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IMPROVED DELIVERY THROUGH BIOLOGICAL MEMBRANES VII. * DERMAL DELIVERY OF CROMOGLYCIC ACID (CROMOLYN) VIA ITS PRODRUGS

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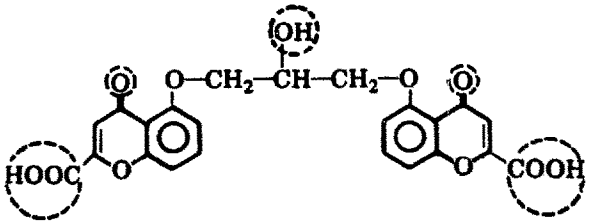
INTRODUCTION

Cromolyn (1) [1,3-bis(2-carboxychromon-5-yloxyl)-propan-2-ol, cromoglycic acid] (Cox, 1967), is used primarily in the prophylactic (Altounyan, 1967) treatment of bronchial asthma. Its mode of action is assumed to be related to its stabilizing effect on the mast cell membrane (Cox, 1971), thus preventing the release of the mediators of anaphylaxis. It is a valuable therapeutic agent against asthmas (Ryo et al., 1976), although it has a number of undesirable physical properties which have a definite effect on the clinical usefulness of 1. It is a highly polar molecule, thus it has poor oral bioavailability whether administered orally, parenterally, or by inhalation. As expected, due to its highly polar properties, 1 is cleared from the body unchanged at a very rapid rate in man (Walker et al., 1972). It was reported that cromolyn has weak anti-inflammatory activity (Cox et al., 1970). More recently, it was found (Haider, 1977) that topically applied sodium cromoglycate was effective in the treatment of atopic eczema in children. Based on its mechanism of action and its structure, it can be expected that cromolyn will have antipruritic and anti-inflammatory activity, but its polar character and short biological half-life make it difficult to have the drug absorbed through and/or concentrate in the skin. This is valid for the variety of analogs (Cairns et al., 1972) having one or two carboxy functions.

It is evident that in order to enhance topical absorption of such a polar drug as cromolyn (1), the most promising route is to develop lipophilic transient derivatives (prodrugs). The main objective of the present work is to design, synthesize and test prodrugs of cromolyn which would be absorbed into and/or through the skin.

There are three types of functions in 1 which were derivatized individually or in combination.

* Part VI of this series: Bodor, N., Kaminski, J.J. and Roller, R.G., Potent sympathomimetic adrenalone derivatives, Int. J. Pharm., 1 (1978) 189.



Cromolyn

The most polar function, the carboxy group, was the first target. The carboxy functions were derivatized, actually, in all prodrugs selected for testing, regardless of whether or not the alcohol or ketone groups were free or derivatized.

The prodrugs were evaluated vs. 1 for their percutaneous absorption characteristics using freshly excised hairless mouse skin mounted in a diffusion cell. HPLC methods were used to analyze in the receptor phase for the prodrug(s) and/or 1 resulting from metabolism in the skin. Selected prodrugs and 1 were also studied in vivo using hairless mice. ^{14}C-radiolabeled compounds were used in the in vivo studies.

EXPERIMENTAL

(A) Synthesis

Chloromethyl pivalate, diethyl chloromalonate, *t*-butyl chloroacetate and benzyl-tri-*n*-butyl ammonium chloride were supplied by Aldrich Chemicals. N,N-diethyl chloroacetamide was supplied by Eastman-Kodak. The ^{3}H- and ^{14}C-radiolabeled cromoglycic acid samples were provided by Fisons.

Chloromethyl hexanoate, α-chloroethyl hexanoate and chloromethyl dodecanoate were prepared according to our earlier work (Bodor and Kaminski, 1980; Bodor et al., 1980).

Chloromethyl-3-cyclopentyl propionate. To a 100 ml round-bottom flask was added 20.8 g 3-cyclopentylpropionic acid chloride (0.13 mol), 3.9 g paraformaldehyde (0.13 mol) and 100 mg anhydrous zinc chloride. The flask was protected from moisture with a drying tube and heated in an oil bath to 90°C until a solution was obtained. The mixture was cooled, slurried in petroleum ether (30–60°C) and filtered. Solvent was removed from the filtrate in vacuo and the residue distilled (bp 68°C at 0.2 mm) to give 14.5 g (59%) of product: IR (neat), 1750 cm^{-1}; PMR ($CDCl_3$, TMS), δ 0.77–3.2 (m, 13 H), 5.7 (s, 2 H).

Analysis: calculated for $C_9H_{15}O_2Cl$: C, 56.69; H, 7.93 – found: C, 56.41; H, 8.10.

1,3-Bis(2-pivaloyloxymethyloxycarbonylchromon-5-yloxy)propan-2-ol (2). Disodium cromoglycate 5 g (9.8 mM) was dissolved in 80 ml H_2O. To this stirred solution was added 6.1 g (19.5 mM) benzyltributylammonium chloride and 80 ml $CHCl_3$. After 5 min stirring, 2.9 g (19.5 mM) chloromethylpivalate was added to the reaction mixture which was then stirred overnight. The $CHCl_3$ was then separated, dried ($MgSO_4$) and the solvent removed in vacuo. The residue was taken up in ethyl acetate and allowed to stand for several days. The resulting precipitate was filtered to give 2.3 g of the title compound. The solvent was removed from the filtrate and the residue chromatographed on silica and eluted with 5% ethyl acetate/$CHCl_3$ to give an additional 1.7 g of the title compound, mp

133–135°C; IR (KBr), 3440, 1740, 1645 cm^{-1}; PMR ($CDCl_3$, TMS), δ 1.27 (s, 18 H), 4.48 (broad s, 5 H), 6.0 (s, 4 H), 6.75–7.31 (m, 6 H), 7.63 (t, 2 H).

Analysis: calculated for $C_{35}H_{30}O_{15}$: C, 60.34; H, 5.21 – found: C, 60.14; H, 5.16.

1,3-Bis(2-hexanoyloxymethyloxycarbonylchromon-5-yloxy)propan-2-ol (3). To a solution of 5 g (9.8 mM) disodium cromoglycate in 100 ml H_2O was added 8.4 g (27 mM) benzyltributylammonium chloride and 50 ml $CHCl_3$. After 10 min stirring 3.2 g (19.5 mM) chloromethyl hexanoate was added and the reaction mixture was stirred overnight. The $CHCl_3$ layer was separated, dried ($MgSO_4$), and the solvent removed in vacuo. The residue was slurried in ethyl acetate and filtered to give 2.2 g of a white solid. One gram of this solid was chromatographed on silica and eluted with 3% ethyl acetate/$CHCl_3$ to give 300 mg of pure product and 400 mg of product with a trace of impurity. The ethyl acetate mother liquor yielded, after standing, 2.3 g of a precipitate which contained large amounts of benzyltributylammonium chloride. This residue was also chromatographed to give 400 mg of the title product and 400 mg of the title product with a trace of impurity. The two impure fractions were combined for rechromatography: mp 124–126°C; IR (KBr), 3400, 1740, 1645 cm^{-1}; PMR ($CDCl_3$, TMS), δ 0.64–2.67 (m, 22 H), 4.47 (broad s, 5 H), 6.0 (s, 4 H), 6.87–7.34 (m, 6 H), 7.6 (t, 2 H).

Analysis: calculation for $C_{37}H_{40}O_{15}$: C, 61.32; H, 5.56 – found: C, 61.16; H, 5.58.

1,3-Bis(2-cyclopentylpropionyloxymethyloxycarbonyl-chromon-5-yloxy)propan-2-ol (4). To a solution of 3.5 ml triethylamine (0.022 mol) in 25 ml DMF was added portionwise 5.0 g 1,3-bis(2-carbonylchromon-5-yloxy)propan-2-ol (0.011 mol). When a solution was obtained, 4.2 g 3-cyclopentylchloromethylpropionate (0.022 mol) was added and the mixture was stirred under nitrogen overnight. The reaction mixture was filtered and the DMF was removed on a rotary evaporator under high vacuum. The residue was slurried with ethyl acetate for 30–60 min with stirring. The ethyl acetate was decanted and the solvent removed in vacuo to give 6.6 g of crude product. Part of this residue was then purified by preparative HPLC to give the pure title product, mp 102–105°C: IR (KBr), 3400, 1750, 1650 cm^{-1}.

Analysis: calculated for $C_{41}H_{44}O_{15}$: C, 63.39; H, 5.71 – found: C, 63.10; H, 5.98.

1,3-Bis(2-dodecanoyloxymethyloxycarbonylchromon-5-yloxy)propan-2-ol (5). To a stirred solution of 3.5 ml triethylamine (0.022 mol) in 25 ml DMF was added portionwise 5.0 g 1,3-bis(2-oxycarbonylchromon-5-yloxy)propan-2-ol (0.011 mol) was added and the mixture was stirred under nitrogen overnight. The reaction mixture was diluted to 150 ml with ethyl acetate and filtered. Washing with additional ethyl acetate made the solution cloudy so the mixture was filtered through Celite the second time. The filtrate was then washed with 250 ml water and with saturated $NaHCO_3$. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo to yield a white solid. This solid was slurried in a small amount of ethyl acetate and filtered to give 300 mg of product as a white solid. After removal of solvent from the filtrate, the residue was slurried with ethanol and filtered to give 800 mg more of product. Analysis of residue remaining in the filtrate by PMR showed the presence of dimer $CH_3(CH_2)_{10}COOCH_2OOC(CH_2)_{10}CH_3$. The products were combined to give 1 g (10%) of the title product: IR (KBr), 3400, 1750, 1650 cm^{-1}; NMR ($CDCl_3$, TMS), δ 0.63–3.63 (m, 46 H), 4.47 (broad s, 5 H), 6.03 (s, 4 H), 6.77–7.90 (m, 8 H).

Analysis: calculated for $C_{49}H_{64}O_{15}$: C, 65.90; H, 7.22 – found C, 65.81; H, 7.34.

Both compounds 4 and 5 were also obtained using the "phase transfer" conditions described for 3, obtaining yields of about 70%.

1,3-Bis[(2-hexanoyloxyethylideneoxycarbonyl)chromon-5-yloxy]propan-2-ol (6). Disodium cromoglycate (5 g, 9.8 mmol) and 6.1 g benzyltributylammonium chloride (19.5 mmol) were dissolved in 80 ml of water. To this solution was added 100 ml of chloroform and the mixture was rapidly stirred for 2 h. The chloroform layer was separated, dried ($MgSO_4$), and added to a 250 ml flask. α-Chloroethyl hexanoate (3.5 g, 19.5 mmol) was added to the solution and the reaction mixture was stirred under nitrogen for 7 days. The chloroform was evaporated and the residue was partitioned between ether and water. The water layer was extracted with a second portion of ether and the organic layers were combined, dried ($MgSO_4$) and evaporated. The residue was then slurried twice with ligroin, was decanted and discarded. The remaining residue was purified by column chromatography on silica and eluting with chloroform to give the title compound as a yellow glass: IR ($CHCl_3$), 3390, 1750, 1650 cm^{-1}; PMR ($CDCl_3$, TMS), δ 0.63–1.97 (m, 24 H), 2.10–2.57 (m, 4 H), 4.20–4.73 (broad s, 5 H), 6.83–7.27 (m, 8 H), 7.62 (t, 2 H).

Analysis: calculated for $C_{39}H_{44}O_{15}$: C, 62.22; H, 5.89 – found: C, 62.08; H, 5.95.

1,3-Bis-(2-N,N-diethylcarbamoylmethyloxycarbonylchromon-5-yloxy)propan-2-ol (7). To a stirred solution of 3.5 ml triethylamine (0.022 mol) in 25 ml DMF was added portionwise 5.0 g 1,3-bis(2-oxycarbonylchromon-5-yloxy)propan-2-ol (0.011 mol). After a solution was obtained, 3.3 g (0.022 mol) N,N-diethylchloroacetamide was added and the mixture was stirred under nitrogen overnight. The DMF was removed on a rotary evaporator under high vacuum and the residue slurried with benzene several times and filtered. The somewhat viscous material was stirred in H_2O and filtered to give a solid. The filtrate from above was concentrated in vacuo, the residue stirred in ethyl acetate and filtered to give a white solid. The solids were combined to give 2.8 g of product. This was stirred with saturated $NaHCO_3$, filtered and washed with H_2O. After drying, 0.8 g of the title product (mp 160–170°C dec) remained: IR (KBr), 3380, 1740, 1640 cm^{-1}; PMR ($CDCl_3$, TMS), δ 0.67–1.33 (m, 12 H), 3.37 (m, 8 H), 4.47 (broad singlet, 5 H), 5.0 (s, 4 H), 7.0 (m, 4 H), 7.27 (d, 2 H), 7.60 (t, 2 H).

Analysis: calculated for $C_{35}H_{38}O_{13}N_2$: C, 60.51; H, 5.51; N, 4.03 – found: C, 59.99; H, 5.52; N, 3.63.

1,3-Bis-(2-t-butyloxycarbonyloxycarbonylchromon-5-yloxy)propan-2-ol (8). Disodium cromoglycate (5 g, 9.8 mmol) was stirred in 20 ml water for 15 min followed by addition of 6.1 g (19 mmol) of benzyltributylammonium chloride. The solution was stirred for 1 h and 25 ml chloroform was added. This two-phase system was stirred for 4 h and the chloroform layer was separated, dried ($MgSO_4$) and the solvent evaporated in vacuo. *t*-Butyl chloroacetate (10 ml) was added to the residue and the mixture was stirred under nitrogen overnight. The mixture was diluted with 150 ml ethyl acetate and this solution was washed twice with water, dried ($MgSO_4$), and the solvent removed in vacuo. The resulting solid was slurried in petroleum ether (30–60°C) and filtered to give 2.3 g of product which was suitably pure for further reaction. Analytically pure material was obtained by chromatography on silica gel and elution with chloroform: mp 149–150°C, IR (KBr) 3385, 1730, 1650, 1600 cm^{-1}; PMR ($CDCl_3$, TMS), δ 1.50 (s, 18 H), 4.43–4.77 (m, 9 H), 7.03 (m, 6 H), 7.61 (t, 2 H).

Analysis: calculated for $C_{35}H_{36}O_{15}$: C, 60.34; H, 5.21 – found: C, 60.48; H, 5.29.

1,3-Bis-(2-carboxymethyloxycarbonylchromon-5-yloxy)propan-2-ol (9). To a solution of 10 ml dichloromethane saturated with hydrogen chloride at −10°C was added 500 mg of the bis-t-butyl acetate ester (8). The mixture was stoppered and stored at −20°C for 24 h. The dichloromethane was decanted and discarded. The remaining solid was slurried in chloroform and filtered to give 268 mg of the title product: mp 278°C dec; IR (KBr), 3380, 1725, 1630, 1590 cm^{-1}; PMR (d_6-DMSO, TMS), δ 4.37 (broad 2, 5 H), 4.73–5.13 (m, 4 H), 6.80 (s, 2 H), 7.0–7.5 (m, 4 H), 7.77 (t, 2 H).

Analysis: calculated for $C_{27}H_{20}O_{15}$: C, 55.48; H, 3.45 – found: C, 54.70; H, 3.35.

bis-Diethyl malonyl ester of 1,3-bis-(2-carboxychromon-5-yloxy)propan-2-ol (10). To 25 ml of water was added 1 g (1.95 mmol) of disodium cromoglycate and 1.2 g (4 mmol) of benzyltributyl ammonium chloride. The solution was stirred for 10 min and 25 ml of chloroform was added. The mixture was stirred rapidly for 3 h and the chloroform layer was separated and dried ($MgSO_4$). The chloroform was filtered and the solvent removed. The residue was dissolved in 20 ml dimethylformamide and 540 mg (2.8 mmol) diethyl chloromalonate was added. The solution was stirred under nitrogen for 2 days and the solvent was evaporated. The residue was slurried in diethyl ether and the ether was decanted and discarded. The residue was dissolved in ethyl acetate and the ethyl acetate was washed with water, dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel and eluted with 3% ethyl acetate/chloroform to give the title product: mp 140–142°C; IR (KBr) 3400, 1740, 1640 cm^{-1}; PMR ($CDCl_3$, TMS), δ 1.33 (t, 12 H), 4.10–4.77 (m, 13 H), 5.7 (s, 2 H), 6.8–7.33 (m, 6 H), 7.63 (t, 2 H).

Analysis: calculated for $C_{37}H_{36}O_{19}$: C, 56.63; H, 4.62 – found: C, 55.88; H, 4.77.

Acetylnitrate reagent was prepared as described earlier (Mantsch and Bodor, 1969). To a 50 ml flask was added 20 ml of acetic anhydride which was cooled to 5°C in an ice bath. To this cooled solution was added 2 ml conc. nitric acid. The mixture was stirred in the cold for 2 h and then used directly in the next reaction.

1,3-Bis(2-pivaloyloxymethyloxycarbonylchromon-5-yloxy)propan-2-nitrate (11). Pivaloyloxymethyl cromolyn (2) (1.0 g) was dissolved in 25 ml of chloroform and 10 ml of acetylnitrate reagent was added. The reaction mixture was stirred for 30 min and was poured into 50 ml of water. The chloroform layer was separated, washed with water, dried ($MgSO_4$) and evaporated. The residue was slurried with Et_2O and filtered to remove final traces of acetic anhydride. The resulting solid was recrystallized from ethyl acetate to give 600 mg of product: mp 156–166°C; IR (KBr) 1750, 1650 cm^{-1}; PMR ($CDCl_3$, TMS), δ 1.23 (s, 18 H), 4.68 (d, 5 H), 5.97 (2, 4 H), 6.80–7.30 (m, 6 H), 7.60 (t, 2 H).

Analysis: calculated for $C_{35}H_{35}NO_{17}$: C, 56.68; H, 4.76; N, 1.89 – found: C, 56.70; H, 4.86; N, 1.62.

1,3-Bis(2-hexanoyloxymethyloxycarbonylchromon-5-yloxy)propan-2-ol nitrate (12). Hexanoyloxymethyl cromolyn (2) (1.0 g) was dissolved in 25 ml of chloroform and 10 ml of acetylnitrate reagent was added. The reaction mixture was stirred for 30 min and poured into 50 ml of water. The chloroform layer was separated, washed with water, dried ($MgSO_4$) and evaporated. The residue failed to crystallize when slurried with ether so the ether was evaporated and the residue was subjected to high vacuum for several hours. The residue was then dissolved in chloroform and the chloroform layer was washed with water, dried ($MgSO_4$), and evaporated. The residue was slurried in ether and the resulting precipitate was isolated by filtration to give 550 mg of product: mp 93–95°C;

IR (KBr) 1750, 1650 cm^{-1}; PMR ($CDCl_3$, TMS), δ 0.65–2.62 (m, 22 H), 4.68 (d, 5 H), 5.95 (s, 4 H), 6.78–7.28 (m, 6 H), 7.62 (t, 2 H).

Analysis: calculated for $C_{37}H_{39}NO_{17}$: C, 57.78; H, 5.11; N, 1.82 – found: C, 57.77; H, 5.20; N, 1.69.

1,3-Bis(2-carbethoxy-4-methoxime-4H-1-benzopyran-5-yloxy)propan-2-ol (13). Cromolyn diethyl ester (2.0 g, 4 mmol) and 1.6 g methoxyamine hydrochloride (16 mmol) were dissolved in 200 ml of pyridine and the reaction mixture was stirred under nitrogen overnight. The solvent was evaporated under high vacuum and the residue was dissolved in chloroform. The chloroform layer was washed twice with water, dried ($MgSO_4$) and evaporated to give a pale yellow solid. This solid was purified by column chromatography using silica and elution with chloroform. The first material eluting from the column gave 300 mg of product: mp 180–182°C; IR (KBr) 3350, 1730, 1640 cm^{-1}; PMR ($CDCl_3$, TMS), δ 1.38 (t, 6 H), 4.0 (s, 6 H), 4.17–4.63 (m, 9 H), 6.63–7.67 (m, 8 H).

Analysis: calculated for $C_{29}H_{30}N_2O_{11}$: C, 59.79; H, 5.19; N, 4.81 – found: C, 59.49; H, 5.01; N, 4.44.

The corresponding mono-methoxime (14) was obtained as the second major fraction eluting from the column (400 mg): mp 195–197°C; PMR ($CDCl_3$, TMS), δ 1.38 (t, 6 H), 4.0 (s, 3 H), 4.17–4.65 (m, 9 H), 6.67–7.65 (m, 8 H).

Analysis: calculated for $C_{28}H_{27}NO_{11}$: C, 60.75; H, 4.92; N, 2.53 – found: C, 60.90; H, 4.88; N, 2.38.

Compounds 6, 7 and 15 were also obtained in radiolabeled form, starting with [^{14}C]-labeled disodium cromoglycate.

(B) Percutaneous absorption studies using fresh excised skin

Animal specifications

Male and female HRS/J hairless mice, 10–15 weeks old; average weight, 20 g; source: Jackson Laboratories, Bar Harbor, Me.

Method

The plexiglass diffusion cells consisted of a lower chamber with a side arm to allow sampling of the receptor phase. A slow moving, teflon-coated, magnetic stirring bar provided efficient mixing. Mice were sacrificed with CO_2 and the whole dorsal skin removed. The skin was gently stretched over the narrow opening of the funnel-shaped plexiglass lid and secured with a rubber gasket. The lid was then placed firmly on the lower chamber and secured with 6 screws. The opening in the lid left exposed an area 2.5 cm in diameter on the epidermis side through which the penetration was measured. A solution containing 0.9% sodium chloride, 0.5% Brij, and 0.01% thimerosal was heated to expel dissolved gases, cooled, and 40 ml was pipetted into each receptor chamber. Air bubbles were carefully removed from the dermal surface of the skin by tipping the cell. Each cell was then placed on a magnetic stirrer in a 32°C incubator and sufficient time was allowed for temperature equilibration.

The test solutions were made up at a concentration of 0.03 M in 100% isopropyl myristate and sonicated for 45 min. The test solutions were pipetted onto the skin surface in 0.25 ml volumes. Samples of 1.0 ml were removed from the receptor chamber via

the side arm at the specified times and the sample volume was replaced with the sodium chloride solution. The receptor phase samples were stored in screw cap tubes at –20°C until they were analyzed for chromoglycic acid and prodrug content in a high pressure liquid chromatography system (Waters Associates).

The chromatography for cromolyn detection was done on a SAX Column (Nr. 1) (Whatman) with mobile phase of 3×10^{-2} M NaH_2PO_4 in 80% water: 20% methanol. A standard of cromolyn was made up at a concentration of 5.00 μg/ml in water and a 100 μl aliquot was injected to obtain a reference peak. All the diffusion cell samples were then analyzed for cromolyn (cromoglycic acid) by injecting 100 μl samples into the HPLC system. The system was then turned over to the prodrug conditions and all the samples were analyzed for prodrug content. The chromatography for prodrug detection was done on a μ Bondapak C18 (Waters Assoc.) with a mobile phase of 80% methanol: 20% water. Standards were made up at a concentration of 4.00 μg/ml in THF and 10 μl aliquots were injected to obtain reference peaks. In most cases studied, only 1 was found in the receptor phase. The applied prodrug was found in the samples in two cases, when 7 and 14 were applied on the skin. Thus, approximately 10% of the cromolyn was in the form of unhydrolyzed prodrug in the case of 7, while in the case of 14, the small amount of prodrug could not be quantitated due to the multiplicity of peaks in the samples.

(C) In vivo absorption studies of topically applied cromolyn and its selected prodrugs

Test solutions and materials

All solutions were made at 0.21 molar in 90% ethanol: 10% isopropyl myristate.

Compound	*Spec. act. of solution*	*Radioactivity applied to each mouse*
[^{14}C]cromolyn	22.15 μCi/ml	1.108 μCi
^{14}C 7	23.36 μCi/ml	1.168 μCi
^{14}C 6	19.700 μCi/ml	0.985 μCi
^{14}C 15	34.180 μCi/ml	1.709 μCi

Econo-cage E110-Metabolism Unit. Maryland Plastics.
Gilson P200 Pipetman; accurate capacity 20–200 μl.
Adhesive Plaster for Patch Test: a 15 mm diameter circle of gauze on a strip of band-aid type adhesive.
Pyrex 7725 Tissue Grinder
Thomas 36609 Tissue Homogenizer
Beckman LS-100C Liquid Scintillation System
Protosol-NEF 935 Tissue and Gel Solubilizer; mixed with equal parts ethanol before using
Biofluor-NEF 961 Emulsifier Cocktail
Aquasol-NEF 934 Universal LSC Cocktail

Method

Each mouse (HRS/J hairless female mice, 10–13 weeks old) was placed in a metabolic cage and allowed to acclimatize for 48 h. After 48 h, the mouse was removed and the

cage was cleaned. A 50 μl portion of the test solution was pipetted onto the gauze pad of the adhesive strip. The adhesive strip was then wrapped securely around the body of the mouse so that the pad remained in airtight contact with the skin. The mouse was then placed back into the metabolic cage. After 24 h, the mouse was anesthetized with ether; the adhesive strip was removed and saved; the body of the mouse was scrubbed with moistened Kimwipes and rinsed with ethanol and water; and the jugular vein was severed, blood was collected in a preweighed test-tube containing 2 drops of 15% EDTA. The mouse was then sacrificed with CO_2 and dissected. The skin, muscle, large intestine, large intestine contents, small intestine, small intestine contents, stomach, stomach contents, fat, bile duct, liver, spleen, kidneys, heart, lungs and brain were all removed and each one placed in a preweighed test-tube. The remaining carcass was placed in a preweighed bottle. The feces were removed from the cage and weighed.

The skin, muscle, and carcass were wrapped in aluminum foil, frozen in liquid nitrogen, and shattered with a hammer. A known amount of water was then added to the shattered tissues and the other organs, and they were homogenized. The feces, stomach contents, and small and large intestine contents were shaken vigorously with a known volume of water until a homogeneous suspension was formed.

Samples of 0.1–0.2 ml of the homogenate or suspension and 0.5 ml of the protosol/ethanol mixture were pipetted into glass LSC vials. The vials were then capped tightly, shaken, and heated at 55°C for 60 min. If necessary, the samples were decolorized with 0.1–0.5 ml of 30% H_2O_2. The vials were capped loosely and heated at 55°C for 30 min. After the vials were cooled to room temperature, 15 ml of Biofluor was added, the vials were capped, shaken, opened, 0.5 ml of 0.5 N HCl was added, and the vials were recapped, shaken, and counted. An internal standard of 24,000 dpm was later added to each vial to determine the counting efficiency.

The urine that had dried on the metabolic cage funnel was rinsed off with water and added to the urine collected in the cup. A 0.2 ml aliquot of this solution was counted in 15 ml of Aquasol.

The gauze pad removed from the mouse and the Kimwipes used to scrub the body were shredded and soaked overnight in 25 ml of 15% NaOH. The NaOH solution was then decanted and the fibers were rinsed 5 times with 5 ml of water. A 0.1 ml aliquot of this solution was neutralized with 1 ml of 0.5 N HCl and counted in 15 ml of Aquasol. The ethanol and water used to rinse the body were collected in a beaker, evaporated, and replaced with 10 ml of 15% NaOH. The NaOH was allowed to sit for several hours, then 0.1 ml was neutralized with 1 ml of 0.5 N HCl and counted in 15 ml of Aquasol.

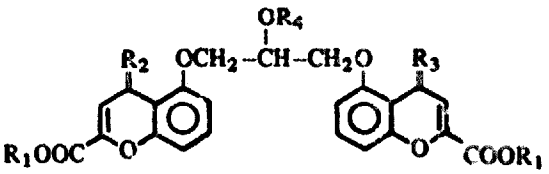
RESULTS AND DISCUSSION

The novel prodrugs of cromolyn selected for testing are shown in Table 1.

In the first set of compounds, the acid was protected in the form of acyloxyalkyl ester, by reacting 1 with selected α-haloalkyl esters (R_1-X) (Bodor and Kaminski, 1980). Although the desired products could be obtained in most cases by reacting triethylammonium salts of 1 with R_1-X in an organic solvent better yields were obtained using "phase-transfer" catalysis (Dehmlow, 1974), i.e. reacting the benzyl-tributylammonium salt of 1 with R_1-X in a heterogeneous (water/chloroform) reaction mixture. Some

TABLE 1

SELECTED PRODRUGS OF CROMOGLYCIC ACID



Compound	R_1	R_2	R_3	R_4
1	H	O	O	H
2	$-CH_2OCC(CH_3)_3$	O	O	H
3	$-CH_2OC(CH_2)_4CH_3$	O	O	H
4	$-CH_2OCCH_2CH_2-$ 	O	O	H
5	$-CH_2OC(CH_2)_{10}CH_3$	O	O	H
6	$-CHOC(CH_2)_4CH_3$ CH_3	O	O	H
7	$-CH_2CN(C_2H_5)_2$	O	O	H
8	$-CH_2COOC(CH_3)_3$	O	O	H
9	$-CH_2COOH$	O	O	H
10	$-CH(COOC_2H_5)_2$	O	O	H
11	$-CH_2OCC(CH_3)_3$	O	O	NO_2
12	$-CH_2OC(CH_2)_4CH_3$	O	O	NO_2
13	$-C_2H_5$	$NOCH_3$	$NOCH_3$	H
14	$-C_2H_5$	$NOCH_3$	O	H
15 [a]	$(n\text{-}C_4H_9)_3\overset{\oplus}{N}CH_2C_6H_5$	O	O	H

[a] The bis-benzyl-tributylammonium salt of the cromoglycic acid.

of the more activated esters, such as the cinnamoyloxybenzyl ($R_1 = C_6H_5\text{-}CH{=}CH\text{-}COOCH\text{-}(C_6H_5)\text{-}$) or benzoyloxybenzyl ($R_1 = C_6H_5COOCH(C_6H_5)\text{-}$) esters of 1 were found to be too unstable and thus could not be obtained in pure form.

The pivalyloxymethyl (2) and hexanoyloxymethyl (3) esters could easily be prepared, however. Other analogous derivatives were also obtained. The main feature of esters of the type 2–5 is removal of the point of attack for ester hydrolysis (i.e. delivery of 1) from the cromoglycic acid portion to the fatty acid. The hydrolysis of the ester results in formation of 1 (in two steps, via the monoester), as the hypothetical hydroxymethyl intermediate is hydrolyzed instantaneously.

The diethylcarbamoylmethyl ester (7) is a different type of compound which should be more water-soluble than the esters 2–5. The glycolate ester 9 was intended to provide a less acidic carboxy derivative (the pK_a of 1 is about 1.2).

The acyloxyalkyl derivative 6 is a stable derivative containing an ethylidene rather than a methylene moiety. This includes additional chiral centers in the molecule, which might explain the much reduced melting point (it is an oil), due to further reduction in the intermolecular forces in the mixture of optical isomers inherently obtained.

Acylation of the secondary alcohol group was the other objective in order to enhance lipophilicity of 1 (and/or its esters). Attempts to obtain the acetate have failed, however. The nitrate esters 11 and 12 could be obtained using acetylnitrate (Mantsch and Bodor, 1969) for the nitration of selected acyloxyalkyl esters.

The protection of the keto function(s) was achieved by formation of the corresponding methoximes.

Finally, the quaternary ammonium salt, 15, was prepared in attempts to enhance topical absorption.

Attempts to prepare 'mono esters' of 1 have failed, probably due to solubility problems: small amounts of the bis esters were obtained even when using 1 : (R_1-X) = 1 : 1 molar ratios.

As expected, the prodrug approach resulted in dramatic changes in the physical properties of 1. The acyloxyalkyl esters are much more lipophilic than 1. Thus, 2 has a mp of 133°C and an octane : water partition coefficient of 30.9. The amide type ester 7 has a partition coefficient of 1.02. The acetaldehyde-containing derivative 6, due to the multiple optically active centers, is a glass, rather than a crystalline material. The prodrug characteristics of the compounds were clearly demonstrated by quantitative conversion of selected derivatives, 2, 3, 6 and 7 to 1 upon incubation with plasma at 37°C for 24 h. No kinetic studies of the conversion were performed due to solubility problems, as well as, because the absorption–metabolism studies in the fresh skin were felt to be more relevant than kinetic studies in isolated plasma. Freshly excised hairless mice skin was used in the skin absorption studies.

For most substances, the limiting region for diffusion across the epidermal barrier is the exterior stratum corneum (Katz and Poulsen, 1971). Several mathematical models have been developed to explain and predict drug permeation through the skin (Scheuplein et al., 1969; Nakagawa et al., 1976). Some of the more recent work, involving prodrugs, has also taken into account the simultaneous metabolism–permeation phenomena (Fox, 1979). The whole skin, and particularly the epidermis, contains many highly active enzyme systems, and some esterase activity has even been found in the stratum corneum of the pig skin (Meyer and Neurand, 1976).

The results of the diffusion studies are summarized in Table 2.

It can be seen that although in only relatively small amounts (0.5% in 12 h), even

TABLE 2

DIFFUSION OF CROMOGLYCIC ACID (1) AND ITS SELECTED PRODRUGS THROUGH HAIRLESS MOUSE SKIN [a]

Compound cromoglycic acid	Material in the receptor phase [b]		
	Conc. (μg/ml)	μg in 40 ml	% of dose diffused
1 [c]	0.478	19.00	0.50
3	2.629	105.16	2.74
6	2.946	117.83	3.07
7	1.789	71.55	1.86
11	2.761	110.45	2.88
12	1.561	62.44	1.63
13	1.957	78.26	2.04
14	1.978	79.10	2.06
15	1.757	70.27	1.83

[a] Sample time = 12 h

[b] Only cromoglycic acid 1 was found in the receptor phase, except in the case of 7 and 14 (see text).

[c] As the disodium salt.

cromoglycic acid does penetrate the intact skin. This observation is consistent with the observed activity (Haider, 1977) of cromolyn in atopic eczema in children. Significantly higher amounts penetrate the skin when the lipoidal prodrugs are used. Thus, the hexanoyloxyethylidene (6), hexanoyloxymethyl (3) and pivalyloxymethyl-nitrate esters (11) all resulted in about 3% of the dose appearing in the receptor phase, in the *form of cromoglycic acid*. It is important to emphasize that the prodrugs did indeed deliver cromoglycic acid, thus significant metabolism in the skin takes place. This metabolism included not only esterase activity, but reductive cleavage as well, since the nitrate ester must cleave by a reductive process. Small amounts (~10% of the diffused amount) of the unchanged prodrug were found only in the case of the less activated ester 7 and in the case of the oxime 14 (just a few per cents of the diffused amount).

The absorption of cromolyn and some selected prodrugs was confirmed in vivo, using hairless mice and [^{14}C]-radiolabeled material, as described in the experimental section. The results are given in Table 3. It is evident that as expected, only negligible amounts are distributed in the various organs and tissues (intestine, bile, fat, etc.).

The only significant amounts were found in the skin, urine and feces, indicating that cromoglycic acid, can, indeed, be delivered topically in vivo. As in the case of the diffusion cell studies, the lipoidal 6 resulted in the highest overall delivery (particularly the excreted part), although cromolyn itself showed an apparently better absorption as in vitro. Small amounts of impurities could, however, account for some radioactivity, which, however, cannot be the case for 6 and 7, as they were thoroughly purified by chromatography when the radioactive derivatives were prepared. The quaternary ammonium salt, 15, apparently results in some enhanced depot effect, but it did not represent significant improvement over cromolyn.

In conclusion, the prodrug approach seems to be promising for topical delivery of such

TABLE 3

IN VIVO DIFFUSION OF TOPICALLY APPLIED CROMOLYN AND ITS SELECTED PRODRUGS

Organ	Compounds (per cent of dose diffused in 24 h)			
	7	6	15	1
No. of animals	n = 2	n = 2	n = 1	n = 4
Skin	0.440	0.840	1.950	0.560
Feces	0.660	1.230	0.820	1.105
Urine	0.310	1.450	0.330	0.645
Small intestine's contents	0.008	0.015	0.003	0.028
Large intestine's contents	0.010	0.020	0.007	0.053
Bile	–	–	0.003	–
Blood	–	0.006	0.002	0.014
Small intestine	–	–	–	0.005
Fat	0.050	–	–	–
Muscle	–	0.002	–	–
Carcass	–	0.030	0.141	0.020
Subtotal	1.478	3.593	3.256	2.430
Wash	98.800	103.050	93.820	104.430
Total recovery	100.278	106.643	97.076	106.860

highly polar compounds as cromolyn. This conclusion is evident based on the quantitative studies given in Table 2. The results for the prodrugs are well supported by the ratio-labeled studies given in Table 3. As mentioned before, the values for the radiolabeled 1 are higher than expected due to some lower molecular weight radioactive impurities.

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