Eur. J. Med. Chem. **22** (1987) 91—100

Original paper

Iodobolpyramine and other iodinated derivatives as ligands for detecting histamine H_1 receptors

Robert C. BLAKEMORE [1], C. Robin GANELLIN [1*•], Monique GARBARG [2], Claude GROS [2], Robert J. IFE [1], Marie KÖRNER [2], Martial RUAT [2], Jean-Charles SCHWARTZ [2], Wasyl TERTIUK [1•] and Colin J. THEOBALD [1]

[1] *Smith Kline and French Research Ltd., The Frythe, Welwyn, Hertfordshire AL6 9AR, U.K., and* [2] *Unité 109 de Neurobiologie, Centre Paul Broca de l'INSERM, 2* ter, *rue d'Alésia, 75014 Paris, France*

(Received June 23 1986, accepted September 1 1986)

Summary — The synthesis is described of potential I-labelled H_1-receptor ligands. Receptor affinity is assessed *in vitro* from inhibition of histamine-stimulated contraction of guinea pig ileum and/or through binding to guinea pig cerebellar membranes.

Direct iodination of mepyramine and of hydroxy analogues of mepyramine and temelastine (SK&F 93944) furnishes derivatives which have lower receptor affinities than the parent compounds. In another approach, novel aminoalkyl derivatives of mepyramine are converted via the [^{125}I]Bolton—Hunter reagent into the corresponding 3-(4-hydroxy-5-[^{125}I]-iodophenyl)propionamides, each of which is separated chromatographically and its affinity determined by a binding assay. The compound of highest affinity is [^{125}I]iodobolpyramine, derived from the aminopentyl analogue, and is among the most potent of known H_1 anti-histamine ligands providing a highly sensitive means of detecting H_1 receptors. A convenient synthesis of [^{125}I]iodobolpyramine from bolpyramine and iodo-gen is also described; the compound is purified by TLC and authenticated by HPLC comparison with non-radiolabelled mono- and di-iodinated bolpyramine derivatives. Cyanobutyl- and acetamidopentyl-analogues of mepyramine are also tested as histamine antagonists and structure—activity relationships are described.

Résumé — **Iodobolpyramine et autres dérivés iodés comme ligands pour les récepteurs H_1 de l'histamine.** *La synthèse de ligands iodés capables de marquer les récepteurs H_1 est présentée. L'affinité de ces ligands pour les récepteurs H_1 a été mesurée* in vitro *par l'inhibition de la contraction de l'iléon de cobaye induite par l'histamine et/ou par la liaison à des membranes de cervelet de cobaye. L'iodation directe de la mépyramine, d'analogues hydroxylés de la mépyramine et de la témelastine (SK&F 93944) a conduit à des dérivés qui possèdent une affinité plus faible que les composés de départ pour les récepteurs H_1. Une autre approche a consisté à transformer de nouveaux dérivés aminoalkylés de la mépyramine en (hydroxy-4-iodo-5-phényl-[^{125}I])-3-propionamides correspondants par le réactif de Bolton—Hunter[^{125}I]. Chacun d'entre eux a été séparé par chromatographie et leur affinité testée par la technique de liaison. Le composé présentant la meilleure affinité a été l'iodobolpyramine-[^{125}I], dérivée de l'analogue aminopentyle. Elle est l'un des plus puissants antagonistes connus de l'histamine pour les récepteurs H_1 et elle permet une détection extrêmement sensible de ces récepteurs.*

Une méthode de synthèse pratique de l'iodobolpyramine[^{125}I] à partir de bolpyramine et d'iodo-gène a été mise au point. Le composé a été purifié par TLC et authentifié en HPLC par comparaison avec les dérivés mono- et di-iodés non radiomarqués de la bolpyramine. Les analogues cyanobutyl et acetamidopentyl de la mépyramine ont aussi été testés comme antagonistes de l'histamine et les relations structure—activité sont décrites.

H_1-receptor binding / anti-histamine / ^{125}I-iodine label / iodobolpyramine / mepyramine derivatives

Introduction

Tritium-labelled ligands have been identified for labelling histamine H_1 receptors, namely [^{3}H]mepyramine [1—3] [^{3}H]doxepin [4, 5] and [^{3}H]mianserin [6], but high affinity ^{125}I-labelled ligands offer technical advantages over ^{3}H-ligands since in theory they can be obtained with 50—100-fold higher specific radioactivity, thereby providing increased sensitivity for receptor assays.

Mepyramine and an analogue of SK & F 93944 (teme-

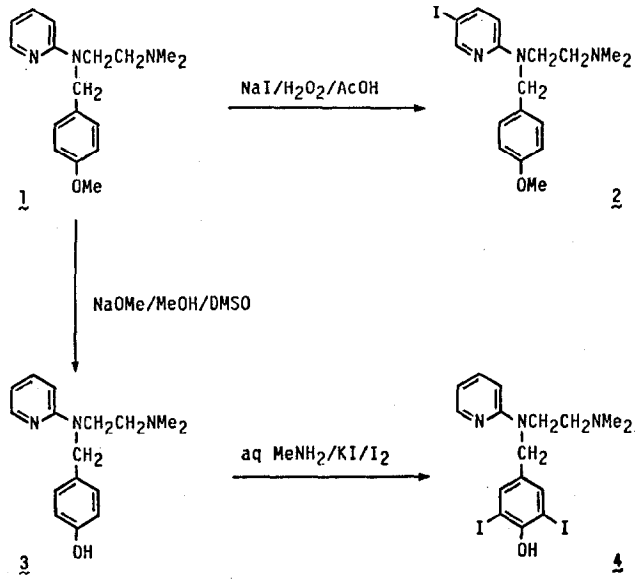
**Author to whom correspondence should be adressed.*
•*Present address:* Department of Chemistry, University College London, 20 Gordon Street, London WCIH OAJ, England.

lastine) [7] were selected as suitable starting material for iodination since they have high selectivity and high affinity as H_1-receptor ligands. Investigative reactions were conducted using non-radioactive reagents (Scheme 1).

Chemistry

The 2-amino group in mepyramine **1** activates the pyridine ring towards halogenation in the 5-position [8], but initial attempts to iodinate mepyramine using I^- and chloramine-T as the oxidising agent in aqueous buffer at pH 7.4 (conditions suitable for ^{125}I-iodination) were unsuccessful, and the only products isolated were various chlorinated derivatives. To avoid chlorination, I^- and hydrogen peroxide were used at pH 7.4; an insoluble brown solid was isolated which, on treatment with aqueous sodium hydroxide according to published procedures [9], only yielded unchanged mepyramine. Use of strongly acidic conditions (40% v/v H_2SO_4) to favour monohalogenation, as described for chlorination of 2-aminopyridine [10], led to cleavage of the benzyl group. In acetic acid, however, using either hydrogen peroxide or *m*-chloroperbenzoic acid as the oxidant, iodomepyramine **2** was obtained (Scheme 1a) which, after column chromatography, was isolated in a high state of purity with relatively low contamination (0.33%) by mepyramine.

As an alternative approach to incorporating iodine into the mepyramine structure, the benzylic methoxyl group was cleaved and the resulting hydroxy analogue **3** of mepyramine was iodinated to furnish the di-iodo-hydroxy derivative **4** (Scheme 1a). Similarly, iodination of a *p*-hydroxybenzyl analogue **5** of the recently described H_1-receptor anti-histamine SK & F 93944 (temelastine) [7] gave the di-iodo-hydroxy compound **6** (Scheme 1b).



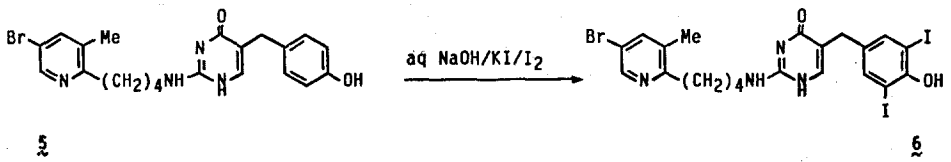
Scheme 1a. Synthesis of iodomepyramine and analogues.

In another approach, a series of aminoalkyl derivatives **13a—f** (x = 2—7) of mepyramine was synthesised (Scheme 2a). The starting material 2-[*N*-(2-aminoethyl)amino]pyridine **7** which has previously been synthesised *via* a multistage route [11] was more conveniently prepared from 2-bromopyridine and 1,2-ethanediamine. It was alkylated with *p*-methoxybenzyl chloride to give the primary amine analogue **8** of mepyramine which was then formylated and reduced to the monomethyl secondary amine **10**. The latter was alkylated with a bromoalkyl *N*-phthalimide or bromoalkyl-nitrile to give the series of phthalimides **11** (x = 2—4) or nitriles **12** (x = 5—7) which were then converted into the respective primary aminoalkyl compounds **13**.

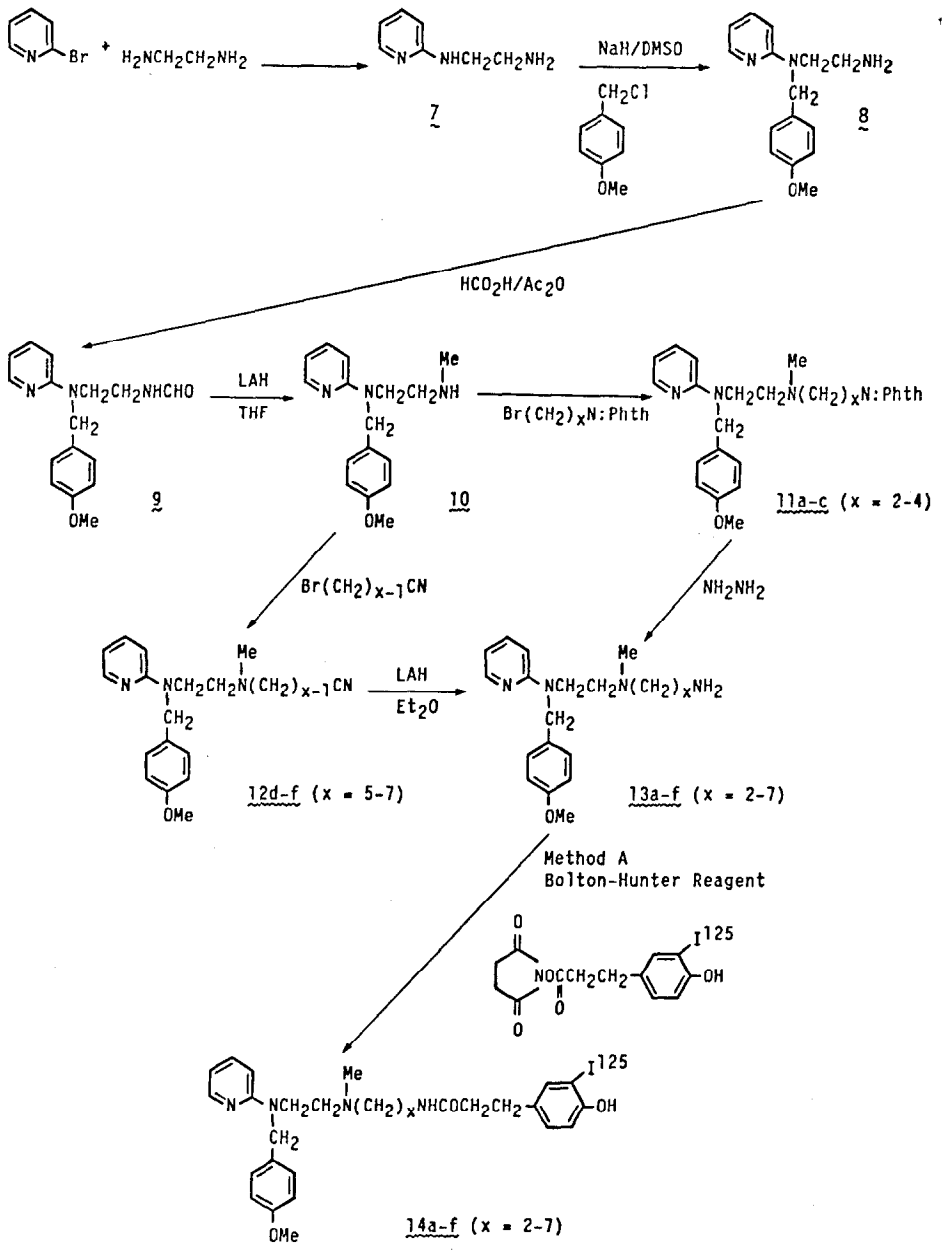
Each of the aminoalkyl derivatives **13** was subjected to the Bolton—Hunter procedure [12] for introducing the 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionyl group to produce the ^{125}I-iodinated ligands **14a—f** (Method A) which were purified by thin—layer chromatography (TLC) and extracted into ethanol for bioassay. In addition, the aminopentyl compound **13d** (SK & F 94461) was converted into the *p*-hydroxyphenylpropionyl derivative, to which we have given the name bolpyramine **15d**, which was then transformed into [^{125}I]iodobolpyramine **14d** [13] with Na^{125}I and iodo-gen [14] (Method B) to provide a more convenient synthesis (Scheme 2b). A trace amount of di-iodobolpyramine was also formed but it was kept to a minimum by using an excess of bolpyramine; it was easily removed by TLC. Bolpyramine was also subjected to non-radioactive iodination to give the mono- and di-iodinated analogues **16d** and **17d** which were separated by column chromatography.

The identity of [^{125}I]iodobolpyramine, obtained by TLC from Methods A and B, was confirmed by comparison with the authentic non-radioactive material **16d** synthesised independently. Evidence for identity and lack of contamination was also obtained by using HPLC to purify the [^{125}I]iodobolpyramine prepared by Method B; in this chromatographic procedure the required [^{125}I]iodobolpyramine was well separated from di-iodobolpyramine and bolpyramine (Fig. 1), and had a retention time identical to that of the authentic non-radioactive material.

The aminopentyl derivative **13d** was also converted into the acetamide **18d**.



Scheme 1b. Synthesis of the iodinated analogue of temelastine.



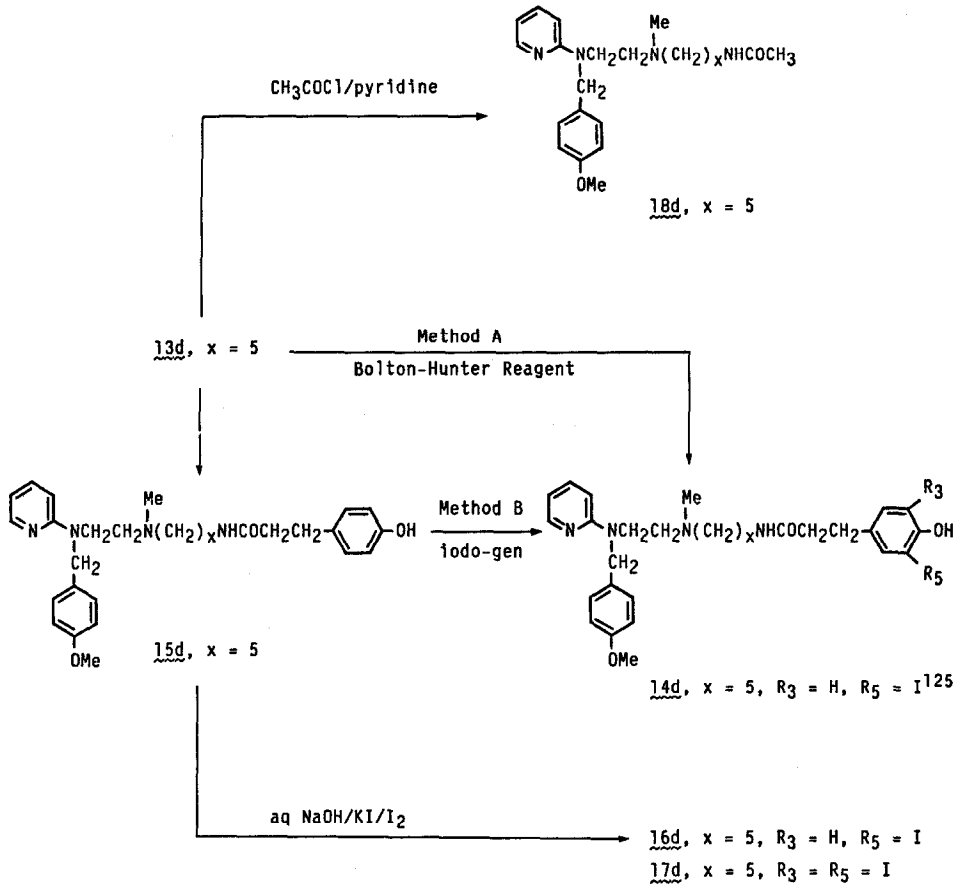
Scheme 2a. Synthesis of aminoalkyl derivatives of mepyramine and corresponding 4-hydroxy-5-[^{125}I]iodophenyl)propionamides (N:Phth = phthalimide).

Results

Compounds assayed *in vitro* on the guinea pig ileum as antagonists of histamine-stimulated contraction are given in Table I. Iodomepyramine **2** appeared to have an affinity (K_B = 6 nM) approximately 1/5 of that of mepyramine (K_B = 1.3 nM) when tested *in vitro* as an antagonist of histamine-induced contractions of the guinea pig ileum. This was an encouraging result since the analogous bromomepyramine compound has been reported [17] to have some 20-times lower affinity than that of mepyramine. However, the slope of the Schild plot for iodomepyramine was significantly lower than unity and iodomepyramine was found to have a much lower affinity (approximately 80-fold; K_i = 38 nM, Table II) than mepyramine, when tested for inhibition of [^{3}H]mepyramine binding to guinea pig cerebellar membranes, suggesting that it would not be a useful ligand for labelling studies of the brain.

The hydroxybenzyl compound **3** derived from mepyramine was substantially less active (K_B = 21 nM) than mepyramine as an H_1-receptor antagonist assayed *in vitro* on the guinea pig ileum, and the introduction of two iodine atoms (structure **4**) led to a considerable further drop in activity (three orders of magnitude).

The hydroxybenzyl analogue **5** of temelastine (SK & F 93944) had a similar antagonist potency (K_B = 2.0 nM)



Scheme 2b. Synthesis of bolpyramine (**15d**), iodo-derivatives, and acetyl analogue.

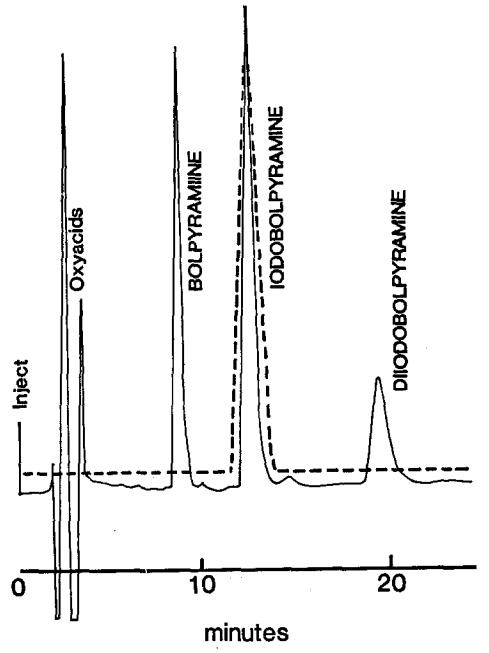


Fig. 1. High—performance liquid chromatogram of a synthetic mixture of bolpyramine **15d**, iodobolpyramine **16d**, and di-iodobolpyramine **17d**, detected at $\lambda = 210$ nm at retention times of respectively 8.5, 12.3 and 19.2 min (see Experimental protocols for conditions). Vertical axis: detector response (optical density); horizontal axis: elution time. The dashed line shows the elution of purified [^{125}I]-iodobolpyramine, fractions counted every 0.3 min, peak height represents approximately 1200 cpm.

to mepyramine determined *in vitro* on the guinea pig ileum but the di-iodo derivative **6** was much less active (K_B = 55 nM).

An alternative approach to introducing a label is to incorporate it into a group attached to an atom chain at a position in the drug structure which does not interfere with drug—receptor interaction (*cf.* the ' congener approach ' to drug design described by Jacobson *et al.* [18]).

Mepyramine has a typical H_1-anti-histamine structure, *viz* a tertiary aliphatic amine linked by a short chain of atoms to two aromatic rings. Although the whole molecule must be considered as the pharmacophore, in the sense that in general for anti-histamines drug affinity for H_1-receptors is very sensitive to changes in chemical structure, the tertiary amino group appears to be able to tolerate the presence of various alkyl groups without having an adverse effect on receptor affinity. On the other hand, affinity appears to be very sensitive to substituent effects in the aromatic rings. Furthermore for chiral anti-histamines, a chiral centre near the aromatic rings induces stereospecificity in receptor antagonism, whereas a chiral centre near the tertiary amino group appears to cause minimal stereospecific discrimination [19]. This sensitivity of drug affinity to the precise stereochemistry clearly indicates that specific molecular interactions must occur between drug and receptor involving, especially, at least one of the aromatic rings (*cf.* models proposed by Nauta and Rekker [20]; see also discussion in ref. [19]).

Table I. H_1-Receptor histamine antagonist activities of compounds assayed *in vitro* on the guinea pig ileum against histamine-stimulated contraction after 8 min of incubation at 30°C.

Compound and salt form*		pA_2	95% limits	slope ± 95% limits	n
**1	M	8.89	8.77 - 9.00	1.16 ± 0.24	12
***2	M	8.22	8.02 - 8.49	0.63 ± 0.12	17
3	M	7.67	7.28 - 7.97	0.89 ± 0.31	9
4		4.70	4.2 - 5.2	0.93 ± 0.38	10
5		8.69	8.42 - 8.92	1.18 ± 0.30	12
6		7.26	6.10 - 7.90	0.79 ± 0.39	12
8	M	6.37	5.96 - 6.97	1.00 ± 0.33	9
10	M	8.47	8.30 - 8.76	0.83 ± 0.33	9
12d	M	8.65	8.60 - 8.72	0.91 ± 0.16	7
13a	M_2	7.64	7.29 - 7.78	0.75 ± 0.18	9
13b	M_2	7.19	7.03 - 7.36	1.23 ± 0.20	9
13c	M_2	7.26	6.17 - 7.74	0.93 ± 0.5	12
#13d	M_2	7.50	7.16 - 7.76	1.23 ± 0.36	9
13e	Ox_2	7.54	6.96 - 8.25	0.79 ± 0.36	7
13f	Ox_2	7.64	7.37 - 7.94	1.15 ± 0.25	6
##15d	Ox_2	8.09	7.83 - 8.34	1.18 ± 0.24	7
##15d	Ox_2	ΔΔ8.42	7.96 - 8.54	1.00 ± 0.38	6
Δ16d	Ox	7.66	7.54 - 7.75	1.69 ± 0.54	7
Δ16d	Ox	ΔΔ8.03	7.75 - 8.36	1.07 ± 0.26	6
17d	OxHI	ΔΔ8.06	7.29 - 8.84	1.03 ± 0.38	9
18d	M	7.88	7.74 - 8.04	0.96 ± 0.37	11

(n = number of observations used in the Schild plot for determining the K_B value and $pA_2 = -\log K_B$). *M = maleate; M_2 = dimaleate; Ox = oxalate; Ox_2 = dioxalate; HI = hydroiodide. **mepyramine. ***iodomepyramine. #SK&F 94461. ##bolpyramine. Δiodobolpyramine. ΔΔAfter 15 min of equilibration.

The foregoing observations led us to explore the effect of siting a labelled atom chain on the tertiary amino function. In the homologous series of aminoalkyl derivatives of mepyramine **13a—f**, (x = 2—7) all the members were found to be similarly less active than mepyramine by some 20—50-fold. Each primary amine in the series was subjected to ^{125}I-iodination using the Bolton—Hunter procedure [12] and the ^{125}I-iodinated product separated chromatographically and assayed for binding to guinea pig cerebellar membranes.

The ^{125}I-iodinated derivatives **14a—f** all showed a considerable increase in potency and, furthermore, a variation in affinity with respect to chain length (Table III) as indicated by the number of specific binding sites measured in the assay. The optimum chain length for highest affinity has x = 5 and this compound, which we have called [^{125}I]-iodobolpyramine **14d**, which also showed the highest percentage of specific binding, has been shown to be a very useful radioligand for membrane binding and receptor autoradiography studies in guinea pig brain [13], where it is among the most potent H_1-receptor antagonists known. [^{125}I]Iodobolpyramine labels H_1-receptors with a high selectivity and relative to [^{3}H]mepyramine it provides a 50-fold increase in sensitivity for detecting H_1-receptors.

[^{125}I]Iodobolpyramine prepared by either method A or B was shown to have similar binding characteristics, indicating that interference from any non-radiolabelled contaminant was unlikely (since different non-radioactive starting materials and reagents were used in the two methods). Furthermore, [^{125}I]iodobolpyramine prepared by Method B and purified by HPLC also showed no significant difference in binding characteristics in comparison with the previous samples.

The K_D for [^{125}I]iodobolpyramine to guinea pig cerebellar membranes was 0.15 nM by Scatchard analysis of the saturation binding curve, or 0.055 nM from the ratio k_1/k_{-1} of association and dissociation rate constants. Binding occurred slowly, however, and at 25°C equilibrium was only reached after approximately 3 h [13].

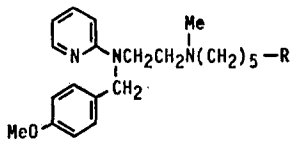
The K_i value for non-radiolabelled iodobolpyramine **16d** for inhibition of [^{125}I]iodobolpyramine binding to guinea pig cerebellar membranes was 0.11 nM (when measured at 0.15 nM) in good agreement with Table II. The K_i value for iodobolpyramine inhibition of [^{3}H]-mepyramine binding was 0.45 nM determined after 30 min of equilibration at a concentration of 0.6 nM, and was not significantly lower after 4 h of incubation. The K_B value for iodobolpyramine determined for inhibition of histamine-stimulated contractions of the isolated guinea pig ileum at 30°C after 15 min of equilibration was significantly higher, *viz* 9.3 nM (Table I).

The K_i values for di-iodobolpyramine (**17d**) and for the acetamide **18d** for inhibition of [^{125}I]iodobolpyramine binding to guinea pig cerebellar membranes were respectively 0.013 and 1.1 nM (Table II). The K_B values for **18d** and other mepyramine derivatives determined on the ileum after 8 min of equilibration are given in Table I.

Discussion

Small changes in the chemical structure of mepyramine may lead to compounds having considerably reduced H_1-antagonist activity as measured on the guinea pig ileum (Table I). Removal of methyl groups, thereby introducing protons which participate in H bonding, leads to pronounced reduction in activity; thus the hydroxy compound **3** has a K_B = 21 nM (relative to mepyramine 1.3 nM), the primary amine **8** has a K_B = 430 nM, and the secondary amine **10** has a K_B = 3.4 nM. Relative to mepyramine, the introduction of an aminoalkyl chain also reduces activity by 20—40-fold, *e.g.* **13d** has a K_B = 32 nM. Although the amino function would be protonated at physiological pH, the loss in activity may be associated with the presence

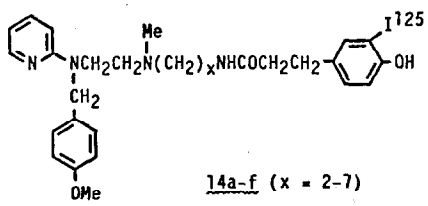
Table II. Inhibition constants (K_I) of compounds as inhibitors of binding of [^{125}I]iodobolpyramine to guinea pig cerebellar membranes.



Structure	Compound Name	R	K_I ± SEM nM	pseudo-Hill coefficient
1	mepyramine		0.33 ± 0.03	1.07 ± 0.10
1	mepyramine*		0.48 ± 0.10	0.92 ± 0.02
2	iodomepyramine*		38 ± 5	1.38 ± 0.15
13d	SK&F 94461	$-NH_3^+$	8.3 ± 0.3	1.03 ± 0.03
18d	acetamide derivative	$-NHCOCH_3$	1.1 ± 0.2	1.29 ± 0.18
15d	bolpyramine	$-NHCOCH_2CH_2$-(4-OH)	0.57 ± 0.09	0.95 ± 0.14
16d	iodobolpyramine	$-NHCOCH_2CH_2$-(4-OH, 3-I)	0.11 ± 0.01	1.11 ± 0.09
17d	di-iodobolpyramine	$-NHCOCH_2CH_2$-(4-OH, 3,5-I)	0.013 ± 0.003	1.10 ± 0.22

Determined after 4 h incubation at 25°C in the presence of 0.1% BSA as described by Körner *et al* [13].
*Determined after 30 min of incubation at 25°C (without BSA) against [^{3}H]mepyramine. (Number of independent experiments, n = 2—3.)

Table III. Specific binding of [^{125}I]mepyramine derivatives (synthesised using the ^{125}I-Bolton—Hunter reagent) to guinea pig cerebellar membranes.*



14a-f (x = 2-7)

[^{125}I]Compounds	x	Ligand concentration nM	Number of sites bound specifically ± SD fmole/mg protein	Specific binding %
14a	2	0.18	4 ± 1	11
14b	3	0.13	7 ± 0.4	35
14c	4	0.13	14 ± 1	57
14d	5	0.11	98 ± 3	85
14e	6	0.11	87 ± 9	75
14f	7	0.12	44 ± 4	67

* Compounds **14a-c**, binding measured at 30°C after 30 min of incubation; compounds **14d-f**, binding measured at 25°C after 4 h of incubation; **14d** = [^{125}I]iodobolpyramine.

of H-bonding groups rather than the additional positive centre since the corresponding acetamide **18d** also has relatively low activity (K_B = 13 nM). The nitrile **12d**, on the other hand, which does not contain additional NH groups, is almost as active (K_B = 2.2 nM) as mepyramine. Comparison of the acetamide **18d** with iodobolpyramine **16d** indicates that the 4-hydroxy-3-iodobenzyl group makes no additional contribution to anti-histaminic activity as determined on the guinea pig ileum.

A different structure—activity pattern emerges, however, from the cerebral binding studies in comparison with the results on the ileum. Iodobolpyramine **16d** does appear to have higher affinity than the acetamide **18d** for binding to cerebral membranes (Table II). Similarly, although iodobolpyramine is apparently less active than mepyramine as an antagonist of histamine-stimulated contraction of the guinea pig ileum, it appears to have a higher affinity than mepyramine for binding to guinea pig cerebellar H_1-receptors. Comparison is complicated, however, by the use of different equilibration times. Whereas iodobolpyramine required about 2 h at 30°C to achieve equilibrium in binding to cerebellar membranes [13], it was not feasible to estimate its K_B value on the guinea pig ileum beyond 15 min of equilibration, since after this time bolpyramine caused considerable depression of the tissue's maximal response to histamine. The K_B value at 15 min (9.3 nM) was, however, significantly lower than that determined after 8 min (22 nM) and it is likely, therefore, that activity on the ileum has been underestimated.

This is further indicated by the results with bolpyramine **15d** where the K_B value determined on the guinea pig ileum after 15 min of equilibration (3.8 nM) decreased by a factor of approximately 2 in comparison with that determined after 8 min of equilibration. For bolpyramine too, there was considerable depression of the maximum after 30 min of equilibration. By contrast, mepyramine reached equilibrium much sooner (within 30 min for [^{3}H]mepyramine binding).

As shown in Table II, the affinities of compounds as inhibitors of [^{125}I]iodobolpyramine binding to cerebellar membranes are sensitive to changes in chemical structure. Thus the acetamide **18d** shows nearly a 10-fold increase in affinity over the amine SK & F 94461 **13d**, although it still has a lower affinity than mepyramine. Introduction of the *p*-hydroxybenzyl group into the acetamide structure to give bolpyramine **15d** is seen to be accommodated without interfering with receptor binding and, indeed, there is a modest increase (nearly 2-fold) in affinity. The introduction of iodine to give iodobolpyramine **16d** increases affinity by a further 5-fold. This is a notable result because the introduction of an iodine atom into a ligand often decreases affinity (as was found for mepyramine, *vide supra*). It is of interest in this connection to note that the introduction of a second iodine atom to give di-iodobolpyramine **17d** affords another 10-fold increase in affinity.

The main interest in [^{125}I]iodobolpyramine is that it provides a highly sensitive means of detecting H_1-receptors due to its high affinity, high specific radioactivity and relatively low non-specific binding [13]. When used at a concentration close to the K_D value, 0.2 fmol of binding sites (corresponding to about 1 μg protein of guinea pig cerebellar membranes) can be reliably assayed: this represents a 50—100-fold increase in sensitivity as compared to [^{3}H]mepyramine. The new ligand may have several valuable applications, *e.g.* for the assay of H_1-receptors in microdissected brain areas, for tissues with low receptor densities, for the autoradiographic mapping of H_1-receptors, and for H_1-receptor purification studies.

Experimental protocols

Chemistry

^{1}H NMR spectra were recorded on a JEOL PFT 100P spectrometer at 100 MHz using TMS as an internal standard (or sodium trimethylsilylpentane sulfonate-d_4 for D_2O solutions) or, if indicated, at 250 MHz or 360 MHz on a Bruker AM 250 or AM 360 pulsed Fourier—transform nuclear magnetic resonance system. High—performance liquid chromatography (HPLC) was carried out with a Perkin—Elmer series 3 pump and Perkin—Elmer LC 75 detector set at 240 or 250 nm, or with a Waters Associates 6000A pump and LKB 2151 detector set at 210 nm, using a C_{18}/μBondapak column from Waters Associates. Preparative column chromatography was conducted using silica gel 60 (70—230 mesh ASTM) from Merck. Elemental analyses are indicated by elemental symbols and were within ± 0.4% of the theoretical values unless otherwise stated; they were provided by Mr. M. J. Graham in the Physical Organic Chemistry Department of Smith Kline and French Research Ltd. Melting points were recorded on an electrothermal apparatus (electrically heated block) and are uncorrected.

N-2-(5-Iodo)pyridinyl-N-(4-methoxybenzyl)-N',N'-dimethyl-1,2-ethanediamine maleate (iodomepyramine, 2)

Sodium iodide (6 g, 40 mmol) in acetic acid (50 ml) was added to a stirred solution of mepyramine maleate (8 g, 20 mmol) in acetic acid (100 ml). *m*-Chloroperbenzoic acid (6.9 g, 40 mmol) was added in portions at 22°C and the mixture was stirred for 1.5 h, then poured into water, and the solution was basified (NaOH). The mixture was extracted with ether, the extracts were concentrated and the resulting residue was chromatographed in chloroform on a silica gel column to yield an oil (3.74 g, 47% yield). The latter in hot ethanol was treated with one equivalent of maleic acid and the product **2** (1.1 g) crystallised out as colourless microprisms, mp 119—120°C. Anal. $C_{17}H_{22}IN_3O$, $C_4H_4O_4$ (C, H, I, N). ^{1}H NMR (D_2O): δ ppm 2.92 (s, 6H, NCH_3); 3.34 (m, 2H, NCH_2); 3.80 (s, 3H, OCH_3); 3.92 (m, 2H, NCH_2); 4.60 (s, $ArCH_2$); 6.30 (s, 2H, =CH); 6.54 (d, 1H, pyridine 3); 6.94, 7.19 (m, 4H, Ar); 7.68 (d, 1H, pyridine 4); 8.31 (m, 1H, pyridine 6). HPLC on C_{18}/μBondapak in 1:2 cyanomethane: 5 mM pentanesulfonic acid (pH 2) at 40°C and 2 ml/min flow rate indicated 99.4% of the total peak area (retention time 7.56 min) detected at λ = 240 nm; mepyramine contaminant was present at relative peak area of 0.33% (retention time 1.89 min).

N-(4-Hydroxy-3,5-di-iodobenzyl)-N', N'-dimethyl-N-2-pyridinyl-1,2-ethanediamine, 4

To mepyramine maleate (2.0 g, 5 mmol) dissolved in dimethyl sulfoxide (10 ml), was added a freshly prepared solution of sodium methoxide (from 0.81 g Na, 35 mmol in 10 ml of methanol) and the mixture was heated under reflux for 48 h. A further portion of methanolic sodium methoxide (15 mmol) was added and heating continued for a further 48 h, and the mixture then concentrated to near dryness. The residual solid was poured into water (100 ml), washed with chloroform (3 × 100 ml), treated with dil HCl to adjust the pH to 9 and extracted with chloroform (4 × 100 ml). The latter combined extracts were dried, concentrated, and the resulting oil dissolved in ethanol and treated with maleic acid to furnish 1.1 g (57% yield) of *N-(4-hydroxybenzyl)-N', N'-dimethyl-N-2-pyridinyl-1,2-ethanediamine maleate*, **3**, which was recrystallised from propan-2-ol and had mp 129—130°C. Anal. $C_{16}H_{21}N_3O$, $C_4H_4O_4$ (C, H, N). ^{1}H NMR (D_2O): δ ppm 2.93 (s, 6H, NCH_3); 3.36, 3.93 (m, 4H, NCH_2); 4.63 (s, 2H, $ArCH_2$); 6.25 (s, 2H, =CH), 6.7—7.15 (m, 6H, Ar, pyridine 3.5); 7.63 (m, 1H, pyridine 4); 8.10 (m, 1H, pyridine 6).

To a stirred solution of the above maleate (**3**, 0.72 g, 1.8 mmol) in 4 ml of 30% aqueous methylamine was added dropwise an aqueous solution of iodine (0.93 g, 3.7 mmol) and potassium iodide (2.4 g, 14 mmol), at 10°C. The mixture was stirred further at 10°C for 3 h and then at 23°C for 10 h, then cooled, neutralised with glacial acetic acid (4.6 ml) to pH 4, treated with sodium metabisulfite (0.1 g) to remove unchanged iodine and stirred for 3 h. The resulting pale-yellow solid was collected, washed with cold water, and crystallised from ethanol to furnish the free base **4**, mp 156—157°C (0.11 g). Anal. $C_{16}H_{19}I_2N_3O$ (C, H, I, N). ^{1}H NMR (Me_2SO-d_6): δ ppm 2.20 (s, 6H, NCH_3); 2.42 (m, 2H, NCH_2); 3.59 (m, NCH_2); 4.59 (s, 2H, $ArCH_2$); 6.55 (m, 2H, pyridine 3,5); 7.47 (m, 1H pyridine 4); 7.59 (s, 2H, Ar); 8.07 (m, 1H, pyridine 6).

Addition of ether to the ethanolic filtrate of **4** furnished the crystalline *hydroiodide monohydrate* of **4**, mp 121—123°C (0.41 g). Anal. $C_{16}H_{19}I_2N_3O$, HI (C, H, N) I found = 55.8%, requires 58.5%.

2-[4-(5-Bromo-3-methylpyridin-2-yl)butylamino]-5-(4-hydroxy-3,5-di-iodobenzyl)-pyrimidin-4-one, 6

2-[4-(5-Bromo-3-methylpyridin-2-yl)butylamino]-5-(4-hydroxybenzyl)-pyrimidin-4-one, **5**, was prepared from 4-(5-bromo-3-methylpyridin-2-yl)butylamine and 5-(4-hydroxybenzyl)-2-methylthio-pyrimidin-4-one in refluxing pyridine as described by Sach [21]. Compound **5** (2.0 g, 4 mmol) was dissolved in 30 ml of 2 N NaOH and then treated dropwise at 10°C with an aqueous solution containing iodine (3.4 g, 9 mmol) and potassium iodide (7.6 g, 46 mmol). The mixture was stirred at 10°C for 3 h and at 23°C for 10 h. It was then again cooled, treated with glacial acetic acid to pH 4, followed by sodium metabisulfite and stirred for a further 3 h. The resulting pale-lemon solid was collected and recrystallised from ethanol: water (2:1) to furnish the free base **6** (2.2 g, 75% yield) which after recrystallisation was obtained as the hemi-hydrate, mp 129—130°C. Anal. $C_{21}H_{21}BrI_2N_4O_2$ 0.5 H_2O, 0.25 C_2H_5OH (C, H, Br, I, N). ^{1}H NMR (Me_2SO-d_6): δ ppm 1.60

(m, 4H, CCH_2CH_2C); 2.27 (s, 3H, CH_3); 2.71 (m, 2H, CH_2); *ca.* 3.4 (m, CH_2N); 3.38 (s, $ArCH_2$); 6.31 (br, 1H, NH); 7.51 (s, 1H, pyrimidone); 7.57 (s, 2H, Ar); 7.76 (m, 1H, pyridine 4); 8.41 (m, 1H, pyridine 6).

N-(4-Methoxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine, ***10***

2-Bromopyridine (154 g, 0.98 mol) and 1,2-ethanediamine (293 g, 4.9 mol) were heated together in 100 ml anhydrous pyridine (dried over KOH) with stirring under reflux for 5 h and then evaporated *in vacuo* as previously described [22]. The resulting oil was dissolved in 200 ml of water, then brought to pH 8 with 10 N HCl and washed with $CHCl_3$ (2 × 100 ml). The aqueous layer was then basified with aq concentrated NaOH and extracted with $CHCl_3$ (4 × 120 ml). The combined latter $CHCl_3$ extracts were dried (K_2CO_3) and concentrated and the resulting oil was distilled *in vacuo* to give N-*2-pyridinyl-1,2-ethanediamine* **7**, bp 106°C at 0.15 mm (80 g, 60% yield). ^1H NMR ($CDCl_3$, 250 MHz): δ ppm 1.41 (br s, 2H, NH_2); 2.93 (t, 2H, NCH_2); 3.36 (m, 2H, CH_2NAr); 4.97 (br s, 1H, NH); 6.40 (d, 1H, pyridine 2); 6.55 (m, 1H, pyridine 5); 7.39 (m, 1H, pyridine 4); 8.07 (m, 1H, pyridine 6).

Compound **7** (41.2 g, 0.3 mol) in dry Me_2SO (50 ml) was added during 5 min to a stirred solution of sodium hydride (0.32 mol) in DMSO (200 ml) at 20°C; 4-methoxybenzyl chloride (50 g, 0.32 mol) in 50 ml of dry DMSO was then added dropwise during 20 min to the mixture with stirring and cooling to maintain the temperature below 40°C. The mixture was stirred for a further 45 min at 22°C, then poured onto 1 kg of cracked ice and extracted with ether (4 × 300 ml). The combined extracts were then extracted with 2 N HCl (2 × 200 ml). The combined acidic extracts were washed with $CHCl_3$ (2 × 100 ml), basified, and then extracted with ether (4 × 200 ml). The latter extracts were combined, dried ($MgSO_4$) and concentrated, to give the oily base, N-*(4-methoxybenzyl)-N-2-pyridinyl-1,2-ethanediamine* **8** (54.9 g, 71% yield); a sample was converted into **8** maleate in EtOH, mp 134—135°C (after recrystallisation). Anal. $C_{15}H_{19}N_3O$, $C_4H_4O_4$ (C, H, N). ^1H NMR (D_2O, 250 MHz): δ ppm 3.30 (t, 2H, NCH_2); 3.78 (s, 3H, OCH_3); 3.93 (t, 2H, NCH_2); 4.68 (s, 2H, $ArCH_2$); 6.20 (s, 2H, =CH); 6.78 (m, 2H, pyridine 3,5); 6.90, 7.16 (m, 4H, Ar); 7.59 (m, 1H, pyridine 4); 8.07 (m, 1H, pyridine 6).

Formic acid (22.2 g, 0.48 mol) and acetic anhydride (43.2 g, 0.42 mol) were heated together at 56°C for 2 h then cooled, and the base **8** (54.6 g, 0.21 mol) in ether (200 ml) was added dropwise during 30 min with stirring and cooling. The mixture was left at 22°C for 16 h and then diluted with water (300 ml) with cooling and basified (40% aqueous NaOH). The oily precipitate was extracted into $CHCl_3$ (3 × 200 ml) and the combined extracts were washed (H_2O), dried ($MgSO_4$) and concentrated. The resulting solid residue was crystallised from $CHCl_3$: Et_2O (1:2) to yield N'-*formyl*-N-*(4-methoxybenzyl)-N-2-pyridinyl-1,2-ethanediamine* **9** (55.1 g, 91% yield) mp 93—94°C. Anal. $C_{16}H_{19}N_3O_2$ (C, H, N). ^1H NMR ($CDCl_3$, 250 MHz): δ ppm 3.52 (m, 2H, NCH_2); 3.78 (s, 3H, OCH_3); 3.85 (m, 2H, NCH_2); 4.59 (s, 2H, $ArCH_2$); 6.49 (d, 1H, pyridine 3); 6.60 (m, 1H, pyridine 5); 6.85, 7.11 (m, 4H, Ar); 7.40 (m, 2H, NH, pyridine 4); 8.12 (m, 2H, CHO, pyridine 6).

A solution of the formyl derivative **9** (54.5 g, 0.19 mol) in dry THF (290 ml) was added dropwise during 75 min to $LiAlH_4$ (14.5 g, 0.38 mol) in THF (130 ml) with stirring and heating under reflux. The mixture was stirred under reflux for 4 h further, cooled and treated with aq saturated Na_2SO_4. The organic layer was decanted, dried ($MgSO_4$) and evaporated to furnish the oily base N-*(4-methoxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine* **10** (48.9 g, 95% yield); a sample was converted into the maleate **10** in propan-2-ol, obtained as needles mp 79—80°C (from EtOH—Et_2O). Anal. $C_{16}H_{21}N_3O$, $C_4H_4O_4$ (C, H, N). ^1H NMR (D_2O, 250 MHz): δ ppm 2.74 (s, 3H, NCH_3); 3.30 (t, 2H, NCH_2); 3.80 (s, 3H, OCH_3); 3.94 (t, 2H, NCH_2); 4.69 (s, 2H, $ArCH_2$); 6.22 (s, 2H, =CH); 6.77 (m, 2H, pyridine 3,5); 6.94, 7.19 (m, 4H, Ar); 7.60 (m, 1H, pyridine 4); 8.08 (m, 1H, pyridine 6).

N'-(4-Cyanobutyl)-N-(4-methoxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine maleate, ***12d***

The amine **10** (18.3 g, 68 mmol), 5-bromopentanonitrile (12.7 g, 78 mmol), and anhydrous potassium carbonate (18.7 g, 135 mmol) in dry DMF (230 ml) were stirred for 2 h at 100°C. The mixture was concentrated under reduced pressure and the residue taken into water (360 ml) and extracted with ether (3 × 200 ml). The combined extracts were washed (H_2O), dried (K_2CO_3) and evaporated to afford the free base **12d** as a pale-yellow oil, which was chromatographed in CH_2Cl_2 on a silica gel column and eluted with 1% MeOH in CH_2Cl_2 (18.8 g, 79% yield). It was converted into the maleate **12d** in ethanol, and recrystallised from propan-2-ol: ether mixture, mp 85—86°C. Anal. $C_{21}H_{28}N_4O$, $C_4H_4O_4$ (C, H, N). ^1H NMR (D_2O, 250 MHz): δ ppm 1.73 (m, 4H, CCH_2CH_2C); 2.52 (t, 2H, CH_2CN); 2.96 (s, 3H, NCH_3); 3.22 (t, 2H, NCH_2); 3.36 (t, 2H, NCH_2); 3.82 (s, 3H, OCH_3); 3.95 (t, 2H, NCH_2); 4.67 (s, 2H, $ArCH_2$); 6.27 (s, 2H, =CH); 6.98, 7.24 (m, 4H, Ar); 6.82, 7.63, 8.14 (m, 4H, pyridine).

In like manner, **10** was treated with 6-bromohexanonitrile or 7-bromoheptanonitrile to afford the cyanopentyl and cyanohexyl analogues (**12e** and **12f**) which were obtained as oily bases.

N'-(2-Aminoethyl)-N-(4-methoxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine dimaleate, ***13a***

The amine **10** (0.27 g, 1 mmol), *N*-(2-bromoethyl)phthalimide (0.28 g, 1.1 mmol), and anhydrous potassium carbonate (0.3 g, 2.2 mmol) in 10 ml DMF were stirred at 100°C for 2.5 h. The mixture was concentrated under reduced pressure and the residue taken into water (50 ml) and extracted with ether (3 × 50 ml). The combined extracts were washed (H_2O), dried (K_2CO_3) and evaporated. The resulting yellow oil (0.35 g) was chromatographed on a column of silica gel (20 g) with CH_2Cl_2 (200 ml), followed by 0.5% MeOH in CH_2Cl_2 to remove an impurity. Elution with 1% MeOH in CH_2Cl_2 then gave the intermediate N-*(4-methoxybenzyl)-N'-methyl-N'-(2-(N-phthalimido)ethyl)-N-2-pyridinyl-1,2-ethanediamine* **11a** (0.14 g, 32% yield).

The phthalimide **11a** (1.32 g, 3 mmol) was heated with hydrazine hydrate (0.45 g, 9 mmol) in ethanol (30 ml) under reflux for 2.5 h. The mixture was cooled, filtered from solid, concentrated, and added to a small amount of 2 N HCl; it was again filtered (from the white solid which had precipitated), basified to pH 14 (aq NaOH) and extracted (× 3) with ether. The combined extracts were dried (K_2CO_3) and concentrated. The resulting oily base (0.83 g) in ethanol (10 ml) was treated with malcic acid (0.63 g, 2 equivalents) in ethanol (5 ml), followed by ether (15 ml). The product, as dimaleate **13a**, crystallised out (1.24 g, 76% yield), mp 144—145°C. Anal. $C_{18}H_{26}N_4O$, $2C_4H_4O_4$ (C, H, N). ^1H NMR (D_2O): δ ppm 2.88 (s, 3H, NCH_3); 3.30 (m, 2H, NCH_2); 3.41 (s, 4H, NCH_2); 3.81 (s, 3H, OCH_3); 3.97 (m, 2H, NCH_2); 4.74 (s, $ArCH_2$); 6.24 (s, 4H, =CH); *ca.* 6.9 (m, 2H, pyridine 3,5); 6.96, 7.24 (m, 4H, Ar); 7.78 (m, 1H, pyridine 4); 8.05 (m, 1H, pyridine 6).

Likewise, the amine **10** was treated with *N*-(3-bromopropyl)phthalimide or *N*-(4-bromobutyl)phthalimide to provide the aminopropyl and aminobutyl analogues **13b** and **13c** which were obtained as dimaleates *viz*: **13b**: mp 146—148°C (EtOH), Anal. $C_{19}H_{28}N_4O$, $2C_4H_4O_4$ (C, H, N); **13c**: mp 123—125°C (EtOH: Et_2O), Anal. $C_{20}H_{30}N_4O$, $2C_4H_4O_4$ (C, H, N).

N'-(5-Aminopentyl)-N-(4-methoxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine dimaleate ***13d***

The nitrile base **12d** (20 g, 57 mmol) in dry ether (200 ml) was added during 1.5 h to lithium aluminium hydride (6.5 g, 170 mmol) in ether (300 ml) with stirring at 15°C. The mixture was stirred at 22°C for a further 18 h, then treated with saturated aq Na_2SO_4 and filtered from the solid aluminium complex. The ethereal filtrate was dried ($MgSO_4$) and evaporated to give the oily base (17.5 g, 86% yield) which was converted into the dimaleate **13d** in ethanol, and recrystallised from propan-2-ol, mp 121—122°C. Anal. $C_{21}H_{32}N_4O$, $2C_4H_4O_4$ (C, H, N). ^1H NMR (D_2O): δ ppm 1.1—1.9 (m, 6H, $C(CH_2)_3C$); 2.95 (s, 3H, NCH_3); 2.9—3.5 (m, 6H, NCH_2); 3.81 (s, 3H, OCH_3); 3.95 (m, 2H, NCH_2); 4.66 (s, 2H, $ArCH_2$); 6.26 (s, 4H, =CH); 6.81, 7.21, (m, 4H, Ar); 6.94, 7.64, 8.09 (m, 4H, pyridine).

In like manner the nitriles **12e** and **12f** were reduced to the aminohexyl and aminoheptyl analogues (**13e** and **13f**) which were isolated as dioxalates *viz*: **13e**: mp 161—162°C (90% EtOH); Anal. $C_{22}H_{34}N_4O$ (C, H, N for 2.15 oxalic acid); **13f**: mp 156—158°C (EtOH); Anal. $C_{23}H_{36}N_4O$, $2C_2H_2O_4$ (C,H,N).

N'-[5-[3-(4-Hydroxyphenyl)propionamido]pentyl]-N-(4-methoxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine dioxalate (bolpyramine ***15d***)

The amine **13d** (0.68 g, 1.9 mmol) was stirred with *N*-succinimidyl-3-(4-hydroxyphenyl)propionate (0.5 g, 1.9 mmol, Fluka) in dry CH_2Cl_2 (16 ml) at 22°C for 2 h, then concentrated. The resulting residue was chromatographed in MeOH on a column of silica gel, discarding the first and last fractions. Evaporation of the eluate furnished the product

free base **15d** as an oil (0.76 g, yield 80%). The latter was treated with oxalic acid in EtOH and diluted with Et_2O to furnish the dioxalate **15d**, mp 77—78°C. Anal. $C_{30}H_{40}N_4O_3$, $2C_2H_2O_4$ (C, H, N for 2.15 oxalic acid). ^1H NMR (D_2O, 360 MHz): δ ppm 0.89, 1.24, 1.45 (m, 6H, $C(CH_2)_3C$); 2.50 (t, 2H, $COCH_2$); 2.84 (t, 2H, $ArCH_2$); 2.88 (s, 3H, NCH_3); 2.98, 3.04, 3.33 (m, 6H, NCH_2); 3.84 (s, 3H, OCH_3); 4.06 (m, 2H, NCH_2); 4.84 (s, $ArCH_2N$); 6.78, 7.10 (m, 4H, hydroxy Ar); 7.02 (m, MeOAr); 7.06 (m, 1H, pyridine 3); 7.27 (m, 3H, pyridine 5, MeOAr); 8.02 (m, 2H, pyridine 4,6); HPLC on C_{18}/μBondapak in cyanomethane: 90 mM H_3PO_4 (pH 2.5 adjusted with KOH) at 40°C in graded proportions of 25—60% cyanomethane as a linear variation over 30 min, and 2 ml/min flow rate indicated 98.6% of the total peak area (retention time 9.97 min) detected at λ = 250 mn.

Iodobolpyramine ***16d*** *and diiodobolpyramine* ***17d***

An aqueous solution of iodine (0.86 g, 3.4 mmol) and KI (5.7 g, 3.4 mmol) was added dropwise to a cooled stirred solution of bolpyramine **15d** (1.82 g, 3.4 mmol) in 2 N NaOH (25 ml) below 10°C. The mixture was then stirred for 4 h at 10°C and 16 h at 22°C, then brought to pH 4 at 10°C with acetic acid, followed by a small amount of sodium metabisulfite (to remove free iodide). The resulting granular solid was collected, dissolved in hot EtOH and diluted with an equal volume of ether. On standing at 0°C, the solution deposited a granular solid consisting of a mixture of the mono- and di-iodinated products **16d** and **17d** (1.83 g), which was chromatographed in methanol on a silica gel column.

The first fractions contained the di-iodo product **17d** (1.42 g) which was treated with oxalic acid (0.33 g) in ethanol. The resulting solid was taken into hot ethanol, filtered from some insoluble solid, and diluted with ether to furnish *N'-[5-[3-(4-hydroxy-3,5-di-iodophenyl)-propionamido]pentyl]-N-(4-methoxybenzyl)-N'-methyl-N-2-pyridinyl-1, 2-ethanediamine hydroiodide oxalate* **17d** (0.66 g) as pale lemon crystals, mp 102—104°C. Anal. $C_{30}H_{38}I_2N_4O_3$, $C_2H_2O_4$, HI (C, H, N; I found 40.7: requires 39.1%). ^1H NMR (Me_2SO-d_6 250 MHz): δ ppm 1.23, 1.39, 1.62 (m, 6H, $C(CH_2)_3C$); 2.31 (t, 2H, $COCH_2$); 2.69 (t, 2H, $ArCH_2$); 2.86 (s, 3H, NCH_3); 3.06 (m, 4H, NCH_2); 3.30 (t, 2H, NCH_2); 3.74 (s, 3H, OCH_3); 3.90 (t, 2H, NCH_2); 4.66 (s, 2H, $ArCH_2N$); 6.68 (m, 2H, pyridine 3,5); 6.90, 7.17 (m, 4H, Ar); 7.52 (m, 1H, pyridine 4); 7.57 (s, 2H, Ar *ortho* to I); 7.68 (m, 1H, NH); 8.14 (m, 1H, pyridine 6).

The later fractions from the column contained the mono-iodo product **16d** (0.77 g) which was treated with oxalic acid (0.21 g) in ethanol and diluted with an equal volume of ether to furnish a crystalline solid; the latter was collected and recrystallised from ethanol: ether mixture to yield *N'-[5-[3-(4-hydroxy-3-iodophenyl)propionamido]pentyl]-N-(4-methoxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine oxalate* **16d** *(iodobolpyramine)* (0.64 g) as a colourless solid mp 80—82°C. Anal. $C_{30}H_{39}IN_4O_3$, 1.5 $C_2H_2O_4$ (C, H, I, N). ^1H NMR (D_2O, 250 MHz): δ ppm 0.85, 1.22, 1.46 (m, 6H, $C(CH_2)_3C$); 2.49 (m, 2H, $COCH_2$); 2.79 (m, 2H, $ArCH_2$); 2.89 (s, 3H, NCH_3); 3.00, 3.35 (m, 6H, NCH_2); 3.83 (s, 3H, OCH_3); 4.06 (m, 2H, NCH_2); 4.85 (s, 2H, $ArCH_2N$); 6.86 (d, 1H, Ar *ortho* to OH); 7.07, 7.29 (m, 7H, Ar, pyridine 3,5); 7.55 (s, 1H, Ar *ortho* to I); 8.04 (m, 2H, pyridine 4,6).

N'-(5-N-Acetamidopentyl)-N-(4-methoxybenzyl)-N'-methyl-N-2-pyridinyl-1,2-ethanediamine maleate ***18d***

Acetyl chloride (1.2 g, 15 mmol) in $CHCl_3$ (10 ml) was added dropwise during 10 min to a stirred mixture of the amine **13d** (1.5 g, 4 mmol) and pyridine (3 g) in dry $CHCl_3$ (50 ml). Stirring was continued at 22°C for 2 h and the mixture was then diluted with water, basified to pH 11 with 2 N NaOH, and extracted with $CHCl_3$ (4 × 100 ml). The combined extracts were dried ($MgSO_4$) and concentrated to furnish the oily base **18d** (1.4 g), which was treated with maleic acid in ethanol and diluted with ether to afford a sticky solid; crystallisation of the latter from propan-2-ol and dilution with ether furnished the required maleate **18d**, mp 69—71°C. Anal. $C_{23}H_{34}N_4O_2$, $C_4H_4O_4$ (C, H, N). ^1H NMR (D_2O, 250 MHz): δ ppm 1.31, 1.50, 1.66 (m, 6H, $C(CH_2)_3C$); 2.00 (s, 3H, $COCH_3$); 3.96 (s, 3H, NCH_3); 3.14 (m, 4H, NCH_2); 3.34 (t, 2H, NCH_2); 3.81 (s, 3H, OCH_3); 3.94 (t, 2H, NCH_2); 4.65 (s, 2H, $ArCH_2$); 6.27 (s, 2H, =CH); 6.80 (m, 2H, pyridine 3,5); 6.96, 7.22 (m, 4H, Ar); 7.61 (m, 1H, pyridine 4); 8.13 (m, 1H, pyridine 6).

Preparation of 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionamides ***14a-f***

Each aminoalkylmepyramine derivative (**13**), as the dimaleate or dioxalate, was converted into the corresponding 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionamide (**14**) with *N*-succinimidyl-3-(4-hydroxy-5-[^{125}I]iodophenyl)propionate (Scheme 2a) based on the method of Bolton and Hunter [12] as follows: the amine dimaleate or dioxalate **13** (10 μg in 10 μl of 0.1 M borate buffer, pH 8.5) was added to the dried [^{125}I]-Bolton—Hunter reagent (1 mCi). After 15 min at room temperature, the mixture was spotted onto a thin—layer chromatography (TLC) silica gel plate (60 F 254 Merck) and the chromatogram was developed for 10 h in butanol:acetic acid:water (4:1:1). After being located by autoradiography (Agfa—Gevaert D7), the [^{125}I]-iodinated spot was scraped from the plate and extracted with 500 μl of ethanol. Solutions were stored at — 20°C before use.

Synthesis of [^{125}*I]iodobolpyramine* ***14d***

Method A [^{125}I]Iodobolpyramine (**14d**) prepared from the amine dimaleate **13d** and 3-(4-hydroxy-5-[^{125}I]iodophenyl)propionamide as described above had an R_f = 0.33; it was scraped from the plate, extracted into 500 μl of EtOH and re-examined by thin—layer chromatography using the system ethyl acetate:methanol:ammonium hydroxide (0.880) (5:1:1) in which it ran as a single spot, R_f = 0.59.

Method B A microvial (0.3 ml, from Pierce or Alltech Europe) was first coated with iodo-gen (1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril from Pierce) by injecting 100 μl of a solution of 2 μg of iodo-gen in dichloromethane (20 μg of iodogen per ml), followed by evaporation of the solvent (**11**). Bolpyramine dioxalate **15d** (10 μg in 10 μl of 50 mM Na_2/K phosphate buffer, pH 7.5) was then injected into the microvial and diluted with 10 μl of the phosphate buffer; 1 mCi of $Na^{125}I$ (2175 Ci/mmol, Amersham) was added (10 μl) and the mixture stirred. After 20—30 min, at room temperature (20°C), the mixture was analysed by TLC (ethyl acetate:methanol:ammonium hydroxide (0.880), 5:1:1) or HPLC (on C_{18}/μBondapak in 1:1 cyanomethane: 10 mM ammonium acetate (pH 4.2) at 20°C and 1 ml/min flow rate). A sample of [^{125}I]iodobolpyramine (**14d**) after purification by HPLC had retention characteristics identical to those of the cold material **15d** analysed as indicated below.

Proof of identity of [^{125}*I]iodobolpyramine* ***14d*** *by TLC and HPLC*

Thin—layer chromatograms of [^{125}I]iodobolpyramine (ethyl acetate: methanol:ammonium hydroxide (0.880), 5:1:1) prepared by either method were compared with bolpyramine **15d** and non-radioactive iodo- and di-iodobolpyramine (**16d** and **17d**) synthesised independently as described above. The radioactive material showed only one spot, and had identical R_f (0.59) with the authentic iodobolpyramine; bolpyramine (R_f = 0.67) and di-iodobolpyramine (R_f = 0.44) were not detected.

For the HPLC comparison, a mixture of 7.14 μg each of bolpyramine dioxalate (**15d**), iodobolpyramine oxalate (**16d**), and di-iodobolpyramine hydroiodide oxalate (**17d**), was prepared in 25 μl of 50 mM Na_2/K phosphate buffer (pH 7.5) and chromatographed on a column of C_{18}/μBondapak in 1:1 cyanomethane: 10 mM ammonium acetate (pH 4.2) at 20°C and 1 ml/min flow rate. Three well separated peaks were detected at λ = 210 nm with respective retention times of 8.5, 12.3, and 19.2 min (Fig. 1). The purified radioactive material showed only one peak, which was superimposable over the iodobolpyramine peak (at 12.3 min).

Biological assay procedures

Guinea pig ileum in vitro *assay for histamine antagonism*

Guinea pigs of either sex weighing between 400—700 g were used. Immediately after an animal was killed, the terminal ileum was removed, washed and mounted in a 15 ml bath containing magnesium-free Tyrode's solution gassed with 95% O_2/5% CO_2 and maintained at 30°C. The tissue was loaded with 0.5 g and contractions in response to histamine were detected by a force transducer and displayed on a potentiometric recorder.

Cumulative histamine dose—response curves were obtained prior to and following 8 min incubations with three different concentrations of the antagonist (9 observations). The dissociation constant (K_B) was calculated from the equation $K_B = B/(x - 1)$, where x is the respective ratio of concentrations of histamine needed to produce half-maximal responses in the presence and absence of different concentrations (B) of antagonists. Slopes of plots of log (x — 1) on log B for these determinations are indicated in Table I and were generally not significantly different from unity within 95% limits.

Membrane preparation

Cerebella of male Hartley guinea pigs (about 300 g, kindly provided by Rhône—Poulenc) were homogenised with a Polytron blender in 40 volumes of cold Na_2/K phosphate buffer (50 mM, pH 7.5). After centrifugation for 1 min at 260 × *g*, the resulting supernatant was recentrifuged (30 min at 20 000 × *g*). The final pellet was rinsed with 2 ml of cold phosphate buffer and stored at —80°C. For binding assays, pellets were resuspended in the phosphate buffer and the protein concentration of the preparation was determined [15] using bovine serum albumin (BSA) as the standard.

Binding assay for ^{125}I-iodinated derivatives

All solutions contained 0.1% BSA to prevent adsorption of the iodinated ligand onto tubes and filters. The pellet suspension (100 µl containing 5—20 µg of protein according to the concentration of the ligand) was incubated with the [^{125}I]ligand in a final volume of 200 µl. Incubations were carried out at 25°C for 4 h or 30°C for 30 min (as indicated). Non-specific binding was determined in the presence of 0.2 µM mianserin. Incubation was stopped by adding 5 × 3 ml of fresh phosphate buffer containing 0.1% BSA and followed by rapid filtration under reduced pressure through glass fibre filters (GF/B) which had been treated previously with 0.3% polyethylenimine [16]. The radioactivity trapped on the filters was measured using an LKB gamma counter, with an efficiency of 82%. Radioactivity bound to the filters in the absence of the membrane preparation represented 0.4% of the total. The concentration of the ligand was corrected for the loss of free radioactivity due to adsorption onto the tubes during the incubation period (5—10% of the initial radioactivity).

To determine drug potency for inhibition of iodobolpyramine binding, [^{125}I]iodobolpyramine (0.12 nM) was incubated with guinea pig cerebellar membranes in the presence of 8—12 different concentrations of the respective compound. IC_{50} values and pseudo-Hill coefficients were determined from pooled data from at least 2 independent experiments, by non-linear regression using a one-site model. IC_{50} values were converted to K_i values using the equation $K_i = IC_{50}/1 + L/K_D$, where L = concentration of [^{125}I]iodobolpyramine, K_D = equilibrium dissociation constant of [^{125}I]iodobolpyramine (0.15 nM).

Acknowledgements

We thank Ms. A. J. Jones and Mr. P. J. Moore for NMR spectral analysis, Ms. J. A. Murphy for HPLC, and Mr. M. J. Graham for elemental analyses. Studies at the Unité de Neurobiologie were supported by a contract from D.R.E.T. and by a grant from SK&F (France).

References

1 Hill S. J., Young J. M. & Marrian D. H. (1977) *Nature (London)* 270, 361
2 Hill S. J., Emson P. C. & Young J. M. (1978) *J. Neurochem.* 31, 997
3 Chang S. L., Tran V. T. & Snyder S. H. (1978) *Eur. J. Pharmacol.* 48, 463
4 Tran V. T., Lebovitz R., Toll L. & Snyder S. H. (1981) *Eur. J. Pharmacol.* 70, 501
5 Taylor J. E. & Richelson E. (1982) *Eur. J. Pharmacol.* 78, 279
6 Peroutka S. J. & Snyder S. H. (1981) *J. Pharmacol. Exp. Ther.* 216, 142
7 Durant G. J., Ganellin C. R., Griffiths R., Harvey C. A., Ife R. J., Owen D. A. A., Parsons M. E. & Sach G. S. (1984) *Br. J. Pharmacol. Proc. Suppl.* 82, 232 P
8 Smith D. M. (1979) *in: Comprehensive Organic Chemistry* (Barton D. R. & Ollis W. D., eds.), Vol. 4, Pergamon, Oxford, p. 19
9 Magidson O. & Menschikoff G. (1925) *Berichte Dtsch. Chem. Ges.* 58, 113
10 Kress T. J., Moore L. L. & Costantino S. M. (1976) *J. Org. Chem.* 41, 93
11 Reynaud P., Tupin T. & Delaby R. (1957) *Bull. Soc. Chim. Fr.* 718
12 Bolton A. E. & Hunter W. M. (1973) *Biochem. J.* 133, 529
13 Körner M., Bouthenet M.-L., Ganellin C. R., Garbarg M., Gros C., Ife R. J., Sales N. & Schwartz J.-C. (1986) *Eur. J. Pharmacol.* 120, 151
14 Salacinski P. R. P., McLean C., Sykes J. E. C., Clement-Jones V.V. & Lowry P. J. (1981) *Anal. Biochem.* 117, 136
15 Lowry O. H., Rosebrough N. J., Farr A. L. & Randall R. J. (1951) *J. Biol. Chem.* 193, 265
16 Bruns R. F., Lawson-Wendling K. & Pugsley T. A. (1983) *Anal. Biochem.* 132, 74
17 Marrian D. H., Hill S. J., Sanders J. K. M. & Young J. M. (1978) *J. Pharm. Pharmacol.* 30, 660
18 Jacobson K. A., Kirk K. L., Padgett W. L. & Daly J. W. (1985) *J. Med. Chem.* 28, 1341
19 Ganellin C. R. See discussion (1982) *in: Pharmacology of Histamine Receptors* (Ganellin C. R. & Parsons M. E., eds.), Wright-PSG, Bristol, pp. 64—69
20 Nauta W. Th. & Rekker R. F. (1978) *in: Histamine II and Anti-Histaminics* (M. Rocha e Silva, ed.) (*Handbook of Exp. Pharmacol.* Vol. 18/2). Springer—Verlag, Berlin, pp. 215—249
21 Sach G. S. (1984) Smith Kline and French Laboratories Limited, Eur. Pat. Appl. 0107914
22 Ife R. J. (1984) Smith Kline and French Laboratories Limited, Eur. Pat. Appl. 0112704