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SYNTHESIS OF ^{14}C-RADIOLABELLED TILMICOSIN

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Summary

Tilmicosin was radiolabelled with carbon-14 on the 3,5-dimethylpiperidinyl sidechain as a requirement for animal metabolism studies. A new radiosynthesis of 3,5-dimethylpiperidine was developed for this purpose. Incorporation into the desmycosin nucleus was accomplished by a reductive amination reaction.

Key Words: Tilmicosin, 3,5-dimethylpiperidine, side-chain, reductive amination, radiolabelled, ^{14}C

Introduction

Tilmicosin (1-4) (EL-870) is an antibacterial for use in treating respiratory diseases in cattle and swine. It is efficacious *in vitro* against a wide variety of Gram positive and certain Gram negative bacteria, anaerobes and mycoplasma species. It is currently under development as a parenterally administered antibacterial agent for the treatment of pneumonic pasteurellosis in calves and for use in feed for the control of pasteurella pneumonia in pigs. Preparation of this semisynthetic macrolide involves reductive amination of the C-20 aldehyde of desmycosin with a mixture of *cis* and *trans* 3,5-dimethylpiperidine (3,5-DMP). Independent analysis of the resulting isomers of tilmicosin confirms the near equivalence of the two isomers in terms of *in vitro* minimum inhibitory concentrations.(3) Desmycosin is readily produced from tylosin by mild acid hydrolysis to remove the terminal sugar mycarose (Scheme 1).

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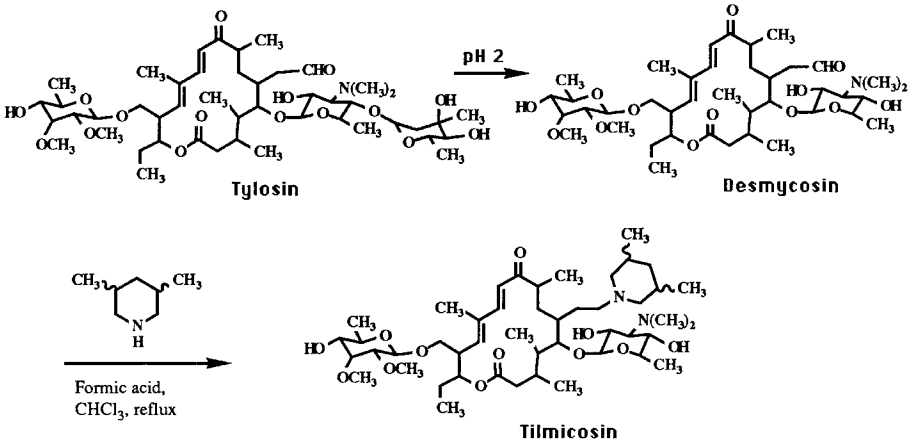
SCHEME I



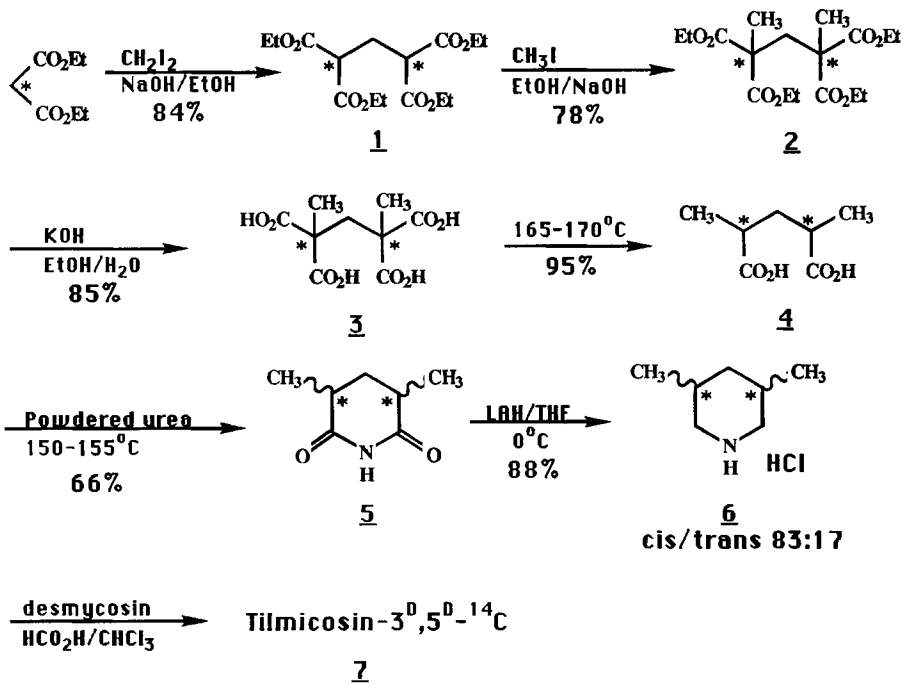
Radiolabelled tilmicosin was required for biochemical studies. Preferably, the label was to be incorporated into the piperidine moiety with as high a specific activity as possible. Specifically required was an 85:15 mixture of cis and trans isomers, which is the ratio obtained commercially by the catalytic hydrogenation of 3,5-lutidine. The preparation of isomerically pure radiolabelled 3,5-lutidine as a precursor to radiolabelled 3,5-DMP was envisioned to be a formidable task, so an alternative synthesis from a readily available radiolabelled precursor was pursued.

Results and Discussion

The most direct nonaromatic precursor to 3,5-DMP (6) was the imide 5 (Scheme II). Lithium aluminum hydride reduction cleanly afforded the amine, which due to high volatility was routinely isolated by formation of the hydrochloride salt. The imide is also capable of thermal equilibration to the more stable cis isomer (5-7), thus providing a means for control of the cis/trans isomer ratio.

The imide 5 was accessible by a number of routes.(8-10) For radiolabelling purposes, the procedure described in Scheme II offered a number of benefits. Foremost was the creation of a doubly labelled molecule from a single radiolabelled precursor.

SCHEME II



Additionally, the ^{14}C labels are incorporated into the piperidine ring rather than in the methyl groups, thereby avoiding concerns about possible metabolic removal. Coupling of two moles of 2-^{14}C-diethyl malonate with methylene iodide under basic conditions (NaOEt) gave the tetraester 1.(11-12) A second dialkylation with iodomethane under basic conditions provided the tetraester 2, which was hydrolyzed with aqueous base to form the corresponding tetraacid potassium salt. Acidification yielded the highly water-soluble tetracarboxylic acid 3 as a mixture of d,l- and meso-isomers. The product could be isolated by azeotropic removal of water followed by solvent extraction. The tetracarboxylic acid was then smoothly decarboxylated by heating to 165-170°C, delivering the requisite doubly-labelled diacid 4. Treatment with powdered urea at elevated temperature (13) then gave the imide 5 directly. NMR analysis of the crystalline imide indicated that both isomers were present, in a ratio of 73.5:26.5 cis:trans. Following treatment with LAH and subsequent formation of the hydrochloride salt, a crystalline product, mp 100°C, was isolated. NMR confirmed that both isomers

of 6 were present, and that the ratio of isomers was now 83:17. Subsequent treatment with desmycosin and formic acid in refluxing chloroform furnished the radiolabelled tilmicosin in an overall radiochemical yield of 19.5%, based on starting radioactive diethyl malonate.

Experimental

Diethyl malonate (2-^{14}C) was purchased from Pathfinder Laboratories, Inc. Radioactivity was determined using a Packard Tri-Carb Liquid Scintillation Counter, Model 300C. Thin-layer radiochromatography, using Fischer Redi-plate GF silica gel plates, was used to determine radioactive purity.

1,1,3,3-Propanetetracarboxylic acid, tetraethylester-1,3-$^{14}C_2$ (1)

Metallic sodium (460 mg, 20.0 mmol) was dissolved in 30 ml of absolute ethanol under nitrogen. The solution was warmed to 60°C and an ethanolic solution of diethylmalonate-2-^{14}C (20 mmol, sp. act. 5 mCi/mmol, act. 100 mCi) was added dropwise. After the solution had returned to ambient temperature, diiodomethane (2.7 g, 10 mmol) was added and the reaction was refluxed for three hours. The solution was evaporated to dryness, treated with water (25 ml) and extracted with ether (2 x 50 ml). The combined extracts were washed with water, dried over $MgSO_4$ and evaporated to dryness to yield (1) (2800 mg, 8.43 mmol, 84% yield) as a colorless oil.

2,2,4,4-Pentanetetracarboxylic acid, tetraethylester-2,4-$^{14}C_2$ (2)

Metallic sodium (388 mg, 16.86 mmol) was dissolved in 30 ml of absolute ethanol under nitrogen. The solution was heated to 60-65°C and an ethanolic solution of methyl iodide (2.63 g, 18.53 mmol) was added. The solution was maintained at the reflux temperature for an additional six hours, then cooled and evaporated to dryness. The residue was treated with water (25 ml) and extracted into ether (50 ml). The organic extract was washed with 10% sodium bisulfite solution, saltwater, and dried ($MgSO_4$). Evaporation of the filtrate yielded (2) (2380 mg, 6.60 mmol, 78% yield) as a colorless oil.

2,2,4,4-Pentanetetracarboxylic acid-2,4-$^{14}C_2$ (3)

The tetraester (2) (2380 mg, 6.60 mmol) was dissolved in 25 ml of ethanol, an aqueous potassium hydroxide solution (4 g KOH in 25 ml H_2O) was added and the solution was stirred at reflux for 20 hours. The solution was then cooled to

0°C, diluted with 25 ml of water, and acidified to pH 2 by the addition of conc. HCl (10 ml). The aqueous solution was evaporated to near dryness in vacuo. The last traces of water were removed azeotropically with benzene, and the solid residue was extracted with ethyl acetate (150 ml). Evaporation of the extract yielded the tetracarboxylic acid (3) (1392 mg, 5.61 mmol, 85% yield) as an orange-colored oil which crystallized upon standing at room temperature.

2,4-Pentanedicarboxylic acid-2,4-$^{14}C_2$ (4)

The tetracarboxylic acid (3) (1392 mg, 5.61 mmol) was heated in an oil bath to 165-170°C for 30 minutes. The resulting oil was cooled to room temperature, dissolved in 25 ml of ethyl acetate and filtered to remove insolubles. Evaporation of the filtrate yielded (4) (854 mg, 5.33 mmol, 95% yield) as a viscous oil which crystallized upon standing.

3,5-Dimethyl-2,6-piperidinedione-3,5-$^{14}C_2$ (5)

The dicarboxylic acid (4) (854 mg, 5.33 mmol) was treated with an excess of powdered urea (8 g) and the mixture was heated in an oil bath under nitrogen at 150-155°C for 24 hours. The mixture was allowed to cool to 90-100°C, then 25 ml of water was added and the aqueous mixture was extracted with ethyl acetate (75 ml). The extract was washed with brine (2 x 50 ml) and dried ($MgSO_4$). Evaporation of the solvent yielded the crude imide. This material was purified by dissolving it in THF, treating the solution with activated carbon, filtering, and evaporating the solvent to yield the imide (5) (500 mg, 3.54 mmol, 66% yield) as a white crystalline solid.

3,5-Dimethylpiperidine hydrochloride-3,5-$^{14}C_2$ (6)

$LiAlH_4$ (277 mg, 7.29 mmol) was suspended with stirring in 25 ml of anhydrous ether under nitrogen. To this suspension was added a solution of the imide (5) (500 mg, 3.54 mmol) dissolved in 5 ml of THF at such a rate that the ether refluxed gently. The reaction was refluxed for an additional 20 hours, then cooled to room temperature, and treated cautiously with 0.5 ml of water. The mixture was filtered, the inorganic solid was rinsed with ether (25 ml), and the filtrate was dried ($MgSO_4$). The filtrate was treated with a solution of ether saturated with gaseous HCl until acidic (pH = 2). Evaporation of the ether yielded a solid product. The solid was purified by dissolving it in 25 ml

of chloroform, treating the solution with activated carbon, filtering and evaporating the solvent in vacuo to yield (6) (465 mg, 3.11 mmol, 88% yield) as a white solid identical (mp) to that of authentic unlabelled 3,5-dimethylpiperidine hydrochloride.(4)

20-Deoxo-20-(3,5-dimethylpiperidin-1-yl-3,5-$^{14}C_2$)-desmycosin (7)

A solution of desmycosin (2401 mg, 3.11 mmol) and 3,5-dimethylpiperidine hydrochloride-3,5-^{14}C (6) (465 mg, 3.11 mmol) dissolved in 50 ml of chloroform was heated to reflux under nitrogen. Over a period of 15-20 minutes a solution of 95-97% formic acid (452 mg, 9.33 mmol) in chloroform (6 ml) was added dropwise. The solution was refluxed an additional two hours, then cooled, evaporated in vacuo, and the residue dissolved in 100 ml of 5% sodium phosphate (monobasic). The aqueous solution was washed twice with ethyl acetate (2 x 75 ml), then the pH was adjusted to 11 by the addition of 5N NaOH. The product separated as a viscous oil and was isolated by extraction into ethyl acetate (2 x 75 ml). The combined organic extracts were dried ($MgSO_4$) and the solvent was evaporated yielding a pale, yellow oil. The oil was treated with n-hexane (125 ml) and the solution was evaporated in vacuo, causing tilmicosin (7) to precipitate as a white powder (2611 mg, specific activity of 6.48 mCi/mmol, total activity of 19.5 mCi) in a yield of 97%.

The overall radiochemical yield for the synthesis was found to be 19.5%. Thin layer radiochromatography (90:10 ethyl acetate:diethylamine, Rf = 0.83) showed no detectable radioactive impurities.

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