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Compounds with Gastric Antisecretory Activity. 1. Phenoxyalkylamines

Peter E. Cross,* Roger P. Dickinson, John E. G. Kemp, Peter R. Leeming, and Laurence G. Pullman

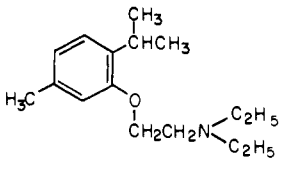
Pfizer Central Research, Pfizer Limited, Sandwich, Kent, United Kingdom. *Received November 15, 1976*

A series of *o*-alkylphenoxyalkylamines, derived from classical H_1 antagonists, has been found to inhibit histamine-induced gastric acid secretion. The most potent compound was *trans*-1-[2-[2-[2-(1-adamantyl)vinyl]-phenoxy]ethyl]pyrrolidine (**54**). The *o*-acylphenol **23** required for the preparation of **54** was obtained by the novel reaction of 1-bromoadamantane (**21**) with 4-hydroxycoumarin (**20**) using diethyl phosphonate as solvent. The product **22** was then hydrolyzed under basic conditions to give **23** in high yield. **54** was not an H_2 antagonist and its mode of action remains unknown. The compound had no significant anticholinergic, antiinflammatory, anticonvulsant, sedative, or H_1-antihistaminic activity.

Since the discovery of the first compound with histamine blocking activity, 929F (I), by Bovet and Staub[1] in 1937, it has been appreciated that compounds of this type do not oppose the gastric secretory actions of histamine. In 1966 Ash and Schild[2] proposed the symbol H_1 for those receptors that were blocked by the antihistamines known at that time. They stressed that further classification of the histamine receptors in the stomach, uterus, and heart must await the discovery of specific antagonists.

The classification of histamine receptors into H_1 and H_2 types is now firmly established following the discovery of selective H_2 agonists and antagonists by Black, Ganellin, and co-workers[3-5] in 1972. Concurrent with this discovery we had been seeking antagonists of histamine-induced gastric acid secretion. We now wish to report a series of compounds, derived from H_1 antagonists, which are capable of antagonizing histamine-induced gastric acid secretion. These compounds, however, are not H_2 antagonists, and their mode of action is at present unknown.

The starting point for our work was the H_1-antihistamine I. Following its discovery in 1937, all synthetic modifications on this compound have been directed toward optimizing H_1-receptor activity. We found that replacement of the isopropyl group of I by large alkyl groups yielded compounds that were capable of inhibiting gastric acid secretion.



I

Chemistry. The majority of compounds listed in Tables II and III were synthesized from the appropriate *o*-alkylphenol **2** as outlined in Scheme I. Treatment of **2** with sodium hydride in an inert solvent such as DMF afforded the alkali metal phenolate which was allowed to react with a dialkylaminoalkyl halide to give the desired product **9** (method A). Alternatively, the phenolate was allowed to react with dibromopropane to give **4** which was then allowed to react with either primary or secondary amines to give **9** (method B). A variation of this route was the reaction of **2** with ethyl chloroacetate to give the ester **3**, followed by hydrolysis, formation of the acyl chloride, and reaction with the appropriate amine to yield the amide. This was then reduced with $LiAlH_4$ to the desired product **9** (method C).

The vinyl compounds of Table IV were prepared in a straightforward manner by treating the metal phenolate of **1** with a dialkylaminoethyl halide to give **5** which was then reduced to the alcohol and dehydrated to give **8** (method D). An alternative route commencing from salicylaldehyde afforded the ether **6**, which upon reaction with an alkyl Grignard gave the alcohol **7**. This was either dehydrated with mild acid or treated with thionyl chloride and warmed to give the vinyl compound **8** (method E).

The *o*-alkylphenols (Table I) were obtained by standard methods. The most favored route involved reacting salicylaldehyde methyl ether with the appropriate alkyl Grignard followed by hydrogenolysis of the secondary alcohol. Demethylation to the phenol was then effected by either pyridine hydrochloride or 48% HBr in acetic acid.

When the position para to the phenolic hydroxyl bore a substituent, the Fries or Friedel–Crafts methods of obtaining the *o*-acylphenols **1** were employed. Reduction to the alkylphenol **2** was then effected by the Clemmensen

Scheme I

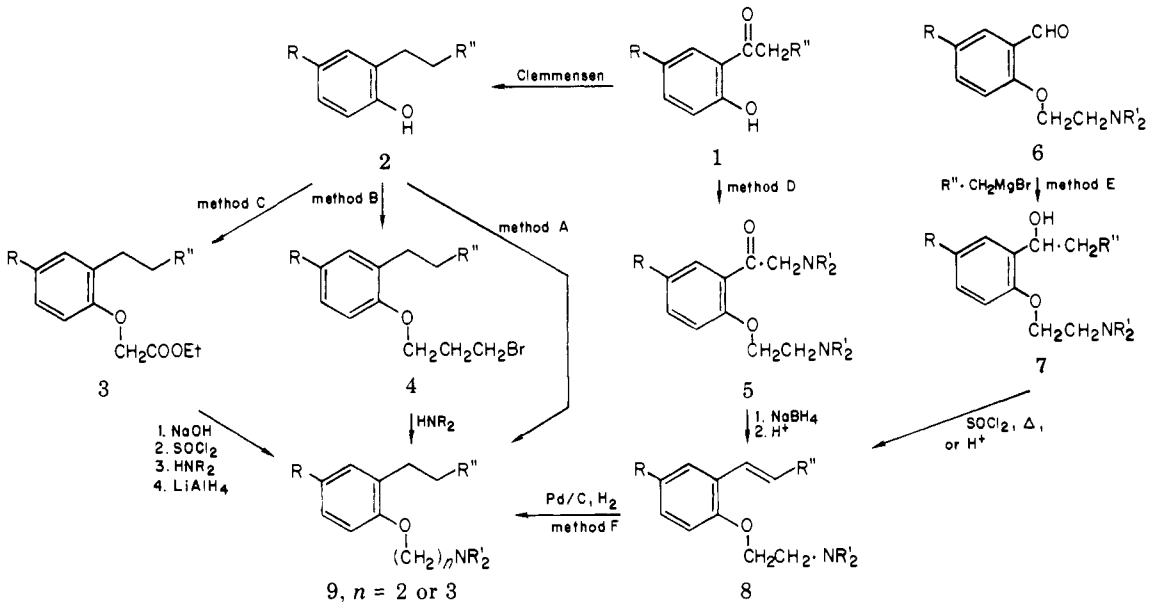
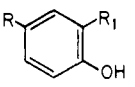


Table I



No.	R	R_1	% yield	Mp or bp (mm), °C	Mol formula
10	H	*n*-Hexyl[a]	61	80 (0.8)	$C_{12}H_{18}O$
11	H	*n*-Heptyl[b]	65	118-120 (1)	$C_{13}H_{20}O$
12	H	*n*-Nonyl[a]	53	123 (0.5)	$C_{15}H_{24}O$
13	H	4,4-Dimethylpentyl[d]	49	106-108 (0.4)	$C_{13}H_{20}O$
14	H	Cyclohexylethyl[c]	86	170-174 (10)	$C_{14}H_{20}O$
15	F	Cyclohexylethyl[d]	48	130-140 (0.6)	$C_{14}H_{19}FO$
16	Cl	Cyclohexylethyl[d]	54	168-170 (1.1)	$C_{14}H_{19}ClO$
17	CH_3O	Cyclohexylethyl[c]	45	93-94	$C_{15}H_{22}O_2$
18	CH_3	*n*-Heptyl[c]	67	120 (1.0)	$C_{14}H_{22}O$
19	CH_3CO	Cyclohexylethyl[c]	57	116-117	$C_{16}H_{22}O_2$

[a] *Chem. Abstr.*, 25, 1228 (1931). [b] "Organic Syntheses", Collect. Vol. III, Wiley, New York, N.Y., 1955, p 444. [c] Anal. C, H. [d] Not analyzed.

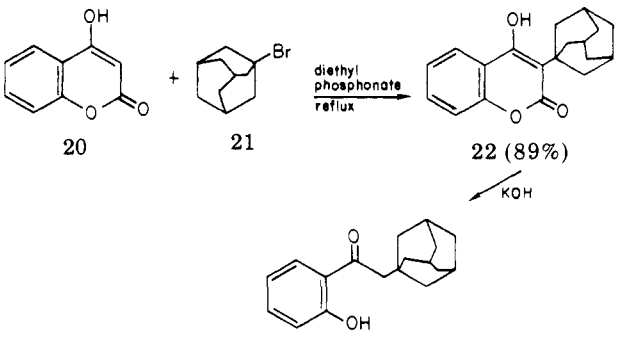
method. Compound **19** (Table I) was obtained by Friedel–Crafts acylation of *o*-cyclohexylethylphenol (**14**).

A new route has been devised for the preparation of the adamantylacetylphenol **23** (Scheme II). This involved condensing 4-hydroxycoumarin (**20**) with 1-bromoadamantane (**21**) in an acid acceptor solvent such as diethyl phosphonate. The resultant 3-adamantyl-4-hydroxycoumarin (**22**) was then cleaved with KOH to give the acylphenol **23** in high yield. The condensation was based on the precedents in the literature for the reaction of 1-bromoadamantane with phenol[6] and of benzyl bromide with 4-hydroxycoumarin.[7] Ketonic hydrolysis of coumarins has long been known[8] but has generally been overlooked as a selective route to *o*-acylphenols except for one brief comment in a paper on coumarins.[9] Since there are many known electrophilic substitutions[10] on **20**, the procedure has the potential for wide usage.

Results and Discussion

Antisecretory activity was encountered when the isopropyl group of I was replaced by *n*-hexyl. Discarding the *m*-methyl of I gave compound **24** (Table II), the activity of which was much improved by lengthening the alkyl chain by another methylene unit to *n*-heptyl (**25**). Further increase in the length of the chain (**26**) or branching at C-4 (compound **27**) was not advantageous. Activity comparable to that shown by compound **25** was found when the *n*-heptyl chain was replaced by cyclohexylethyl (**28**).

Scheme II

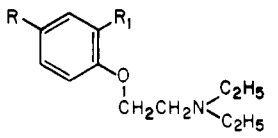


However, the most dramatic increase in potency occurred when the cyclohexyl group was replaced by 1-adamantyl as in compound **44** (Table III).

Substitution para to the oxygen atom afforded only minor changes in activity. Fluorine (**29**) was the most favored substituent with chlorine, methoxy, and methyl giving activity comparable to that of the parent structure **28**.

Activity was retained, but not improved, when the basic chain was lengthened by one methylene unit, and therefore the length of the chain was retained at dialkylaminoethoxy, in similar manner to that of the H_1 antihistamines I and phenyltoloxamine.[11] Replacement of the diethylamino

Table II



No.	R	R_1	Salt	Mp, °C	Mol formula[e]	Crystn solvent	Synth method[a]	% yield	Dose, mg/kg[b] iv	% inhibn of histamine-induced gastric acid secretion,[c] rat
24	H	*n*-Hexyl	Citrate	126–127	$C_{18}H_{31}NO\cdot C_6H_8O_7$	EtOAc	A	74	5.0	48
25	H	*n*-Heptyl	Citrate	129–130	$C_{19}H_{33}NO\cdot C_6H_8O_7$	EtOAc	A	68	5.0	82
26	H	*n*-Nonyl	HCl	114–115	$C_{21}H_{37}NO\cdot HCl$	Toluene	A	84	5.0	25
27	H	4,4-Dimethylpentyl	Citrate	130–131	$C_{19}H_{33}NO\cdot C_6H_8O_7$	H_2O	A	60	5.0	50
28	H	Cyclohexylethyl	Citrate	130–131	$C_{20}H_{33}NO\cdot C_6H_8O_7$	IPA[d]	A	71	5.0	74
29	F	Cyclohexylethyl	Citrate	129–130	$C_{20}H_{32}FNO\cdot C_6H_8O_7$	EtOAc	A	69	5.0	95
30	Cl	Cyclohexylethyl	Citrate	145–146	$C_{20}H_{32}ClNO\cdot C_6H_8O_7$	H_2O	A	75	5.0	75
31	CH_3O	Cyclohexylethyl	Citrate	118–119	$C_{21}H_{35}NO_2\cdot C_6H_8O_7$	H_2O	A	70	5.0	78
32	CH_3	*n*-Heptyl	Citrate	135–136	$C_{20}H_{35}NO\cdot C_6H_8O_7$	H_2O	A	73	5.0	61
33	CH_3CO	Cyclohexylethyl	Citrate	119–120	$C_{22}H_{35}NO_2\cdot C_6H_8O_7$	EtOAc	A	66	5.0	36

[a] See Scheme I. [b] mg/kg of the free base. [c] Mean of two determinations. [d] Isopropyl alcohol. [e] All compounds were analyzed for C, H, and N.

moiety of **28** by other basic moieties was examined, and pyrrolidino (**37**), piperidino (**38**), and *N*-methyl-2-piperidinylmethyl (**48**) were found to be the groups of choice. Replacement of tertiary amino by secondary amino (**42** and **43**) was decidedly unfavorable since these compounds proved lethal to the rat after intravenous injection at the standard dose of 5 mg/kg.

Modification of the alkyl group of compounds **28** and **37** from cyclohexylethyl to *trans*-cyclohexylvinyl (**50** and **51**) produced a significant increase in antisecretory activity. Curiously, this potency improvement was not shown by the open-chain compounds **52** and **53**. The most potent compound of the whole series was the *trans*-adamantylvinyl compound **54**. This at an iv dose of 0.078 mg/kg caused virtual total inhibition of histamine-induced gastric acid secretion in the rat perfused stomach preparation. The cis isomer was also active but less so than **54**.

Using the fragmentation constants, described by Nys and Rekker,[12] the log *P* value for compound **51** was calculated as 5.53. This was in close agreement with the experimental value of 5.38 obtained by the octanol–water partition technique. However, the experimental figure of 5.54 for compound **28** was 0.80 units lower than the calculated value. Even larger discrepancies were sometimes encountered, and in the case of compound **54** it was not possible to obtain a reliable experimental log *P* value. Lipophilicity measurements, in this highly lipophilic series, encountered theoretical[13] and practical[14] problems. Thus, attempts to obtain a deeper insight into the SAR by multiple regression analysis did not succeed.

The *f* values of the *n*-heptyl, cyclohexylethyl, *n*-nonyl, and adamantylethyl side chains of compounds **25**, **37**, **26**, and **44** were calculated as 3.86, 3.92, 4.92, and 5.10, respectively. The marked increase in activity of compound **44** over that of **37** suggests that a steric factor is playing a role in determining the level of antisecretory activity. This is also supported by the observation that activity actually declines when going from the open-chain *n*-heptyl compound **25** to the *n*-nonyl compound **26**, even though the calculated lipophilicity of the side chain of the latter compound is comparable to that of **44**.

Table V shows the po and iv ED_{50} values of **54** in the rat, with the H_2 antagonist cimetidine for comparison. Unlike cimetidine, neither **54** nor any of its analogues competitively antagonized the effect of histamine on either the guinea-pig spontaneously beating right atrium or on rat uterine contractions evoked by carbachol. Thus the compounds are not H_2 antagonists; their mode of action is at present unknown. Investigation of the general pharmacological properties in the anesthetized cat in vivo showed that the compounds had a very weak α-adrenergic blocking action and, as a consequence, reduced the carotid occlusion reflex, the contraction of the nictitating membrane caused by electrical stimulus of the preganglonic cervical sympathetic trunk and the pressor effects of adrenaline and noradrenaline. The vascular effects of 5-HT, acetylcholine, and histamine were not modified. Similarly, on the guinea pig ileum in vitro there was no atropine-like activity against acetylcholine.

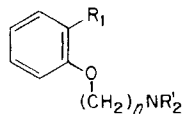
Experimental Section

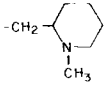
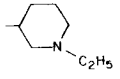
Pharmacology. The inhibition of histamine-induced gastric acid secretion was determined using the perfused rat stomach preparation first described by Ghosh and Schild.[15] The preparation was sensitized by an intravenous injection of carbachol (1 μg). The time course for the completion of the gastric acid secretory response to this sensitization was between 30 and 45 min. Repeated doses of histamine (250–500 μg) were then given in order to obtain a standard response for a particular preparation. Each gastric acid secretory response to histamine took approximately 25–35 min to completion and the preparation was judged stable only after two to three histamine responses were obtained that differed by no less than 0.25 pH units. The compound under test was injected iv, usually at a standard dose of 5 mg/kg. At least 15 min were then allowed to elapse in order to assess the effects per se on blood pressure and gastric acid secretion. Histamine was then injected in order to ascertain whether or not the compound had modified the secretory response. The results in Tables II–IV are the percentage inhibition of histamine-induced gastric acid secretion 45 min after iv injection of the test compound.

The oral efficacy was determined using a rat ligated stomach preparation.[16] The compound was administered 1 h prior to the ligation of the pylorus and subcutaneous injection of 20 mg/kg of histamine. Acid output was then measured for a further 1 h by titration of pH 7.0.

Chemistry. All melting points are uncorrected and were obtained using an Electrothermal capillary melting point apparatus. The structures of all compounds were confirmed by their IR and NMR spectra, the latter of which were determined as solution in either $CDCl_3$ or Me_2SO-d_6. The IR spectra were

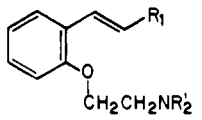
Table III



No.	R_1	n	NR'_2	Salt	Mp or bp (mm), °C	Mol formula[g]	Crystn solvent	Synth method[a]	% yield	Dose, mg/kg[b] iv	% inhibn of histamine-induced gastric acid secretion,[c] rat
34	Cyclohexylethyl	2	$-NMe_2$	HCl	164	$C_{18}H_{29}NO\cdot HCl$	EtOAc	A	76	5.0	29
35	Cyclohexylethyl	3	$-NMe_2$	Citrate	127-128	$C_{19}H_{31}NO\cdot C_6H_8O_7$	EtOAc	A	64	5.0	42
36	Cyclohexylethyl	3	$-NEt_2$	Citrate	135-136	$C_{21}H_{35}NO\cdot C_6H_8O_7$	H_2O	B	60	5.0	66
37	Cyclohexylethyl	2	c-NC_4H_8	Citrate	97-98	$C_{20}H_{31}NO\cdot C_6H_8O_7$	H_2O	A	59	5.0	95
38	Cyclohexylethyl	2	c-NC_5H_{10}	Oxalate	151-152	$C_{21}H_{33}NO\cdot C_2H_2O_4$	H_2O	A	44	2.5	50
39	Cyclohexylethyl	2	c-$N(CH_2CH_2)_2O$	Citrate	106-107	$C_{20}H_{31}NO_2\cdot C_6H_8O_7$	EtOAc	A	22	5.0	10
40	Cyclohexylethyl	2	$-NPr_2$	Citrate	95-96	$C_{22}H_{37}NO\cdot C_6H_8O_7$	EtOAc	A	66	5.0	62
41	Cyclohexylethyl	2	$-N\text{-}i\text{-}Pr_2$	Base	202 (1.0)	$C_{22}H_{37}NO$		A	42	5.0	32
42	*n*-Heptyl	2	$-NHEt$	Oxalate	189-190	$C_{17}H_{29}NO\cdot C_2H_2O_4$	H_2O	C	47	5.0	*f*
43	*n*-Heptyl	3	$-NH\text{-}i\text{-}Pr$	Oxalate	137-138	$C_{19}H_{33}NO\cdot C_2H_2O_4$	H_2O	B	69	5.0	*f*
44	1-Adamantylethyl	2	c-NC_4H_8	HCl	175-177	$C_{24}H_{35}NO\cdot HCl$[d]	Toluene	F	63	1.25 0.078	100 83
45	Cyclohexylethyl	2	c-$N(CH_2CH_2)_2N\text{-}4\text{-}CH_3$	Citrate	159	$C_{21}H_{34}N_2O\cdot C_6H_8O_7$	EtOAc	A	57	5.0	0
46	Cyclohexylethyl	2	c-$N(CH_2CH_2)_2N\text{-}4\text{-}Ph$	2HCl	187-188	$C_{26}H_{36}N_2O\cdot 2HCl$	2 N HCl	C	56	5.0	0
47	Cyclohexylethyl	2	c-$N(CH_2CH_2)_2N\text{-}4\text{-}CH_2CHMe_2$	2HCl	255-256	$C_{24}H_{40}N_2O\cdot 2HCl$	IPA[e]	C	52	5.0	51
48	Cyclohexylethyl	0		HCl	170	$C_{21}H_{35}NO\cdot HCl$	EtOAc	A	60	5.0 2.5	100 64
49	Cyclohexylethyl	0		HCl	159-160	$C_{21}H_{33}NO\cdot HCl$	EtOAc	A	41	5.0	57

[a] See Scheme I. [b] mg/kg of the free base. [c] Mean of two determinations. [d] Hemihydrate. [e] Isopropyl alcohol. [f] Lethal to the animal at this dose level. [g] All compounds were analyzed for C, H, and N.

Table IV



trans

No.	R_1	$-NR'_2$	Salt	Mp, °C	Mol formula[h]	Crystn solvent	Synth method[a]	% yield	Dose, mg/kg[b] iv	% inhibn of histamine-induced gastric acid[c] secretion, rat
50	Cyclohexyl	$-NEt_2$	HCl	169-170	$C_{20}H_{31}NO \cdot HCl$	EtOAc	E	65	1.25	82
51	Cyclohexyl	c-NC_4H_8	HCl	163-164	$C_{20}H_{29}NO \cdot HCl$	EtOAc	E	41	1.25	90
52	*n*-Pentyl	c-NC_4H_8	Citrate	102-103	$C_{19}H_{29}NO \cdot C_6H_8O_7$	EtOH	E	43	5.0	71
53	*n*-Hexyl	c-NC_4H_8	Citrate	106-108	$C_{20}H_{31}NO \cdot C_6H_8O_7$	EtOH	E	39	5.0	50
54	1-Adamantyl	c-NC_4H_8	HCl	211	$C_{24}H_{33}NO \cdot HCl$[d]	MIBK[f]	D	95	0.078	97
55	1-Adamantyl (cis)	c-NC_4H_8	HCl	180	$C_{24}H_{33}NO \cdot HCl$[e]	EtOH	D[g]	92	0.078	65
56	1-Adamantyl	$-NMe_2$	Oxalate	168-169	$C_{22}H_{29}NO \cdot C_2H_2O_4$	H_2O	D	68	0.078	52

[a] See Scheme I. [b] mg/kg of the free base. [c] Mean of two determinations. [d] Monohydrate. [e] Hemihydrate. [f] Methyl isobutyl ketone. [g] Formed by irradiation of the trans isomer **54**. [h] All compounds were analyzed for C, H, and N.

Table V. Antagonism of Histamine-Induced Gastric Acid Secretion in the Rat

Compd	Route of admin	ED_{50}, mg/kg	*n*
54	iv[a]	0.018	2
	po[b]	1.5	10
Cimetidine[c]	iv[a]	0.3	2
	po[b]	21.5	10

[a] Rat perfused stomach preparation. [b] Rat ligated stomach preparation. [c] The sample of cimetidine was synthesized in these laboratories.

obtained with a Perkin-Elmer 237 spectrophotometer and the NMR spectra with a Varian Associates Spectrometer, Model A-60A. Where analyses are indicated only by symbols of elements, analytical results obtained for those elements are within 0.4% of the theoretical values.

2-(2-Cyclohexylethyl)phenol (14). Cyclohexylmethyl bromide (453 g, 2.60 mol) in dry ether (900 mL) was cautiously added to magnesium turnings (63.4 g, 2.64 mol) covered by dry ether (250 mL) and the mixture cooled and stirred. When all the magnesium had reacted to form the Grignard reagent, *o*-methoxybenzaldehyde (348 g, 2.56 mol) in dry ether (900 mL) was added dropwise at a rate sufficient to maintain a gentle reflux of the reactants. The mixture was then refluxed for 3 h before addition of 5 N HCl to decompose the reaction intermediates. Water (650 mL) was added and the ether layer separated; the aqueous layer was extracted with more ether and the combined extracts were dried, filtered, and evaporated to give a yellow solid. Recrystallization from petroleum ether (bp 80–100 °C) gave the pure alcohol (314 g, 49%), mp 97–99 °C.

The alcohol (314 g) was dissolved in EtOH (730 mL) and concentrated HCl (36.5 mL) added before transference of the solution to a 2-L Parr bomb. Pd/C catalyst (10%) was then added and the mixture hydrogenated at 50 psi and 50 °C until uptake ceased. The catalyst was then filtered off, sodium carbonate solution added, and the mixture extracted with ether; the organic extracts were washed, dried, filtered, and evaporated to give a colorless oil. Distillation under reduced pressure afforded pure 2-(2-cyclohexylethyl)anisole (261 g, 90%), bp 134 °C (1.5 mm).

Freshly prepared pyridine hydrochloride (320 g, 2.5 mol) was added to 2-(2-cyclohexylethyl)anisole (63.4 g, 0.29 mol) and the mixture stirred very vigorously to ensure homogeneity during heating to reflux (~220 °C). Heating was continued for 1.5 h and a pale yellow solution was obtained and cooled, dilute HCl added, and the mixture extracted with ether.

Evaporation of the organic solvent afforded a colorless oil that was distilled under reduced pressure to give the product 14 that solidified on standing (51.1 g, 86%), bp 170–174 °C (10 mm).

This method was also used for the preparation of the phenols **10–13** (Table I) starting from the appropriate alkyl halides.

2-*n*-Heptyl-4-methylphenol (18). *p*-Cresol (54 g, 0.5 mol) and *n*-heptyl chloride (79 g, 0.53 mol) were mixed and stirred together at room temperature and heated to reflux on the steam bath for a further 1 h. The mixture was cooled, washed with H_2O until no longer acidic, dried, and distilled to give the ester.

Powdered $AlCl_3$ (40 g, 0.3 mol) was added portionwise to the ester (60 g, 0.27 mol) and the mixture heated slowly to 135 °C and kept at this temperature for 2 h. While still hot the mixture was cautiously poured onto ice and acidified with concentrated HCl. The oil was separated off and basified, the solid sodium salt was filtered off, washed with water, and reacidified, and the oil was extracted with ether and worked up as usual to give the *o*-acylphenol (36 g).

The acylphenol (30 g) was added dropwise to zinc amalgam (100 g), H_2O (150 mL), and concentrated HCl (100 mL). The mixture was heated to reflux for 20 h before cooling and separation to the oily layer. The aqueous layer was extracted twice with toluene and the combined toluene extracts were added to the oil, dried, and evaporated to afford a dark brown oil as the product. This was distilled to give 18 as a clear colorless oil (18.9 g, 67%), bp 120 °C (1.0 mm).

This method was also used for preparation of the phenols **15–17** (Table I).

3-(1-Adamantyl)-4-hydroxycoumarin (22). A solution of 4-hydroxycoumarin (40.5 g, 0.27 mol) and 1-bromoadamantane (53.75 g, 0.25 mol) in diethyl phosphonate (34.5 g) was heated and stirred at 170–180 °C for 45 min. The mixture was then cooled to 60 °C and diluted with MeOH (250 mL). After stirring for 1 h at room temperature, the product was filtered, washed with further methanol (25 mL), and dried in vacuo at 50 °C: yield 66 g (89%) of the desired product; mp 118–119 °C. Anal. ($C_{19}H_{20}O_3$) C, H.

2-(1-Adamantyl)-2-hydroxyacetophenone (23). A solution of 3-(1-adamantyl)-4-hydroxycoumarin (**22**, 275 g, 0.97 mol) and potassium hydroxide (255 g, 4.55 mol) in IMS (industrial methylated spirits) (2.75 L) and water (540 mL) was stirred and refluxed for 4 h. The mixture was cooled and added to 5% hydrochloric acid (16.5 L). After stirring for a further 2 h the product was filtered off, washed with water (2 L), and dried in vacuo at 60 °C: yield 228 g (92%) of the desired product; mp 81–83 °C. Anal. ($C_{18}H_{22}O_2$) C, H.

General Methods. Route A (Scheme I). *N,N*-Diethyl-2-[2-(2-cyclohexylethyl)phenoxy]ethylamine (28). A 50% dispersion of sodium hydride (1.2 g, 0.025 mol) was added in portions to a stirred solution of 2-(2-cyclohexylethyl)phenol (**14**, 5.0 g, 0.025 mol) in dry DMF (75 mL) and the mixture then heated to 100 °C for 0.5 h. 2-Diethylaminoethyl chloride (3.3 g, 0.025 mol) in DMF (25 mL) was slowly added, the mixture was refluxed for 3 h and then cooled, and water (10 mL) was added. After evaporation of most of the solvent under reduced pressure, water (100 mL) was added to the residue and mixture extracted twice with ether. The organic extract was washed with water, dried over sodium sulfate, and filtered. Evaporation of the solvent then afforded the product as a pale yellow oil. Addition of an ethereal solution of citric acid gave a white precipitate of the citrate salt of the product, **28** (9.1 g, 71%), which was recrystallized from

isopropyl alcohol: mp 130–131 °C. Anal. C, H, N.

Route B (Scheme I). 1. **1-Bromo-3-(2-*n*-heptylphenoxy)propane.** 2-*n*-Heptylphenol (11, 48 g, 0.25 mol) was slowly added to a stirred solution of KOH (14 g, 0.25 mol) in MeOH (100 mL). 1,3-Dibromopropane (202 g, 1.0 mol) was then added in one portion and the mixture refluxed on the steam bath for 2 h before cooling and filtering the precipitated potassium bromide. Evaporation of the solvent left a residual oil which was distilled under reduced pressure, the pure product being the fraction (36 g) collected between 138 and 146 °C at 0.6 mmHg pressure.

2. ***N*-Isopropyl-3-(2-*n*-heptylphenoxy)propylamine (43).** 1-Bromo-3-(2-*n*-heptylphenoxy)propane (5.0 g) was added to isopropylamine (50 mL) and the mixture refluxed on the steam bath for 6 h. The excess isopropylamine was then evaporated off to leave a solid residue that was dissolved in water, basified, and extracted with ether. The ether was dried and evaporated to give the product as an oil. This was converted to the oxalate salt and recrystallized from H_2O to give pure product, **43** (4.2 g, 69%), mp 137–138 °C. Anal. C, H, N.

Method C (Scheme I). 1. Sodium hydride (50% dispersion, 4.8 g, 0.1 mol) was added portionwise to a solution of 2-(2-cyclohexylethyl)phenol (14, 20 g, 0.098 mol) in dry DMF (100 mL) and the mixture heated to 80 °C for 1 h. Ethyl chloroacetate (12.1 g, 0.098 mol) was then added and the mixture refluxed for 4 h, cooled, poured into water, extracted with ether, evaporated, and dried in the usual manner. High-vacuum distillation gave 14.1 g of ethyl 2-(2-cyclohexylethyl)phenoxyacetate as a clear liquid.

2. This ester (25 g) was added to ethanolic sodium hydroxide (150 mL) and warmed overnight on the steam bath. It was then evaporated to one-third, added to water, extracted with ether, acidified with hydrochloric acid, and extracted three times with chloroform (50 mL), and the extract was evaporated to give 18.5 g of crude 2-(2-cyclohexylethyl)phenoxyacetic acid.

3. The acid was treated with thionyl chloride, and the acid chloride so formed (4.0 g) was slowly added in benzene (30 mL) to a solution of 1-isobutylpiperazine (2.0 g) in benzene (40 mL). A rapid reaction took place and a gelatinous precipitate was formed. The mixture was refluxed for 1 h, cooled, and then evaporated to near dryness under reduced pressure. Water (100 mL) was added to the residue; after basification with NaOH, the mixture was extracted with $CHCl_3$ (50 mL). Working up the chloroform extract in the usual manner afforded 4.6 g of 1-isobutyl-4-[2-(2-cyclohexylethyl)phenoxyacetyl]piperazine as a dark brown oil.

4. This amide (4.6 g) was slowly added to $LiAlH_4$ (1.1 g) in dry ether (200 mL) and the mixture stirred and refluxed for 6.5 h. Aqueous NaOH solution (5 N, 1.8 mL) was then added to decompose the excess $LiAlH_4$ and the mixture filtered. The filter cake was well washed with ether; the filtrates were combined, dried, and evaporated to give a mobile yellow oil. Addition of ethereal HCl gave 2.8 g (52%) of **1-isobutyl-4-[2-(2-cyclohexylethyl)phenoxy]ethyl]piperazine dihydrochloride (47)** which was recrystallized from 2-propanol: mp 255–256 °C. Anal. C, H, N.

Method D (Scheme I). 1. **2-(1-Adamantyl)-2′-[2-(1-pyrrolidinyl)ethoxy]acetophenone Hydrochloride (57).** To a solution of 2-(1-adamantyl)-2′-hydroxyacetophenone (**23**, 0.25 mol) in IMS (270 mL) was added a solution of potassium hydroxide (14 g) in IMS (67.5 mL). The solution was then refluxed for 1 h and evaporated to dryness. The potassium salt was washed well with ether (500 mL) and dried in vacuo.

Anhydrous K_2CO_3 (38 g) was added to a solution of *N*-(chloroethyl)pyrrolidine hydrochloride (42.5 g) in H_2O (85 mL), and the free base extracted into methyl isobutyl ketone. To this dried solution was added the potassium salt prepared above (74 g) and the reaction mixture was stirred and refluxed for 4 h.

After cooling, H_2O (200 mL) was added, and the organic layer was separated and dried. Hydrogen chloride (12 g) was then passed into the solution and the product obtained after evaporation to dryness was washed with ether and dried: 91 g (93.8%); mp 176–178 °C. Anal. ($C_{24}H_{33}NO_2$·HCl) C, H, N.

2. **2-(1-Adamantyl)-1-[2-[2-(1-pyrrolidinyl)ethoxy]phenyl]ethanol.** A solution of 2-(1-adamantyl)-2′-[2-(1-pyrrolidinyl)ethoxy]acetophenone hydrochloride (**57**, 891 g, 2.3 mol) in water (4.45 L) was basified by addition of 1 N sodium hydroxide (315 mL) and extracted with ethyl acetate. Evaporation gave the free base. This was dissolved in IMS (3.4 L) and sodium borohydride (180 g, 4.86 mol) was added in portions. After refluxing for 12 h the mixture was evaporated to dryness and the residue partitioned between water and ethyl acetate. Evaporation of the organic layer gave the desired alcohol product as an oil: yield 655 g (85%).

3. ***trans*-1-[2-[2-[2-(1-Adamantyl)vinyl]phenoxy]ethyl]-pyrrolidine Monohydrochloride Monohydrate (54).** 2-(1-Adamantyl)-1-[2-[2-(1-pyrrolidinyl)ethoxy]phenyl]ethanol (50 g) was added to a solution of oxalic acid (50 g) in H_2O (500 mL) and the mixture refluxed for 2.5 h. The solid obtained on cooling was filtered off and dispersed in H_2O (600 mL) at 60 °C. A solution of KOH (30 g) in H_2O (150 mL) was added and the mixture stirred for 0.5 h. Extraction with EtOAc (480 mL), dilution with ether (960 mL), and the passage of hydrogen chloride gave the desired product, **54**. This was washed with ether and dried in vacuo: yield 52.2 g (95.2% g); mp 211 °C.

Method E (Scheme I). 1. ***N*,*N*-Diethyl-2-[2-(2-cyclohexyl-1-hydroxyethyl)phenoxy]ethylamine (58).** Cyclohexylmethylmagnesium bromide (35.4 g, 0.179 mol) was prepared in ether (610 mL) in the usual manner from cyclohexylmethyl bromide and magnesium. 2-Diethylaminoethoxybenzaldehyde (44.0 g, 0.137 mol) in ether (60 mL) was added slowly to the cooled Grignard reagent. At the completion of the addition the mixture was heated under reflux for 3 h and then decomposed with 5 N hydrochloric acid in the presence of ice. The free base of the product was liberated by the addition of ammonium chloride (42.8 g) and diluted ammonia and the separated ethereal layer was removed. The aqueous phase was extracted twice more with ether (150 mL total) and the ether extracts were pooled and washed with dilute ammonia. Ether was removed by evaporation on a water bath and the residual oil distilled in vacuo. The fraction of bp 180–186 °C (0.3 mm) (29.8 g) was the required alcohol **58**. A small quantity (3.19 g) of the free base was dissolved in ether (75 mL) and treated with ethanolic HCl (1 mL, 1.0 N) to convert it to the hydrochloride (3.3 g), mp 169–170 °C. Anal. ($C_{20}H_{33}NO_2$·HCl) C, H, N.

2. ***N*,*N*-Diethyl-2-[2-(2-cyclohexylvinyl)phenoxy]ethylamine (50).** The alcohol **58** (25.0 g as the free base) was dissolved in benzene (50 mL) and $SOCl_2$ (10.4 g) was added dropwise with cooling. The solution was heated under reflux for 1 h, cooled, and shaken briefly with 10% Na_2CO_3 solution (80 mL). Benzene was removed on the water bath and the residual oil heated in vacuo to 160 °C for about 15 min, during which time the contents of the flask partially solidified. The product was dissolved in water (150 mL) adjusted to pH 10.0 with dilute ammonia and the separated oil was recovered by extraction with ether (3 × 50 mL). Ether was removed on the water bath and the resulting oil distilled in vacuo, the fraction (18.0 g), bp 160–165 °C (0.5 mm), being collected. Of this fraction, 17.0 g was dissolved in ether (350 mL) and the solution was treated with ethanolic HCl (5.5 mL, 9.5 N), yielding 17.4 g (65%) of the crude hydrochloride **50**. Recrystallization from EtOAc gave the pure hydrochloride, mp 169–170 °C. Anal. C, H, N.

Method F (Scheme I). ***N*-[2-[2-[2-(Adamant-1-yl)ethyl]phenoxy]ethyl]pyrrolidine Hydrochloride Hemihydrate (44).** *trans*-*N*-[2-[2-[2-(Adamant-1-yl)vinyl]phenoxy]ethyl]pyrrolidine hydrochloride (**54**), prepared as in method C (1.2 g), in ethanol (50 mL), was hydrogenated at 25 °C and 15 psi over 5% palladium on carbon for 1.5 h. The mixture was then filtered and the catalyst washed with ethanol. The filtrate and catalyst washings were combined and evaporated to give the product hydrochloride hemihydrate (**24**) as a crude solid which was recrystallized from a mixture of EtOAc and isopropyl ether and then from toluene to give a white solid (0.825 g), mp 175–178 °C (with prior softening). Anal. C, H, N.

***cis*-1-[2-[2-[2-(1-Adamantyl)vinyl]phenoxy]ethyl]-pyrrolidine Monohydrochloride Hemihydrate (55).** Ultraviolet irradiation of **54** (10 g) in methanol (1 L) for 2 h and evaporation of the solvent gave the impure desired product. Crystallization from ethanol–ether gave the pure cis isomer **55** (9.2 g, 92%): mp 180 °C; UV (MeOH) λ_{max} 278 nm. Anal. C, H, N.

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Methotrexate Analogues. 9. Synthesis and Biological Properties of Some 8-Alkyl-7,8-dihydro Analogues

Michael Chaykovsky,* Margaret Hirst, Herbert Lazarus, Jack E. Martinelli,

The Sidney Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115

Roy L. Kisliuk, and Yvette Gaumont

Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts 02111.
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A series of 8-alkyl-7,8-dihydromethotrexate analogues was prepared by direct alkylation of 7,8-dihydromethotrexate, after pilot studies were performed with simpler pteridines. These compounds were tested for in vitro inhibitory activity against *Lactobacillus casei* and as enzyme inhibitors against dihydrofolate reductase and thymidylate synthetase derived from this organism. All of the analogues were less inhibitory toward dihydrofolate reductase than was methotrexate but were more inhibitory toward thymidylate synthetase. The analogues were also evaluated for in vitro inhibitory activity against the CCRF-CEM human lymphoblastic leukemia cells. In vivo against the L-1210 leukemia in mice, several of the analogues exhibited some antileukemic activity.

Dihydro- and tetrahydropteridines, in which the pyrazine ring is the reduced site, are intermediates in many biological reactions in diverse living organisms.[1] An important series of reactions is the biosynthetic reduction of folic acid and 7,8-dihydrofolic acid to tetrahydrofolic acid which is subsequently converted into metabolites that play a role in biochemical one-carbon transfer reactions, including the biosynthesis of nucleotides. Antimetabolites which inhibit these reactions are useful in the treatment of neoplastic diseases, a classical example of which is the antitumor agent methotrexate[2] (4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid; amethopterin; MTX), which binds very strongly to the enzyme dihydrofolate reductase and inhibits the formation of tetrahydrofolate. In doing so, MTX blocks the formation of most, if not all, of the coenzymes involved in folate metabolism, and this probably accounts for its high toxicity. An important goal in the search for new antifolates has been the design of agents which would be less inhibitory toward dihydrofolate reductase than is MTX, but which would inhibit other folate enzymes, such as thymidylate synthetase. This could lead to antineoplastic agents with more therapeutic specificity for tumor cells.[3]

Since all of the enzymes involved in folate metabolism utilize reduced folate coenzymes in their biosynthetic transformations, it became apparent that dihydro- and tetrahydrofolate analogues might inhibit these enzymes and might be useful as antineoplastic agents. The first work in this area began in the late 1950's and it continues to be an important area of research in cancer chemotherapy. Several studies[4-8] have shown that certain reduced folate analogues exhibit significant inhibition against thymidylate synthetase derived from *Escherichia coli*. For example, it was found[8] that 7,8-dihydromethotrexate was about 40 times more potent an inhibitor of this enzyme than was MTX and that it was only one-half as potent as MTX against dihydrofolate reductase (from L-1210 mouse leukemic cells).

It has long been recognized that the study of the chemical and biological properties of reduced pteridines and reduced folates is complicated by the susceptibility of these compounds to oxidative degradation, even upon standing in air.[1] However, their chemical stability can be greatly improved by substitution of alkyl groups at N^8 in 7,8-dihydropteridines[9] and at N^5 and/or N^8 in 5,6,7,8-tetrahydropteridines.[10] Direct chemical substitution at N^5 in tetrahydropteridines proceeds rather easily under mild conditions,[10-13] but N^8 has been found to be resistant to direct alkylation.[12] In a recent communication,[14] we described a method by which 2,4-diamino-7,8-dihydropteridines can be directly monoalkylated at N_8, without substitution either in the pyrimidine ring or on the 2- or 4-amino groups. The alkylated derivatives, unlike the parent compound, were stable under mild oxidative conditions.

With this chemical method in hand, we applied it to the preparation of several 8-alkyl-7,8-dihydromethotrexate analogues. Although these compounds are structurally more complex than the smaller pteridines, their preparation by direct alkylation of 7,8-dihydromethotrexate