PREPARATION OF SODIUM CROMOGLYCATE (INTAL^{R}) LABELLED WITH ISOTOPIC HYDROGEN

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SUMMARY

Procedures for the labelling of the anti-allergic drug, sodium cromoglycate, with both tritium and deuterium are described. Sodium [6,6',8,8'-^{2}H]cromoglycate was prepared by acid catalysed exchange with deuterosulphuric acid. The 2-^{2}H and 2-^{3}H isotopomers were prepared via borodeuteride and borotritide reductions of an oxo-precursor. Sodium [3,3'-^{3}H]cromoglycate was prepared at high specific radioactivity by rhodium(III) chloride catalysed exchange with high atom % abundance tritiated water.

Key Words: Intal^{R}, Sodium cromoglycate, Cromolyn sodium, Deuterium, Tritium, Isotope-exchange

INTRODUCTION

Sodium cromoglycate 1a (disodium 5,5'-[(2-hydroxy-1,3-propanediyl)-dioxy]bis[4-oxo-4H-1-benzopyran-2-carboxylate; Intal^{R}) is a prophylactic anti-allergy agent widely used in the treatment of asthma and other diseases with an allergic basis such as seasonal rhinitis (1). This publication describes the synthesis of various isotopomers of sodium cromoglycate which have been utilised in the course of microautoradiographic studies of tissue distribution (2), investigations of receptor binding, the development of radioimmunoassay procedures (3) and in studies of the mesophase properties of the compound (4). The above investigations have required the synthesis of tritium-labelled sodium cromoglycate at various specific radioactivities and of deuterium-labelled compound in which the label was specifically introduced in either the aromatic or aliphatic portions of the molecule.

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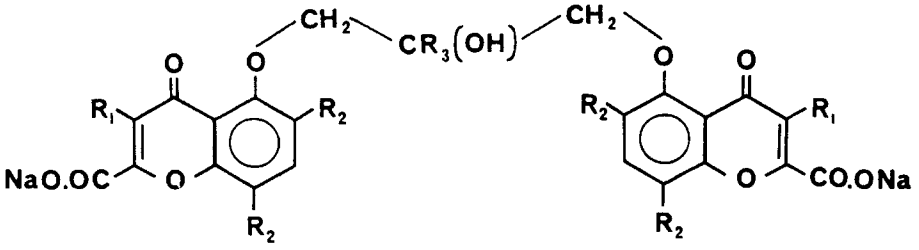
DISCUSSION

Studies of the molecular orientation in mesophases of sodium cromoglycate required the synthesis of the compound labelled, initially, with deuterium in the 4-oxo-4H-1-benzopyran-2-carboxylate moiety. Since the aromatic rings are susceptible to electrophilic substitution, labelling by exchange with a suitable deuteron donor was feasible. Accordingly, deuteration with concentrated deuterosulphuric acid was studied, since either sodium cromoglycate or the corresponding diacid 2a readily dissolve in this medium via formation of the pyrilium salt. Exchange proceeded smoothly at 85° and was not complicated by concomitant sulphonation, often a problem with the use of deuterosulphuric acid. The extent and position of deuterium labelling was assessed by ^{1}H-nmr spectroscopy. Whilst the resonances at δ 6.6 and 4.4 ppm assignable to the 3,3' olefinic protons and to the aliphatic protons were unaffected by the deuteration procedure, both pairs of overlapping doublet resonances at δ 6.9 and 7.0 ppm assignable to the protons at the 6,6',8,8' positions showed ca 70% reduction in intensity. In addition, the resonance at δ 7.6 ppm assignable to the 7 and 7' protons, although unreduced in intensity now comprised overlapping triplet, doublet and singlet resonances consistent with the successive replacement of ^{1}H-^{1}H ortho couplings from the 6,6' and 8,8' protons with unresolved ^{2}H-^{1}H couplings. Thus a single cycle of exchange yielded sodium [6,6',8,8'-^{2}H] cromoglycate 1c in which the deuterium abundance at the 6 and 6' positions and 8 and 8' positions (ca. 70% at each position) was sufficiently high for the purposes in hand.

Subsequent studies of mesophase behaviour required sodium cromoglycate labelled with deuterium in the aliphatic chain. Accordingly, the ethyl ester 2b was oxidised with Jones reagent (5) to yield a ketone 2c which after hydrolysis of the ester groups gave an oxo-derivative 2d of sodium cromoglycate. Sodium boro[^{2}H]hydride reduction of this compound gave



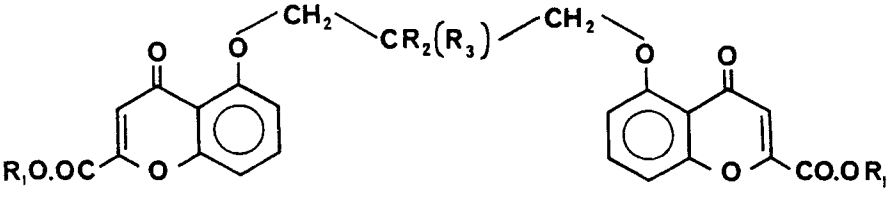
1a $R_1 = R_2 = R_3 = {}^1H$

1b $R_1 = {}^3H$, $R_2 = R_3 = {}^1H$

1c $R_2 = {}^2H$, $R_1 = R_3 = {}^1H$

1d $R_3 = {}^2H$, $R_1 = R_2 = {}^1H$

1e $R_3 = {}^3H$, $R_1 = R_2 = {}^1H$

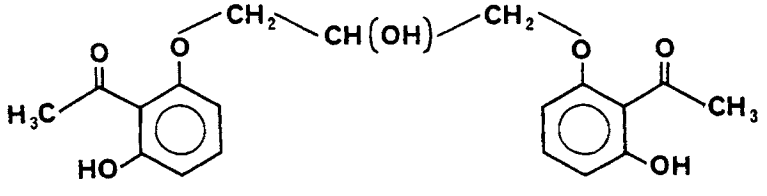


2a $R_1 = H$, $R_2 = H$, $R_3 = OH$

2b $R_1 = Et$, $R_2 = H$, $R_3 = OH$

2c $R_1 = Et$, $R_2 + R_3 = O$

2d $R_1 = H$, $R_2 + R_3 = O$



sodium [2-^{2}H]cromoglycate 1d in good yield. The position of labelling was confirmed by ^{1}H-nmr. The aliphatic proton resonances in the ^{1}H-nmr spectrum of unlabelled sodium cromoglycate occur as a five-proton AA'BB'C multiplet at δ 4.3 to 4.6 ppm. These resonances are assignable to the two pairs of magnetically non-equivalent methylene protons and to the methine proton at the 2 position. This complex resonance was absent from the ^{1}H-nmr spectrum of the labelled compound. Instead the aliphatic proton resonances consisted of a simpler AA'BB' four-proton signal at δ 4.3 to 4.5 ppm. The apparent simplification arises from the absence in the deuterated compound of the easily-resolved ^{1}H-^{1}H vicinal couplings arising from the methine proton at position 2 and their replacement by small and unresolved ^{2}H-^{1}H couplings.

A similar reaction employing sodium boro[^{3}H]hydride rather than the deuterium analogue yielded sodium [2-^{3}H]cromoglycate 1e. Excess sodium boro[^{3}H]hydride was employed in this reaction to ensure complete reduction of the oxo-precursor 2d which was not separable from sodium cromoglycate by the thin-layer chromatographic system most suited to purification of the crude tritiated sodium cromoglycate.

Since sodium boro[^{3}H]hydride is not routinely commercially available carrier-free, tritium-labelled sodium cromoglycate with a specific radioactivity suitable for use in receptor binding studies and in radioimmunoassay procedures was prepared by an alternative route.

Isotopic exchange between α,β -unsaturated or aromatic carboxylic acids and isotopically labelled water in the presence of rhodium(III) chloride yields species labelled predominantly or exclusively at positions beta to the carboxylic acid group (6,7). This technique was utilised to prepare sodium [3,3'-^{3}H]cromoglycate 1b by rhodium(III) chloride catalysed exchange with tritium oxide at high atom percent abundance. Although no attempt was made to optimise the reaction, the specific radioactivity

achieved was over eight times higher than that from boro[^{3}H]hydride reduction of 2d and was well suited to the proposed use of the compound. Since the tritium was introduced via an exchange reaction, the site of tritiation was investigated by alkaline degradation of 1b to yield the bisethanone derivative 3. This process was accompanied by loss of 99.6% of the tritium-label which, since no exchange of the aromatic protons with deuterium oxide occurred under the degradation conditions used, suggests that the labelling reaction was highly regioselective (8).

All the labelling procedures detailed above were simple to perform and yielded sodium cromoglycate isotopomers of good quality without the need for extensive purification. In addition, the syntheses orginate, initially, from unlabelled sodium cromoglycate and are therefore suitable for the preparation of the labelled drug without the need for specialised precursors.

EXPERIMENTAL

Authentic samples of sodium cromoglycate 1a, the diacid derivative 2a, the diethyl ester derivative 2b and 1,1'-[(2-hydroxy-1,3-propanediyl)bis [oxy-(6-hydroxy-2,1-phenylene)]bisethanone 3 were obtained from Fisons plc, Pharmaceutical Division, Loughborough, Leics, UK.

Deuterosulphuric acid (98%, 99.5 atom % ^{2}H), sodium boro[^{2}H]hydride (98 atom % ^{2}H) and rhodium(III) chloride trihydrate were obtained from Aldrich Chemical Company Ltd, Gillingham, Dorset, UK. Sodium boro[^{3}H]hydride (312 GBq mmol^{-1}) was obtained from Amersham International plc, Amersham, Bucks, UK, who also carried out the initial tritium oxide exchange of sodium cromoglycate via the TR-8 Tritium Labelling Service. All the sodium cromoglycate isotopomers prepared were identical with authentic unlabelled material by thin-layer chromatography using the following systems: Merck pre-coated silica gel F_{254}; ethyl acetate/propan-2-ol/water, 10:7:6 by volume; butan-1-ol/acetic acid/water, 12:3:5 by

volume; ethyl acetate/acetic acid/water, 7:3:1 by volume; propan-2-ol/aqueous ammonia solution 0.880 g cm^{-3}/water, 6:3:1 by volume; butanone/ethyl acetate/formic acid/water, 3:5:1:1 by volume; Merck pre-coated RP18S, methanol/water/formic acid, 40:60:1 by volume.

Preparation of disodium 5,5'-[(2-hydroxy-1,3-propanediyl)dioxy]bis[[3-^{3}H] 4-oxo-4H-1-benzopyran-2-carboxylate]; (sodium [3,3'-^{3}H]cromoglycate) 1b

A solution of the diacid 2a (5 mg) and rhodium(III) chloride trihydrate (1 mg) in N,N-dimethylformamide (0.4 cm^3) and tritium oxide (> 90 atom % abundance, 925 GBq, 25 Ci) was heated at 90° for 24 hours. After cooling and removal of exchangeable tritium by lyophilisation, a portion of the crude product (40% of the total) was purified by gradient high performance liquid chromatography using two Spherisorb 5 ODS 250 x 4.5 mm columns linked in series and a mobile phase gradient comprising increasing amounts of methanol in ammonium acetate solution (65 mmol dm^{-3}). The purified sodium [3,3'-^{3}H]cromoglycate 1b (2.18 GBq, 59 mCi, 0.61 TBq $mmol^{-1}$, 16.5 Ci $mmol^{-1}$) was stored in the mobile phase at 4°. The radiochemical purity of the material was > 97% after storage for an 18 month period.

Degradation of disodium 5,5'-[(2-hydroxy-1,3-propanediyl)dioxy]bis[[3-^{3}H] 4-oxo-4H-1-benzopyran-2-carboxylate]; (sodium [3,3'-^{3}H]cromoglycate) 1b

Sodium [3,3'-^{3}H]cromoglycate 1b was diluted with unlabelled sodium cromoglycate so as to yield a final specific radioactivity of 40 MBq $mmol^{-1}$ (1.08 mCi $mmol^{-1}$). To the resulting low specific radioactivity sodium [3,3'-^{3}H]cromoglycate (150 mg) dissolved in water (2 cm^3), sodium hydroxide solution (50 g dm^{-3}, 1 cm^3) was added.

After heating at 95° for 1.5 hour the solution was cooled, poured into hydrochloric acid (4 mol dm^{-3}, 20 cm^3) and extracted with dichloromethane (60 cm^3). The dichloromethane layer was washed with sodium hydrogen carbonate solution (50 g dm^{-3}, 20 cm^3) and with water (20 cm^3). After

removal of the dichloromethane under reduced pressure the crude product was purified by preparative thin-layer chromatography (one Macherey-Nagel 200 x 200 x 2 mm preparative-layer chromatography plate developed in ethyl acetate/chloroform, 1:1 by volume) to yield the bisethanone derivative, 3, which was further purified by two recrystallisations from a mixture of chloroform and hexane (2:1 by volume). The purified 3 had a specific radioactivity of 0.168 MBq $mmol^{-1}$ (0.00454 mCi $mmol^{-1}$) and mp 168°, undepressed upon admixture with authentic unlabelled material (9).

Preparation of disodium 5,5'-[(2-hydroxy-1,3-propanediyl)dioxy]bis[[6,8-^{3}H] 4-oxo-4H-1-benzopyran-2-carboxylate]; (sodium [6,6'8,8'-^{2}H]cromoglycate) 1c

The diacid 2a (500 mg) was dissolved in concentrated deuterosulphuric acid (5 cm^3) and the solution heated at 85° for two days. After cooling the mixture was poured into saturated sodium hydrogen carbonate solution, re-acidified with hydrochloric acid and extracted well with butan-1-ol. After removal of the butan-1-ol under reduced pressure the crude deuterated diacid was recrystallised from boiling N,N-dimethylformamide to yield purified deuterated 2a (360 mg). A portion (297 mg) of this diacid was converted into the sodium salt by dissolution in sodium hydrogen carbonate solution (106.6 mg in 3 cm^3 of water). Precipitation with acetone (15 cm^3) followed by filtration and drying under vacuum yielded sodium [6,6',8,8'-^{2}H]cromoglycate 1c (310 mg), δ (80 MHz, $^{2}H_2O$) 7.5 to 7.7 (2H, 5 line m, 7-H), 6.8 and 7.1 (partially deuterated, overlapping d, 6-H and 8-H), 6.6 (2H, s, 3-H), 4.4 (5H, broad s, CH_2CHCH_2) ppm; MS (fast atom bombardment, M/Z) 539, 538, 537, 536, 535 (M + Na + up to four ^{2}H atoms), 517, 516, 515, 514, 513 (M + H + up to four ^{2}H atoms). Since sodium cromoglycate decomposes extensively prior to melting, a small sample of the product was esterified with ethanol and sulphuric acid. The diethyl ester derivative had mp 182 - 183° undepressed upon admixture with authentic material (9).

Preparation of diethyl 5,5'-[(2-hydroxy-1,3-propanediyl)dioxy]bis[4-oxo-4H-1-benzopyran-2-carboxylate] 2b

Sodium cromoglycate (2.4 g) was dissolved in concentrated sulphuric acid (10 cm^3) and ethanol (90 cm^3) added. The resulting yellow solution was boiled under reflux for 2.5 hours and allowed to cool to room temperature when the crude diethyl ester crystallised. After filtration the crystals were washed with a small volume of ethanol and recrystallised from boiling ethanol to yield the purified diester 2b (2.0 g, 81%, mp 183°, literature (9) mp 180 - 182°).

The material was inseparable from an authentic sample of the diethyl ester (9) using Merck silica gel F_{254} tlc plates developed in acetone/hexane, 1:1 by volume and in ethyl acetate/chloroform, 1:1 by volume.

Preparation of diethyl 5,5'-[(2-oxo-1,3-propanediyl)dioxy]bis[4-oxo-4H-1-benzopyran-2-carboxylate] 2c

To a solution of 2b (1.5 g) in a mixture of dichloromethane and acetone (1:1 by volume, 800 cm^3) was added Jones reagent (5) until a faint yellow colouration persisted. The resulting suspension was filtered and the filtrate washed well with water. After drying over anhydrous magnesium sulphate the solution was filtered and evaporated to dryness under reduced pressure. The residual crude oxo-derivative 2c was recrystallised twice from a mixture of chloroform and ethyl acetate to yield the purified oxo-diester, 2c (1.1 g, 74%, mp 212°). Found: C, 61.49; H, 4.18. $C_{27}H_{22}O_{11}$ requires C, 62.07; H, 4.21%. ν_{max} (KBr), 1750, 1670, 1618, 1480, 1280, 1260 and 800 cm^{-1}; δ (80 MHz, C^2HCl_3), 7.6 (2H, dd, 7-H), 7.2 and 6.8 (4H, 2 x d, 6-H and 8-H), 6.9 (2H, s, 3-H), 5.2 (4H, s, 2 x CH_2), 4.4 (4H, q, 2 x ester CH_2), 1.5 (6H, t, 2 x CH_3) ppm; λ_{max} 262 nm (ε 16,300) and 320 nm (ε 8560).

Preparation of 5,5'-[(2-oxo-1,3-propanediyl)dioxy]bis[4-oxo-4H-1-benzopyran-2-carboxylic acid) 2d

To a suspension of the oxo-diester, 2c (522 mg) in boiling ethanol (75 cm^3) was added sodium hydroxide (80 mg) in a mixture of ethanol and water (5:1 by volume, 10 cm^3). The mixture became clear within one minute and was then cooled and acidified with hydrochloric acid (4 mol dm^{-3}) until the pH was < 1. The precipitated diacid was filtered, washed with water and dried under vacuum overnight. Recrystallisation from boiling N,N-dimethylformamide followed by drying under vacuum yielded the purified oxo-diacid 2d (320 mg as a 1:1 N,N-dimethylformamide solvate, 59%, mp 215° (decomp)). Thermogravimetric analysis shows a weight loss of 13.4 ± 0.5% between 150 - 200°; 1 mole of DMF requires a loss of 13.6%. ν_{max} (KBr) 3480, 1750, 1660, 1610, 1480, 1280, 800 cm^{-1}; δ (360 MHz, $(C^2H_3)_2SO$) 7.9 (1H, DMF), 7.7 (2H, dd, 7-H), 7.2 and 7.0 (4H, 2 x d, 6-H and 8-H), 6.8 (2H, s, 3-H), 5.3 (4H, s, 2 x CH_2), 2.7 and 2.9 (6H, DMF) ppm; δ (^{13}C, 90.56 MHz, $(C^2H_3)_2SO$) 30.6, 35.7, 71.9, 108.5, 111.0, 114.3, 115.0, 135.0, 151.2, 157.1, 161.3, 162.2, 176.5, 201.3 ppm; λ_{max} 318 nm (ε 9042).

Preparation of disodium 5,5'-[([2-^{2}H]2-hydroxy-1,3-propanediyl)dioxy]bis[4-oxo-4H-1-benzopyran-2-carboxylate]; (sodium [2-^{2}H]cromoglycate) 1d

To the oxo-diacid 2d (100 mg of the DMF solvate) dissolved in sodium hydrogen carbonate solution (25 g dm^{-3}, 4 cm^3) was added excess sodium boro[^{2}H]hydride (3.44 mg) and the resulting solution allowed to stand for ten minutes. After acidification to pH < 1 with hydrochloric acid (4 mol dm^{-3}) the precipitated diacid was filtered, dried under vacuum and recrystallised from boiling N,N-dimethylformamide. The sodium salt was prepared by dissolution of the diacid in sufficient sodium hydrogen carbonate solution (36 mg cm^{-3}) as to yield a final pH, after degassing under vacuum, of 7. Precipitation with acetone (4 cm^3) followed by filtration and drying under vacuum yielded sodium [2-^{2}H]cromoglycate 1d

(62.8 mg, 66%). ν_{max} (KBr) 3400, 1640, 1480, 1410, 1360, 1310, 1050, 805 cm^{-1}; δ (360 MHz, ^2H_2O), 7.6 (2H, dd, 7-H), 7.0 and 6.9 (4H, 2 x d, 6-H and 8-H), 6.5 (2H, s, 3-H), 4.3 to 4.5 (4H, q, 2 x CH_2) ppm; MS (fast atom bombardment, M/Z) 536 (M + Na + one ^2H atom), 514 (M + H + one ^2H atom); λ_{max} 324 nm (ε 7465). A small portion of the product was esterified by treatment with ethanol and sulphuric acid. The resulting diethyl ester derivative had mp 181 - 182°, undepressed on admixture with authentic unlabelled material (9).

Preparation of disodium 5,5'-[([2-^{3}H]2-hydroxy-1,3-propanediyl)dioxy]bis[4-oxo-4H-1-benzopyran-2-carboxylate]; (sodium [2-^{3}H]cromoglycate) 1e

The oxo-diacid, 2d (20.1 mg as the 1:1 DMF solvate) was dissolved in sodium hydrogen carbonate (30 g dm^{-3}, 0.5 cm^3) and the resulting solution added to sodium boro[^{3}H]hydride (3.7 GBq, 100 mCi, 312 GBq $mmol^{-1}$, 8.43 Ci $mmol^{-1}$). After stirring, the solution was allowed to stand for 15 minutes. Excess sodium boro[^{3}H]hydride was destroyed by the addition of hydrochloric acid (4 mol dm^{-3}, 100 mm^3) and the pH re-adjusted to neutrality with sodium hydrogen carbonate solution (30 g dm^{-3}). Preparative thin-layer chromatography (four Merck 200 x 200 x 0.5 mm pre-coated silica gel F_{254} tlc plates developed in ethyl acetate/propan-2-ol/water, 10:7:6 by volume) yielded the purified ^{3}H-labelled product which was eluted from the silica gel with sodium hydrogen carbonate solution (30 g dm^{-3}, 25 cm^3). Acidification with hydrochloric acid (4 mol dm^{-3}) and solvent extraction with a mixture of chloroform and propan-2-ol (1:1 by volume, 3 x 10 cm^3) yielded a solution of the ^{3}H-labelled diacid which was evaporated to dryness under reduced pressure. After dissolution in a mixture of ethanol and water (5:1 by volume, 28 cm^3), neutralisation with sodium hydrogen carbonate solution (3 g dm^{-3}) yielded sodium [2-^{3}H]cromoglycate 1e (1.42 GBq, 38.4 mCi, 73.6 GBq $mmol^{-1}$, 1.99 Ci $mmol^{-1}$). The radiochemical purity was > 98%.

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