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(47) Acid-washed AU-4 charcoal, purchased from Barneby-Cheney, Columbus, Ohio.
(48) Distilled in glass, purchased from Mallinckrodt Chemicals, St. Louis, Mo.

4-Hydroxy-3-nitro-2-quinolones and Related Compounds as Inhibitors of Allergic Reactions

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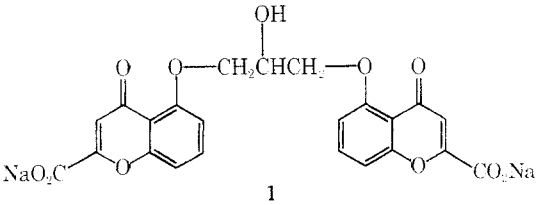
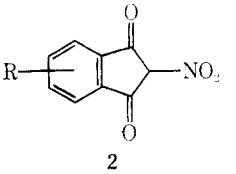
The synthesis and biological activity of a number of 4-hydroxy-3-nitro-2-quinolones are discussed and compared with their related hydroaromatic analogs. Antiallergic activity has been assessed by their ability to inhibit the homocytotropic antibody–antigen induced passive cutaneous anaphylaxis reaction in the rat.

In 1967 disodium cromoglycate (**1**) was introduced as a treatment for asthma and it was shown that it could inhibit the release of spasmogens induced by antigen challenge of tissue sensitized with immunoglobulin E.[1] Since the introduction of compound **1**, a variety of compounds have been claimed to possess a similar activity. Many of these possess an acidic function attached to a carbon atom linked by ethylenic conjugation to a carbonyl group. This acidic function is often a carboxyl group although this can be replaced by the tetrazolyl group.[2] We have described compounds which show a similar type of activity to disodium cromoglycate and yet are of a somewhat different type in that they possess the α-nitro-β-dicarbonyl moiety, a system which again confers acidity.[3]



1



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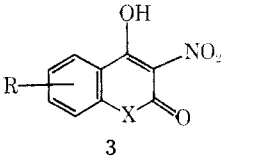
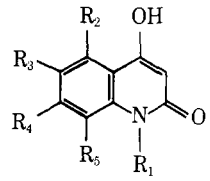


3

Following the observation that activity was shown by 2-nitro-1,3-indandiones (**2**)[3,4] and to a lesser extent in their reduced derivatives,[5] we have embarked on a program of ring-expanded systems of type **3**. As part of this program we have reported on the activity of 4-hydroxy-3-nitrocoumarins[6] (**3**, X = O) and currently wish to report our work on the analogous nitrogen compounds (**3**, X = NR).

Compounds have been compared with disodium cromoglycate for antiallergic activity by assessing their relative

Table I. Physical Properties of Substituted 4-Hydroxy-2-quinolones

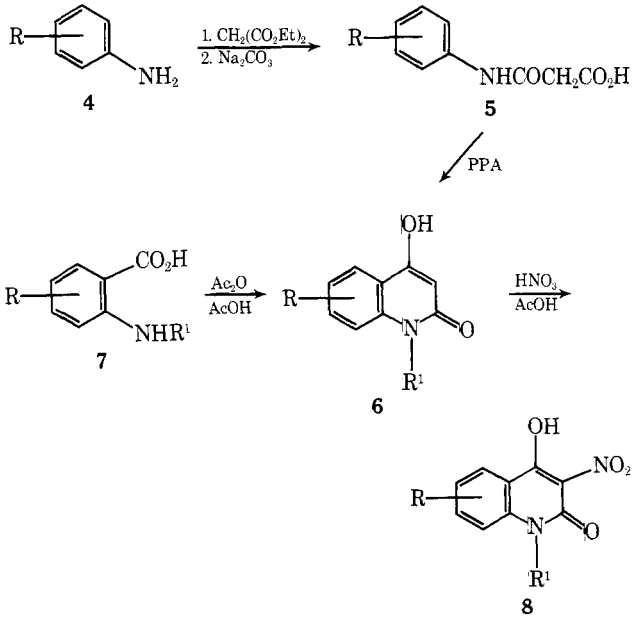


Compd	R_1	R_2	R_3	R_4	R_5	Mp, °C	Formula	Analyses	Lit. mp, °C	Yield, %
6a	H	H	H	H	H	>320	$C_9H_7NO_2$		360[a]	70
6b	H	Me	H	Me	H	>365	$C_{11}H_{11}NO_2$	C, H, N		67
6c	H	Me	H	H	Me	>340	$C_{11}H_{11}NO_2$	C, H, N		90
6d	H	H	Me	H	H	340–345 dec	$C_{10}H_9NO_2$	C, H, N	342[a]	68
6e	H	H	Et	H	H	328	$C_{11}H_{11}NO_2$	H, N; C[b]		64
6f	H	H	Br	H	H	>340	$C_9H_6BrNO_2$	C, H, Br, N		94
6g	H	H	Cl	H	H	>360	$C_9H_6ClNO_2$	C, H, Cl, N	370[a]	78
6h	H	H	Me	Me	H	>340	$C_{11}H_{11}NO_2$	C, H, N	345[a]	100[c]
6i	H	H	Me	H	Me	342–350	$C_{11}H_{11}NO_2$	C, H, N		84
6j	H	H	Et	Et	H	>300	$C_{13}H_{15}NO_2$	C, H, N		38[c]
6k	H	H	H	Me	H	>330	$C_{10}H_9NO_2$	C, H, N	388[a]	69[d]
6l	H	H	H	Me	Me	348 dec	$C_{11}H_{11}NO_2$	C, H, N		74
6m	H	H	H	H	Me	>330	$C_{10}H_9NO_2$	C, H, N	360[a]	99
6n	H	H	H	H	Br	271	$C_9H_6BrNO_2$	C, H, Br, N		100
6o	H	H	H	H	Cl	305	$C_9H_6ClNO_2$	C, H, Cl, N		100
6p	Me	H	H	H	H	270–273	$C_{10}H_9NO_2$	C, H, N	252–262[e]	46
6q	Et	H	H	H	H	273–275	$C_{11}H_{11}NO_2$	C, H, N		49
6r	Ph	H	H	H	H	298–300	$C_{15}H_{11}NO_2$	H, N; C[f]		15
6s	Me	H	Me	H	H	290–298	$C_{11}H_{11}NO_2$	C, H, N		47
6t	Et	H	Me	H	H	275–280	$C_{12}H_{13}NO_2$	C, H, N		34
6u	Me	H	Cl	H	H	317–318	$C_{10}H_8ClNO_2$	C, H, Cl, N		37
6v	Et	H	Cl	H	H	305–308	$C_{11}H_{10}ClNO_2$	C, H, Cl, N		39
6w	Me	H	Me	Me	H	300–305 dec	$C_{12}H_{13}NO_2$	C, H, N		67
6x	Me	H	H	Cl	H	308–309	$C_{10}H_8ClNO_2$	C, H, Cl, N	291–305[e]	37

[a] G. H. Patel and C. M. Mehta, *J. Sci. Ind. Res., Sect. B*, 436 (1960). [b] C: calcd, 69.84; found, 68.94. [c] Mixture of 5,6 and 6,7 isomers with the latter predominating. [d] A mixture of 5 and 7 isomers with the latter predominating. [e] R. E. Lutz, J. F. Codington, R. J. Rowlett, A. J. Deinet, and P. S. Bailey, *J. Am. Chem. Soc.*, **68**, 1810 (1946). [f] C: calcd, 75.94; found, 75.40.

abilities to inhibit the rat passive cutaneous anaphylaxis reaction mediated by the rat IgE or rat PCA test.

Scheme I

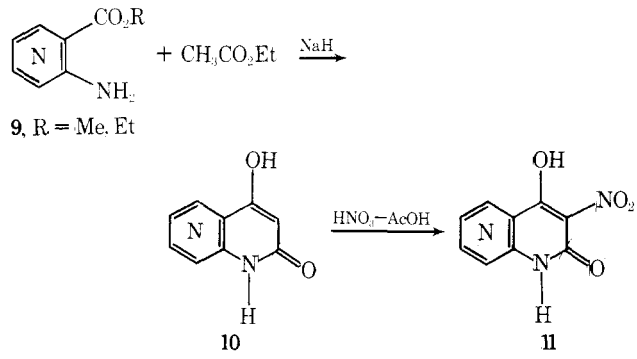


R^1 = H, alkyl, or aryl

Chemistry. A variety of routes for the synthesis of 4-hydroxy-2-quinolones (**6**) are known, most of which utilize readily available anilines or anthranilic acids. We have chosen two procedures (Scheme I) depending on the nature of the nitrogen substituent. Acylation of the appropriate aniline **4** with diethyl malonate and hydrolysis afforded the malonic acid monoanilides **5**,[7] which cyclized in polyphosphoric acid (PPA) to give the quinolones **6** (R = H) in high yield. For those derivatives substituted at nitrogen (**6**, R = alkyl or aryl) the synthesis was effected by acetylation and concomitant cyclization of the appropriate anthranilic acid **7** using a mixture of acetic acid and anhydride.[8] Alternatively, lower *N*-alkyl homologs of **6** may be prepared by taking advantage of the preferential attack on nitrogen using alkaline dialkyl sulfate.[9] Nitration of **6** with a mixture of nitric and acetic acids was accomplished at 100° in good yield. As expected, for asymmetric anilines the conversion of **5** to **6** resulted in the formation of two isomers when possible. In our hands these were inseparable as the hydroxy quinolones **6** but the major isomers were readily isolable by fractional recrystallization after nitration. In general, the product derived by the least sterically hindered route was preferentially formed (Table I).

The 4-hydroxy-3-nitronaphthyridines or azanitro-2-quinolones **11a–d** were prepared as shown in Scheme II. Condensation of the appropriate aminopyridine carboxylate (**9**) with ethyl acetate[10] followed by nitration of the resulting naphthyridine **10** gave **11a–d.** The bridgehead de-

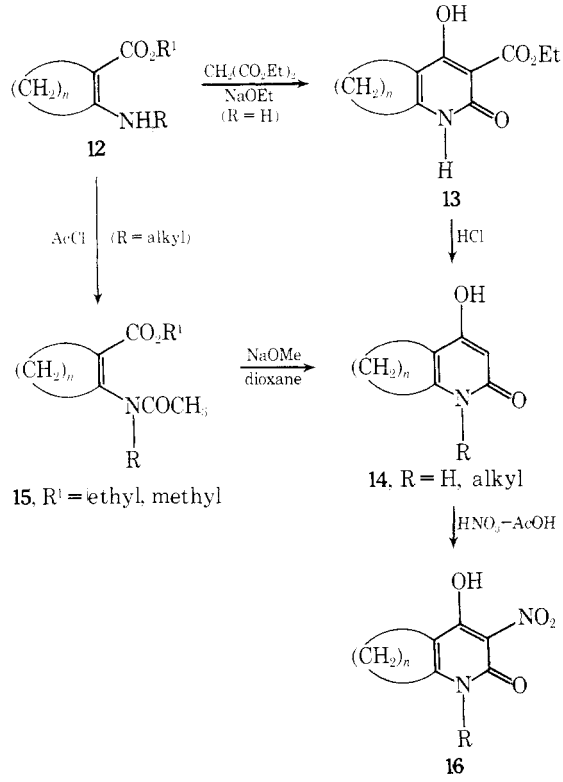
Scheme II



rivative **11e**, which is not a true member of this class, was obtained by nitration of the condensation product from 2-aminopyridine and diethyl malonate.[11]

Synthesis of the hydroaromatic derivatives **16** was accomplished as shown in Scheme III. The requisite 2-amino

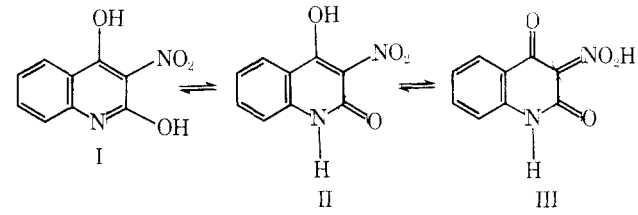
Scheme III



cycloalkenoate **12** (R = H) gave the 2-pyridone esters **13** on reaction with diethyl malonate which were quantitatively decarboxylated to the 4-hydroxy-2-pyridones (**14**, R = H) in refluxing acid.[12] Derivatives bearing *N*-alkyl substituents (**14**, R = alkyl) were prepared by acetylation of the amino esters **12** (R = alkyl) followed by ring closure of the resulting amides **15** with sodium methoxide in dioxane.[13] Nitration as before afforded the 3-nitro derivatives **16**.

Results and Discussion

Within the 2-nitroindandione system **2** the adverse biological effects encountered on modification of the 2-nitro-1,3-dicarbonyl moiety have been noted, as too has the need to retain this unit within a cyclic system.[3,4] Following these observations we have expanded the functional ring to incorporate both oxygen[6] and nitrogen atoms (**3**, X = O or NR, respectively) and have observed a retention of activity. Apparently neither the electronic effects of the heteroatom nor the increase in size of the functional ring seriously affect the biological efficacy of this system. To some extent this result may be reflected in the preferred tautomeric forms of these systems, which from physical evidence appear to be similar. Both the nitroindandiones **2** and nitroquinolones **3** (X = NR) are strong acids ($pK_a \approx 1$ for 2-nitroindandione) and from the ir spectrum of the parent nitroquinolone **8a** strong bands in the region 1670–1680 cm^{-1} indicate carbonyl absorption. A broad band at 2800 cm^{-1} suggests the presence of a hydrogen bonded hydroxyl group. On this evidence alone the existence of the dihydroxy quinoline tautomer I in the solid phase may be rejected. From ^{13}C NMR a similar conclusion may be drawn but differentiation between II and III is difficult.



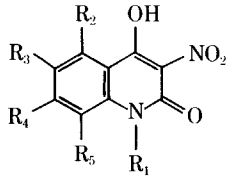
Evidence for tautomeric forms akin to II and III has been found in the nitroindandione series. A similar result has been found in the *N*-alkyl derivatives **8** which cannot generate the dihydroxyquinoline tautomer I and it is probably on account of this similarity of preferred tautomeric forms that the biological properties of the *N*-methyl derivatives compare favorably with their nonalkylated analogs. With the larger *N*-ethyl substituent, however, and more especially with *N*-phenyl, a more noticeable drop in activity occurs. Possibly the reduction in these cases is a result of steric crowding at the active site.

Within the group as a whole (Table II) greatest activity is found with alkyl groups at both C-6 and C-7 (**8j** and **8l**) which are of the same order of activity as disodium cromoglycate (ED_{50} 6.7 mg/kg). Substitution at C-8 with either alkyl or halogeno groups also results in favorable activity (ED_{50} 7–15 mg/kg) but other substitutions in general give a diminished activity.

Replacement of the carbocyclic support ring by pyridine residues (Table III) results in good activity only when nitrogen is at position 7 (**11c**), the compound then being as active as the parent **8a**. The bridgehead nitrogen derivative **11e** is inactive.

Finally we studied a selection of hydroaromatic 3-nitro-2-quinolones (Table VI) in which planarity at the ring junction was preserved. Within the nitroindandione system reduced forms having a cis-fused ring junction were markedly less active than their aromatic counterparts[5] and it was therefore of interest to note a similar level of activity in the hydroaromatic 2-quinolones not alkylated at nitrogen (**16a–d**) to that of their aromatic analogs. This would suggest that it is indeed planarity at the ring junction which is of importance with regard to biological efficacy and not some function of the aromatic support ring. With increasing size of the N-substituents in the hydroaromatic 2-quinolones PCA activity was found to fall off rapidly, a result which parallels that found in the aromatic series. Furthermore, biological activity was influenced by variation of the support ring size (Table VI, **16i–k**). Although activity is maximal in the cyclohexyl derivative **16a**, it is present in both the cycloheptyl and cyclooctyl homologs, **16j** and **16k**, respectively. The cyclopentyl derivative **16i**, however, was inactive in doses up to 100 mg/kg. These differences are probably best rationalized in terms of the high angular strain in **16i** in which the internal angles made by the cyclopentyl ring at the ring junction are ca. 108° com-

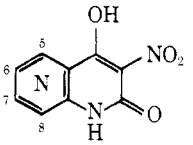
Table II. 4-Hydroxy-3-nitro-2-quinolones



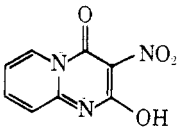
Compd	R_1	R_2	R_3	R_4	R_5	Mp, °C	Formula	Analyses	Lit. mp, °C	Yield, %	Act. in rat PCA test, ED_{50},[a] mg/kg sc at T max[b]	T max[b]
1	Disodium cromoglycate										6.7 (4.9–9.3, 14.7, 56)	10
8a	H	H	H	H	H	216 dec	$C_9H_6N_2O_4$	C, H, N	216–218[c]	89	17 (*e*, 41, 18)	10
8b	H	Me	H	H	H	231–233 dec	$C_{10}H_8N_2O_4$	C, H, N		94	*d*	
8c	H	Me	H	Me	H	226 dec	$C_{11}H_{10}N_2O_4$	C, H, N		100	38 (*e*, 40, 12)	30
8d	H	Me	H	H	Me	270–272 dec	$C_{11}H_{10}N_2O_4$	C, H, N		97	50 (*e*, 59, 30)	30
8e	H	H	Me	H	H	245 dec	$C_{10}H_8N_2O_4$	C, H, N		75	78 (*e*, 54, 18)	30
8f	H	H	Et	H	H	220–222 dec	$C_{11}H_{10}N_2O_4$	C, H, N		63	14 (*e*, 50, 30)	10
8g	H	H	Br	H	H	213–214 dec	$C_9H_5BrN_2O_4$	C, H, Br, N		76	>100	
8h	H	H	Cl	H	H	200 dec	$C_9H_5ClN_2O_4$	C, H, Cl, N		68	50 (*e*, 46, 36)	45
8i	H	H	NO_2	H	H	199–200 dec	$C_9H_5N_3O_6$	C, H, N	200 dec[f]	60	>50	
8j	H	H	Me	Me	H	275–277	$C_{11}H_{10}N_2O_4$	C, H, N		60	3.8 (1.0–14, 68, 60)	10
8k	H	H	Me	H	Me	290 dec	$C_{11}H_{10}N_2O_4$	C, H, N		64	*d*	
8l	H	H	Et	Et	H	248–250 dec	$C_{13}H_{14}N_2O_4$	C, H, N		98	1.5 (0.5–4.0, 63, 24)	10
8m	H	H	H	Me	H	224–227 dec	$C_{10}H_8N_2O_4$	C, H, N		94	*d*	
8n	H	H	H	Me	Me	284–285 dec	$C_{11}H_{10}N_2O_4$	C, H, N		97	4.9 (1.7–15.7, 97, 18)	10
8o	H	H	H	H	Me	260	$C_{10}H_8N_2O_4$	C, H, N		64	8.8 (4.5–17.3, 105, 18)	10
8p	H	H	H	H	Br	191 dec	$C_9H_5BrN_2O_4$	C, H, Br, N		84	13 (5.6–31, 86, 24)	10
8q	H	H	H	H	Cl	204–205 dec	$C_9H_5ClN_2O_4$	C, H, Cl, N		97	10 (1.3–95, 73, 18)	20
8r	Me	H	H	H	H	159–161	$C_{10}H_8N_2O_4$	C, H, N		65	23 (*e*, 52, 17)	10
8s	Et	H	H	H	H	154–155 dec	$C_{11}H_{10}N_2O_4$	C, H, N		88	27 (12–64, 134, 17)	20
8t	Ph	H	H	H	H	167–169	$C_{15}H_{10}N_2O_4$	C, H, N		38	>100	
8u	Me	H	Me	H	H	185	$C_{11}H_{10}N_2O_4$	C, H, N		80	19 (8.0–45, 84, 22)	10
8v	Et	H	Me	H	H	192–194	$C_{12}H_{12}N_2O_4$	C, H, N		93	*d*	
8w	Me	H	Cl	H	H	171 dec	$C_{10}H_7ClN_2O_4$	C, H, Cl, N		58	90 (*e*, 38, 18)	60
8x	Et	H	Cl	H	H	205–206 dec	$C_{11}H_9ClN_2O_4$	H, Cl; C,[g] N[h]		69	>100	
8y	Me	H	Me	Me	H	220–224 dec	$C_{12}H_{12}N_2O_4$	C, H, N		34	9.3 (4.5–19, 87, 17)	10
8z	Me	H	H	Cl	H	164–165 dec	$C_{10}H_7ClN_2O_4$	C, H, Cl, N		90	56 (*e*, 33, 23)	45

[a]Figures in parentheses are 95% confidence limits, slope of inhibition/log dose line, number of animals used. [b]T max is the time between sc administration of the drug and challenge to give maximum activity. [c]S. Gabriel, *Chem. Ber.*, **51**, 1500 (1918). [d]Insufficient data for complete analysis. [e]Insufficient data to calculate confidence limits. [f]J. N. Ashley, W. H. Perkin, and R. Robinson, *J. Chem. Soc.*, 382 (1930). [g]C: calcd, 49.18; found, 49.91. [h]N: calcd, 10.43; found, 9.79.

Table III. Azanitro-2-quinolones



11a-d

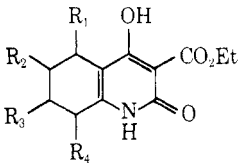


11e

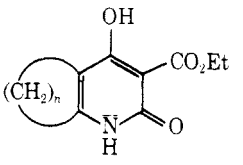
Compd	Position of N	Mp, °C	Formula	Analyses	Yield, %	Act. in rat PCA test, ED_{50},[a] mg/kg sc at *T* max[b]	*T* max[b]
11a	5	>350 dec	$C_8H_5N_3O_4$	C, H, N	30	>100[c]	
11b	6	>370	$C_8H_5N_3O_4$	C, H, N	44	>100[c]	
11c	7	Dec at 344	$C_8H_5N_3O_4$	C, H, N	78	7.2 (5.7–232, 52, 30)	10
11d	8	288–289 dec	$C_8H_5N_3O_4$	C, H, N	99	>100[c]	
11e		293 dec	$C_8H_5N_3O_4$	C, H, N	81	Inactive[c]	

[a]Figures in parentheses are 95% confidence limits, slope of inhibition/log dose line, number of animals used. [b]*T* max is the time between sc administration of the drug and challenge to give maximum activity. [c]24 rats.

Table IV. 3-Carboethoxy-4-hydroxy-2-pyridones



13a-d

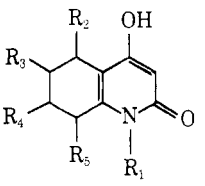


13e-g

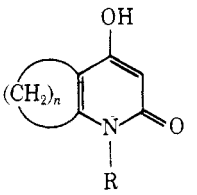
Compd	R_1	R_2	R_3	R_4	*n*	Mp, °C	Formula	Analyses	Yield, %
13a	H	H	H	H		236–237 dec[a]	$C_{12}H_{15}NO_4$	C, H, N	35
13b	Me	H	Me	H		264	$C_{14}H_{19}NO_4$	C, H, N	30
13c	H	Me	H	H		214–216 dec	$C_{13}H_{17}NO_4$	C, H, N	45
13d	H	H	Me	H		247–248 dec	$C_{13}H_{17}NO_4$	C, H, N	33
13e					3	227–229 dec[b]	$C_{11}H_{13}NO_4$	C, H, N	48
13f					5	213–216	$C_{13}H_{17}NO_4$	C, H, N	46
13g					6	232–234	$C_{14}H_{19}NO_4$	C, H, N	23

[a]V. Prelog and S. Szpilfogel, *Helv. Chim. Acta*, **28**, 1684 (1945), quote mp 234° dec. [b]V. Prelog and S. Szpilfogel, *ibid.*, **28**, 1684 (1945), quote mp 221° dec.

Table V. 4-Hydroxy-2-pyridones



14a-h

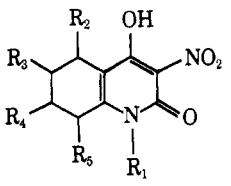


14i-k

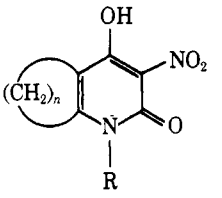
Compd	R_1	R_2	R_3	R_4	R_5	*n*	Mp, °C	Formula	Analyses	Yield, %
14a	H	H	H	H	H		>300	$C_9H_{11}NO_2$	C, H, N	100
14b	H	Me	H	Me	H		>300	$C_{11}H_{15}NO_2$	C, H, N	94
14c	H	H	Me	H	H		>300	$C_{10}H_{13}NO_2$	C, H, N	100
14d	H	H	H	Me	H		>360	$C_{10}H_{13}NO_2$	C, H, N	98
14e	Et	H	H	H	H		355–358 dec	$C_{11}H_{15}NO_2$	C, H, N	67
14f	Et	H	H	Me	H		335–338	$C_{12}H_{17}NO_2$	C; H,[a] N[b]	47
14g	*n*-Pr	H	H	H	H		277–278	$C_{12}H_{17}NO_2$	C, H, N	52
14h	*i*-Pr	H	H	H	H		304–307 dec	$C_{12}H_{17}NO_2$	C, N; H[c]	44
14i	H					3	>310	$C_8H_9NO_2$	C, H, N	100
14j	H					5	>355	$C_{10}H_{13}NO_2$	C, H, N	100
14k	H					6	>300	$C_{11}H_{15}NO_2$	C, H, N	100

[a]H: calcd, 8.27; found, 8.70. [b]N: calcd, 6.76; found, 7.21. [c]H: calcd, 8.27; found, 8.92.

Table VI. 4-Hydroxy-3-nitro-2-pyridones



16a-h



16i-k

Compd	R_1	R_2	R_3	R_4	R_5	n	Mp, °C	Formula	Analyses	Yield, %	Act. in rat PCA test, ED_{50},[a] mg/kg sc at T max[b]	T max[b]
16a	H	H	H	H	H		241 dec[c]	$C_9H_{10}N_2O_4$	C, H, N	61	23 (9.9–53, 114, 18)	10
16b	H	Me	H	Me	H		235–236 dec	$C_{11}H_{14}N_2O_4$	C, H, N	86	9.1 (4.7–17.6, 111, 17)	10
16c	H	H	Me	H	H		243–244	$C_{10}H_{12}N_2O_4$	C, H, N	91	23 (16–33, 140, 17)	10
16d	H	H	H	Me	H		239–240 dec	$C_{10}H_{12}N_2O_4$	C, H, N	64	14 (4.1–46, 64, 24)	10
16e	Et	H	H	H	H		152–154 dec	$C_{11}H_{14}N_2O_4$	C, H, N	51	>50	
16f	Et	H	H	Me	H		137–140 dec	$C_{12}H_{16}N_2O_4$	C, H, N	69	>50	
16g	n-Pr	H	H	H	H		139–141 dec	$C_{12}H_{16}N_2O_4$	C, H, N	82	~100	
16h	i-Pr	H	H	H	H		171–173	$C_{12}H_{16}N_2O_4$	C, H, N	75	>100	
16i	H					3	243 dec	$C_8H_8N_2O_4$	C, H, N	63	Inactive	
16j	H					5	233–235 dec	$C_{10}H_{12}N_2O_4$	C, H, N	70	>100	
16k	H					6	221 dec	$C_{11}H_{14}N_2O_4$	C, H, N	74	>100	

[a] Figures in parentheses are 95% confidence limits, slope of inhibition/log dose line, number of animals used. [b] T max is the time between sc administration of the drug and challenge to give maximum activity. [c] F. 1,369,634 (1963) quote mp 244° dec.

pared to 120° in strain-free SP_2 hybridized carbon atoms and the cyclohexyl derivative **16a**. The cycloheptyl and cyclooctyl homologs, and all larger ring systems, can easily accommodate the ring junction olefin without strain by folding of the carbon structure. A less likely alternative is the decreased partition coefficient, π, in the cyclopentyl homolog **16i**. Although this would undoubtedly influence activity to some extent, the variation of 0.5 between **16a** and **16i** would be insufficient to explain the pronounced reduction noted.

Experimental Section

The melting points were determined on a Büchi apparatus and are recorded uncorrected. All compounds had spectral data consistent with the assigned structures, ir spectra being measured either as liquid films or as suspensions in Nujol and NMR spectra as solutions in $CDCl_3$, DMSO-d_6, or CF_3CO_2H. Elemental analyses where represented by symbols fall within ±0.4% of the theoretical values.

Malonic Acid Monoanilides. These were prepared using known procedures[7] from readily available anilines and where documented had melting points in agreement with cited values.

4-Hydroxy-2-quinolones. The 4-hydroxy-2-quinolones were synthesized by two distinct procedures; those having no substituent on nitrogen (**6a–o**) were prepared by polyphosphoric acid (PPA) cyclization of their respective malonic acid monoanilides[7] whereas those bearing nitrogen substituents (**6p–x**) were prepared by acylation and cyclization of the appropriate anthranilic acid.[8] The yields and physical data of each are listed in Table I. Typical reaction conditions are outlined below.

Method A (Compounds 6a–o). The malonic acid monoanilide (0.1 mol) and 85% PPA (200 g) were stirred for 3 hr at 140° and cooled and the clear solution was poured onto 1 N HCl (300 ml). After adjustment to pH 4 with 2.5 N NaOH (ca. 1 l.) the hydroxyquinolone separated as a white solid which was filtered, washed well with water, and recrystallized from glacial AcOH.

Method B (Compounds 6p–x). A solution of the N-alkylanthranilic acid (0.1 mol) in a mixture of glacial AcOH (55 ml) and Ac_2O (55 ml) was refluxed 4–12 hr, cooled, and poured onto crushed ice. After bringing to pH 14 with 5 N NaOH the solution was filtered free of insoluble material and the filtrate adjusted to pH 4–5. Separation of the precipitated solid followed by charcoalization from glacial AcOH gave the product.

Derivatives by both procedures were dried over both P_2O_5 and NaOH; analytical samples often required elevated temperatures (ca. 100–120°) to remove residual AcOH.

Aza-4-hydroxy-2-quinolones. Aminopyridinecarboxylic acids[14,15] were esterified with ethanolic HCl and cyclized according to the procedure outlined below.

A solution of the amino ester (0.1 mol) in EtOAc (100 ml) was added to a 50% dispersion of NaH in mineral oil (9.5 g, 0.2 mol) and after the initial vigorous reaction ceased the mixture was heated for 2 hr at 100°. After cooling the precipitated solid was taken up in water and extracted once with Et_2O, and the aqueous phase was acidified with AcOH. The azaquinolones separated as white solids which were purified by recrystallisation from glacial AcOH. All had mp >300° and were characterized by ir and NMR spectroscopy and elemental analysis.

3-Carboethoxy-4-hydroxy-2-pyridones (Compounds 13a–g, Table IV). 2-Carboalkyloxycycloalkanones (commercially available or prepared by acylation of the appropriate enamines[16] or cycloalkanones[17]) were converted with gaseous NH_3 in the presence of NH_4NO_3 to their enamines **12** (R = H) according to the procedure of Prelog and Szpilfogel.[12] The unpurified enamines on condensation with diethyl malonate at 110° in the presence of ethanolic NaOEt then gave the corresponding 3-carboethoxy-4-hydroxy-2-pyridones (**13**). A typical procedure is given below.

A mixture of the enamine **12** (R = H, 0.1 mol), diethyl malonate (16 g, 0.1 mol), and ethanolic NaOEt [from Na (2.4 g) and EtOH (50 ml)] was stirred at 110° in a glass-lined autoclave for 30 hr and cooled and the white suspension acidified to pH 4 with 5 N HCl. After dilution with water the white precipitate was filtered off, washed well with water, and recrystallized from EtOH in the presence of charcoal to yield pure **13**.

4-Hydroxy-2-pyridones (Table V). Method A (14a–d,i–k). A suspension of the 3-carboethoxypyridone **13** (0.03 mol) in 2 N HCl

(100–200 ml) was refluxed until the solution cleared (2–3 days) and evaporated to dryness under reduced pressure. Dissolution or suspension of the residual white hydrochloride in water (100 ml) followed by adjustment to pH 4 with 2 *N* NaOH gave **14** as a white solid in high purity. Analytical samples were recrystallized from glacial AcOH.

Method B (14e–h). A mixture of the amine **12** (R = alkyl,[18] 0.24 mol) and acetyl chloride (0.25 mol) was heated to 150° for 10 min, cooled, poured into water, and extracted into $CHCl_3$. The dried ($MgSO_4$) extracts were evaporated and the residual amide **15** was distilled. Without further purification a solution of **15** (0.1 mol) in dry dioxane (250 ml) was added to dry NaOMe (6.3 g, 0.117 mol) and heated to 100° for 2–6 hr. The precipitated solid was filtered, dissolved in a minimum of water, and brought to pH 4 with concentrated HCl. The product separated as a white crystalline solid which after filtration was recrystallized from glacial AcOH.

Nitro Products. Apart from **11e**, the preparation of which is given below, all nitro products were prepared by the direct nitration of a suspension of the ketoamide in glacial AcOH using HNO_3 (*d* 1.42–1.52). Compounds **6a–x** and **14a–k** were nitrated with HNO_3 of *d* 1.42, whereas the aza derivatives **10** required fuming HNO_3 (*d* 1.52).

General Procedure. Nitric acid (2.5 ml, *d* 1.42) was added in one portion to a suspension of the ketoamide (0.009 mol) in glacial AcOH (10 ml) and the mixture heated on a steam bath until the vigorous reaction ensued (2–7 min). After rapidly cooling, water (40 ml) was added and the precipitated yellow product filtered off, washed well with water, and recrystallized from AcOH–EtOH (**8a–z, 11a–d**) or EtOH (**16a–k**) (Table VI). All were dried in vacuo over both P_2O_5 and NaOH.

2-Hydroxy-3-nitro-4-oxo-2*H*-pyrido[1,2-*a*]pyrimidine (11e). Fuming HNO_3 (1.43 ml, *d* 1.52) was cooled in ice and stirred during the portionwise addition of 2-hydroxy-4-oxo-2*H*-pyrido[1,2-*a*]pyrimidine (1.00 g, 0.0061 mol) over 2 hr. After a further 1 hr at 0° the 3-nitro derivative separated. Water (10 ml) was added and the off-white solid filtered, washed well with water, and recrystallized from DMSO to give 1.02 g (81%), mp 293° dec. Anal. ($C_8H_5N_3O_4$) C, H, N.

Passive Cutaneous Anaphylaxis. Serum containing heat-labile homocytotropic antibody was raised in rats to crystallized ovalbumin XOA by the method of Mota[19] using Bordettela pertussis vaccine as adjuvant.

Passive cutaneous anaphylaxis (PCA) was carried out by a method based on that of Ovary and Bier[20] as modified by Goose and Blair.[21]

Male Wistar rats of 250–300 g were given 0.1 ml of each of six twofold serial dilutions of pooled antiserum in 0.9% saline injected intradermally into separate sites on their shaved backs. Later (72 hr) the animals were challenged by intravenous injection of 0.3 ml of a 1% solution of ovalbumin in PBS mixed with 0.2 ml of a 5% solution of Pontamine Sky Blue (6 BX. C.I. 24410, Raymond A. Lamb, London) in isotonic saline. The rats were killed after 20 min and the diameter of the blue wheals at the antibody injection sites was measured on the outer surface of the skin. The starting dilution of the serum was adjusted so that there was no response, after challenge, at the injection site of the highest dilution and a maximum response at the lowest dilutions. Typically six twofold serial dilutions of the serum from $\frac{1}{4}$ to $\frac{1}{128}$ were used.

Compounds were tested for their ability to reduce the diameter of the wheals at those intradermal sites which in control animals gave less than maximum response. Each dose of the compound was administered to an animal at a measured time prior to intravenous challenge with ovalbumin. Control groups of six animals were given the same volume of carrier fluid at the same time.

The results were calculated as follows. % inhibition of PCA = $100\ (1 - a/b)$ where a = the sum of the diameters of the wheals produced in the test animal at the sites of antibody dilutions as used in control groups and b = the mean sum of the diameters of the wheals produced in the control group of animals at those antibody sites where at least five out of six of the animals gave less than maximum response. A typical variation in the control group of animals was SEM ± 6%.

T max was the time between sc administration of a compound and iv challenge at which the compound showed maximum activity. It was determined by injecting sc into rats the approximate ED_{50} of the compound, when given just prior to iv challenge, and challenging the rats in groups of six after 0, 10, 20, 30, 60, and 120 min. The line of best fit for the log dose–response curve and the 95% confidence limits were calculated as described in ref 22.

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