

(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 filt'd 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- $\alpha$ -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  $CH_3N_2$  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- $\alpha$ -methylfluorene-2-acetate (23). Esterification of 19 with  $CH_3N_2$  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- $\alpha$ -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,<sup>11</sup> and gave 28 after crystn from EtOH (mp 172-174°, 15%). *Anal.* ( $C_{15}H_{12}O_2$ ) C, H.

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

$\alpha$ -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.

$\alpha$ -Methylfluorene-9-acetic Acid (31). A mixt of 9-bromo-fluorene (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.

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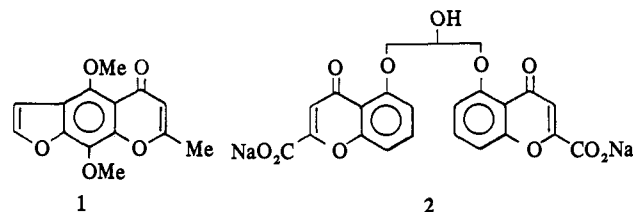
## Xanthone-2-carboxylic Acids, a New Series of Antiallergic Substances<sup>†</sup>

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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.<sup>1</sup> Starting from this observation, the Fisons group was able to develop disodium cromoglycate (2), which has become an exceptionally im-



<sup>†</sup>Publication No. 403 from the Syntex Institute of Organic Chemistry.

portant agent in the prophylactic treatment of the allergic condition underlying bronchial asthma.<sup>2,3</sup> Our work

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 <sup>a</sup>	No. of rats	PCA assay
2	H					1 (standard)
3 <sup>b</sup>	H					0.5
6	1-CH <sub>3</sub> O	I	211-213	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	41	<1
10a	3-CH <sub>3</sub> O	II	287-288	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	15	0.3
10b	4-CH <sub>3</sub> O	II	320-322	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	10	~0.1
10c	8-CH <sub>3</sub> O	II	295-297	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	15	0.4
14a	5-CH <sub>3</sub> O	III	329-331	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub>	60	3
14b	7-CH <sub>3</sub> O	III	280-281	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub> <sup>c</sup>	94	3
18b	6-CH <sub>3</sub> O	IV	>300	C <sub>15</sub> H <sub>10</sub> O <sub>5</sub> <sup>d</sup>	15	0.3
22a	7-CH <sub>3</sub>	V	300-302	C <sub>15</sub> H <sub>10</sub> O <sub>4</sub>	38	0.5
22b	7-C <sub>2</sub> H <sub>5</sub>	V	269-271	C <sub>16</sub> H <sub>12</sub> O <sub>4</sub>	45	3
22c	7- <i>n</i> -C <sub>3</sub> H <sub>7</sub>	V	254-255	C <sub>17</sub> H <sub>14</sub> O <sub>4</sub>	29	~1
22d	7- <i>i</i> -C <sub>3</sub> H <sub>7</sub>	V	274-276	C <sub>17</sub> H <sub>14</sub> O <sub>4</sub>	101	7
22e	7- <i>sec</i> -C <sub>4</sub> H <sub>9</sub>	V	240	C <sub>18</sub> H <sub>16</sub> O <sub>4</sub> <sup>e</sup>	30	2
22f	7- <i>n</i> -C <sub>4</sub> H <sub>9</sub>	V	242-244	C <sub>19</sub> H <sub>18</sub> O <sub>4</sub>	15	<0.3
22g	5- <i>i</i> -C <sub>3</sub> H <sub>7</sub>	V	277-279	C <sub>17</sub> H <sub>14</sub> O <sub>4</sub>	32	~3
22h	7-F	V	>300	C <sub>14</sub> H <sub>7</sub> FO <sub>4</sub>	27	~0.2
24	7-OH	VI	>300	C <sub>14</sub> H <sub>8</sub> O <sub>5</sub>	45	~1
25a	7-C <sub>2</sub> H <sub>5</sub> O	VI	287-289	C <sub>16</sub> H <sub>12</sub> O <sub>5</sub>	45	1
25b	7- <i>n</i> -C <sub>3</sub> H <sub>7</sub> O	VI	263-265	C <sub>17</sub> H <sub>14</sub> O <sub>5</sub>	45	1
25c <sup>f</sup>	7- <i>i</i> -C <sub>3</sub> H <sub>7</sub> O	VI	>300	C <sub>17</sub> H <sub>13</sub> NaO <sub>5</sub>	100	8
25d	7- <i>n</i> -C <sub>4</sub> H <sub>9</sub> O	VI	243-245	C <sub>18</sub> H <sub>16</sub> O <sub>5</sub> <sup>g</sup>	50	0.2
25e	5- <i>i</i> -C <sub>3</sub> H <sub>7</sub> O	VI	265-266	C <sub>17</sub> H <sub>14</sub> O <sub>5</sub>	45	4
26 <sup>h</sup>	7-CO <sub>2</sub> H		>300	C <sub>15</sub> H <sub>8</sub> O <sub>6</sub>	58	2

<sup>a</sup>All compds were analyzed for C and H. <sup>b</sup>Ref 11. <sup>c</sup>C: calcd, 66.67; found, 66.15. <sup>d</sup>C: calcd, 66.67; found, 66.26. <sup>e</sup>C: calcd, 72.96; found, 72.43. <sup>f</sup>Sodium salt. <sup>g</sup>C: calcd, 69.22; found, 69.72. <sup>h</sup>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,<sup>4</sup> led to the finding of significant antiallergic activity in xanthenes 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.

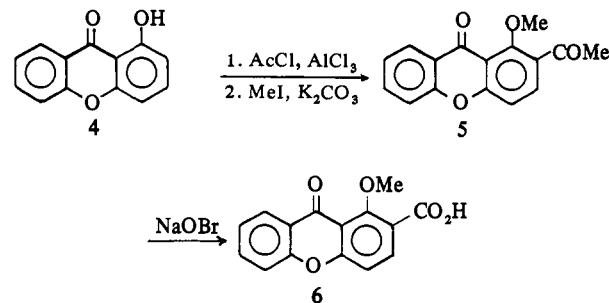
The relationship between activity and substitution pattern on the xanthone nucleus was initially determined using the methoxyl substituent. At positions 1, 3, 4, 6, or 8 (Table I, compds 6, 10a, 10b, 18b, and 10c) substitution was accompanied by either a decrease or by little change in activity relative 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 sig-

nificance 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<sup>5</sup> 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 non-challenged control rats 42 ± 5% of these cells degranulated.

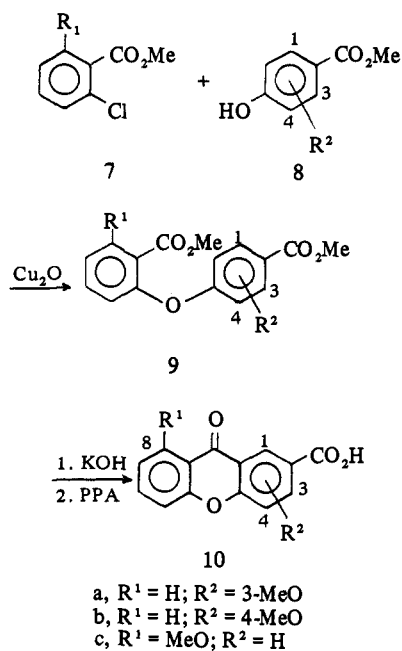
## Scheme I



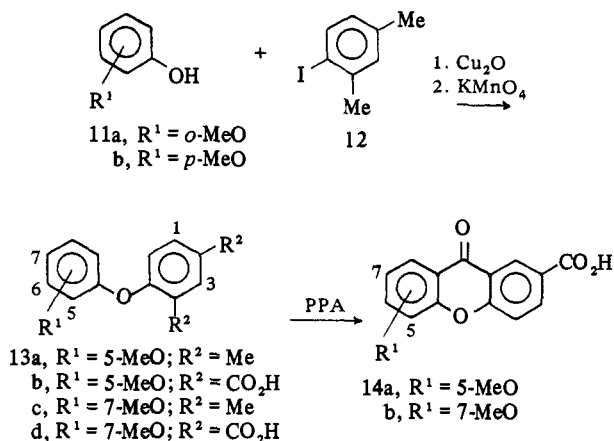
(This control reflects possible damage of the mast cells during tissue preparation.) Compound 25c significantly inhibited mast cell degranulation since only 29 ± 5% of the mast cells degranulated in challenged rats which were given 0.1 mg of 25c iv. Disodium cromoglycate and the xanthenes described here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

Since the work reported above was completed, claims<sup>6</sup> of antiallergy activity for some substituted xanthone-2-

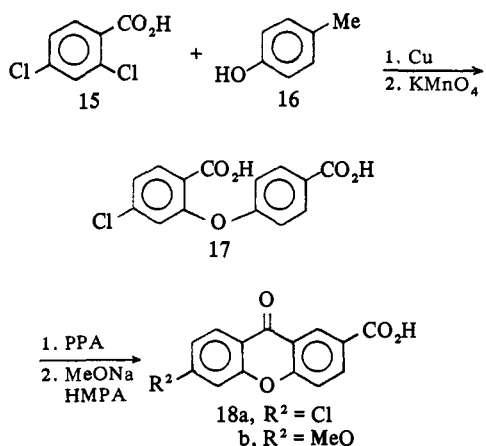
## Scheme II



## Scheme III



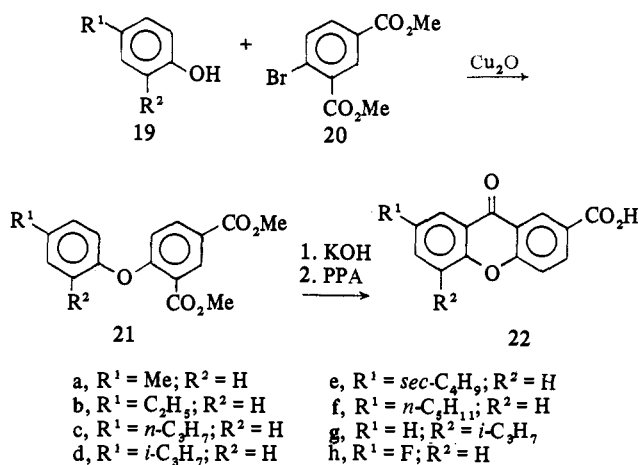
## Scheme IV



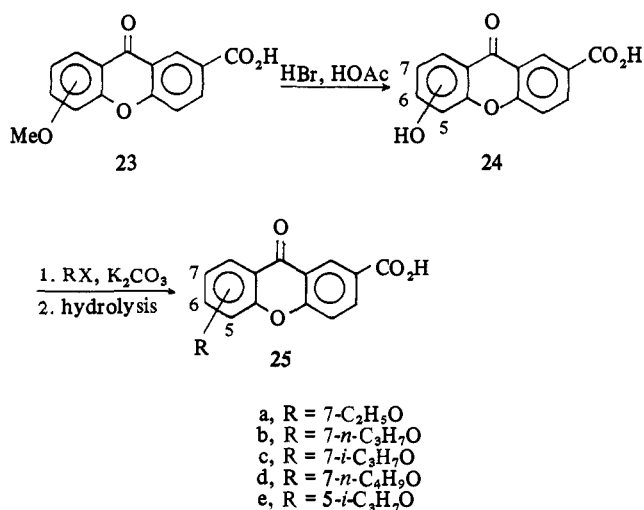
carboxylic acids have appeared in the patent literature but quantitative biological data have not been published.

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

## Scheme V



## Scheme VI



**Scheme I.** 1-Methoxyxanthone-2-carboxylic acid (6) was prepared by Friedel-Crafts acylation<sup>7</sup> 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<sup>8</sup> of methyl 2-chlorobenzoate (7a) with the phenol 8a forming the ether 9a, which was saponified and then cyclized<sup>9</sup> 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<sup>8</sup> 2,4-dimethyliodobenzene (12) with the phenol 11a forming the ether 13a, which was oxidized by KMnO<sub>4</sub> to the dicarboxylic acid 13b and cyclized by polyphosphoric acid or concd H<sub>2</sub>SO<sub>4</sub> 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<sup>9</sup> 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  $\text{Cu}_2\text{O}$  in dimethylacetamide (Schemes II, III, and V) and copper powder<sup>10</sup> with anhydrous potassium carbonate in dimethylformamide (Scheme IV). Dicarboxylic acids were cyclized to xanthone-carboxylic acids by polyphosphoric acid<sup>9</sup> in tetramethylene sulfone or with concd  $\text{H}_2\text{SO}_4$ . Methyl diphenyl ethers were oxidized to the corresponding diphenylcarboxylic acids by  $\text{KMnO}_4$  in *tert*-BuOH- $\text{H}_2\text{O}$ . Methanolysis of 6-chloroxanthone-2-carboxylic acid (18a) was performed as described by Goldberg and Wragg,<sup>9</sup> except that HMPA was used as solvent.

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E. Schuler for the biological data, and Dr. Howard Ringold for helpful comments.

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

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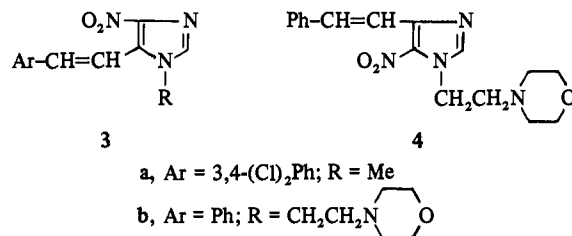
A series of 1-substituted-2-styryl-5-nitroimidazoles was prepared by condensing 1-substituted-2-methyl-5-nitroimidazoles with aryl aldehydes in the presence of sodium methoxide. The aryl aldehydes were substituted with aryl, alkyl, alkoxy, and chloro groups while the 1 substituent on the imidazole nucleus 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 arylolethynyleneimidazoles 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**,<sup>1-3</sup> and the subsequent introduction of metronidazole,<sup>4</sup> **2**, as a clinically effective trichomonicide, many papers<sup>5</sup> have appeared on the biological properties of nitroimidazoles.

Although several papers and patents<sup>6-8</sup> have described the preparation of styrylimidazoles and their antitrichomonal and antiamebic 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.*,<sup>6</sup> 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<sup>7</sup> 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.

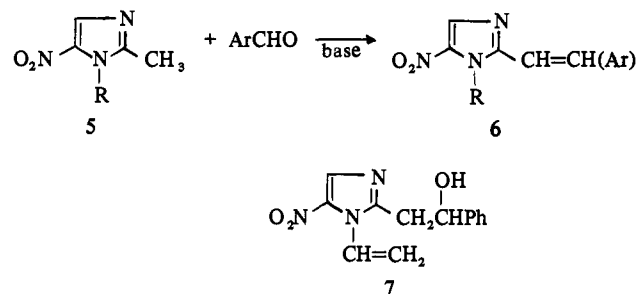
**Chemistry.** The 2-styryl-5-nitroimidazoles were all prepared by the general route shown in Scheme I followed by modification of the N substituent in appropriate cases.



The *N*-vinyl series (Table IV) could be prepared by condensing the aromatic aldehyde with **5** (R = CH=CH<sub>2</sub>) or by base elimination of the tosylate derived from **6** (R = CH<sub>2</sub>CH<sub>2</sub>OH).

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

### Scheme I



R = alkyl, hydroxyalkyl, or vinyl