$^{37}Cl_2$]dichloro-N-[β-hydroxy-α-(hydroxymethyl)-4-nitrophenyl]acetamide ([$^{37}Cl_2$]chloramphenicol)

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Summary

[$^{37}Cl_2$]Chloramphenicol was synthesized from D-threo-2-amino-1-(4-nitrophenyl)-1,3-propanediol and ethyl[$^{37}Cl_2$]dichloroacetate. Ethyl[$^{37}Cl_2$]dichloroacetate was prepared by chlorination with $^{37}Cl_2$ of ethyldiazoacetate. $^{37}Cl_2$ was obtained by reaction of a solution of $Na^{37}Cl$ in 30% hydrogen peroxide with fuming sulfuric acid. The overall yield (from $Na^{37}Cl$) was 36%.

Key words: sodium[^{37}Cl]chloride, [$^{37}Cl_2$]chloramphenicol, chlorination, [$^{37}Cl_2$]chlorine

Introduction

Chloramphenicol is a potent broad-spectrum antibiotic possessing toxic properties (1). The most serious (toxic) effect is its bone marrow toxicity. Therefore, the use of chloramphenicol in laying hens and milk producing cattle is prohibited in The Netherlands (2,3). In order to monitor the illegal use of chloramphenicol sensitive and specific methods to detect and quantify this antibiotic in edible tissues are required.

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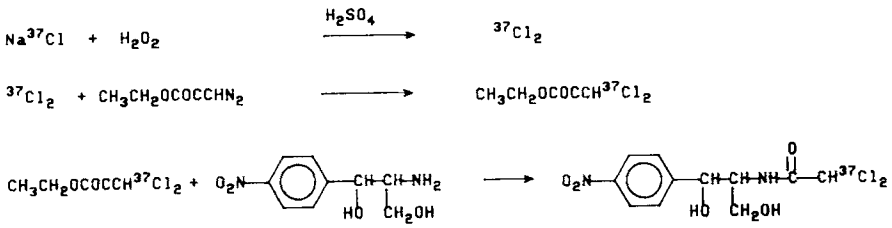
Although radioimmunoassay or enzyme-linked immunosorbent assay offer sensitive and fast screening methods, a reliable supplementary system is needed to verify the results obtained with these methods. Isotope-dilution-gas-chromatography-mass-spectrometry (ID-GC-MS) offers a sensitive and reliable tool excellently suited for this purpose. In this connection an isotopically labelled internal standard is needed. This paper desribes the synthesis of [$^{37}Cl_2$]chloramphenicol as a labelled internal standard.

Results and discussion

[$^{37}Cl_2$]Chloramphenicol was prepared according to the following scheme:



$^{37}Cl_2$ was prepared from Na^{37}Cl according to Woeber (4). The [$^{37}Cl_2$]chlorine was passed into dichloromethane, and the resulting solution was used in the chlorination of ethyldiazoacetate. During the preparation of $^{37}Cl_2$ a quantity of hydrogen[$^{37}Cl_2$]chloride was also produced which resulted in the formation of ethyl[^{37}Cl]chloroacetate next to ethyl$^{37}Cl_2$]dichloroacetate. In the final step this mixture of mono- and dichloro-ester was treated with an excess of D-threo-2-amino-1-(4-nitrophenyl)-1,3-propanediol in refluxing ethanol (5). The excess amine was removed by washing with acid. The crude product, which was contaminated with ca 10% of 2-[^{37}Cl]chloro-N-[β-hydroxy-α(hydroxymethyl)-4-nitrophenyl]acetamide was obtained in 36% overall yield from Na^{37}Cl. The product was purified by flash-chromatography to give pure [$^{37}Cl_2$]chloramphenicol. The compound was characterised by chromatographic

(TLC and GC) and spectroscopic (IR, NMR, and MS) methods. From MS measurements the isotopic purity was estimated to be > 95 atom%. This labelled material will be used as internal standard in an isotope-dilution gas-chromatographic mass-spectrometric (ID-GC-MS) method to quantify chloramphenicol in biological samples (6).

Experimental

IR spectra were taken using a Bruker FTIR type IFS 85. NMR spectra were recorded on a Varian FT80A instrument in $CDCl_3$ or CD_3COCD_3 using tetramethylsilane (TMS) as an internal standard (δ=0.0 ppm). Melting points were determined on a Mettler FP62 and are uncorrected. GC-MS (CI) experiments of TMS-derivatives were performed on a Finnigan 4021 GC-MS system (column DB 1701 (1μm), (CH_4)).
$Na^{37}Cl$ (95.7 atom% ^{37}Cl) was obtained from Isotec Inc. (Miamisburg, USA). D-threo-2-amino-1-(4-nitrophenyl)-1,3-propanediol was obtained from Aldrich.

[$^{37}Cl_2$]Chlorine

$Na^{37}Cl$ (1.0 g) was dissolved in 30% H_2O_2 (17 ml) and the solution stirred and cooled in an ice bath. To this solution, H_2SO_4 (30% SO_3, 25 ml) was slowly added from a dropping funnel. The [$^{37}Cl_2$]chlorine evolved was collected in two washing bottles each filled with CH_2Cl_2 (75 ml). The chlorine solution was dried over Na_2SO_4.

[$^{37}Cl_2$]Chloramphenicol

To the [$^{37}Cl_2$]chlorine solution was added a solution of ethyldiazoacetate in dichloromethane until the yellow colour disappeared. The solvent was removed at reduced pressure using a rotary evaporator. A mixture was obtained of ethyl[$^{37}Cl_2$]dichloroacetate and ethyl[^{37}Cl]chloroacetate (ca 3:2 as judged by NMR). This mixture was dissolved in ethanol (25 ml), and treated with D-threo-2-amino-(4-nitrophenyl)-1,3-propanediol (2 g). The mixture was refluxed for two hours. Ethanol was removed at reduced pressure. The solid residue was dissolved in ethylacetate (50 ml), the solution washed with 1N HCl (100 ml), 5% $NaHCO_3$ (100 ml), water (100 ml),

dried over Na_2SO_4, and evaporated. The oily residue was triturated with diethylether to give 1.0 g (36%) of [$^{37}Cl_2$]chloramphenicol (mp 137-141°C). Part of the product was purified by flash-chromatography on silica (elution 5% CH_3OH in $CHCl_3$) to give material melting at 145-147.5°C. This material was homogenous on TLC (SiO_2, $CHCl_3$/ CH_3OH (95/5)).

NMR (CD_3COCD_3): δ3.75 (m, 2H), δ4.19 (m, 2H), δ5.29 (m, 2H), δ6.35 (s, 1H), δ7.65 (bs, 1H), δ7.72-8.25 (AB, 4H).

GC-MS (bis TMS-derivative, CI (CH_4), m/z (%)): 471 (80), 381 (100), 225 (40).

The IR spectra (KBr) of labelled and natural chloramphenicol were identical.

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References

1 Allen, E.H., J. Assoc. Off. Anal. Chem. 68: 990 (1985)

2 Commodity Board for Poultry and Eggs, 16 November 1985, Prohibition Chloramphenicol Administration

3 Diary Produce Commodity Board, Dairy-regulation 1985, chloramphenicol

4 Woeber, H.H., J. Am. Chem. Soc., 74: 1354 (1952)

5 Controulis, J., Rebstock, M.C., and Crooks, H.M., J. Am. Chem. Soc., 71: 2463 (1949)

6 Manuscript in preparation