SYNTHESIS OF NEDOCROMIL SODIUM LABELLED WITH TRITIUM, DEUTERIUM AND CARBON-14

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SUMMARY

Nedocromil sodium labelled with carbon-14 has been prepared by a Claisen reaction between diethyl [1,2-^{14}C]-ethanedioate and methyl 6-ethanoyl-1-ethyl-1,4-dihydro-7-hydroxy-4-oxo-8-propylquinoline-2-carboxylate. The ^{2}H- and ^{3}H-labelled materials are readily prepared by rhodium(III) chloride catalysed exchange with the appropriate isotopically labelled water.

Key Words: Nedocromil sodium, synthesis, isotopic exchange, carbon-14, tritium, deuterium, rhodium(III) chloride.

INTRODUCTION

Nedocromil sodium (disodium 9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate, I; figure 1) is a novel compound synthesized by Fisons as a modulator of inflammation currently undergoing clinical trials for a number of inflammatory conditions such as reversible obstructive airways disease (1,2).

The requirement for suitably labelled derivatives to assist pharmacokinetic studies and the development of assay procedures for the compound, prompted us to prepare a number of labelled nedocromil sodium derivatives. Pharmacokinetic and metabolism studies with nedocromil sodium required the synthesis of a derivative possessing a high degree of label stability and to meet this requirement, the carbon-14 labelled compound was prepared by chemical synthesis. Outstanding label stability was not, however, required of the labelled compound to be used in the development of an assay method for nedocromil sodium. Here the requirement was for a high

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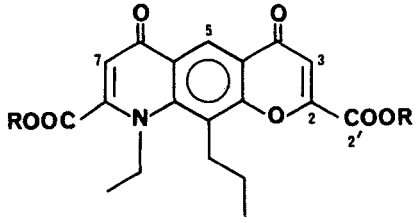


I R= Na
II R= H
III R= C_2H_5

Figure 1

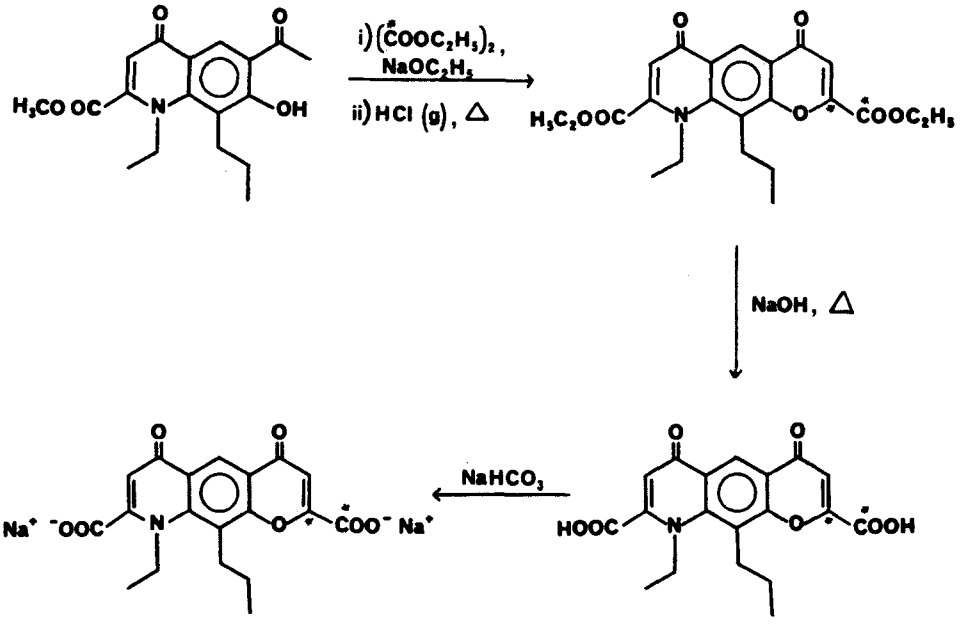
specific radioactivity label for a radioimmunoassay of the compound, and so the stability of labelling needed only to be sufficient for the purpose in hand. For this reason, therefore, isotopic exchange labelling with tritiated water was employed because of the low cost and convenience of such a synthesis.

DISCUSSION AND RESULTS

Labelling of nedocromil sodium (I) with carbon-14 was performed via the route outlined in Scheme 1; the label being introduced via the isotopically labelled ethanedioate precursor. A Claisen condensation between diethyl [1,2-^{14}C]ethanedioate and methyl 6-ethanoyl-1-ethyl-1,4-dihydro-7-hydroxy-4-oxo-8-propylquinoline-2-carboxylate gave a β-diketoester which was cyclised without purification to yield the diester (III). Subsequent purification by preparative liquid chromatography gave the pure diethyl ester which was hydrolysed with sodium hydroxide solution. Acidification of the hydrolysis solution precipitated the crude ^{14}C-labelled diacid (II). The diacid was purified by preparative reversed-phase high performance liquid chromatography (hplc) and converted into nedocromil sodium by reaction with sodium hydrogen carbonate.

The above procedure yielded [2,2'-^{14}C]nedocromil sodium (284 MBq mmol^{-1}) in an overall radiochemical yield of 28% from diethyl [1,2-^{14}C]ethanedioate. The radiochemical purity was examined by tlc/autoradiography/radiochromatogram scanning and by reversed-phase hplc and found to be > 99%.

The preparation of tritiated nedocromil sodium at high specific radioactivity involved preliminary experiments designed to investigate the suitability of the proposed labelling procedure. In these experiments, deuterium rather than tritium was employed for reasons of economy and convenience.



SCHEME 1

Nedocromil sodium proved unstable in the presence of deuterated Raney nickel at room temperature (3) and underwent partial decarboxylation at the temperatures required for labelling in the presence of platinum and palladium blacks. Homogeneous deuteration proved possible, however, in the presence of both palladium(II) chloride and rhodium(III) chloride (4,5), with the latter catalyst proving the most active. Investigation of the regioselectivity of labelling in the rhodium(III) chloride case was performed by ^{2}H-nmr spectroscopy which showed that labelling was confined to three molecular sites. Both the 3 and 7 positions were extensively labelled as predicted from previous labelling studies with the structurally related anti-allergy agent sodium cromoglycate (6). In addition, a small amount of deuteration also occurred at position 5 i.e. β to the quinolone and pyranone carbonyl groups. Possibly this labelling reflects a small degree of directive ability by the quinolone system, itself a vinologous amide (5).

The subsequent tritiation, which was performed by Amersham International utilising the TR-8 service, yielded a product which after purification had a specific radioactivity, as determined by ultraviolet spectrophotometry/liquid scintillation counting, of 796 GBq mmol^{-1}. The radiochemical purity was examined by tlc/radiochromatogram scanning and found to be greater than 95%.

EXPERIMENTAL

Authentic samples of nedocromil sodium and methyl 6-ethanoyl-1-ethyl-1,4-dihydro-7-hydroxy-4-oxo-8-propylquinoline-2-carboxylate were obtained from the Department of Process, Research and Development, Fisons plc, Pharmaceutical Division, Loughborough, Leics., UK. Rhodium(III) chloride trihydrate and deuterium oxide (99.8 atom % ^{2}H) were obtained from Aldrich Chemical Co. Ltd., Gillingham, Dorset, UK. All other chemicals were of reagent quality.

Diethyl [1,2-^{14}C]ethanedioate was obtained from ICI Physics and Radioisotope Services, Labelled Compounds Section, Billingham, Cleveland, UK. The tritiation was performed by Amersham International plc, Amersham, Bucks., UK via the TR-8 Tritium Labelling Service.

Thin layer chromatography (tlc)

Merck pre-coated silica gel F_{254} 100 x 50 x 0.25 mm tlc plates were used throughout this work. The solvent systems employed for this work were:

System A.	Trichloromethane/propanone,	19:1 by volume
System B.	Trichloromethane/ethyl ethanoate,	9:1 by volume
System C.	Trichloromethane/diethyl ether/methanoic acid,	14:4:2 by volume
System D.	Butan-1-ol/ethanoic acid/water,	12:3:5 by volume
System E.	Ethyl ethanoate/propan-2-ol/water,	10:7:6 by volume
System F.	Ethyl ethanoate/butanone/methanoic acid/water,	14:6:2:2 by volume
System G.	Butan-1-ol/water,	9:1 by volume

Inspection of chromatograms was performed under short (254 nm) and long (360 nm) wavelength ultraviolet light.

Synthesis of diethyl [2,2'-^{14}C]-9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate (III)

Methyl 6-ethanoyl-1-ethyl-1,4-dihydro-7-hydroxy-4-oxo-8-propylquinoline-2-carboxylate (643 mg, 1.94 mmol), diethyl [1,2-^{14}C]ethanedioate (740 MBq, 100.6 mg in 4 cm^3 of dry ethanol; 0.69 mmol) and diethyl ethanedioate (215.2 mg, 1.47 mmol) in 25% v/v dry benzene/ethanol (40 cm^3) was dried by slow azeotropic distillation through a 10 cm column of Fenske helices. After approximately 15 cm^3 of the higher boiling benzene/ethanol azeotrope had been collected, the apparatus was converted for reflux and a solution of sodium (223 mg, 9.70 mmol) in dry ethanol (8 cm^3) was added and the

resulting orange-red solution refluxed for two hours. After cooling to 4°, dry hydrogen chloride gas was passed through the solution for 15 minutes, before resuming the reflux for a further 30 minutes. After this period the solution was cooled, poured into water, and extracted with ethyl ethanoate. The organic layer was washed with water, dried over anhydrous magnesium sulphate, filtered and the solvent removed under reduced pressure to yield the crude diethyl ester (III, 855 mg).

The ester was purified by preparative liquid chromatography using a Merck Lobar Lichroprep Si60 (40-63 μm) 310 x 25 mm column and a mobile phase of trichloromethane to yield the pure diethyl ester (III, 560 mg, 319 MBq $mmol^{-1}$) with a radiochemical purity of $>$ 97% as determined by tlc/autoradiography/radiochromatogram scanning in solvent systems A, B and C.

Synthesis of [2,2'-^{14}C]-9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylic acid (II)

The diethyl ester (III, 560 mg, 1.311 mmol) was dissolved in hot methanol and sodium hydroxide solution (5 mol dm^{-3}, 725 mm^3, 3.625 mmol) was added in portions over 120 minutes. Upon cooling, the methanol was removed to leave a solid which was partially purified by means of an ion-pair extraction.

The solid was dissolved in sodium citrate/hydrochloric acid buffer (50 g dm^{-3}, pH 6.5, 80 cm^3) to which tetrabut-1-ylammonium phosphate solution (0.46 mol dm^{-3}, pH 6.5, 3 cm^3) was added and the resulting solution extracted with trichloromethane/methylbenzene, 3:2 by volume (4 x 80 cm^3) after which, tlc of the aqueous layer in system C revealed complete removal of the less polar impurities.

The aqueous phase was acidified with concentrated hydrochloric acid and the precipitated ^{14}C-labelled diacid collected by centrifugation and dried

under vacuum at 55° to yield the crude diacid (II). The diacid was further purified, in 30 mg portions, by preparative high performance liquid chromatography using a Spherisorb 5ODS 500 x 10 mm i.d. column eluted with increasing amounts of methanol in aqueous ammonium ethanoate solution (0.26 mol dm^{-3}). The fractions containing the purified diacid were extracted with acidic trichloromethane/propan-2-ol (1:1, by volume) and the organic phases bulked and evaporated under reduced pressure to yield the pure ^{14}C-labelled diacid (II, 404 mg, 277 MBq $mmol^{-1}$). The radiochemical purity of the purified diacid was assessed by tlc/autoradiography/radiochromatogram scanning in systems C, D, E and F and found to be greater than 99%.

Disodium [2,2'-^{14}C]-9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano-[3,2-g]quinoline-2,8-dicarboxylate (I)

The entire batch of purified diacid (404 mg, 1.09 mmol) was suspended in water (4 cm^3) and sodium hydrogen carbonate (183 mg, 2.18 mmol) was added in small portions until a clear solution was obtained (pH = 7.0). The solution was filtered and propanone (ca. 30 cm^3) was added causing the sodium salt to crystallize. After standing at 4° for three hours, the disodium salt was filtered and dried under vacuum to yield disodium [2,2'-^{14}C]-9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate (I, 271 mg, 284 MBq $mmol^{-1}$; overall radiochemical yield from diethyl [1,2-^{14}C]ethanedioate = 28%). ^{1}H-nmr, δ(80 MHz, ^2H_2O), 8.9 (1H, s, 5-H), 7.0 (1H, s, 7-H), 6.5 (1H, s, 3-H), 4.5 (2H, q, $N\underline{CH_2}CH_3$), 3.3 (2H, t, $Ar\underline{CH_2}CH_2CH_3$), 1.8 (2H, m, $ArCH_2\underline{CH_2}CH_3$), 1.1 (3H, t, $NCH_2\underline{CH_3}$), 1.0 (3H, t, $ArCH_2CH_2\underline{CH_3}$) ppm; ν_{max} 2970, 1655, 1620, 1475, 1375 and 1365, 800 cm^{-1}.

The radiochemical purity of the batch was determined by tlc/autoradiography/radiochromatogram scanning in eleven solvent systems and found

to be greater than 99% in all systems. Reversed-phase hplc determinations showed chemical and radiochemical purities of 99.7% and 99.6% respectively.

Synthesis of disodium [3,5,7-^{2}H]-9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate (I)

Nedocromil sodium (150 mg) and rhodium(III) chloride trihydrate (75 mg) were dissolved in a mixture of N,N-dimethylformamide (3 cm^3) and deuterium oxide (99.8 atom % ^{2}H, 1 cm^3) and heated at 90° for 24 hours. The reaction mixture was cooled, poured into hydrochloric acid (4 mol dm^{-3}) and the resulting suspension extracted into butan-1-ol, washed with water and reduced to dryness under vacuum to yield the deuterated diacid (II, 92.4 mg, 69%); m/z 286, 285, 284, 283 (M-2CO_2 + deuterium), 257, 256, 255, 254 (M-C_2H_5-2CO_2 + deuterium); λ_{max} 374, 343, 285, 254 nm; ν_{max} 2970, 2300, 1730, 1630, 1600, 1405, 1260, 800 cm^{-1}.

The diacid (37 mg) was converted into deuterated nedocromil sodium by dissolution in sufficient sodium hydrogen carbonate solution (1% w/v) to render the solution neutral to pH paper (pH 6.5 to 7.0). Addition of propanone (five volumes) led to crystallisation of the salt which was washed with propanone and dried under vacuum to yield [3,5,7-^{2}H]nedocromil sodium (I, 38.2 mg, 92%); ^{1}H-nmr δ(360 MHz, ^2H_2O) 8.8 (0.62H, s, residual 5-H), 7.0 and 6.5 (singlets, residual traces of 7-H and 3-H), 4.5 (2H, q, NCH_2CH_3), 3.3 (2H, t, $ArCH_2CH_2CH_3$), 1.8 (2H, m, $ArCH_2CH_2CH_3$), 1.1 (3H, t, NCH_2CH_3), 1.0 (3H, t, $ArCH_2CH_2CH_3$) ppm; ^{2}H-nmr δ (55.3 MHz, ^1H_2O) 8.8 (0.3^{2}H, bs, 5-^{2}H), 6.9 (1^{2}H, bs, 3-^{2}H), 6.5 (1^{2}H, bs, 7-^{2}H) ppm; λ_{max} 376, 344, 286, 253 nm; ν_{max} 2970, 2300, 1650, 1620, 1480, 1370, 800 cm^{-1}; found, C 47.25%, H 2.88%, H_2O (Karl Fischer 13.1%); required for $C_{19}H_{13}D_2NO_7Na_2.3.5\ H_2O$, C 47.46%, N 2.92%, H_2O 13.13%.

Synthesis of disodium [3,5,7(n)-^{3}H]-9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano[3,2-g]quinoline-2,8-dicarboxylate (I)

Tritiated nedocromil sodium was prepared by catalytic exchange labelling of the disodium salt with tritiated water. The labelling reaction was performed by Amersham International plc using the following procedure: To disodium 9-ethyl-6,9-dihydro-4,6-dioxo-10-propyl-4H-pyrano[3,2-g]-quinoline-2,8-dicarboxylate (I, 2 mg) and rhodium(III) chloride trihydrate (1 mg) in N,N-dimethylformamide solution (400 mm^3) was added tritium oxide (9 mm^3, 0.93 TBq). The reaction flask was sealed and the mixture heated at 90° for 24 hours. After this period the flask was opened and labile tritium removed by lyophilisation of an ethanolic solution. The non-volatile residue (19.2 GBq) was dissolved in ethanol (25 cm^3) and returned to our laboratories.

Upon receipt, a 10 cm^3 aliquot was reduced to dryness under a stream of nitrogen and the resulting residue purified by gradient hplc using two Spherisorb 5ODS 250 x 4.6 mm i.d. columns in series, eluted with increasing amounts of methanol in aqueous ammonium ethanoate (65 mmol dm^{-3}). The fractions containing the ^{3}H-labelled nedocromil sodium were collected and pooled for storage.

The specific radioactivity of the ^{3}H-labelled nedocromil sodium was determined by ultraviolet spectrophotometry/liquid scintillation counting and found to be 796 GBq $mmol^{-1}$. The radiochemical purity was assessed by tlc/radiochromatogram scanning in systems D, E and G and found to be greater than 95% in all three systems.

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