Mrs. M. D. Napoli for generously providing the pharmacological information listed in Table I and discussed here.

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Antiasthma Agents. 1. 4-Oxo-4*H*-[1]benzothieno-[3,2-*b*]pyran-2-carboxylic Acid and 4-Oxo-4*H*-[1]benzofuro[3,2-*b*]pyran-2-carboxylic Acid

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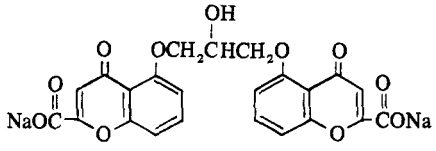
Disodium cromoglycate (**1**) is a substance which has shown very promising antiasthmatic properties.[1,2] We were interested in investigating other compounds for this type of activity, particularly those compounds that possessed a fused γ-pyrone ring, similar to that found in the chromone ring of disodium cromoglycate. We chose for synthesis and for biological study 4-oxo-4*H*-[1]benzothieno[3,2-*b*]pyran-2-carboxylic acid (**2**) and 4-oxo-4*H*-[1]benzofuro[3,2-*b*]pyran-2-carboxylic acid (**3**).



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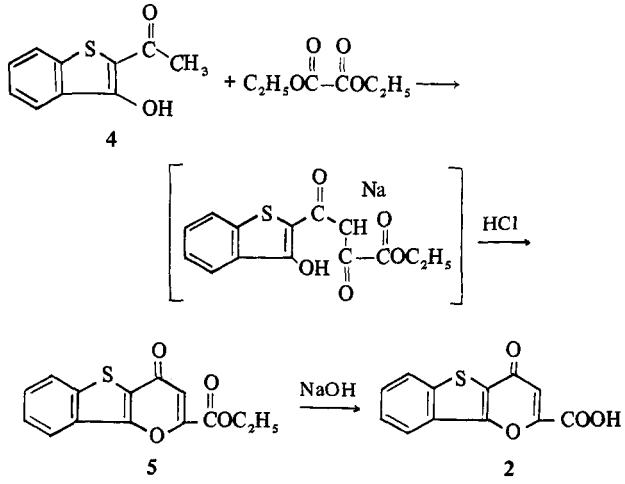
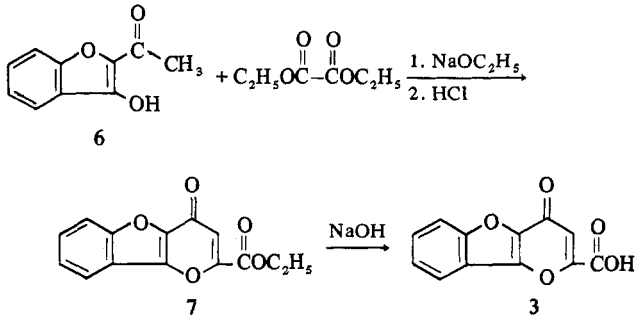
Chemistry. The only reference in the literature to the synthesis of 4-oxo-4*H*-[1]benzothieno[3,2-*b*]pyrans is that of Mustafa[3] who prepared 2-aryl derivatives by treatment of 3-hydroxybenzothienyl-2-methyl ketone[4] (**4**) with aromatic aldehydes followed by treatment of the resulting chalcone with selenium dioxide. We found that the desired 4-oxo-4*H*-[1]benzothieno[3,2-*b*]pyran-2-carboxylic acid (**2**) could be obtained readily in good yield from 3-hydroxybenzothienyl-2-methyl ketone[4] (**4**) by treatment with diethyl oxalate in the presence of sodium ethoxide to give the corresponding ester **5** (obtained in 71% yield) which, upon hydrolysis, gave the desired acid **2**.



4 **5** **2**

A review of the literature disclosed no examples of the related 4-oxo-4*H*-[1]benzofuro[3,2-*b*]pyran heterocyclic system. We found that the desired 4-oxo-4*H*-[1]benzofuro-[3,2-*b*]pyran-2-carboxylic acid (**3**) could be obtained in an analogous way from 3-hydroxy-2-benzofuranyl methyl ketone[5] (**6**).



6 **7** **3**

Biological Methods and Results. (A) Rats. **2** and **3** were tested in rats for their ability to inhibit the passive cutaneous anaphylaxis (PCA) reaction in animals passively sensitized to egg albumin as follows.[6] Rat homocytotropic antibody was elicited to egg albumin (EA) by the injection of 0.5 mg of EA + 0.5 cc of *H. pertussis* vaccine per rat. After 18–20 days the serum was collected and frozen until use. The antibody was shown to be of the 72-hr latency, heat labile type Five 0.1-ml vol of an appropriate dilution of this serum were inoculated into the shaved dorsal surface of a 200-g Sprague-Dawley rat. Saline controls were run also. After 72 hr the rat was challenged iv with 4 mg of EA + 0.5% Evans blue dye. In the case of drug-treated animals the materials were given iv at the time of antigen challenge or the materials were given ip 30 min before challenge with antigen. Results were reported as the inhibition of the number of spots per animal (regardless of size) that were seen at five dilutions of serum. The number of spots from a number of sensitization sites in drug-treated animals was compared with the spot score (number of total spots divided by the number of animals) obtained from the same number of sites in untreated animals. The per cent inhibition of the PCA reaction was then calculated.

The significance of the difference between treated and control in this PCA test has been analyzed and found to be significant with a P value of <0.001. The procedure is to find the highest dilution of the controls for which some of the animals do not have spots and some of the animals have spots. The number of animals not having spots at the next higher dilution of serum is counted and added to past control data. A new average is taken. We then graphed the probability of not getting a spot at this dilution *vs.* the sam-

Table I

Compd no.	Rat PCA inhibitory dose 50, mg/kg	Monkey PK ID_{50}, mg/kg	Monkey ACA ID_{50}, mg/kg
2	5.0	0.085	0.0028
3	4.0	0.950	
Disodium cromoglycate	2.5	0.270	0.05

ple size and got a probability of about 0.1. This probability $p = (0.1)^3$ or N for the number of animals gives a degree of certainty of assuming there is a difference in control and treated. This was done for disodium cromoglycate and the P value was <0.001. Both compounds **2** and **3** were approximately half as active as disodium cromoglycate in this PCA test (Table I).

(B) Primates. One of the compounds **(2)** was chosen for further study in the Rhesus monkey (*Macaca mulatta*) for its ability to inhibit two reactions: (a) the Prausnitz-Kustner (PK) reaction using serum from humans, sensitive to ragweed, to sensitize the skin of the animal followed by a challenge id with antigen E, and (b) the active cutaneous anaphylaxis (ACA) reaction using id extracts of hog *Ascaris suum*. **3** was tested only in the PK reaction.

PK. Human serum obtained from subjects with skin reactivities of 4+ or greater was used. Monkeys were given 4 0.1-ml id injections of 1:10–1:40 dilutions of serum. Forty-eight hours later the id site was challenged with 0.1-ml vol of dilutions of antigen E from ragweed. In the cases of drug additions, compounds were made up in appropriate solvents and added to these dilutions of antigen E. The drug did not constitute more than 10% of the volume of the antigen. The sites of mediator release were visualized by iv bluing of the animal under "Sernylan" tranquilization with 2 ml of 2% "Coomaassie Blue" made up in 0.85% saline.

ACA. A water 1:20 extract of *Ascaris suum* was obtained from R. Patterson (Northwestern University). This material was further diluted (10^{-3}–10^{-7}) and injected id into sensitive primates with or without drugs in 0.1 ml of saline into the monkey shaved dorsal area. The areas of bluing were measured after 30 min, and the amounts of inhibition were calculated by comparing the diameter of the drug-treated site with control site diameters on each animal. Dose-response curves were constructed for disodium cromoglycate and for each compound and I_{50} determinations were read off the dose-response curves.

In the former test (PK reaction) **2** was approximately 3.2 times as active as disodium cromoglycate and in the latter test (ACA) it was approximately 18 times as active (Table I). **3** was less active than disodium cromoglycate in the PK reaction.

Experimental Section

Ethyl 4-Oxo-4*H*-[1]benzothieno[3,2-*b*]pyran-2-carboxylate (5). To a stirred solution of 19.32 g (0.825 mol) of sodium in 2 l. of anhydrous ethanol was added 57.6 g (0.3 mol) of 3-hydroxy-2-benzothienyl methyl ketone[4] and then, dropwise over the course of about 5 min, 120.3 g (0.825 mol) of diethyl oxalate. The mixture was heated under reflux for 15 hr and allowed to cool, and the red solid was removed by filtration.

The precipitate was added to a mixture of 360 ml of ethanol and 88.5 ml of concentrated hydrochloric acid, and the resulting mixture was heated under reflux for 30 min and filtered hot. From the ethanolic filtrate on cooling there was obtained 51.3 g of material. Additional material could be obtained by dissolving the hot ethanolic insoluable material, obtained above, in water and extracting the mixture with methylene chloride. Evaporation of the solvent gave 6.85 g of material. The total weight of the product was thus 58.15 g (71%), mp 152–154°.

A sample was purified for analysis by dissolving it in a 1:1 benzene–ether mixture and extracting the resulting solution with dilute sodium bicarbonate solution and then water. The solvent was removed by distillation and the residue was recrystallized from ethanol. There were obtained green flat prisms melting at 158–159°; ir (Nujol) 1725 cm^{-1} (C=O); $\delta_{DMSO\text{-}d_6}$ (TMS) 1.48 (t, 3, CH_3), 4.48 (q, 2, CH_2), 7.03 (s, 1, CH=C), and 7.50–8.30 (m, 4, aryl H); λ max (CH_2Cl_2) 237 (21,600), 251 (17,400), 259 (13,800), 291 (13,900), 301 mμ (13,300). *Anal.* ($C_{14}H_{10}SO_4$) C, H, S.

4-Oxo-4*H*-[1]benzothieno[3,2-*b*]pyran-2-carboxylic Acid (2). A mixture of 44.1 g (0.162 mol) of ethyl 4-oxo-4*H*-[1]benzothieno-[3,2-*b*]pyran-2-carboxylate **(5)** and 650 ml of a 1% sodium hydroxide solution (0.162 mol) was stirred for 30 min. The mixture was extracted with methylene chloride. Evaporation of the solvent gave 2.70 g of unreacted ester. The aqueous extract was acidified with concentrated hydrochloric acid and the resulting precipitate was removed by filtration. There was obtained 35.2 g (100%, based on ester not recovered) of material melting at 257° dec. A sample recrystallized from a large volume of ethanol gave golden brown prisms melting at 267° dec after darkening at about 253°: ir (Nujol) 2440 (COOH), 1725 cm^{-1} (C=O); $\delta_{DMSO\text{-}d_6}$ (TMS) 7.1 (s, 1, CH=C), 7.5–8.5 (m, 4 H, aryl H); λ max (DMF) 291 (14,000), 312 (6450), and 325 mμ (700); mass spectrum, found *m/e* 246. *Anal.* ($C_{12}H_6SO_4$) C, H, S.

Ethyl 4-Oxo-4*H*-[1]benzofuro[3,2-*b*]pyran-2-carboxylate (7). To a solution of 11.5 g (0.498 g-atom) of sodium in 500 ml of anhydrous ethanol was added 29.5 g (0.166 mol) of 3-hydroxy-2-benzofuranyl methyl ketone.[5] To the stirred mixture was then added, dropwise over the course of 5 min, 73.5 g (0.498 mol) of diethyl oxalate. The mixture was heated under reflux for 17 hr. A large excess of anhydrous ether was added. The precipitate was removed by filtration and added to a mixture of 40 ml of concentrated hydrochloric acid and 200 ml of ethanol. The mixture was heated under reflux for 1 hr and allowed to cool; the precipitate was removed by filtration and the filtrate evaporated to dryness. The solid and the residue were combined and dissolved in methylene chloride and the resulting solution was extracted with a sodium bicarbonate solution and then with water. The methylene chloride was removed by distillation, and the residue was recrystallized from ethanol. There was obtained 6.78 g of material melting at 134–135°. Additional recrystallization from ethanol gave brown needles melting at 135–136°; ir (Nujol) 1745, 1655 [-C(=O)-], 1625 (sh), 1590, 1565, 1495 cm^{-1} (C=C); δ_{CDCl_3} (TMS) 1.52 (t, 3, CH_3), 4.58 (q, 2, CH_2), 7.48–8.18 (m, 5, aromatic H). *Anal.* ($C_{14}H_{10}O_5$), C, H.

4-Oxo-4*H*-[1]benzofuro[3,2-*b*]pyran-2-carboxylic Acid (3). A mixture of 1.26 g (0.0049 mol) of ethyl 4-oxo-4*H*-[1]benzofuro-[3,2-*b*]pyran-2-carboxylate **(7)** and 20 ml of a 1% sodium hydroxide solution was stirred for 30 min. The solution was extracted with ether and the aqueous layer was acidified by the addition of dilute hydrochloric acid. The resulting precipitate was removed by filtration. There was obtained 1.16 g of material melting at 291° dec. Recrystallization from a large volume of ethanol gave material melting at 289° dec; ir (Nujol) 2900, 2720, 2580, 2540, 2430 (acid OH), 1740 (C=O), 1620, 1575, 1555, 1495 cm^{-1} (C=O/C=C); $\delta_{DMSO\text{-}d_6}$ (TMS) 7.2 (s, 1, CH=C); λ max (dioxane) 215 (sh, 21,900), 218 (22,250), 294 (15,750), 299 mμ (16,100). *Anal.* ($C_{12}H_6O_5$) C, H.

Acknowledgments. We are indebted to Fisons Pharmaceuticals, Ltd., for a generous sample of disodium cromoglycate. We are indebted also to Mr. Norman Knight and his coworkers of these laboratories for microanalytical data and to Mr. Paul Meulman for infrared data. We are especially indebted to Miss Christine VanHout and to Mr. Nelson Major for excellent technical assistance.

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