(ATCC-9142) [the slant containing as a nutrient medium: glucose (10 g)-yeast extract (2.5 g)-K_2HPO_4 (1 g)-agar (20 g) in distd H_2O (*q.s.* to 1 l.)] was suspd in 0.01% aqueous sodium lauryl sulfate. One-milliliter portions of this suspension were used to inoculate three 250-ml erlenmeyer flasks, each containing 50 ml of the following sterilized medium: glucose (30 g)-soy bean meal (20 g)-soy bean oil (2.0 g)-$CaCO_3$ (2.5 g) in distd H_2O (*q.s.* to 1 l.). After a 96-hr incubation at 25° with continuous rotary agitation (280 cpm; 2-in. stroke), 5% (v/v) transfers were made to 20 250-ml erlenmeyer flasks, each containing 50 ml of the following sterilized medium: corn steep liquor (6 g)-$NH_4H_2PO_4$ (3 g)-yeast extract (2.5 g)-dextrose (10 g)-$CaCO_3$ (2.5 g) in distd H_2O (*q.s.* to 1 l.). After 24 hr of further incubation, as described above, 0.25 ml of a sterile soln of 4 in DMF (40 mg/ml) was added. A total of 200 mg (0.00089 mole) was fermented. After 6 days of further incubation as described above, the contents of the flasks were combined, and the broth was adjusted to pH 2.5 with 12 *N* H_2SO_4. The broth was filtd through a Seitz clarifying pad, and the flasks, mycelium, and pad were washed with warm H_2O. The filtrate and washings (1.5 l.) obtained in this way were extd (EtOAc), and the exts were washed (8% aq NaCl), dried, and evapd. Crystn from EtOAc gave **18** (42 mg, mp 235.5-237.5°, mmp, 20%).

7-Hydroxy-α-methylfluorene-2-acetic acid (19) was prepared by reduction of **14** with Zn and $CaCl_2$ to form **16** (93%), which was diazotized and hydrolyzed as described for the preparation of **18** to give **19** (31%). The analytical sample was prepared by sublimation: mp 218-219°. *Anal.* ($C_{16}H_{14}O_3$) C, H. Fermentation of **9** (300 mg, 0.00126 mole) with *C. blakesleeana* (ATCC 8688a), as described above, gave **19** (98 mg, mp 218-220°, mmp, 30%).

Methyl 7-Hydroxyfluorene-2-acetate (20). Esterification of **18** with CH_2N_2 and crystn from C_6H_6 gave **20** (mp 139-140°, 71%). *Anal.* ($C_{16}H_{14}O_3$) C, H.

Methyl 7-Methoxyfluorene-2-acetate (22). Treatment of **20** with MeI-K_2CO_3 in Me_2CO and crystn from EtOAc-*i*-Pr_2O gave **22** (mp 114.5-115.5°, 65%). *Anal.* ($C_{17}H_{16}O_3$) C, H.

Methyl 7-Methoxy-α-methylfluorene-2-acetate (23). Esterification of **19** with CH_2N_2 and treatment of the ester **21** with MeI-K_2CO_3 in Me_2CO, followed by crystn from Me_2CO-*i*-Pr_2O, gave **23** (mp 101-103°, 44%). *Anal.* ($C_{18}H_{18}O_3$) C, H.

7-Methoxyfluorene-2-acetic Acid (24). Hydrolysis of **22** with EtOH-45% aqueous KOH and crystn from EtOH gave **24** (mp 204-205°, 97%). *Anal.* ($C_{16}H_{14}O_3$) C, H.

7-Methoxy-α-methylfluorene-2-acetic Acid (25). Hydrolysis of **23** with EtOH-40% aqueous KOH and crystn from EtOAc gave **25** (mp 183-184°, 80%). *Anal.* ($C_{17}H_{16}O_3$) C, H.

Fluorene-1-acetic Acid (28). Fluorene-1-carboxylic acid was homologated under the Arndt-Eistert conditions employed in the synthesis of **26** and **27**,[11] and gave **28** after crystn from EtOH (mp 172-174°, 15%). *Anal.* ($C_{15}H_{12}O_2$) C, H.

α-Methylfluorene-1-acetic acid (29) was prepared from fluorene-1-carboxylic acid (3.0 g, 0.014 mole) by Arndt-Eistert homologation[17] with diazoethane.[18] Crystn from MeOH gave **29** (1.4 g, mp 189-191°, 41%). *Anal.* ($C_{16}H_{14}O_2$) C, H.

α-Methylfluorene-4-acetic acid (30) was prepared, as described for **29**, from fluorene-4-carboxylic acid and gave, after sublimation and crystn from MeCN, **30** (mp 176-178°, 62%). *Anal.* ($C_{16}H_{14}O_2$) C, H.

α-Methylfluorene-9-acetic Acid (31). A mixt of 9-bromofluorene (24.5 g, 0.1 mole) and potassium methylmalonic ester [prepared from diethyl methylmalonate (17.2 g) and *tert*-BuOK (11.2 g) in *tert*-BuOH (80 ml)] was refluxed for 4 hr. The mixt was evapd and the residue was dissolved in H_2O and extd (Et_2O). The exts were washed (H_2O), dried, and evaporated. The residue was hydrolyzed with EtOH-40% aqueous KOH to the diacid (2-hr reflux). The diacid was heated at 170-180° until the evolution of CO_2 had ceased. Sublimation of the product gave **31** (3.0 g, mp 103-104°, 13%). *Anal.* ($C_{16}H_{14}O_2$) C, H.

Acknowledgments. The authors wish to thank our Immunology Section for the biological results and Mr. J. Alicino and his staff for the microanalyses.

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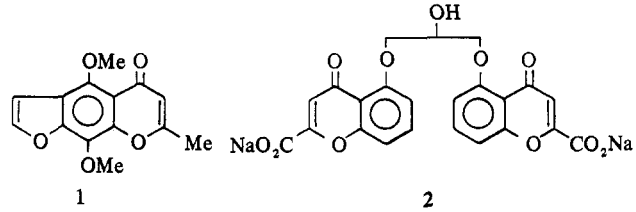
Xanthone-2-carboxylic Acids, a New Series of Antiallergic Substances†

J. R. Pfister, R. W. Ferraresi, I. T. Harrison,* W. H. Rooks, A. P. Roszkowski, A. Van Horn, and J. H. Fried

Syntex Research, Stanford Industrial Park, Palo Alto, California 94304. *Received April 24, 1972*

Substituted xanthone-2-carboxylic acids are shown to be highly active in antiallergic bioassays and are, therefore, of possible value in the treatment of asthma.

We wish to report a new series of antiallergic substances based on xanthone-2-carboxylic acid. It has long been known that khellin (1), a chromone isolated from the fruit and seeds of the plant *Ammi visnaga*, exhibits antiasthma properties of clinical value.[1] Starting from this observation, the Fisons group was able to develop disodium cromoglycate (2), which has become an exceptionally important agent in the prophylactic treatment of the allergic condition underlying bronchial asthma.[2,3] Our work



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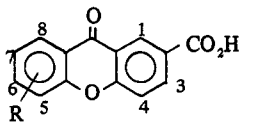
†Publication No. 403 from the Syntex Institute of Organic Chemistry.

Table I. Physical Properties and Activities in the Iv Rat Passive Cutaneous Anaphylaxis (PCA) Assay of Xanthone-2-carboxylic Acids (Disodium Cromoglycate = 1)



Compd	R	Method	Mp, °C	Formula[a]	No. of rats	PCA assay
2						1 (standard)
3[b]	H				41	0.5
6	1-CH_3O	I	211–213	$C_{15}H_{10}O_5$	15	<1
10a	3-CH_3O	II	287–288	$C_{15}H_{10}O_5$	10	0.3
10b	4-CH_3O	II	320–322	$C_{15}H_{10}O_5$	10	~0.1
10c	8-CH_3O	II	295–297	$C_{15}H_{10}O_5$	15	0.4
14a	5-CH_3O	III	329–331	$C_{15}H_{10}O_5$	60	3
14b	7-CH_3O	III	280–281	$C_{15}H_{10}O_5$[c]	94	3
18b	6-CH_3O	IV	>300	$C_{15}H_{10}O_5$[d]	15	0.3
22a	7-CH_3	V	300–302	$C_{15}H_{10}O_4$	38	0.5
22b	7-C_2H_5	V	269–271	$C_{16}H_{12}O_4$	45	3
22c	7-*n*-C_3H_7	V	254–255	$C_{17}H_{14}O_4$	29	~1
22d	7-*i*-C_3H_7	V	274–276	$C_{17}H_{14}O_4$	101	7
22e	7-*sec*-C_4H_9	V	240	$C_{18}H_{16}O_4$[e]	30	2
22f	7-*n*-C_5H_{11}	V	242–244	$C_{19}H_{18}O_4$	15	<0.3
22g	5-*i*-C_3H_7	V	277–279	$C_{17}H_{14}O_4$	32	~3
22h	7-F	V	>300	$C_{14}H_7FO_4$	27	~0.2
24	7-OH	VI	>300	$C_{14}H_8O_5$	45	~1
25a	7-C_2H_5O	VI	287–289	$C_{16}H_{12}O_5$	45	1
25b	7-*n*-C_3H_7O	VI	263–265	$C_{17}H_{14}O_5$	45	1
25c[f]	7-*i*-C_3H_7O	VI	>300	$C_{17}H_{13}NaO_5$	100	8
25d	7-*n*-C_4H_9O	VI	243–245	$C_{18}H_{16}O_5$[g]	50	0.2
25e	5-*i*-C_3H_7O	VI	265–266	$C_{17}H_{14}O_5$	45	4
26[h]	7-CO_2H		>300	$C_{15}H_8O_6$	58	2

[a] All compds were analyzed for C and H. [b] Ref 11. [c] C: calcd, 66.67; found, 66.15. [d] C: calcd, 66.67; found, 66.26. [e] C: calcd, 72.96; found, 72.43. [f] Sodium salt. [g] C: calcd, 69.22; found, 69.72. [h] Ref 12.

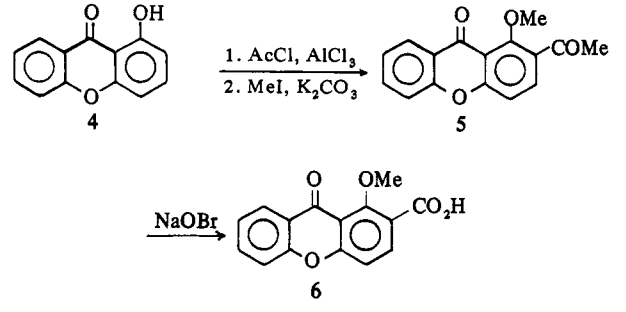
started at this point and was based on our supposition that the OC=CC=O grouping was the major structural parameter responsible for the antiallergy activity of khellin and disodium cromoglycate. Bioassay of a variety of compounds containing the OC=CC=O grouping, using the rat passive cutaneous anaphylaxis (PCA) assay with homocytotropic reaginic antibody,[4] led to the finding of significant antiallergic activity in xanthones and indandiones. Additionally activity was observed with 1,4-naphthaquinones which contain the O=CC=CC=O group. Further work in the xanthone series established that a carboxylic acid substituent at the 2 position was essential for high levels of activity. In this report we describe the relationship of structures to biological activity for these xanthonecarboxylic acids.

The relationship between activity and substitution pattern on the xanthone nucleus was initially determined using the methoxyl substituent. At positions 1, 3, 4, 6, or 8 (Table I, compds **6**, **10a**, **10b**, **18b**, and **10c**) substitution was accompanied by either a decrease or by little change in activity relative to the unsubstituted xanthone-2-carboxylic acid (**3**). At the two remaining positions, 5 and 7 (compds **14a** and **14b**), introduction of the methoxyl group resulted in a sixfold enhancement of activity. Other small alkoxy substituents at the 5 or 7 position, including those with branched chains, also gave more active compounds (**25a–c** and **25e**). A rather similar correlation is evident in the 5- or 7-alkyl-substituted series (**22a–g**). An unusually wide range of lipophilic–hydrophilic character is tolerated in the substituent group. Thus, *i*-propyl (**22d**), *i*-propoxy (**25c**), hydroxyl (**24**), and carboxyl groups (**26**) substituted at the 7 position all give highly active compounds.

Further studies have shown that compounds **22c**, **22d**, **22g**, and **25c** are active not only by iv and ip routes, but also have marked oral activity. This is of particular significance in view of the lack of oral activity observed with disodium cromoglycate which must be administered by inhalation.

The anaphylactic response to the antigen–antibody combination is primarily due to an induced degranulation of mast cells. The resulting release of histamine leads to an increased capillary permeability. Both xanthonecarboxylic acids and disodium cromoglycate[5] protect mast cells against this degranulation. Thus in a typical experiment 87 ± 4% (mean ± se of 8 rats) of the mast cells in the subcutaneous connective tissue from the site of the anaphylactic reaction had degranulated. In similar tissue from nonchallenged control rats 42 ± 5% of these cells degranulated.

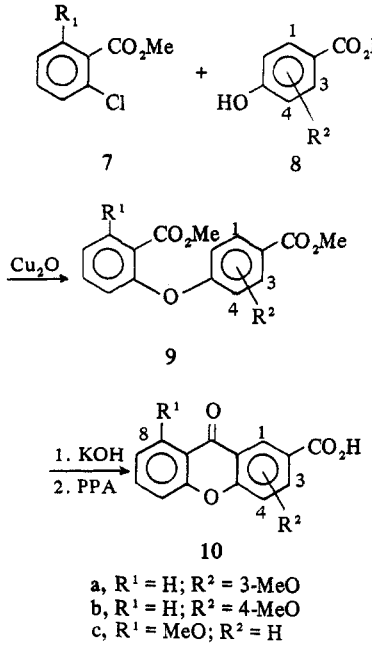
Scheme I



(This control reflects possible damage of the mast cells during tissue preparation.) Compound **25c** significantly inhibited mast cell degranulation since only 29 ± 5% of the mast cells degranulated in challenged rats which were given 0.1 mg of **25c** iv. Disodium cromoglycate and the xanthones described here are unique among the known inhibitors of anaphylaxis in their mechanism of action.

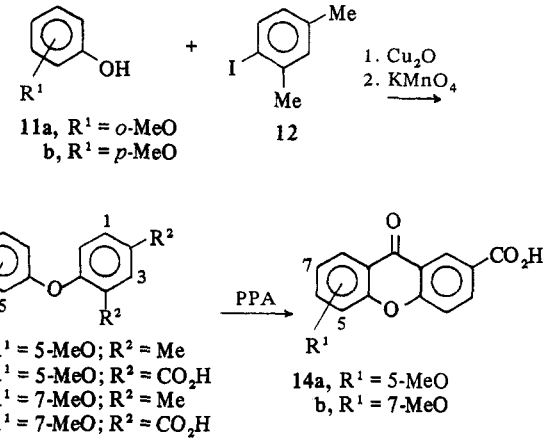
Since the work reported above was completed, claims[6] of antiallergy activity for some substituted xanthone-2-

Scheme II



a, R^1 = H; R^2 = 3-MeO
b, R^1 = H; R^2 = 4-MeO
c, R^1 = MeO; R^2 = H

Scheme III

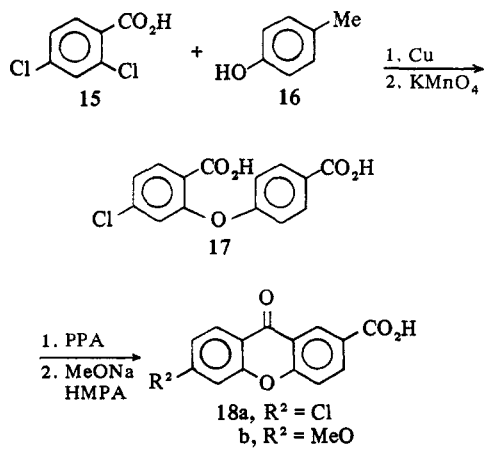


11a, R^1 = *o*-MeO
b, R^1 = *p*-MeO

13a, R^1 = 5-MeO; R^2 = Me
b, R^1 = 5-MeO; R^2 = CO_2H
c, R^1 = 7-MeO; R^2 = Me
d, R^1 = 7-MeO; R^2 = CO_2H

14a, R^1 = 5-MeO
b, R^1 = 7-MeO

Scheme IV

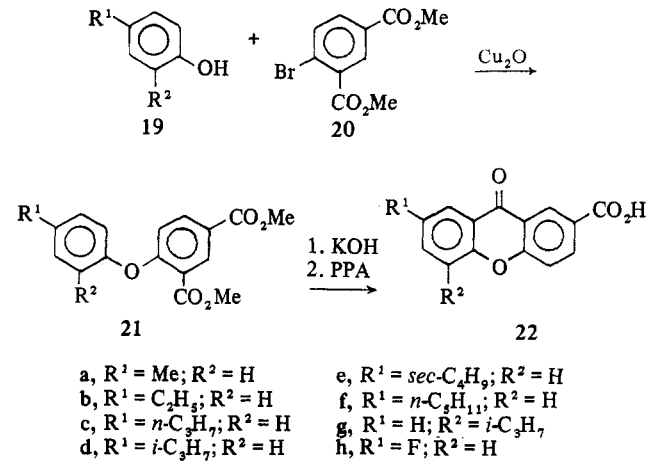


18a, R^2 = Cl
b, R^2 = MeO

carboxylic acids have appeared in the patent literature but quantitative biological data have not been published.

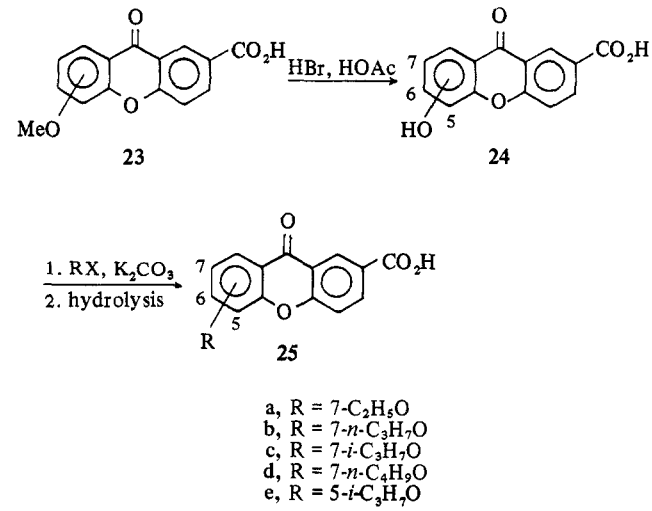
Chemistry. A variety of synthetic schemes were utilized for the preparation of the substituted xanthonecarboxylic acids reported in this study.

Scheme V



a, R^1 = Me; R^2 = H
b, R^1 = C_2H_5; R^2 = H
c, R^1 = *n*-C_3H_7; R^2 = H
d, R^1 = *i*-C_3H_7; R^2 = H
e, R^1 = *sec*-C_4H_9; R^2 = H
f, R^1 = *n*-C_5H_{11}; R^2 = H
g, R^1 = H; R^2 = *i*-C_3H_7
h, R^1 = F; R^2 = H

Scheme VI



a, R = 7-C_2H_5O
b, R = 7-*n*-C_3H_7O
c, R = 7-*i*-C_3H_7O
d, R = 7-*n*-C_4H_9O
e, R = 5-*i*-C_3H_7O

Scheme I. 1-Methoxyxanthone-2-carboxylic acid (**6**) was prepared by Friedel-Crafts acylation[7] of 1-hydroxyxanthone (**4**) followed by methylation forming **5** and hypobromite oxidation to the acid **6**.

Scheme II. 3-Methoxyxanthone-2-carboxylic acid (**10a**) was prepared by the coupling[8] of methyl 2-chlorobenzoate (**7a**) with the phenol **8a** forming the ether **9a**, which was saponified and then cyclized[9] by polyphosphoric acid to the acid **10a**. Similarly, 4-methoxyxanthone-2-carboxylic acid (**10b**) was prepared from the phenol **8b** *via* **9b**. 8-Methoxyxanthone-2-carboxylic acid (**10c**) was prepared from methyl 2-chloro-6-methoxybenzoate (**7c**) *via* **9c**.

Scheme III. 5-Methoxyxanthone-2-carboxylic acid (**14a**) was prepared by coupling[8] 2,4-dimethyliodobenzene (**12**) with the phenol **11a** forming the ether **13a**, which was oxidized by $KMnO_4$ to the dicarboxylic acid **13b** and cyclized by polyphosphoric acid or concd H_2SO_4 to the acid **14a**. 7-Methoxyxanthone-2-carboxylic acid (**14b**) was similarly prepared from the phenol **11b** *via* **13c** and **13d**.

Scheme IV. 6-Methoxyxanthone-2-carboxylic acid (**18b**) was prepared by methanolysis[9] of 6-chloroxanthone-2-carboxylic acid (**18a**).

Scheme V. 5-Alkyl- (**22g**), 7-alkyl- (**22a-f**), and 7-fluoro- (**22h**) substituted xanthone-2-carboxylic acids were prepared by Scheme V, a modification of Scheme III.

Scheme VI. 5-Methoxy- and 7-methoxy-substituted

xanthone-2-carboxylic acids (23) were converted into alkoxyxanthone carboxylic acids (25a–e) by ether cleavage with HBr in acetic acid followed by alkylation of the resulting phenol.

Experimental Section

General Methods. Phenols were coupled with aromatic halides using Cu_2O[8] in dimethylacetamide (Schemes II, III, and V) and copper powder[10] with anhydrous potassium carbonate in dimethylformamide (Scheme IV). Dicarboxylic acids were cyclized to xanthonecarboxylic acids by polyphosphoric acid[9] in tetramethylene sulfone or with concd H_2SO_4. Methyl diphenyl ethers were oxidized to the corresponding diphenylcarboxylic acids by $KMnO_4$ in *tert*-BuOH–H_2O. Methanolysis of 6-chloroxanthone-2-carboxylic acid (18a) was performed as described by Goldberg and Wragg,[9] except that HMPA was used as solvent.

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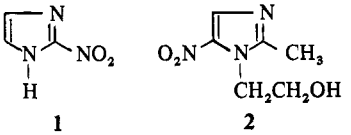
Antiparasitic Nitroimidazoles. 1. Some 2-Styryl-5-nitroimidazoles

William J. Ross,* William B. Jamieson, and Max C. McCowen

Lilly Research Centre Ltd., Erl Wood Manor, Windlesham, Surrey, England. *Received March 22, 1972*

A series of 1-substituted-2-styryl-5-nitroimidazoles was prepared by condensing 1-substituted-2-methyl-5-nitroimidazoles with aryl aldehydes in the presence of sodium methoxide. The aryl aldehydes were substituted with aryl, alkyl, alkoxyl, and chloro groups while the 1 substituent on the imidazole nucleus varied from alkyl to hydroxyalkyl to alkylene. The alkylene substituent was introduced by base elimination of the tosylates of the hydroxyalkyl compounds. A number of arylethynyleneimidazoles were also prepared by bromination of the styryl compound followed by didehydrobromination with DBN. All the compounds were tested against *Trichomonas vaginalis* and *Entamoeba histolytica in vitro* and *in vivo* and against various *Trypanosoma* species *in vivo*. Structure–activity relationships are discussed and comparisons of biological activity made with established drugs.

Since the discovery of the antibacterial and antiprotozoal activity of the antibiotic azomycin, **1**,[1-3] and the subsequent introduction of metronidazole,[4] **2**, as a clinically effective trichomonicide, many papers[5] have appeared on the biological properties of nitroimidazoles.

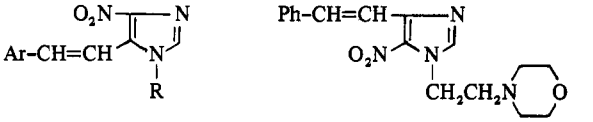


1 **2**

Although several papers and patents[6-8] have described the preparation of styrylimidazoles and their antitrichomonal and antiamoebic properties *in vitro* none of the compounds so far discussed appear to have *in vivo* activity against these protozoa. In an early paper, Ellis, *et al.*,[6] described the preparation of **3a** and its *in vitro* activity against *Trichomonas vaginalis* but no *in vivo* activity was reported. Similarly, during the course of our work, Giraldi and his coworkers[7] described a series of styrylimidazoles, exemplified by **3b** and **4**, which, although very active against *T. vaginalis* and *Entamoeba histolytica in vitro*, were devoid of *in vivo* activity.

In this paper we describe a series of 2-styryl-5-nitroimidazoles which exhibit a wide range of antiprotozoal activity both *in vitro* and *in vivo* against *T. vaginalis, E. histolytica,* and various *Trypanosoma* species.

Chemistry. The 2-styryl-5-nitroimidazoles were all prepared by the general route shown in Scheme I followed by modification of the N substituent in appropriate cases.



3 **4**

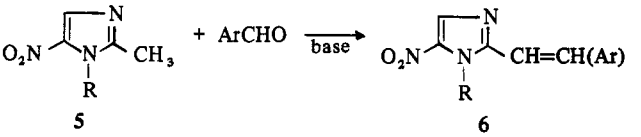
a, Ar = 3,4-$(Cl)_2$Ph; R = Me

b, Ar = Ph; R = CH_2CH_2NO

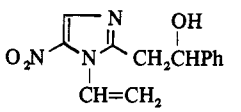
The *N*-vinyl series (Table IV) could be prepared by condensing the aromatic aldehyde with **5** (R = $CH{=}CH_2$) or by base elimination of the tosylate derived from **6** (R = CH_2CH_2OH).

In general, it was better to prepare the *N*-vinyl series by the latter method because compounds of type **6** (R = vinyl)

Scheme I



5 **6**



7

R = alkyl, hydroxylalkyl, or vinyl