which equilibrium between enzyme and subtrate is rapidly attained, while equilibrium between the enzyme and inhibitor (tightly or weakly binding) is slow. All of the compounds in this study (for bovine enzyme at both pH values and for murine enzyme at pH 7.20) were assayed at at least eight inhibitor concentrations (each in quadruplicate) by assay procedure 1 of ref 2: i.e., the reaction was initiated by mixing a solution of inhibitor (if any) and FAH_2 with a solution of enzyme which had been preincubated with NADPH. With this assay procedure, initial reaction velocities were found to decrease as the reaction proceeds, leveling off at a constant velocity within about 5–30 s. It was assumed that this observed effect was a result of the slow (relative to the time scale of the assay) attainment of a final equilibrium between enzyme, FAH_2, NADPH, and inhibitor. V_i (reaction velocities in presence of I) were then taken once this equilibrium had been established. V_0 (initial reaction velocity in absence of inhibitor) was linear from the start of the assay. All compounds were also assayed (for both enzymes at pH 7.20) at at least one concentration (each in quadruplicate) by assay procedure 2 of ref 2: i.e., the reaction was initiated by mixing a solution of FAH_2 with a solution of enzyme which had been preincubated with NADPH and inhibitor (if any). With this assay procedure, initial reaction velocities were found to increase as the reaction proceeds, leveling off at a constant velocity within about 5–30 s. V_i values were, as above, taken once equilibrium appeared to be established. Each compound (for both enzymes at pH 7.20) exhibited equal activities when assayed by these two procedures, thereby confirming that sufficient time was being allowed for equilibrium to be established.

As in our previous studies,[2,6-8] we have assumed that, under our assay conditions (i.e., saturating NADPH concentration), the triazine inhibitors of type I are acting as competitive inhibitors for FAH_2; hence, the log $(1/C)$ values were calcuated as log $[1/K_{i(app)}]$ values as in our previous study,[2] utilizing the jackknife procedure.[18] These $K_{i(app)}$ values are, for this study, equal to I_{50}, the concentration of inhibitor necessary for 50% inhibition of the enzyme, since the enzyme concentration was negligible compared to $K_{i(app)}$ (as determined by methotrexate titration of the enzymes; see ref 2).

No attempt was made in this study to further analyze the kinetics of the slow DHFR/NADPH/FAH_2/inhibitor equilibrium or how the structural features of the 2-substituents of I influence this equilibrium (as has been done with other inhibitors of DHFR[15]). As mentioned above, for the three disubstituted compounds (15–17, Table III), the rate of attainment of equilibrium was too slow (compared to the time scale of the assay) to permit reliable estimation of their log $[1/K_{i(app)}]$ values at this point. In this study we did observe, however, that the rate of approach to equilibrium does appear qualitatively to be inversely related to the bulk and/or number of X substitutents of I (e.g., especially the disubstituted compounds just mentioned).

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Synthesis and Antiallergy Activity of 4-Oxopyrano[3,2-*b*]indoles

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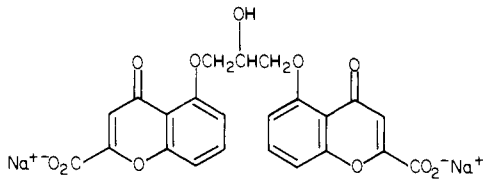
A series of 4-oxopyrano[3,2-*b*]indole carboxylic acids, tetrazoles, and carboxamidotetrazoles has been prepared and tested for antiallergy potential in the rat passive cutaneous anaphylaxis (PCA) assay. Many of the compounds showed activity comparable or superior to that of cromolyn sodium or doxantrazole. Several compounds were orally active.

Allergic reactions initiated by antigen–antibody interactions are thought to result from the release of mediators [histamine, slow-reacting substance of anaphylaxis (SRS-A), and others] of immediate hypersensitivity. The antiallergy agent cromolyn sodium (1) appears to act by inhibition of mediator release.[2]

Numerous research groups have reported antiallergy properties for additional compounds in a variety of chemical classes.[3] In some instances, a common structural feature is the presence of an acidic function located on, or in conjugation with, an aromatic or heteroaromatic ring.



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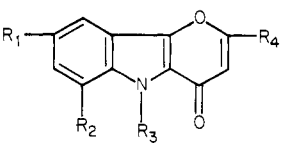
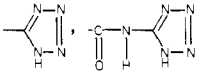


2, R_4 = CO_2H, 

A novel chemical series of this type is represented by acidic 4-oxopyrano[3,2-*b*]indoles, **2**.

We now report preliminary results concerning the preparation and antiallergic activity of a series of compounds of general structure **2**. Many of these compounds show high potency in the standard rat passive cutaneous

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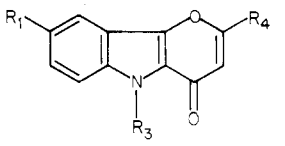
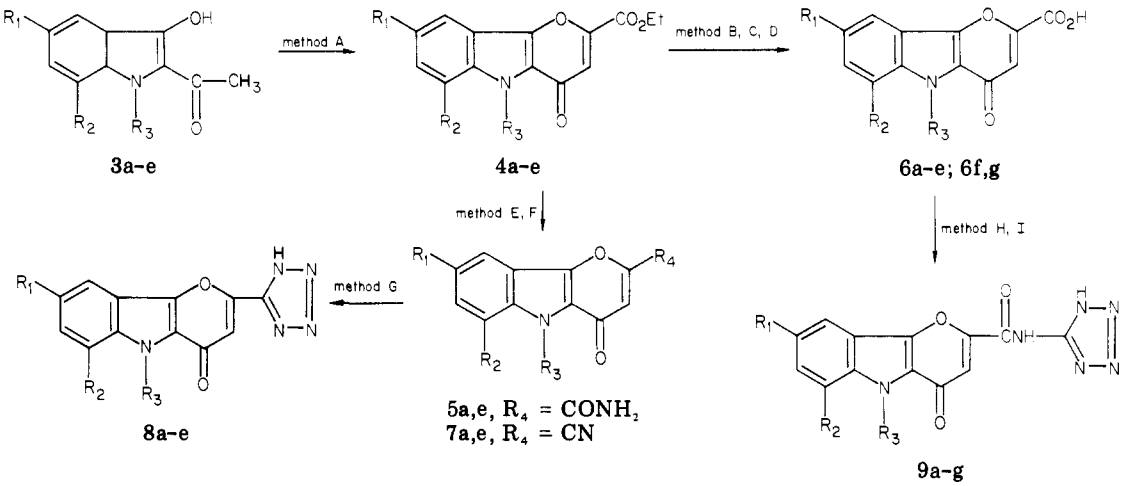
Table I. 4-Oxopyrano[3,2-*b*]indole Synthetic Intermediates



compd	R_1	R_3	R_4	formula	mp, °C	method[a]	yield,[b] %	crystn solvent	anal.
4a	H	CH_3	CO_2Et	$C_{15}H_{13}NO_4$	160-162	A	40	MeOH-H_2O	C, H, N
4b	H	C_2H_5	CO_2Et	$C_{16}H_{15}NO_4$	169-171	A	20	MeOH-H_2O	C, H, N
4c	H	C_6H_5	CO_2Et	$C_{20}H_{15}NO_4$	192-194	A	27	MeOH-H_2O	C, H, N
4d	Cl	CH_3	CO_2Et	$C_{15}H_{12}ClNO_4$	170-172	A	33	MeOH	C, H, Cl, N
4e	CH_3	CH_3	CO_2Et	$C_{16}H_{15}NO_4$	147-149	A	21	MeOH-H_2O	C, H, N
5a	H	CH_3	$CONH_2$	$C_{13}H_{10}N_2O_3$	>295	E	70	DMF-H_2O	C, H, N
5b	H	C_2H_5	$CONH_2$	$C_{14}H_{12}N_2O_3$	224-226	E	65	DMF-H_2O	C, H, N
5c	H	C_6H_5	$CONH_2$	$C_{18}H_{12}N_2O_3$	263-265	E	75	DMF-H_2O	C, H, N
5d	Cl	CH_3	$CONH_2$	$C_{13}H_9ClN_2O_3$	292-295	E	74	DMF-H_2O	H, Cl, N; C[c]
5e	CH_3	CH_3	$CONH_2$	$C_{14}H_{12}N_2O_3$	285 dec	E	85[d]		
7a	H	CH_3	CN	$C_{13}H_8N_2O_2$	190-192	F	85	EtOH	C, H, N
7b	H	C_2H_5	CN	$C_{14}H_{10}N_2O_2$	184-186	F	75	EtOH	C, H, N
7c	H	C_6H_5	CN	$C_{18}H_{10}N_2O_2$	180 dec	F	82	CH_3CN	C, H; N[e]
7d	Cl	CH_3	CN	$C_{13}H_7ClN_2O_2$	239-242	F	75	EtOAc-EtOH	C, H, Cl, N
7e	CH_3	CH_3	CN	$C_{14}H_{10}N_2O_2$	218-221	F	69	CH_3CN	C, H, N

[a] General preparative procedures A-I are given under Experimental Section. [b] Yields are not maximized. [c] C: calcd, 56.43; found, 55.93. [d] This material was dehydrated to the nitrile without prior recrystallization. [e] N: calcd, 9.79; found, 10.23.

Scheme I



anaphylaxis (PCA) test, when compared to the clinically effective antiallergy agents cromolyn sodium (1) and doxantrazole.[2,4]

Chemistry. Table I lists the various synthetic intermediates prepared. The 4-oxopyrano[3,2-*b*]indole esters, **4a**–e, were prepared (Scheme I) by cyclization of a series of indolylethanones,[5] **3a**–**e**, with diethyl oxalate and NaOEt.[3a] The esters were hydrolyzed to the carboxylic acids **6a**–**e** under either acidic or basic conditions. Two additional carboxylic acids **6f**,**g** were prepared by direct nitration of acid **6a**.

The carboxamidotetrazoles **9a**–**g** were obtained from the corresponding carboxylic acids **6a**–**g** and 1*H*-tetrazol-5-amine monohydrate. Amide formation was facilitated by the use of the coupling reagents 1,1′-carbonylbis(1*H*-imidazole) and "EEDQ" [ethyl 2-ethoxy-1(2*H*)-quinolinecarboxylate].

Intermediate amides **5a**–**e** were obtained by treating a suspension of the corresponding esters **4a**–**e** in EtOH at 0 °C with NH_3 and then stirring overnight at room temperature. The crude amides obtained by filtration could usually be dehydrated directly to the nitriles **7a**–**e** with tosyl chloride and pyridine in DMF.[6] Tetrazoles **8a**–**e** were then prepared from the corresponding nitriles and $NaN_3/AlCl_3$ in THF.

Details of the above preparative procedures are described under Experimental Section (general methods A–I and preparation of **6f**,**g**).

Biological Results and Discussion

The acidic 4-oxopyrano[3,2-*b*]indoles prepared and tested are listed in Table II. Many of the compounds tested showed antiallergic activity in the rat PCA test comparable to that of cromolyn sodium and doxantrazole, upon parenteral administration. In addition, several compounds (**6a**, **8b**, and **8e**) also exhibited significant oral activity.

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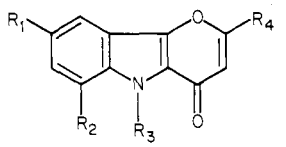
Table II. Inhibition of Rat PCA by Acidic 4-Oxopyrano[3,2-*b*]indoles



compd[a]	R_1	R_3	R_4	formula	mp, °C	method[b]	yield,[c] %	crystn solvent	anal.	rat PCA test, % inhibn 5.0 mg/kg ip	1.0 mg/kg ip	5.0 mg/kg po
6a	H	CH_3	CO_2H	$C_{13}H_9NO_4$	280 dec	B	62	DMF-H_2O	C, H, N	100	30	96
6b	H	C_2H_5	CO_2H	$C_{14}H_{11}NO_4$	275 dec	C, D	20	EtOAc	C, H, N	100[d]	0	22[e]
6c	H	C_6H_5	CO_2H	$C_{18}H_{11}NO_4$	>300	C	26	DMF-H_2O	C, H, N	100[d]	13	0[e]
6d	Cl	CH_3	CO_2H	$C_{13}H_8ClNO_4$	290 dec	C	31	DMF-H_2O	C, H, Cl, N	23		2
6e	CH_3	CH_3	CO_2H	$C_{14}H_{11}NO_4$	>300	C, D	22	DMF-H_2O	H, N; C[f]		18	11[e]
6f	NO_2	CH_3	CO_2H	$C_{13}H_8N_2O_6$	295 dec		56	DMF-H_2O	C, N; H[g,k]	100	1	0
6g	NO_2	CH_3	CO_2H	$C_{13}H_7N_3O_8$	265 dec		54	DMF-H_2O	C, H, N[h]	0		0
8a	H	CH_3	tet[i]	$C_{13}H_9N_5O_2$	270 dec	G	30	DMF-H_2O	C, H, N	100	87	39
8b	H	C_2H_5	tet	$C_{14}H_{11}N_5O_2$	255 dec	G	63	DMF-H_2O	C, H, N	100	100	70
8c	H	C_6H_5	tet	$C_{18}H_{11}N_5O_2$	245 dec	G	55	DMF-H_2O	C, H, N	100	61	32
8d	Cl	CH_3	tet	$C_{13}H_8ClN_5O_2$	265 dec	G	65	DMF-H_2O	C, H, N	100	46	33
8e	CH_3	CH_3	tet	$C_{14}H_{11}N_5O_2$	257 dec	G	35	DMF-H_2O	C, H, N	100	25	56
9a	H	CH_3	CONHtet	$C_{14}H_{10}N_6O_3$	295 dec	H	30	DMF	H, N; C[j]	100	28	0
9b	H	C_2H_5	CONHtet	$C_{15}H_{12}N_6O_3$	288 dec	H	45	DMF	C, H, N	100	12	1
9c	H	C_6H_5	CONHtet	$C_{19}H_{12}N_6O_3$	280 dec	I	54	DMF-H_2O	C, H, N[k]	0		7
9d	Cl	CH_3	CONHtet	$C_{14}H_9ClN_6O_3$	300 dec	H	34	DMF	C, H, Cl, N	100	38	6
9e	CH_3	CH_3	CONHtet	$C_{15}H_{12}N_6O_3$	290 dec	H	29	DMF	C, H, N	100	33	0
9f	NO_2	CH_3	CONHtet	$C_{14}H_9N_7O_5$	295 dec	I	25	DMF-H_2O	C, H, N[k]	100	42	15[l]
9g	NO_2	CH_3	CONHtet	$C_{14}H_8N_8O_7$	294 dec	I	52	DMF-H_2O	C, H, N	48		
1 (cromolyn sodium)										89[m]	50[n]	*o*
doxantrazole										97[p]	33	50

[a] For compounds **6g** and **9g**, R_2 = NO_2; all others, R_2 = H. [b] See footnote *a*, Table I. [c] See footnote *b*, Table I. [d] Tested at 1.0 mg/kg iv. [e] Tested at 2.0 mg/kg po. [f] C: calcd, 65.36; found, 64.71. [g] H: calcd, 3.05; found, 3.48. [h] Calculated as ·1DMF (C_3H_7NO). [i] tet = (1*H*-tetrazol-5-yl). [j] C: calcd, 54.19; found, 53.56. [k] Calculated as ·0.5H_2O. [l] Tested at 1.0 mg/kg po. [m] Tested at 10.0 mg/kg ip; see ref 9. [n] Tested at 2.0 mg/kg, ip; see ref 10. [o] Inactive. [p] Tested at 10.0 mg/kg ip.

Replacement of a carboxylic acid function with a 5-tetrazolyl ring has sometimes resulted in improved antiallergic potency.[3d,6,7] Such an improvement was generally evident in our chemical series; for example, tetrazoles **8b–e** were more potent than the corresponding carboxylic acids **6b–e**. In addition, the activity upon oral administration, with one exception, was significantly increased. Tetrazole **8a** was less potent upon oral administration than the corresponding acid **6a**; however, some oral activity was retained.

In contrast, the effect of replacement of the carboxyl function by the carboxamidotetrazole group[8] is less clear. Comparison of the potency of the carboxylic acids **6a–g** and the corresponding carboxamidotetrazoles **9a–g** shows both enhanced and diminished antiallergic activity throughout the series.

The substituent (R_3) on the indole nitrogen of the acidic 4-oxopyrano[3,2-*b*]indoles was changed from methyl to ethyl and phenyl with no obvious potency trend evident. The phenyl-substituted compound **9c**, however, was one of the few inactive compounds.

When the aromatic ring substituent in the 8 position (R_1) was hydrogen, the activity of the carboxylic acids **6a,b** and the tetrazoles **8a,b** was moderately increased over that of the compounds with R_1 = Cl, CH_3, or NO_2. A similar improvement was not evident with the carboxamidotetrazoles **9a–f**. Compounds **6g** and **9g**, containing two nitro substituents, exhibited significantly lower activity than their unsubstituted analogues **6a** and **9a**.

We have described the preparation and preliminary biological activity of acidic 4-oxopyrano[3,2-*b*]indoles, a new chemical series possessing significant antiallergic activity. Additional biological evaluation and the synthesis of other analogues are in progress.

Experimental Section

Rat Reaginic Passive Cutaneous Anaphylaxis (PCA) Test.[11] The PCA test involved immunization of rats with 1 mg of ovalbumin intramuscularly and approximately 10^{11} *B. pertussis* organisms as pertussis vaccine intraperitoneally. Fourteen days

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later, the rats were bled and the serum was prepared. Suitable dilutions of antiserum were injected intradermally at various sites on the back of rats 48 h before an intravenous injection of 1 mg of ovalbumin in 1 mL of physiological saline and 0.25% Evans blue. Thirty minutes after antigen challenge, the animals were killed in ether, the dorsal skin was reflected, and the mean orthogonal diameter of the wheal was measured. For oral or intraperitoneal dosing, the drugs were suspended in 1% gum tragacanth in physiological saline and given 10–15 min before intravenous antigen challenge. If necessary, the compounds were first dissolved in a slight molar excess of sodium bicarbonate and then diluted into the antigen solution. Groups of five animals were used for all dose levels and control groups.

To quantitate the PCA test, the mean diameter of each wheal spot was graphed as a function of the relative antiserum concentration. The line, fitted by the least-squares equation, was extrapolated to the value at "zero" antiserum concentration (base value). The following equation was then used to calculate the percent inhibition:

$$\% \text{ inhibn} = 1 - \left(\frac{\text{diameter of drug} - \text{base value}}{\text{diameter of control} - \text{base value}}\right) \times 100$$

The statistical significance of the results was determined by Student's t test ($p \leq 0.05$). An inhibition of 15% was significant.

Chemistry. Melting points were determined in a Mel-Temp capillary apparatus and are uncorrected. Spectra were recorded for all compounds and were consistent with the assigned structure. NMR spectra were recorded on a Varian EM-390 spectrometer at 90 MHz, with tetramethylsilane as an internal standard. IR spectra were recorded on a Digilab FTS-14 pulsed Fourier-transform spectrophotometer as Nujol mulls or KBr disks. UV spectra were determined in MeOH on a Cary Model 118 spectrophotometer. All compounds had elemental analyses within ±0.4% of the theoretical value, except where noted.

Method A. Ethyl 4,5-Dihydro-5-methyl-4-oxopyrano[3,2-*b*]indole-2-carboxylate (4a). To a solution of 6.0 g (0.26 mol) of sodium in 600 mL of EtOH was added 18.0 g (0.095 mol) of 1-(3-hydroxy-1-methyl-1*H*-indol-2-yl)ethanone,[5] followed by 37.6 g (0.26 mol) of diethyl oxalate, added over 10 min. The mixture was stirred at reflux for 17 h and cooled, and the reddish disodium salt was filtered and washed well with cold hexane. The crude salt was added to a solution of 28 mL of concentrated HCl and 120 mL of EtOH and stirred at reflux for 20 min, and the mixture was filtered while hot. The cooled filtrate yielded a gray solid, which was filtered and then dissolved in 400 mL of $CHCl_3$. The $CHCl_3$ solution was washed with 5% aqueous $NaHCO_3$ (3 × 200 mL) and H_2O (1 × 200 mL), dried ($MgSO_4$), and evaporated. Recrystallization of the residue from aqueous MeOH yielded yellow needles of ester **4a** (10.3 g, 40%), mp 160–162 °C.

The disodium salt intermediate of ester **4b** was obtained by evaporation (vacuum) of the bulk of the EtOH reaction solvent, followed by the addition of a large excess of cold hexane to the residue.

Method B. 4,5-Dihydro-5-methyl-4-oxopyrano[3,2-*b*]indole-2-carboxylic Acid (6a). A mixture of 5.4 g (0.02 mol) of the ester **4a** in 80 mL of 1% aqueous NaOH was stirred at room temperature for 30 min. (In some cases EtOH was added before extraction to aid in dissolution of the acid salt.) The nearly one-phase mixture was washed with CH_2Cl_2 (2 × 50 mL), and the aqueous layer was cooled in ice and acidified with 4 N HCl. The gelatinous crude acid was filtered, digested for a few minutes with 100 mL of hot H_2O, and refiltered warm. Recrystallization from EtOH gave yellow needles of acid **6a** (3.0 g, 62%), mp 280 °C dec.

Method C. 8-Chloro-4,5-dihydro-5-methyl-4-oxopyrano-[3,2-*b*]indole-2-carboxylic Acid (6d). A mixture of 3.5 g (0.012 mol) of the ester **4d** in 50 mL of glacial AcOH and 10 mL of concentrated HCl was stirred at reflux for 3 h, then cooled, and added to 300 g of ice/H_2O. The crude product was suspended in 75 mL of $CHCl_3$, stirred for 90 min, and then recovered by filtering. Recrystallization from DMF–H_2O yielded the acid **6d** as yellow flakes (1.0 g, 31%), mp 290 °C dec.

Method D. 4,5-Dihydro-5-methyl-4-oxopyrano[3,2-*b*]indole-2-carboxylic Acid (6b). A mixture of 8.5 g (0.025 mol) of the red disodium salt intermediate obtained from ester **4b** (method A) and 50 mL of glacial AcOH plus 10 mL of concentrated HCl was stirred at reflux for 90 min. After the solution was cooled and 500 g of ice/H_2O was added, the crude solid was filtered and washed with ice water. The crude acid was dissolved in 40 mL of 1 N NaOH, and an additional 20 mL of H_2O was added. The solution was washed with CH_2Cl_2 (3 × 40 mL), cooled in ice, and acidified with 4 N HCl. The brown solid was filtered, washed with H_2O, and recrystallized twice from EtOAc to yield the acid **6b** as a tan solid (1.3 g, 20%), mp 275 ° C dec.

Method E. 4,5-Dihydro-5-methyl-4-oxopyrano[3,2-*b*]indole-2-carboxamide (5a). A suspension of 11.0 g (0.041 mol) of the ester **4a** in 500 mL of EtOH was cooled in ice while gaseous NH_3 was added slowly until the reaction mixture temperature rose to 20 °C (ca. 10 min). The ice bath was removed, and stirring was continued for 24 h. The light yellow solid was filtered, washed with a little cold EtOH, and recrystallized from DMF/H_2O to yield the amide **5a** (6.9 g, 70%), light yellow needles of mp >295 °C.

Method F. 4,5-Dihydro-5-methyl-4-oxopyrano[3,2-*b*]indole-2-carbonitrile (7a). A mixture of 10.5 g (0.043 mol) of the amide **5a**, 12.6 g (0.066 mol) of *p*-toluenesulfonyl chloride, and 10.3 g (10.5 mL, 0.13 mol) of pyridine in 55 mL of DMF was heated under N_2 on a steam bath for 4 h. The cooled mixture was added to 500 g of ice/H_2O and stirred for 20 min, and the pink solid was filtered and washed with cold H_2O. Two recrystallizations from EtOH yielded light pink needles of nitrile **7a** (8.2 g, 85%), mp 190–192 °C.

Method G. 5-Methyl-2-(1*H*-tetrazol-5-yl)pyrano[3,2-*b*]indol-4(5*H*)-one (8a). To a mixture of 4.0 g (0.018 mol) of the nitrile **7a** and 4.0 g (0.062 mol) of NaN_3 in 500 mL of THF under N_2 was added 5.3 g (0.040 mol) of anhydrous $AlCl_3$ in portions over 20–30 min. The mixture was stirred at reflux for 21 h, cooled, and an additional 2.0 g (0.031 mol) NaN_3 and 4.8 g (0.036 mol) $AlCl_3$ were similarly added. Stirring at reflux was continued for a total of 61 h. The reaction mixture was cooled in ice and then added cautiously to ca. 2.0 L of ice/H_2O. Acidification with 4 N HCl (**caution: HN_3 is evolved**) at ≤5 °C yielded a tan solid, which was filtered, digested on the steam bath for 30 min with 200 mL of H_2O, and then refiltered warm. The crude product was stirred for 30 min in 150 mL of Me_2CO and then recovered by filtering. Recrystallization from DMF–H_2O yielded yellow plates of tetrazole **8a** (1.4 g, 30%), mp 270 °C dec.

Method H. 8-Chloro-4,5-dihydro-5-methyl-4-oxo-*N*-(1*H*-tetrazol-5-yl)pyrano[3,2-*b*]indole-2-carboxamide (9d). A mixture of 2.5 g (0.0091 mol) of acid **6d** and 4.8 g (0.020 mol) of ethyl 2-ethoxy-1(2*H*)-quinolinecarboxylate (EEDQ) in 100 mL of benzene was stirred for 45 min under nitrogen. To the reaction mixture was added 1.1 g (0.011 mol) of 1*H*-tetrazol-5-amine monohydrate, and stirring was continued for a total of 88 h. The precipitate that had formed was filtered, stirred overnight in 50 mL of MeOH, and refiltered. Two recrystallizations from DMF yielded the carboxamidotetrazole **9d** as a yellow solid (1.1 g, 34%) of mp 300 °C dec.

Method I. 4,5-Dihydro-5-methyl-8-nitro-4-oxo-*N*-(1*H*-tetrazol-5-yl)pyrano[3,2-*b*]indole-2-carboxamide (9f). A mixture of 4.7 g (0.016 mol) of acid **6f** and 3.8 g (0.023 mol) of 1,1′-carbonylbis(1*H*-imidazole) in 100 mL of DMF was heated at 60 °C for 1 h and then cooled to room temperature. In a separate flask, a mixture of 1.8 g (0.018 mol) 1*H*-tetrazol-5-amine monohydrate and 5.4 g (0.053 mol) of Et_3N were combined in 50 mL of DMF. This mixture was cooled in an ice bath while 5.8 g (0.053 mol) of chlorotrimethylsilane was added in one portion. Stirring in ice was continued for 1 h, followed by an additional 2 h with the ice bath removed. The Et_3N mixture was then added to the original carboxylic acid mixture, and the new mixture was heated on a steam bath for 1 h. The mixture was cooled, and the precipitated Et_3N·HCl was filtered and discarded. The filtrate was added to 500 g of ice/H_2O, the mixture was filtered by gravity, and the filtrate was acidified with 4 N HCl. The precipitated crude product was filtered, washed with H_2O, and recrystallized twice from DMF–H_2O to yield the carboxamidotetrazole **9f** as a hemihydrate (1.5 g, 25%), yellow solid of mp 295 °C dec.

4,5-Dihydro-5-methyl-8-nitro-4-oxopyrano[3,2-*b*]indole-2-carboxylic Acid (6f). Acid **6a** (8.2 g, 0.034 mol) was added over a few minutes to 80 mL of concentrated H_2SO_4 cooled in ice. When solution was complete, 3.5 g (0.035 mol) of KNO_3 was added in portions over 30 min. The mixture was stirred in ice for a total

of 5 h and then poured over 750 g of ice/H_2O. The crude product was filtered, stirred briefly in 200 mL of 50% aqueous EtOH, and refiltered. Two recrystallizations from DMF–H_2O yielded the mononitro acid **6f** as a hemihydrate (5.7 g, 56%), fine yellow needles of mp 295 °C dec.

4,5-Dihydro-5-methyl-6,8-dinitro-4-oxopyrano[3,2-*b*]-indole-2-carboxylic Acid (6g). Acid **6a** (6.0 g, 0.025 mol) was added over a few minutes to 22 mL of concentrated H_2SO_4 cooled in ice. Concentrated HNO_3 (3.0 mL, 0.048 mol) was then added in one portion, and the mixture was stirred and heated on the steam bath for 30 min. The cooled mixture was added to 300 g of ice/H_2O, and the crude product was filtered and washed with cold H_2O. Two recrystallizations from DMF–H_2O yielded the dinitro acid **6g** as a DMF complex (5.5 g, 54%), yellow needles of mp 265 °C dec.

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Note added in proof: After this manuscript had been submitted, the preparation of several of the compounds described was reported by other workers.[12]

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Antiarrhythmic Activity of Amitriptyline Analogues in Conscious Dogs after Myocardial Infarction: Cyproheptadinium Methiodide[1]

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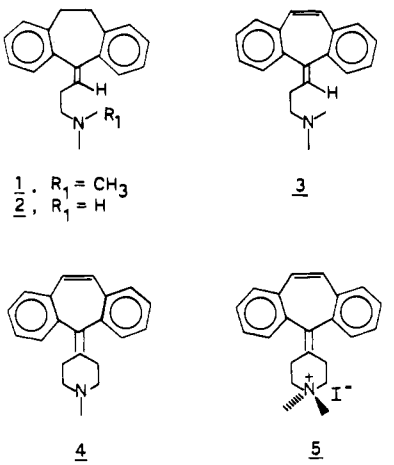
The antiarrhythmic effects of amitriptyline (1), its secondary amine metabolite nortriptyline (2), as well as cyclobenzaprine (3) and cyproheptadine (4), tertiary amine analogues of 1, were studied in conscious dogs 24 h after myocardial infarction. Since the sedative side effect of 4 presents a potential problem for its clinical use, a quarternary derivative of 4, cyproheptadine methiodide (5), was prepared and its effects also studied in this model. Complete conversion to a normal sinus rhythm occurred in all animals studied after cumulative doses of 1700 μg/kg (6.17 μmol/kg) of 3, 1300 μg/kg (4.69 μmol/kg) of 1, 300 μg/kg (1.04 μmol/kg) of 4, and 25 μg/kg (0.058 μmol/kg) of 5. While 2 significantly decreased ventricular ectopic activity, it did not convert any of the animals studied to a sinus rhythm at doses up to 3000 μg/kg. Thus, the order of potency for conversion to a normal sinus rhythm appears to be 5 ≫ 4 > 1 > 3 ≫ 2. These data suggest that 5 is very potent in converting ventricular arrhythmias associated with myocardial infarction.

Although the cardiotoxic effects of tricyclic antidepressant drugs have been thoroughly described,[2-4] recent evidence suggests that these drugs may exert a potentially beneficial effect on cardiac rhythm abnormalities.[5,6] This antiarrhythmic action also has been observed in a limited number of patients with ventricular and supraventricular arrhythmias being treated for depression with imipramine.[7]

Recently, it has been demonstrated that imipramine exerts cardiac electrophysiologic effects similar to other antiarrhythmic drugs.[8,9] Imipramine decreased the rate of phase 0 depolarization and shortened the action potential duration in Purkinje fibers.[8,9] Imipramine also decreased membrane responsiveness and conduction velocity in isolated Purkinje preparations.[9] Unlike classical antiarrhythmic drugs, however, imipramine did not decrease the rate of diastolic depolarization in concentrations sufficient to render Purkinje fibers unresponsive to external stimuli.[8]

Chart I



Antiarrhythmic activity of tricyclic antidepressant drugs demonstrated against arrhythmias induced by ouabain administration in anesthetized dogs suggested that tertiary amine tricyclics were considerably more potent than secondary amine members of this group.[5] This may be related to the fact that tertiary amine tricyclics are less effective inhibitors of norepinephrine uptake than secondary amines,[10] since inhibition of catecholamine uptake and the

(1) Portions of this work were presented at the 179th ACS National Meeting; see: "Abstracts of Papers", 179th National Meeting of the American Chemical Society, Houston, TX, Mar 24–27, 1980, American Chemical Society: Washington, D.C., 1980; Abstr MEDI 35.
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