Synthesis and Antiallergic Activity of Some Mono- and Disubstituted Xanthone-2-carboxylic Acids¹

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A series of xanthone-2-carboxylic acids, substituted mainly with electron-withdrawing groups, has been synthesized and assayed for antiallergic activity, using the passive cutaneous anaphylaxis (PCA) reaction in the rat. The effect of substituent type and substitution pattern on PCA neutralizing capacity is presented.

Khellin,² a naturally occurring oxygen heterocycle, has served as a basis for the development of the antiasthma drug disodium cromoglycate.^{3,4} Recent investigations in this field have focused on the chemistry and pharmacology of a variety of other chromones⁵ as well as xanthones⁶ and annellated γ -pyrones.⁷

Continuing our work on xanthone-2-carboxylic acids,⁸ we have prepared a number of compounds with increased potency in the PCA assay by selecting substituents with mainly -I/-E character and determining the influence of size, shape, and substitution pattern of these groups on antiallergic activity.

7-Methylsulfinylxanthone-2-carboxylic acid (11a; tixanox, USAN) has been shown to be orally active against exercise-induced asthma in man.⁹

Chemistry. Since xanthone-2-carboxylic acid (1) cannot be acylated directly under Friedel–Crafts conditions, it was first converted into xanthene-2-carboxylic acid (2) by a Huang-Minlon reduction and then esterified with Li_2CO_3 -MeI in DMF. Reaction of the resulting methyl ester **3** with an acyl halide and AlCl₃ in 1,2-dichloroethane, followed by oxidation with Jones reagent in DMF and base hydrolysis, provided the desired 7-acylxanthone-2carboxylic acids **4a-e** (Scheme I).

Introduction of a thio function into the 7 position of the xanthone nucleus was achieved through thermal rearrangement¹⁰ of the dimethylthiocarbamate 6 derived from the hydroxy ester 5 (Scheme II). Base hydrolysis of the resulting S-aryl derivative 7 provided the mercapto acid 8.

Alkylation of 8 with MeI or *i*-PrBr in the presence of K_2CO_3 gave the alkylthio esters 9a,b, which were converted into the corresponding carboxylic acids 10a,b by saponification with NaOH. Oxidation of 9a and 9b with *m*-chloroperoxybenzoic acid, followed by base hydrolysis, furnished the alkylsulfinyl derivatives 11a,b. The sulfone 12 was obtained by oxidation of 10a with H_2O_2 in refluxing AcOH.

The disubstituted xanthone-2-carboxylic acids 15a-gwere prepared by coupling¹¹ the required phenols with dimethyl 4-bromoisophthalate in the presence of Cu₂O, hydrolyzing the ester groups of the intermediate diphenyl ethers 13a-f, and cyclizing the resulting dicarboxylic acids 14a-f with PPA (Scheme III). Starting from 3,4-dimethylphenol, isomers 15e and 15f were obtained as a mixture which was esterified and separated by column chromatography. Structures were readily assigned on the basis of the ¹H NMR spectra (see the Experimental Section).

O-Demethylation of 15g with HI-AcOH (\rightarrow 16) followed by esterification with Li₂CO₃-MeI in DMF provided the phenolic intermediate 17, which was converted into the desired 5-alkoxy-7-methylsulfinylxanthone-2-carboxylic acids 18a-h by alkylation, oxidation, and saponification (Scheme IV).

Discussion and Conclusions

As has already been noted earlier,⁸ introduction of a





Scheme II



substitutent only into the 5 or 7 position of the xanthone nucleus leads to compounds with increased PCA activity.

 $CH_3CO C_2H_5CO$

i-C,H,S

CH,SO

CH₃SO

i-C,H,SO

 $5,7 \cdot (CH_{3}O)_{2}$

6.7-(CH₃O)₂

6,8-(CH₃O)₂

5,7-(CH₃)₂

6.7 (CH₃)₂

7,8-(CH₃)₂ 5-C₂H₅O-7-CH₃SO

5-n-C,H,SO

5-i-C₃H₇O-7-CH₃SO

 $5-n-C_{4}H_{9}O-7-CH_{3}SO$ $5-n-C_{5}H_{11}O-7-CH_{3}SO$

Table I. Rat Passive Cutaneous Anaphylaxis Assay of Xanthone-2-carboxylic Acids^a

>300

>350

>320

>300

>300

365

335

363-365

306-309

273-274

265-267

280-282

269-270

263-265



 $C_{15}H_{10}O_{5}S$

 $C_{17}H_{14}O_5S$

 $C_{15}H_{10}O_{0}$

C16 H12 O6

C16 H12 O6

 $C_{16}H_{12}O$

7H14O

 $C_{18}H_{16}O_6S$

 H_{20}

 $_{18}H_{16}O_{6}S$

 $H_{18}O_6S$

O₆S

C 6H12O

C $_{16}H_{12}O_{4}$

C

C

C

H12O6

 68^i

63ⁱ

 70^{i}

54

61^j

 57^{j}

 48^{j}

56^{j,l}

 $56^{j,l}$

 82^m

 75^m

 71^m

 68^m

 71^{m}

	100		200 200	O_{20} 1120 $O_{6}O$	1 -	100	00		
	18 f	5-i-C,H, O-7-CH,SO	271 - 273	$C_{20}H_{20}O_{s}S$	75^{m}	94	100		
	18g	5-C,H _a Ö-7-CH _a SO ^h	274 - 276	$C_{20}H_{10}O_{0}S$	81^m	59	100		
	18ĥ	5- <i>n-</i> C ₈ H ₁₇ O-7-CH ₃ SO	258 - 260	$C_{23}H_{26}O_{6}S$	74^m	35	35		
^a All compounds exhibited IR and 'H NMR spectra consistent with the assigned structures. ^b All single substituents at									
C-7. ^c All compounds were analyzed for C and H. ^d Total number of rats receiving the test material. ^e Potency of the									
ç	compounds in the rat passive cutaneous anaphylaxis assay, iv (see Experimental Section); disodium cromoglycate = 1.								
f	^f Yield based on 3. ^g C_3H_5 is cyclopropyl. ^h C_5H_5 is cyclopentyl. ⁱ Yield based on 8. ^j Yield based on dimethyl 4-bromo-								
i	sophthalate. k	C: calcd, 71.63; found, 72.0)8. ¹ Yield of mix	ture of 15e and 15t	f. ^m Yield b	based on 17.			

Scheme III

Compd

4a

4b

4c

4d

4e

10a

10b

11a

11b

12

15a

15b

15c

15d

15e

15f

18a

18b

18c

18d

18e



Additionally, a wide range of lipophilic-hydrophilic character is tolerated in the substituent, whereas there seems to exist an optimum regarding its size. In contrast to the compounds containing mostly electron-releasing groups like alkyl and alkoxy, described before,⁸ we have now focused our attention on substituents with electron-withdrawing properties as well as combinations of two substituents.

The results of the PCA assay¹² in the 7-acyl series (Table I) show maximum activity for the acetyl compound 4a. Although increased chain length causes a reduction of activity (see 4b vs. 4a), branching of the side chain at the α -carbon is generally consistent with maintaining some PCA activity, an effect which has also been observed in the 7-alkyl and 7-alkoxy series.8 Therefore, one can find only a limited correlation between potency and parameters characterizing only substituent bulk like molar refraction¹³ or the steric substituent constant $E_{\rm s}$.¹⁴

A group of compounds carrying sulfur-containing substituents with widely dissimilar electronic as well as hydrophobic character was examined next (Table I). In contrast to the alkoxy series, the methylthio and methylsulfinyl analogues 10a and 11a display higher PCA activity than their isopropyl counterparts 10b and 11b, which may be a reflection of the larger bulk of the sulfur atom compared to oxygen. A rather unexpected enhancement of activity is achieved by introduction of the hydrophilic methylsulfinyl and methylsulfonyl groups (compounds 11a and 12), which are both characterized by strongly negative π values.¹³ Interestingly, MeS, MeSO, and MeSO₂ are groups of very similar size (molar refraction $MR = 13.28, 13.70, and 13.49, respectively^{13}$).

In an attempt to further define the structural requirements for increased PCA activity observed so far in this series, we were interested in determining how this activity would be influenced by introduction of two substituents. In particular, we were hoping to find an additive effect with groups occupying both the 5 and 7

25

4

7 6

2 ~0.1

1

0.2

0.2

80

50

190

260

90

54

15

33

15

15

49

39

40

59

59

69

60

108

4

Scheme IV



positions, monosubstitution of which has already been shown to lead to active compounds.

Again, using the methoxy group as a probe,⁸ three different dimethoxy analogues were examined (Table I). While the 6,8 isomer 15c was found to be considerably less active than either of the corresponding monosubstituted compounds, an enhancement of activity was observed on moving one methoxy group from the 8 to the 7 position (15b). In the case of the 5,7-substitution pattern, as represented by 15a, PCA activity increased by a factor of 3 relative to 15b. A rather similar correlation is evident in the dimethyl series (Table I), where the 5,7 combination (15d) is superior to 6,7-Me₂ (15e) or 7,8-Me₂ (15f).

In order to determine whether it would be possible to increase PCA activity by combining a hydrophilic substituent at C-7 with a lipophilic group at C-5, a number of 5-alkoxy-7-methylsulfinyl derivatives were prepared (Table I). Surprisingly high activities were observed in this case, with a maximum potency of 260 times disodium cromoglycate elicited by the butoxy compound 18d. Even the lipophilic *n*-octyloxy analogue 18h still surpasses the unsubstituted 7-sulfoxide 11a. Apparently, the stringent requirements as to bulk and shape of a given substituent, noted previously, do not dominate this particular series and can be discerned only when comparing *n*-propoxy with isopropoxy groups (compounds 18b and 18c).

Experimental Section

Melting points are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within $\pm 0.4\%$ of theoretical values. All compounds exhibited IR and ¹H NMR spectra consistent with the assigned structures. Thin-layer chromatograms were eluted with a benzene-THF-AcOH (30:3:1) mixture. For filtrations, "Woelm" alumina (activity II, neutral) was used.

Xanthene-2-carboxylic Acid (2). Huang-Minlon reduction¹⁵ (hydrazine, KOH, and triethylene glycol) of xanthone-2-carboxylic acid¹⁶ (1) provided the desired compound 2 in 85% yield: mp 257–258 °C (THF–EtOH). Anal. ($C_{14}H_{10}O_3$) C, H.

Methyl Xanthene-2-carboxylate (3). A mixture of 43.5 g (0.192 mol) of 2, 35 g (0.47 mol) of Li_2CO_3 , 30 mL (0.485 mol) of MeI, and 500 mL of DMF was stirred for 16 h at room temperature. AcOH-H₂O (400 mL, 1:1) containing 10 g of NaHSO₃ was added in small portions, followed by dilution with 2 L of H₂O. Ester 3 was filtered off, washed with H₂O, and dried: 41.2 g (89.5%); mp 131-133 °C. Anal. (C₁₅H₁₂O₃) C, H.

7-Acylxanthone-2-carboxylic Acids 4a-e. A solution of 3.0 g (12.5 mmol) of 3, 5.0 g (37.5 mmol) of AlCl₃, and 15.0 mmol of the corresponding acyl chloride in 60 mL of ClCH₂CH₂Cl was stirred at room temperature for 3 h and then poured on ice-HCl. Extraction with CH₂Cl₂, followed by filtration of the dried $(MgSO_4)$ extracts through alumina and crystallization from CH₂Cl₂-MeOH, provided the desired 7-acylxanthene-2-carboxylates, which were dissolved in 100 mL of DMF and treated with excess Jones reagent (8 N CrO₃ in 8 N H₂SO₄) until TLC indicated the absence of starting material. Water (400 mL) was then added, and the resulting precipitate was filtered off and washed thoroughly with warm water. These esters were refluxed for 30 min with 600 mg (15.0 mmol) of NaOH in 100 mL of 80% aqueous EtOH. After acidification with 2 N HCl, the 7-acylxanthone-2-carboxylic acids 4a-e (Table I) were isolated by suction filtration and recrystallized from DMF-AcOH.

Methyl 7-Hydroxyxanthone-2-carboxylate (5). Esterification of 7-hydroxyxanthone-2-carboxylic acid⁸ with Li₂CO₃-MeI in DMF as described for $2 \rightarrow 3$ gave a 91.3% yield of 5, mp 280-282 °C (AcOH). Anal. (C₁₅H₁₀O₅) C, H.

Methyl 7-Dimethylthiocarbamoyloxyxanthone-2carboxylate (6). Treatment of 5 with dimethylthiocarbamoyl chloride and NaH in DMF as described¹⁰ provided the O-arylthiocarbamate 6 in 82% yield: mp 236-238 °C (CHCl₃-AcOEt). Anal. ($C_{18}H_{15}NO_5S$) C, H.

Methyl 7-(Dimethylcarbamoylthio)xanthone-2-carboxylate (7). A solution of 8.0 g (22.4 mmol) of 6 in 150 mL of sulfolane was stirred and heated at 230–240 °C under N₂ for 6 h, during which time a single new compound (more polar on TLC) had been formed. After cooling to 80 °C, 600 mL of water was added, and the resulting precipitate was filtered off, washed with water, dried, and recrystallized from CHCl₃-EtOH to give 7.5 g (87.5%) of 7, mp 237–238 °C. Anal. ($C_{18}H_{15}NO_{5}S$) C, H, S.

7-Mercaptoxanthone-2-carboxylic Acid (8). 7 (7.5 g, 21.0 mmol) was refluxed under N₂ with 10 g (0.18 mol) of KOH in 250 mL of 80% aqueous EtOH for 4 h. Water (250 mL) and charcoal (2.0 g) were added, and refluxing was continued for 15 min. After filtration through Celite, the filtrate was acidified with excess 2 N HCl to give 5.3 g (93%) of the thiol acid 8, mp >400 °C. Anal. (C₁₄H₈O₄S) C, H, S.

Alkyl 7-(Alkylthio)xanthone-2-carboxylates 9a,b. Treatment of 1.36 g (5.0 mmol) of 8 with 3.45 g (25.0 mmol) of K_2CO_3 and 25.0 mmol of MeI (or *i*-PrBr) in 40 mL of DMF for 20 h at room temperature, followed by dilution with 2 N HCl, extraction with CH₂Cl₂, and filtration of the dried extracts through a short column of alumina, provided the esters 9a and 9b in nearly quantitative yields.

7-(Alkylthio)xanthone-2-carboxylic Acids 10a,b (Table I). Refluxing 10.0 mmol of 9a or 9b with 600 mg (15.0 mmol) of NaOH in 75 mL of 80% aqueous EtOH for 30 min, followed by acidification with 2 N HCl, gave the acids 10a and 10b, which were recrystallized from DMF-AcOH.

7-Alkylsulfinylxanthone-2-carboxylic Acids 11a,b (Table I). To a solution of 5.0 mmol of 9a or 9b in 50 mL of CH_2Cl_2 , cooled to 0 °C, a solution of 1.06 g (5.25 mmol) of 85% m-chloroperoxybenzoic acid in 50 mL of CH_2Cl_2 was added dropwise with stirring. The reaction mixture was washed with 2 N NaHCO₃ and water. Evaporation of the dried CH_2Cl_2 solution gave a crude ester which was directly saponified with NaOH as described for $9 \rightarrow 10$ to provide acids 11a and 11b. In this case, aqueous DMF was used to recrystallize the crude products.

7-Methylsulfonylxanthone-2-carboxylic Acid (12) (Table I). A mixture of 1.62 g (5.66 mmol) of 10a, 6.42 mL (56.6 mmol) of 30% H₂O₂, and 65 mL of AcOH was refluxed for 90 min. The

hot solution was diluted with 75 mL of H_2O and cooled and the resulting precipitate isolated by suction filtration. An analytical sample was prepared by recrystallization from AcOH.

Dimethyl 4-Phenoxyisophthalates 13a–f. A mixture of 12.0 g (44.0 mmol) of dimethyl 4-bromoisophthalate,¹⁷ 50.0 mmol of the desired disubstituted phenol, 3.46 g (24.2 mmol) of Cu₂O, and 200 mL of DMA was refluxed under N₂ for 16 h. The cooled reaction mixture was poured into 1.5 L of H₂O containing 100 mL of 2 N HCl and extracted with ether. The organic phase was washed five times with H₂O, twice with 2 N NaOH, and again with H₂O. The dried ether solution was evaporated and the residue dissolved in CH₂Cl₂ and filtered through alumina. The crude, oily esters obtained in this manner contained dimethyl isophthalate (GLC; formed by reductive debromination) as the only major impurity. They were not purified or characterized further.

4-Phenoxyisophthalic Acids 14a–f. A mixture of 30.0 mmol of one of the foregoing diesters (13a–f), 3.6 g (90.0 mmol) of NaOH, and 300 mL of 80% aqueous EtOH was refluxed for 30 min. The resulting clear solution was acidified with 2 N HCl, diluted with 200 mL of H₂O, and concentrated on a rotary evaporator. In some instances, crystalline diacids, contaminated with isophthalic acid (TLC), were obtained. Oily reaction products were extracted with AcOEt. To avoid any loss of material at this stage, no further attempt to purify these compounds was made.

Xanthone-2-carboxylic Acids 15a-g (Table I). The desired dicarboxylic acid 14a-f (25.0 mmol) was dissolved in 150 mL of sulfolane with overhead stirring at 90 °C on a steam bath. After adding 100 g of PPA, the reaction mixture was stirred at 90 °C for 1 h and then slowly diluted with 450 mL of H_2O . The resulting precipitate was filtered off and washed with 50% aqueous EtOH. The dried, crude products were recrystallized from DMF-AcOH.

Cyclization of 14e in this manner gave a 1:1 mixture of the two isomers 15e and 15f, which was esterified (MeI–Li₂CO₃) and then separated by chromatography on silica gel (benzene). The methyl ester of 15e showed mp 202–204 °C (THF–EtOH): NMR (CDCl₃; δ , ppm) 2.32 (s, 3 H, Me-7), 2.35 (s, 3 H, Me-6), 7.18 (s, 1 H, H-5), 7.95 (s, 1 H, H-8). The Me ester of isomer 15f had mp 164–166 °C (THF–EtOH): NMR (CDCl₃) 2.32 (s, 3 H, Me-7), 2.78 (s, 3 H, Me-8), 7.15 (d, J = 9 Hz, 1 H, H-5), 7.43 (d, J = 9 Hz, 1 H, H-6). Both esters were converted back into the corresponding acids by hydrolysis with NaOH (80% aqueous EtOH, 30 min of reflux). Starting with 2-methoxy-4-(methylthio)phenol,¹⁸ 15g was obtained in 46% yield based on dimethyl 4-bromoisophthalate: mp 304–305 °C (DMF–AcOH). Anal. (C₁₆H₁₂O₅S) C, H, S.

5-Hydroxy-7-(methylthio)xanthone-2-carboxylic Acid (16). HI (116 mL, 47%) was added dropwise to 380 mL of ice-cooled Ac₂O with vigorous stirring. After adding 17.0 g (53.8 mmol) of 15g, the resulting suspension was refluxed for 20 h. The hot reaction mixture was diluted with 400 mL of H₂O and cooled and the product (16) isolated by suction filtration: 14.3 g (88%); mp 364-366 °C dec. Anal. ($C_{15}H_{10}O_5S$) C, H, S.

Methyl 5-Hydroxy-7-(methylthio)xanthone-2-carboxylate (17). Esterification of 13.8 g (45.7 mmol) of 16 with MeI-Li₂CO₃ in DMF as for $2 \rightarrow 3$ gave 12.6 g (87%) of 17, mp 272-274 °C (AcOH). Anal. (C₁₆H₁₂O₅S) C, H, S.

5-Alkoxy-7-methylsulfinylxanthone-2-carboxylic Acids 18a-h (Table I). Alkylation of 17 with an alkyl halide, followed by oxidation of the MeS group with *m*-chloroperoxybenzoic acid and base-catalyzed hydrolysis of the ester moiety (cf. $8 \rightarrow 9 \rightarrow$ 11) furnished the desired compounds, which were recrystallized from THF-EtOH.

Biological Assay. Female Sprague–Dawley derived albino rats (Simonsen Labs., Gilroy, Calif.) weighing 140–160 g were used. Rat reaginic antibody was produced using the method of Mota.¹⁹ The rats were immunized with 1.25 mg of egg albumin (General Biochemicals, twice crystallized and salt-free) sc and 1.0 mL of *Hemophilus pertussis* vaccine (Eli Lilly and Co.) ip. Eleven days later the rats were bled and the serum obtained was pooled and

frozen. This serum met the requirements for reaginic antiserum; namely, it was heat labile (1 h at 56 °C) and could elicit a passive cutaneous anaphylaxis (PCA) reaction 75 h after intradermal injection. The PCA test was based on the method of Goose and Blair.¹² The rats were passively sensitized with two dilutions (right and left flanks) of the antiserum given intradermally in 0.1 mL of normal saline. These rats were challenged 24 h later iv with 1.0 mL of buffered saline (pH 7.2) containing 1.0 mg of albumin, 0.5% Evans blue dye, and the compound to be evaluated. The rats were sacrificed 15-25 min after challenge, at which time the abdominal skin was reflected and the diameter of the dermal bluing (mean of two perpendicular axes) was determined. The relative potencies of the test materials were determined graphically, using dose-response curves obtained from semilog plots of the data, expressed as percent inhibition of the PCA response relative to challenge animals not receiving drug. One or more tests were performed, comparing each test agent simultaneously with cromolyn sodium. Usually three doses of each compound were used (fivefold increments) in each test, each dosage group usually having at least five rats.

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References and Notes

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