Pharmacologically Active Sulfoximides: 5-Hexyl-7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic Acid, a Potent Antiallergic Agent

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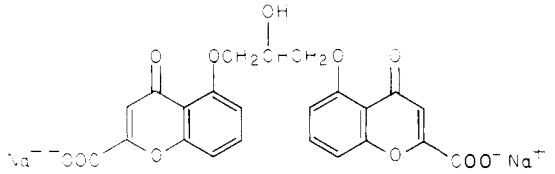
The antiallergic activity of some xanthone derivatives containing a sulfoximide substituent has been investigated. While 2-(*S*-methylsulfonimidoyl)xanthone itself was found to be inactive, a series of 7-(*S*-methylsulfonimidoyl)-xanthone-2-carboxylic acids showed good levels of activity in the passive cutaneous anaphylaxis screen. N-Substituted sulfoximide derivatives were, without exception, less active than the corresponding unsubstituted compounds. The activity of the 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic acids could be enhanced by the introduction of an alkyl or alkoxy substituent at C-5. As a result of these studies, 5-hexyl-7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic acid has been selected for further investigation as an antiasthmatic agent.

The sulfoximide moiety[1] has until recent years received little attention as a potential synthon in medicinal chemistry, although recent examples[2] have shown that structures containing this grouping can have pronounced biological activities. As part of a program aimed at discovering new series of therapeutically active compounds, we have investigated the capacity of the sulfoximide group to modify the activity of parent structures with established activity. Our investigations were intended, firstly, to establish whether the sulfoximide group could act as a biological isostere for the carboxylic acid group (or its equivalents) in known classes of therapeutic agents, and, secondly, to determine the scope for modification of the biological activity of compounds by substitution with a sulfoximide group, which may then undergo further derivatization.

The antiasthmatic drug disodium cromoglycate (DSCG, 1)[3] is thought to act by inhibition of the release of me-



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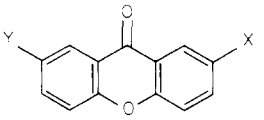
diators of the allergic reaction following antigen–antibody interaction but suffers from the major disadvantage of being ineffective orally. For our investigations in this area, we chose to investigate the xanthone-2-carboxylic acid system (**2**) which was reported[4] in 1972 to possess



2, X = –COOH; Y = H
3, X = –COOH; Y = –SO(=NH)Me
4a, X = –SO(=NH)Me; Y = H
4b, X = –SO(=NH)Me·HCl; Y = H
5, X = –SMe; Y = H
6, X = –SOMe; Y = H

DSCG-like activity on oral administration. Pfister et al.[4] had shown that, while xanthone-2-carboxylic acid (**2**) was only half as active as disodium cromoglycate when administered intravenously in the standard passive cutaneous anaphylaxis test,[5] the introduction of certain alkyl or alkoxy substituents into positions 5 or 7 of the xanthone nucleus led to compounds several times more active than cromoglycate. It was our hope that compounds such as **3** containing a sulfoxamide substituent might also show enhanced activity.

Chemistry. The first part of our investigation entailed the synthesis of the simplest xanthone substituted in the 2 position with a sulfoximide group, namely, 2-(*S*-methylsulfonimidoyl)xanthone (**4a**). Oxidation of 2-(methylthio)xanthone (**5**)[6] with sodium metaperiodate gave the corresponding sulfoxide **6**, which was treated with sodium azide in polyphosphoric acid to give the required sulfoximide **4a**. This was converted to the water-soluble hydrochloride **4b** for pharmacological testing.

Two routes of synthesis have been used for the preparation of xanthone-2-carboxylic acids containing a 7-sulfoximide group. In the first route (Scheme I), a *p*-(methylthio)phenol (**7**) was coupled with dimethyl 4-bromoisophthalate (**8**) to give, after hydrolysis, the ether **9**. Cyclization of **9** using polyphosphoric acid gave 7-(methylthio)xanthone-2-carboxylic acid (**10**), the acid group of which was protected as the methyl ester **11** prior to oxidation. This was accomplished with sulfuryl chloride in the presence of wet silica,[7] giving the sulfoxide **12** exclusively. Since the commencement of this work, a similar route has been described in a recent patent[8] for the preparation of 7-(methylsulfinyl)xanthone-2-carboxylic acids, as an alternative to the earlier route[9] for the synthesis of **13**.

Reaction of the sulfoxide **12** with sodium azide in polyphosphoric acid or alternatively with *O*-mesitylenesulfonylhydroxylamine (MSH)[10] gave the corresponding methyl 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (**14**) which was then hydrolyzed to the carboxylic acid **15**.

The second route (Scheme II) which has been developed reverses the order in which the formation of the xanthone and that of the sulfoximide is carried out. The diphenyl ether **9b** was esterified to give the ethyl diester **16**, which on treatment with sulfuryl chloride in the presence of wet silica[7] gave the sulfoxide **17**. Reaction of **17** with sodium azide in polyphosphoric acid at 40 °C gave the sulfoximide **18**. At this point it was convenient not to isolate this material but, instead, to rapidly heat the reaction mixture to 150 °C until cyclization to form the xanthone ethyl ester **14d**, the homologue of methyl ester **14b**, occurred. Furthermore, it was found that in the addition of water to the polyphosphoric acid solution sufficient heat was generated to hydrolyze partially the ester **14d** to the acid **15b**. In practice, the diluted reaction mixture was heated for a short time to ensure hydrolysis was complete. Thus, the diphenyl ether **9b** was converted in three stages to the 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic acid (**15b**) by this method, compared with the five steps required by the former method.

Some N-substituted sulfoximidoxanthonecarboxylic acids, which are listed in Table I, were prepared by reacting

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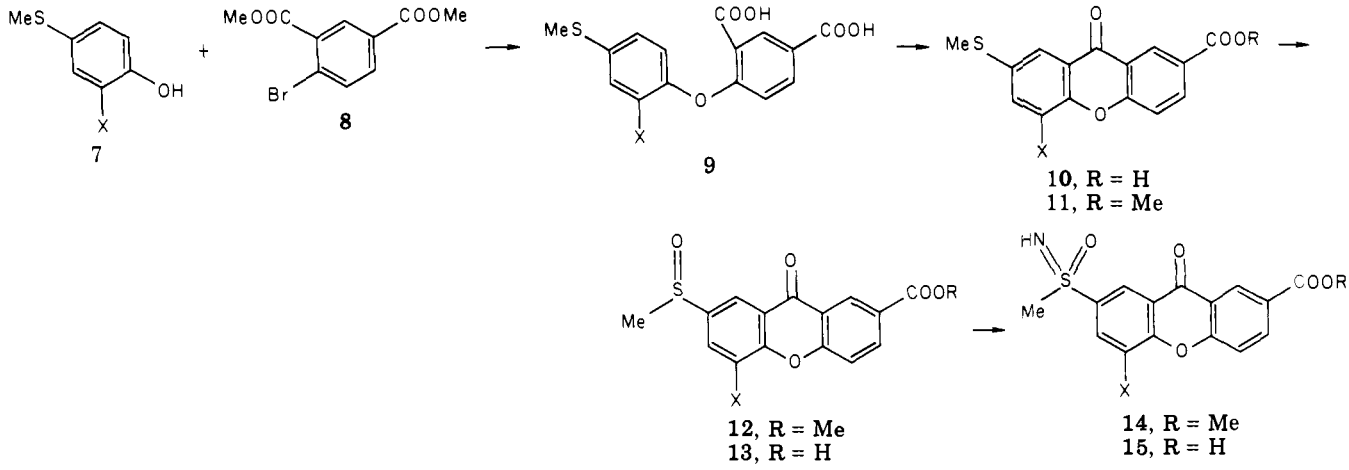
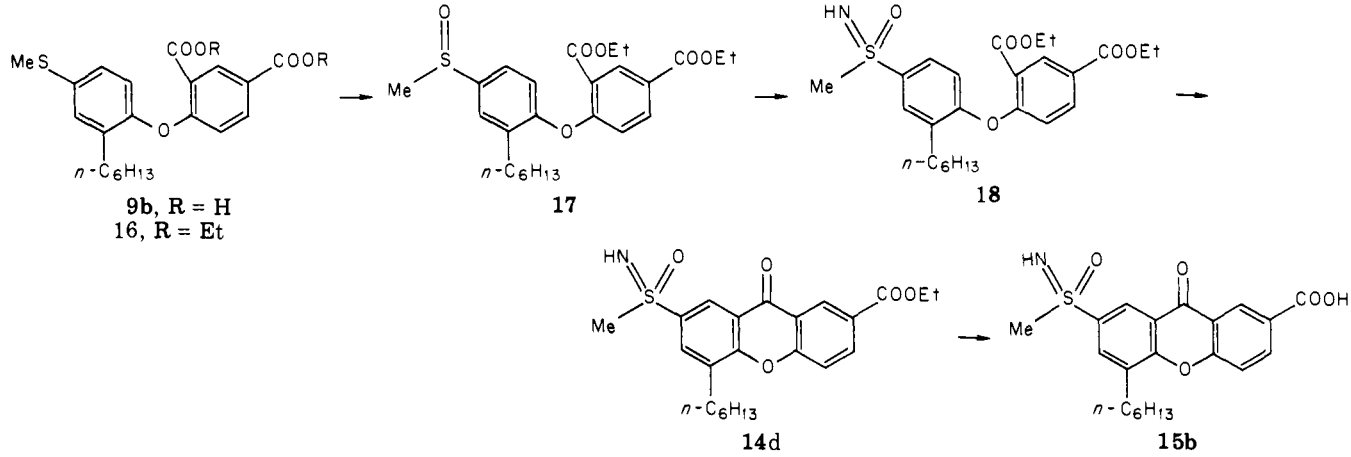
Scheme I

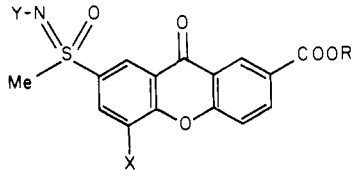


a, X = H; b, X = $-(CH_2)_5Me$; c, X = $-O(CH_2)_4Me$

Scheme II



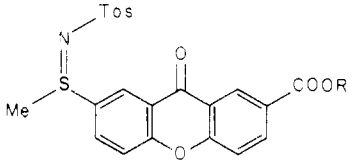
the unsubstituted sulfoximidoxanthone esters **14a** or **b** with an appropriate reagent, followed by mild hydrolysis of the ester group with 1.2 equiv of sodium hydroxide in aqueous ethanol. Thus, the *N*-carbamoyl derivatives **19** and **20** were prepared using sodium cyanate in glacial acetic acid, and the *N*-benzoyl compounds **21** and **22** were synthesized using benzoyl chloride. The *N*-acetyl compound **23** proved more difficult to prepare, since it was necessary to use very mild conditions to hydrolyze the ester **24** without cleaving the *N*-acetyl group. The preparation was carried out successfully using 1.1 equiv of sodium bicarbonate as the hydrolyzing agent. A similar mild hydrolysis was necessary for the conversion of the ester of the ethoxycarbonyl derivative **25**, prepared by reaction of **14a** with ethyl chloroformate, to the corresponding acid **26**. Reaction of **14a** with chloroacetyl chloride led to the *N*-chloroacetyl derivative **27**, which was subsequently converted to the morpholinoacetyl (**28**) and piperidinoacetyl (**29**) compounds by treatment with the appropriate amine. Unfortunately, and perhaps surprisingly, all attempts to hydrolyze the ester group selectively in these compounds led to partial cleavage of the *N*-acyl group.



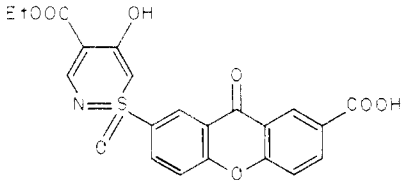
19a, R = Me; X = H; Y = $-CONH_2$
19b, R = Me; X = n-C_6H_{13}; Y = $-CONH_2$
20a, R = H; X = H; Y = $-CONH_2$
20b, R = H; X = n-C_6H_{13}; Y = $-CONH_2$
21, R = Me; X = H; Y = -COPh
22, R = H; X = H; Y = -COPh
23, R = H; X = H; Y = -COMe
24, R = Me; X = H; Y = -COMe
25, R = Me; X = H; Y = -COOEt
26, R = H; X = H; Y = -COOEt
27, R = Me; X = H; Y = $-COCH_2Cl$
28, R = Me; X = H; Y = $-COCH_2$-c-$N(CH_2CH_2)_2O$
29, R = Me; X = H; Y = $-COCH_2$-c-NC_5H_{10}
30, R = Me; X = H; Y = $-SO_2$-C_6H_4-p-Me
31, R = H; X = H; Y = $-SO_2$-C_6H_4-p-Me
35, R = Me; X = H; Y = $-CH{=}C(COOEt)_2$

Reaction of **14a** with *p*-toluenesulfonyl chloride gave the *N*-(*p*-toluenesulfonyl)sulfoximidoxanthone ester **30** which was hydrolyzed in the usual manner to the corresponding carboxylic acid **31**. The comparison of the sulfoximide, in the form of this tosyl derivative, with the sulfimide **32** was of interest. The preparation of **32** was achieved from the reaction between the methylthio compound **11a** and chloramine-T (*N*-chloro-4-methylbenzenesulfonamide, sodium salt) to give the ester **33**, which was then hydrolyzed to **32**.

Finally, a cylic sulfoximide **34** having a thiazine 1-oxide partial structure was prepared. The synthesis[11] involves the reaction of the sulfoximide **14a** with diethyl ethoxymethylenemalonate to give the diethoxycarbonylethylene adduct **35**. Cyclization was affected with sodium hydride in dimethyl sulfoxide when, during the acid workup procedure, the methyl ester in the 2 position of the



32, R = H
33, R = Me



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xanthone was hydrolyzed to the corresponding carboxylic acid, while the ethyl ester in the 4 position of the thiazine ring remained intact.

Biological Methods and Results. The xanthonecarboxylic acids synthesized as above were tested for their ability to inhibit the passive cutaneous anaphylaxis (PCA) reaction in rats passively sensitized to ovalbumen. The method[12] followed established procedures. Donor rats (male CFHB animals weighing 180–200 g) were sensitized with ovalbumen (1 mg) intramuscularly and *Bordatella pertussis* vaccine (2×10^{10} organisms) intraperitoneally. Ten days later the rats were injected with *Nippostrongylus brasiliensis* (4×10^3 larvae) subcutaneously and, after a further 14 days, the serum was collected. The serum contains both IgE and IgG anaphylactic antibodies. In order to manifest the IgE-mediated reaction, rats were injected with 0.1 mL of diluted (1:25) serum into four sites on the shaved back. The animals were challenged intravenously 72-h later (by which time the IgG antibodies are inactive) with ovalbumen (1 mg) together with 1% Evans blue dye (0.5 mL). Thirty-minutes later the animals were killed, and the severity and area of each blue spot, when viewed from the reverse side of the skin, was scored on an arbitrary scale from 0 to 4.

The test compounds were dissolved in Tris buffer and administered in saline solution for intravenous testing or in water for oral testing. Compounds were administered either 30 s before challenge when given intravenously or 10 min before challenge when given orally.

Each compound was tested at three or more dose levels in groups of animals (at least seven), with control groups also containing the same number of animals. The percentage of inhibition of the PCA reaction was calculated from the difference between the scores assigned to the test group and the control group. A dose–response curve was obtained, and from this the dose required to produce 50% inhibition (ED_{50}) was read off. The results are shown in Table II.

2-(*S*-Methylsulfonimidoyl)xanthone (**4a**) was devoid of activity at doses up to 10 mg/kg iv. Since xanthone-2-carboxylic acid (**2**) is known to possess half the activity of DSCG,[4] suggesting an ED_{50} value of 2–3 mg/kg iv in our PCA test, it was quite apparent that the sulfoximide moiety is not a good biological isostere for the carboxylic acid group in this series. Having established this, our attention was turned to the use of the sulfoximide substituent as a means of enhancing activity within the series of xanthone-2-carboxylic acids. The target compound, 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic acid (**15a**) was discovered to be some 30–40 times more potent than DSCG when given intravenously. However, unlike DSCG, the compound exhibited potent oral activity, although by this route the drug was about 50 times less efficient than by the intravenous route. Moreover, introduction of a hexyl or pentyloxy substituent into the C-5 position of the xanthone nucleus produced a sixfold increase in activity measured intravenously, as seen in compounds **15b** and **15c**, respectively. The oral activity of the two sulfoximides **15b** and **15c** was less predictable. While the 5-(pentyloxy) compound **15c** showed no significant improvement upon the parent compound **15a**, the 5-hexyl derivative **15b** was nearly ten times as potent.

Substitution on the sulfoximide nitrogen in all cases resulted in decreased antiallergic activity. Marginal reductions in potency were seen in the ureas **20a** and **20b** compared to **15a** and **15b**, respectively, and in the *N*-acetyl derivative **23** relative to **15a**. Much greater decreases in activity were seen in the *N*-benzoyl (**22**) and *N*-(ethoxycarbonyl) (**26**) compounds. The *N*-tosyl derivative **31** was some 100 times less potent than the unsubstituted derivative **15a**, but the corresponding *N*-tosylsulfimide **32** was better, being only ten times less potent than **15a**. Finally, the bulky cyclic sulfoximide **34** was found to be devoid of activity at up to 10 mg/kg iv. While it is difficult to draw any firm conclusion from these results, there does seem to be a trend for the activity of the series to decrease as the substituent on the sulfoximide nitrogen increases in size. Hence, the optimum activity lies in those compounds with the smallest substituent, notably the unsubstituted sulfoximides **15a–c**. The greater activity of the sulfimide **32** compared with the corresponding sulfoximide **31** is significant, and, except for the problems in their preparation and lack of stability, the sulfimides might prove to possess greater levels of activity than those seen in the corresponding sulfoximides described here.

On the basis of the above results, the 5-hexyl derivative **15b** was selected for further biological evaluation as a potential orally active antiallergic agent in man.[12]

Experimental Section

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Infrared spectra were measured on a Pye-Unicam SP 1000 instrument as KBr wafers in the case of solids and as thin films in the case of liquids.

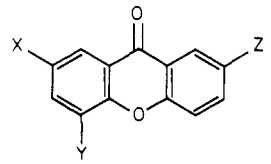
NMR spectra were obtained on solutions in either $CDCl_3$ or Me_2SO-d_6 using a Perkin-Elmer R12A instrument. Infrared and NMR spectra were run on all compounds and are consistent with the assigned structure in each case. Elemental analysis was carried out on all new compounds, and the results were within ±0.4% of the expected value, except where noted.

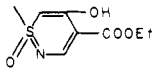
2-(Methylsulfinyl)xanthone (6). Sodium metaperiodate (2.0 g, 9.3 mmol) was added to a solution of 2-(methylthio)xanthone (**5**) (2.0 g, 8.3 mmol) in methanol–water (4:1, 250 mL). The reaction mixture was stirred at room temperature for 18 h and then the methanol was removed under reduced pressure. The aqueous residue was extracted with chloroform (2 × 100, mL) and, after drying the residue over magnesium sulfate, the organic extract was evaporated to dryness, leaving the crude product as a yellow crystalline solid (2.0 g).

This product was chromatographed on alumina (BDH neutral, grade 1; 150 g), first eluting less polar impurities with petroleum ether (bp 60–80 °C) and then 2-(methylsulfinyl)xanthone (**6**; 1.74 g, 82%) with ethyl acetate. Recrystallization from ethyl acetate gave the pure compound as a pale yellow crystalline solid melting at 161–164 °C. Anal. ($C_{14}H_{10}O_3S$) C, H, S.

2-(*S*-Methylsulfonimidoyl)xanthone (4a). Sodium azide (1.0 g, 15.4 mmol) was added in small portions to a stirred solution of 2-(methylsulfinyl)xanthone (**6**) (1.0 g, 3.9 mmol) in polyphosphoric acid (50 mL) maintained at 70 °C. After 2 h, the reaction mixture was cooled and cautiously diluted with water (100 mL) before being neutralized with 2 N sodium hydroxide solution. A pale yellow crystalline solid (0.92 g) separated out and was filtered off and dried before being chromatographed on

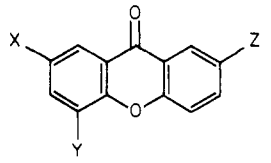
Table I

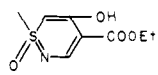


compd	X	Y	Z	mp	formula	mol wt	anal.
6	-H	-H	-SO-Me	161-164	$C_{14}H_{10}O_3S$	258.3	C, H, S
4a	-H	-H	-SO(=NH)Me	198-201	$C_{14}H_{11}NO_3S$	273.3	C, H, N, S
14a	-SO(=NH)Me	-H	-COOMe	229-231	$C_{16}H_{13}NO_5S$	331.3	C, H, N, S
15a	-SO(=NH)Me	-H	-COOH	285-286	$C_{15}H_{11}NO_5S$	317.3	C, H, N, S
14b	-SO(=NH)Me	$-C_6H_{13}$	-COOMe	145-146	$C_{22}H_{25}NO_5S$	415.5	C, H, N, S
15b	-SO(=NH)Me	$-C_6H_{13}$	-COOH	194-196	$C_{21}H_{23}NO_5S$	401.5	C, H, N, S
14c	-SO(=NH)Me	$-OC_5H_{11}$	-COOMe	157-159	$C_{21}H_{23}NO_6S$	417.5	
15c	-SO(=NH)Me	$-OC_5H_{11}$	-COOH	253-255	$C_{20}H_{21}NO_6S$	403.5	*a*
19a	$-SO(=NCONH_2)Me$	-H	-COOMe	217-220	$C_{17}H_{14}N_2O_6S$	374.4	C, H, N, S
20a	$-SO(=NCONH_2)Me$	-H	-COOH	227-279	$C_{16}H_{12}N_2O_6S$	360.3	C, H, N, S
19b	$-SO(=NCONH_2)Me$	$-C_6H_{13}$	-COOMe	225-226	$C_{23}H_{26}N_2O_6S$	458.5	C, H, N, S
20b	$-SO(=NCONH_2)Me$	$-C_6H_{13}$	-COOH	214-216	$C_{22}H_{24}N_2O_6S$	444.5	
21	-SO(=NCOPh)Me	-H	-COOMe	217-221	$C_{23}H_{17}NO_6S$	435.5	C, H, N, S
22	-SO(=NCOPh)Me	-H	-COOH	310-313	$C_{22}H_{15}NO_6S$	421.4	*b*
23	-SO(=NCOMe)Me	-H	-COOH	260-264	$C_{17}H_{13}NO_6S$	359.3	C, H, N, S[c]
24	-SO(=NCOMe)Me	-H	-COOMe	254-256	$C_{18}H_{15}NO_6S$	373.4	
25	-SO(=NCOOEt)Me	-H	-COOMe	191-193	$C_{19}H_{17}NO_7S$	403.4	C, H, N, S
26	-SO(=NCOOEt)Me	-H	-COOH	261-263	$C_{18}H_{15}NO_7S$	389.4	C, H, N, S
27	$-SO(=NCOCH_2Cl)Me$	-H	-COOMe	214-216	$C_{18}H_{14}ClNO_6S$	407.8	C, H, Cl, N, S
28	$-SO[=NCOCH_2\text{-}c\text{-}N(CH_2CH_2)_2O]Me$	-H	-COOMe	216-218	$C_{22}H_{22}N_2O_7S$	458.5	C, H, N, S[c]
29	$-SO(=NCOCH_2\text{-}c\text{-}NC_5H_{10})Me$	-H	-COOMe	169-173	$C_{23}H_{24}N_2O_6S$	456.5	H, N, S[c,d]
30	-SO(=N-Tos)Me	-H	-COOMe	224-227	$C_{23}H_{19}NO_7S_2$	485.5	C, H, N, S
31	-SO(=N-Tos)Me	-H	-COOH	276-278	$C_{22}H_{17}NO_7S_2$	471.5	C, H, N, S[c]
32	-S(=N-Tos)Me	-H	-COOH	~300	$C_{22}H_{17}NO_6S_2$	455.5	C, H, N, S[c]
33	-S(=N-Tos)Me	-H	-COOMe	226-229	$C_{23}H_{19}NO_6S_2$	469.5	C, H, N, S
34		-H	-COOH	~300	$C_{21}H_{15}NO_8S$	477.9	C, H, N, S[e]
35	$-SO[=NCH=C(COOEt)_2]Me$	-H	-COOMe	225-230	$C_{24}H_{23}NO_9S$	501.5	H, N, S[f]

[a] m/e = 403.107 ± 0.004. MS ($C_{20}H_{21}NO_6S$): calcd, 403.109. [b] m/e = 421.062 ± 0.006. MS ($C_{22}H_{15}NO_6S$): calcd, 421.062. [c] Satisfactory analysis for compound + $0.5H_2O$. [d] Anal. ($C_{23}H_{24}N_2O_6S \cdot 0.5H_2O$) C: calcd, 59.34; found, 59.81. [e] Analyzed as the HCl salt. [f] Anal. ($C_{24}H_{23}NO_9S$) C: calcd, 57.49; found 57.03.

Table II



compd	X	Y	Z	PCA, ED_{50} (mg/kg)[a] iv	PCA, ED_{50} (mg/kg)[a] po
1	disodium cromoglycate			1.21 (1.04-1.42)	inactive
4a	-H	-H	-SO(=NH)Me	no inhibn[b]	
15a	-SO(=NH)Me	-H	-COOH	0.033 (0.026-0.037)	2.72 (1.44-4.74)
15b	-SO(=NH)Me	$-C_6H_{13}$	-COOH	0.005 (0.004-0.006)	0.19 (0.07-0.30)
15c	-SO(=NH)Me	$-OC_5H_{11}$	-COOH	0.005 (0.004-0.006)	1.62 (1.00-2.63)
20a	$-SO(=NCONH_2)Me$	-H	-COOH	0.11 (0.08-0.16)	8.6 (3.9-25.6)
20b	$-SO(=NCONH_2)Me$	$-C_6H_{13}$	-COOH	0.009 (0.005-0.016)	
22	-SO(=NCOPh)Me	-H	-COOH	0.68 (0.38-1.49)	
23	-SO(=NCOMe)Me	-H	-COOH	0.065 (0.036-0.089)	
26	-SO(=NCOOEt)Me	-H	-COOH	89% inhibn[b]	
31	-SO(=N-Tos)Me	-H	-COOH	3.93 (2.23-5.71)	
32	-S(=N-Tos)Me	-H	-COOH	0.43 (0.33-0.56)	
34		-H	-COOH	no inhibn[b]	

[a] Figures in parentheses represent 95% confidence limits. [b] At 10 mg/kg.

alumina (BDH neutral, grade 1; 100 g). Elution with ethyl acetate–petroleum ether (bp 60–80 °C, 1:1) removed less polar impurities, and then elution with chloroform gave 2-(*S*-methylsulfonimidoyl)xanthone (**4a**) (0.72 g, 68%), which was recrystallized from ethanol, giving white needles melting at 198–201 °C. Anal. ($C_{14}H_{11}NO_3S$) C, H, N, S. The hydrochloride salt **4b**, a white crystalline solid, has a melting point of 160–164 °C.

4-[2-Hexyl-4-(methylthio)phenoxy]isophthalic Acid (9b). To a solution of dimethyl 4-bromoisophthalate (8; 5.5 g, 0.02 mol) and 2-hexyl-4-(methylthio)phenol (**7b**; 4.5 g, 0.02 mol) in nitrobenzene (40 mL) were added copper powder (0.4 g) and potassium carbonate (5.5 g, 0.04 mol). The mixture was then heated at 140 °C under an atmosphere of nitrogen for 3 h. After cooling, a solution of sodium hydroxide (3.2 g, 0.08 mol) in ethanol–water (3:1, 60 mL) was added, and the resulting mixture was refluxed for 1 h before being poured into ice–water. Extraction with dichloromethane (3 × 50 mL) removed the nitrobenzene, and then the aqueous solution was acidified with dilute hydrochloric acid. The solid was filtered off (or alternatively extracted into ethyl acetate) and recrystallized from methanol to give 4-[2-hexyl-4-(methylthio)phenoxy]isophthalic acid (**9b**; 5.8 g, 74%) melting at 135–140 °C.

5-Hexyl-7-(methylthio)xanthone-2-carboxylic Acid (10b). 4-[2-Hexyl-4-(methylthio)phenoxy]isophthalic acid (**9b**; 3.9 g, 0.01 mol) was dissolved in sulfolane (40 mL) and heated to 80 °C. Polyphosphoric acid (40 mL) was added and the solution stirred for 1 h before being cooled and poured into water. The crude product was filtered off and washed thoroughly with water. It was then recrystallized from ethanol to give 5-hexyl-7-(methylthio)xanthone-2-carboxylic acid (**10b**; 2.8 g, 75%) melting at 164–170 °C.

Methyl 5-Hexyl-7-(methylthio)xanthone-2-carboxylate (11b). 5-Hexyl-7-(methylthio)xanthone-2-carboxylic acid (**10b**; 2.0 g, 0.0054 mol) was added to a solution of concentrated sulfuric acid (2 mL) in methanol (40 mL). The solution was refluxed for 3 h before being poured into water. The product was isolated in chloroform, and the chloroform solution was then washed with sodium bicarbonate solution and water before being dried ($MgSO_4$) and evaporated to leave a gummy solid (2.0 g), which was recrystallized from petroleum ether (40–60 °C), giving pale yellow crystals of methyl 5-hexyl-7-(methylthio)xanthone-2-carboxylate (11b; 1.64 g, 79%) melting at 63–64 °C. Anal. ($C_{22}H_{24}O_4S$) C, H, S.

Methyl 5-Hexyl-7-(methylsulfinyl)xanthone-2-carboxylate (12b). A solution of sulfuryl chloride (0.57 g, 4.2 mmol) in dichloromethane (5 mL) was added dropwise at room temperature to a stirred mixture of wet silica [prepared by shaking silica (Kieselgel 60, 70–230 mesh; 1.2 g) with water (1.2 g) such that the water is totally absorbed] and methyl 5-hexyl-7-(methylthio)xanthone-2-carboxylate (**11b**; 1.54 g, 4.0 mmol) in dichloromethane (25 mL). Stirring was continued for 2 h after the addition was complete. After being filtered, the reaction mixture was washed with sodium bicarbonate solution and then dried ($MgSO_4$) before being evaporated to dryness. The crude product (1.62 g) was recrystallized from methanol, giving methyl 5-hexyl-7-(methylsulfinyl)xanthone-2-carboxylate (**12b**; 1.52 g, 95%) melting at 130–132 °C. Anal. ($C_{22}H_{24}O_5S$) C, H, S.

Methyl 5-Hexyl-7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (14b). A solution of methyl 5-hexyl-7-(methylsulfinyl)xanthone-2-carboxylate (**12b**; 1.2 g, 3.0 mmol) dissolved in polyphosphoric acid (100 mL) was stirred at 45–50 °C while sodium azide (0.24 g, 3.7 mmol) was added in small portions. Stirring was continued for an additional hour before the mixture was poured onto ice. The resulting mixture was neutralized by the addition of 30% ammonia solution and then extracted with ethyl acetate. The organic layer was washed with water, then dried ($MgSO_4$), and evaporated to yield a yellow solid (1.12 g), which was recrystallized from ethyl acetate, giving methyl 5-hexyl-7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (**14b**; 0.85 g, 68%) as white crystals melting at 145–146 °C. Anal. ($C_{22}H_{25}NO_5S$) C, H, N, S.

5-Hexyl-7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic Acid (15b). Methyl 5-hexyl-7-(*S*-methylsulfonimidoyl)-xanthone-2-carboxylate (**14b**; 0.42 g, 1.0 mmol) was dissolved in ethanol (10 mL), and 0.1 N sodium hydroxide solution (12 mL) was added. The resulting solution was refluxed for 1 h and then poured onto ice. Acidification with dilute hydrochloric acid produced a solid, which was filtered off, washed well with water, and dried. Recrystallization from ethanol gave 5-hexyl-7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic acid (**15b**; 0.36 g, 89%) melting at 194–196 °C. Anal. ($C_{21}H_{23}NO_5S$) C, H, N, S.

Diethyl 4-[2-Hexyl-4-(methylthio)phenoxy]isophthalate (16). A solution of 4-[2-hexyl-4-(methylthio)phenoxy]isophthalic acid (**9b**; 6.75 g, 0.017 mol) in ethanol (200 mL) containing concentrated sulfuric acid (15 mL) was heated under reflux for 3 h. The reaction mixture was poured into water and the product isolated in ethyl acetate. The ethyl acetate solution was washed with sodium bicarbonate solution and then with water before being dried ($MgSO_4$) and evaporated to leave diethyl 4-[2-hexyl-4-(methylthio)phenoxy]isophthalate (**16**; 7.36 g, 95%) as a red viscous oil. Anal. ($C_{25}H_{32}O_5S$) C, H, S.

Diethyl 4-[2-Hexyl-4-(methylsulfinyl)phenoxy]isophthalate (17). A solution of sulfuryl chloride (2.40 g, 0.18 mol) in dichloromethane (25 mL) was added dropwise at room temperature to a stirred mixture of wet silica gel [prepared by shaking silica (Kieselgel 60, 70–230 mesh; 10 g) with water (10 mL)] and diethyl 4-[2-hexyl-4-(methylthio)phenoxy]isophthalate (**16** 7.25 g, 0.16 mol) in dichloromethane (100 mL). The reaction mixture was stirred for 3 h and then filtered. The dichloromethane solution was washed with sodium bicarbonate solution and then with water before being dried ($MgSO_4$) and evaporated to leave a red oil (7.6 g). This material was chromatographed on silica (Kieselgel 60, 70–230 mesh; 250 g) eluting with chloroform. Diethyl 4-[2-hexyl-4-(methylsulfinyl)phenoxy]isophthalate (**17**; 4.38 g, 58%) was obtained as a pale red viscous oil. Anal. ($C_{25}H_{32}O_6S$) C, H, S.

Diethyl 4-[2-Hexyl-4-(methylsulfonimidoyl)phenoxy]isophthalate (18). Sodium azide (0.10 g, 154 mmol) was added in small portions to a stirred solution of diethyl 4-[2-hexyl-4-(methylsulfinyl)phenoxy]isophthalate (**17**; 0.62 g, 1.35 mmol) in polyphosphoric acid (50 mL) at room temperature. After the addition was complete, stirring was continued for 1 h before water was added. The product was extracted into chloroform, and this solution was washed with sodium bicarbonate solution and then with water. After being dried ($MgSO_4$), the solution was evaporated to leave diethyl 4-[2-hexyl-4-(*S*-methylsulfonimidoyl)phenoxy]isophthalate (**18**; 0.58 g, 91%) as a colorless viscous oil. Anal. ($C_{25}H_{33}NO_6S$) C, H, N, S.

5-Hexyl-7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic Acid (15b) from Diethyl 4-[2-Hexyl-4-(methylsulfinyl)-phenoxy]isophthalate (17). Sodium azide (0.16 g, 2.46 mmol) was added in small portions to a stirred solution of diethyl 4-[2-hexyl-4-(methylsulfinyl)phenoxy]isophthalate (**17**; 1.00 g, 2.18 mmol) in polyphosphoric acid (100 mL) at 40 °C. After the addition was complete, stirring was continued for a further hour at the same temperature. The white frothy reaction mixture was then plunged into an oil bath at 150 °C and stirred until the bubbles dispersed and a clear brown solution remained (about 1 h). When the reaction mixture had cooled to ca. 50 °C, water was added with care and the resulting mixture was kept at ca. 100 °C for 15 min. Ammonia was added to the cooled solution until the pH was approximately 4, and then the product was isolated in chloroform. The chloroform solution was washed with water, then dried ($MgSO_4$), and evaporated, leaving a pale yellow solid (0.72 g). Recrystallization from ethanol gave 5-hexyl-7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylic acid (**15b**; 0.64 g, 73%) as white needles, melting at 193–195 °C. The material was identical with that synthesized using the earlier method, by IR and NMR spectra, TLC, mp and mmp.

Methyl 7-(*N*-Carbamoyl-*S*-methylsulfonimidoyl)-xanthone-2-carboxylate (19a). Methyl 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (**14a**; 500 mg, 1.50 mmol) was dissolved in acetic acid (20 mL). Sodium cyanate (1.0 g, 15.0 mmol) was added and the resulting solution stirred at room temperature overnight before being poured into water. The crude mixture was extracted into ethyl acetate, and the ethyl acetate solution was then washed with sodium bicarbonate solution and water before being dried ($MgSO_4$) and evaporated to leave a yellow solid. Recrystallization from chloroform–methanol gave pure methyl 7-(*N*-carbamoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylate (**19a**; 345 mg, 61%) as white needles melting at 217–220 °C. Anal. ($C_{17}H_{14}N_2O_6S$) C, H, N, S.

7-(*N*-Carbamoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylic Acid (20a). Methyl 7-(*N*-carbamoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylate (**19a**; 300 mg, 0.80 mmol) was hydrolyzed in the manner described for the preparation of compound **15b** from **14b**. Recrystallization of the crude product from dimethylformamide–chloroform gave 7-(*N*-carbamoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylic acid (**20a**; 235 mg,

81%) as white crystals melting at 277–279 °C. Anal. ($C_{16}H_{12}N_2O_6S$) C, H, N, S.

Methyl 7-(*N*-Benzoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylate (21). Benzoyl chloride (1.0 mL, 8.6 mmol) and triethylamine (1.4 mL, 1.02 g, 10.0 mmol) were added to a solution of methyl 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (14a; 1.0 g, 3.0 mmol) in chloroform (50 mL). The resulting solution was heated under reflux for 4 h before being poured into water. The chloroform layer was separated and washed first with dilute hydrochloric acid, then with sodium bicarbonate solution, and finally with water. After being dried ($MgSO_4$), the solution was evaporated to dryness, leaving a yellow solid (760 mg). Recrystallization from chloroform–ethanol gave methyl 7-(*N*-benzoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylate (21; 645 mg; 49%) as white crystals melting at 217–221 °C. Anal. ($C_{23}H_{17}NO_6S$) C, H, N, S.

7-(*N*-Benzoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylic Acid (22). Methyl 7-(*N*-benzoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylate (21; 0.5 g, 1.15 mmol) was hydrolyzed in the usual manner to give, after recrystallization from dimethylformamide–ether, 7-(*N*-benzoyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylic acid (22; 0.37 g, 76%) melting at 310–313 °C dec. Anal. ($C_{22}H_{15}NO_6S$) requires M^+ 421.062; found 421.062 ± 0.006.

Methyl 7-(*N*-Acetyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylate (24). Methyl 7-(*S*-methylsulfonimidoyl)-xanthone-2-carboxylate (14a; 1.0 g, 3.0 mmol) was dissolved in pyridine (50 mL) with warming, and then acetic anhydride (25 mL) was added. The solution was warmed on a steam bath and then allowed to stand overnight before being poured into water. The crude mixture was extracted twice with chloroform, and the combined chloroform extracts were then washed with dilute hydrochloric acid, followed by water. After being dried ($MgSO_4$), the solution was evaporated, leaving a pale yellow solid. Recrystallization from chloroform–petroleum ether (40–60 °C) gave methyl 7-(*N*-acetyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylate (24; 0.72 g, 64%) melting at 254–256 °C. Anal. ($C_{18}H_{15}NO_6S$) C, H, N, S.

7-(*N*-Acetyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylic Acid (23). Methyl 7-(*N*-acetyl-*S*-methylsulfonimidoyl)xanthone-2-carboxylate (24; 560 mg, 1.5 mmol) was suspended in ethanol–water (1:1, 50 mL) containing sodium bicarbonate (126 mg, 1.5 mmol). The mixture was heated under reflux for 1 h before being cooled and filtered to remove any remaining insoluble material. The resulting solution was acidified to pH 4 by careful addition of dilute hydrochloric acid. Filtration of the mixture gave 7-(*N*-acetyl-*S*-methylsulfonimidoyl)-xanthone-2-carboxylic acid (23; 256 mg, 47%) as a white powder, melting at 260–264 °C. Anal. ($C_{17}H_{13}NO_6S \cdot 0.5\ H_2O$) C, H, N, S.

Methyl 7-[*N*-(Ethoxycarbonyl)-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (25). Ethyl chloroformate (2.0 mL, 2.27 g, 20.9 mmol) was added to a solution of methyl 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (14a; 1.5 g, 4.5 mmol) in pyridine (100 mL). The mixture was heated at 100 °C for 3 h before being poured into ice–water. After acidification of the mixture to pH 4 with dilute hydrochloric acid, the solid product was collected and washed with water. Recrystallization from chloroform–methanol gave methyl 7-[*N*-(ethoxycarbonyl)-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (25; 0.94 g, 51%) melting at 191–193 °C. Anal. ($C_{19}H_{17}NO_7S$) C, H, N, S.

7-[*N*-(Ethoxycarbonyl)-*S*-methylsulfonimidoyl]-xanthone-2-carboxylic Acid (26). Methyl 7-[*N*-(ethoxycarbonyl)-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (25; 0.4 g, 1 mmol) was hydrolyzed with sodium bicarbonate (0.090 g, 1.1 mmol) in aqueous ethanol by the method described in the preparation of 23. 7-[*N*-(Ethoxycarbonyl)-*S*-methylsulfonimidoyl)xanthone-2-carboxylic acid (26; 0.235 g, 61%) was obtained as an off-white powder melting at 261–263 °C. Anal. ($C_{18}H_{15}NO_7S$) C, H, N, S.

Methyl 7-[*N*-(Chloroacetyl)-*S*-methylsulfonimidoyl]-xanthone-2-carboxylate (27). Methyl 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (14a; 2.5 g, 7.50 mmol) in dry dimethylformamide (80 mL) was stirred with chloroacetic anhydride (2.6 g, 15.0 mmol) at room temperature for 3 h. The reaction mixture was poured into water and then extracted with chloroform. The chloroform extract was washed first with dilute hydrochloric acid and then with water before being dried ($MgSO_4$) and evaporated to leave a yellow solid (2.7 g). Recrystallization from chloroform–methanol gave pale yellow crystals of methyl 7-[*N*-(chloroacetyl)-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (27; 2.25 g, 73%) melting at 214–216 °C. Anal. ($C_{18}H_{14}ClNO_6S$) C, H, Cl, N, S.

Methyl 7-[*S*-Methyl-*N*-(morpholinoacetyl)sulfonimidoyl]xanthone-2-carboxylate (28). Methyl 7-[*N*-(chloroacetyl)-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (27; 500 mg, 1.23 mmol) and morpholine (0.5 mL, 500 mg, 5.81 mmol) were dissolved in dichloromethane (25 mL), and the mixture was stirred for 24 h before being poured into water. The dichloromethane extract was separated, dried ($MgSO_4$), and evaporated to leave a light brown solid (480 mg). Recrystallization from chloroform–methanol gave methyl 7-[*S*-methyl-*N*-(morpholinoacetyl)sulfonimidoyl]xanthone-2-carboxylate (28; 375 mg, 67%) as a white powder melting at 216–218 °C. Anal. ($C_{22}H_{22}N_2O_7S \cdot 0.5\ H_2O$) C, H, N, S.

Methyl 7-[*S*-Methyl-*N*-(piperidinoacetyl)sulfonimidoyl]xanthone-2-carboxylate (29). Methyl 7-[*N*-(chloroacetyl)-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (27; 500 mg, 1.23 mmol) was reacted in the manner described above using piperidine instead of morpholine, to give, after recrystallization from chloroform–ethanol, 7-[*S*-methyl-*N*-(piperidinoacetyl)-sulfonimidoyl]xanthone-2-carboxylate (29; 405 mg, 72%) as a white powder melting at 169–173 °C. Anal. ($C_{23}H_{24}N_2O_6S \cdot 0.5\ H_2O$) H, N, S; C: calcd, 59.34; found, 59.81.

Methyl 7-[*S*-Methyl-*N*-(*p*-toluenesulfonyl)sulfonimidoyl]xanthone-2-carboxylate (30). Methyl 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (14a; 1.65 g, 5.0 mmol) was dissolved in dry pyridine (30 mL) with warming. *p*-Toluenesulfonyl chloride (1.0 g, 5.2 mmol) was added and the solution heated on a water bath for 10 min before being stood overnight at room temperature. The reaction mixture was poured into ice-cold dilute hydrochloric acid, and the product was then extracted with ethyl acetate. After being washed with water and dried ($MgSO_4$), the solution was evaporated to dryness, leaving an off-white solid (2.01 g). Recrystallization from chloroform–methanol gave methyl 7-[*S*-methyl-*N*-(*p*-toluenesulfonyl)sulfonimidoyl]xanthone-2-carboxylate (30; 1.84 g, 76%) melting at 224–227 °C. Anal. ($C_{23}H_{19}NO_7S_2$) C, H, N, S.

7-[*S*-Methyl-*N*-(*p*-toluenesulfonyl)sulfonimidoyl]-xanthone-2-carboxylic Acid (31). Methyl 7-[*S*-methyl-*N*-(*p*-toluenesulfonyl)sulfonimidoyl]xanthone-2-carboxylate (30; 0.95 g, 2.0 mmol) was hydrolyzed in the usual manner to give after recrystallization from chloroform–methanol, 7-[*S*-methyl-*N*-(*p*-toluenesulfonyl)sulfonimidoyl]xanthone-2-carboxylic acid (31; 0.75 g, 81%) as white crystals melting at 276–278 °C. Anal. ($C_{22}H_{17}NO_7S_2 \cdot 0.5H_2O$), C, H, N, S.

Methyl 7-[*S*-Methyl-*N*-(*p*-toluenesulfonyl)sulfimidoyl]xanthone-2-carboxylate (33). Methyl 7-(methylthio)-xanthone-2-carboxylate (11a; 1.2 g, 4.0 mmol) and chloramine-T (1.2 g, 4.3 mmol) were heated under reflux in dioxane–water (2:1, 100 mL) for 1 h. The reaction mixture stood at 0 °C overnight and then filtered, giving methyl 7-[*S*-methyl-*N*-(*p*-toluenesulfonyl)-sulfimidoyl]xanthone-2-carboxylate (33; 1.0 g, 53%) as a white crystalline solid melting at 226–229 °C. Anal. ($C_{23}H_{19}NO_6S_2$) C, H, N, S.

7-[*S*-Methyl-*N*-(*p*-toluenesulfonyl)sulfimidoyl]xanthone-2-carboxylic Acid (32). Methyl 7-[*S*-methyl-*N*-(*p*-toluenesulfonyl)sulfimidoyl]xanthone-2-carboxylate (33; 0.85 g, 1.80 mmol) was hydrolyzed in the usual manner. The product was not recrystallized. 7-[*S*-Methyl-*N*-(*p*-toluenesulfonyl)sulfimidoyl]xanthone-2-carboxylic acid (32; 0.69 g, 84%) was obtained as a white powder melting at 330 °C with decomposition. Anal. ($C_{22}H_{17}NO_6S_2 \cdot 0.5H_2O$) C, H, N, S.

Methyl 7-[*N*-[2′,2′-Bis(ethoxycarbonyl)ethenyl]-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (35). Methyl 7-(*S*-methylsulfonimidoyl)xanthone-2-carboxylate (14a; 4.0 g, 12.0 mmol) was added to diethyl ethoxymethylenemalonate (35 mL), and the mixture was heated at 125 °C for 24 h. The total crude product was dissolved in hot chloroform–methanol, and on cooling, white crystals of methyl 7-[*N*-[2′,2′-bis(ethoxycarbonyl)-ethenyl]-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (35; 3.05 g, 50%) separated out, mp 225–230 °C. Anal. ($C_{24}H_{23}NO_9S$) H,

N, S; C: calcd, 57.49; found, 57.03.

7-[4-(Ethoxycarbonyl)-5-hydroxy-1-oxo-1,2-thiazin-1-yl]xanthone-2-carboxylic Acid (34). Sodium hydride (80% dispersion in oil, 0.30 g, 10.0 mmol) was added in small portions to a stirred solution of methyl 7-[*N*-[2',2'-bis(ethoxycarbonyl)-ethenyl]-*S*-methylsulfonimidoyl]xanthone-2-carboxylate (**35**; 2.5 g, 5.0 mmol) in dry dimethyl sulfoxide (150 mL) at 0 °C. After being stirred for 4 h, the reaction mixture was poured onto ice-cold dilute hydrochloric acid. Chloroform was added, and the chloroform extract was washed with water and dried ($MgSO_4$) before being evaporated to leave a brown semisolid. Trituration with ethanol gave a buff crystalline solid which was recrystallized from chloroform to give white crystals of 7-[4-(ethoxycarbonyl)-5-hydroxy-1-oxo-1,2-thiazin-1-yl]xanthone-2-carboxylic acid (**34**) as the hydrochloride (0.43 g, 18%) melting at 300 °C with decomposition. Anal. ($C_{21}H_{15}NO_8S \cdot HCl$) C, H, N, S.

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Biological Properties of Transition-Metal Organometallic Compounds. 3. β-Ferrocenylalanine[1]

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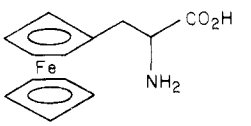
We have investigated the effects of DL-β-ferrocenylalanine (1), five DL-halophenylalanines (*p*-F, **2**; *m*-F, **3**; *o*-F, 4; *p*-Cl, **5**; *p*-Br, **6**), L-β-(2-pyridyl)alanine (**7**), and DL-β-(6-methyl-2-pyridyl)alanine (**8**) on the growth of *Leuconostoc meserentoides* and as inhibitors of phenylalanine decarboxylase from *S. Fecalis*, hog kidney aromatic L-amino acid decarboxylase, and rat liver phenylalanine hydroxylase. None of the compounds supported bacterial growth in the absence of L-Phe, but they inhibited growth in the presence of L-Phe in the order **2** ≫ 4 > 3 ≃ 7 > 5 > 6; **8** and 1 were inactive. Both decarboxylases were inhibited by the analogues to a similar extent, the inhibition decreasing in the order 1 > 6 > 5 > 2 > 8 ≃ 7. Compound 1 was a competitive inhibitor of the hog kidney enzyme with a K_i of 7.2 mM; L-Phe had a K_M of 48.8 mM. Inhibition of the phenylalanine hydroxylase ($DMPH_4$ cofactor) decreased in the order **2** > 1 > 3 > 4 > 5 > 6 > 7 > 8 over a range from 73 to 2% inhibition. Compound 1 gave *noncompetitive* inhibition with respect to L-Phe and mixed inhibition with respect to $DMPH_4$ cofactor. This pattern of inhibition kinetics is apparently unique, as other known inhibitors either compete for substrate (e.g., **2** and **5**) or with cofactor (e.g., norepinephrine or other catechol compounds). It was suggested that 1 may affect the enzyme through interaction at an allosteric or regulatory site previously proposed to account for the activation observed upon preincubation of the enzyme with its normal substrate L-Phe.

Two very well established approaches to modifying the biological activity of molecules containing an aromatic ring involve the placing of substituents at various positions around the perimeter of the ring and/or the introduction of various heterocyclic modifications into the ring itself. An alternative type of substitution which has received relatively little attention involves replacing the aromatic moiety by an aromatic *organometallic* moiety such as ferrocene or benzenechromiumtricarbonyl. The biological activity of ferrocene analogues of several drugs[2-6] has generally been disappointing from a pharmacological or therapeutic point of view. However, ferrocene itself is known to be an effective hematinic agent having very low toxicity in animals and man.[7-11] Recent studies in our laboratory have shown that ferrocene is extensively metabolized in rats by "aromatic hydroxylation" followed by conjugation with glucuronic acid or sulfate. The release of iron in vivo for hemoglobin synthesis apparently occurs via spontaneous decomposition of hydroxyferrocene prior to conjugation.[1a]

Because the enzymatic hydroxylation of phenylalanine to tyrosine shows mechanistic similarities to "aromatic hydroxylation", we have now investigated the interaction of β-ferrocenylalanine (1) with phenylalanine hydroxylase.



1

A series of halophenyl- and pyridylanines were included in the testing, and two other phenylalanine-utilizing test systems, *Leuconostoc mesenteroides* and phenylalanine decarboxylase, were also investigated with these compounds.

As detailed below, 1 behaved similar to other "organic" analogues of phenylalanine in the latter two systems but was found to have unique kinetic properties as an inhibitor of phenylalanine hydroxylase.

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