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A convenient synthesis of [^{3}H]mepyramine and certain related [^{3}H]antihistamines

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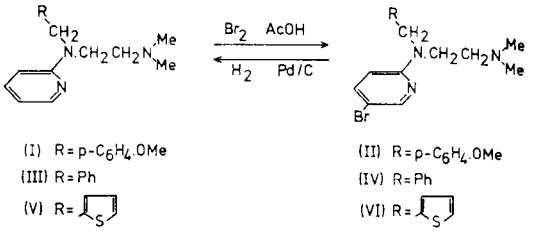
In recent years valuable information on the numbers, distribution and biochemical properties of a wide range of drug receptors has been obtained from studies on the binding of receptor ligands. The success of this approach is dependent on the availability of high-affinity receptor-specific ligands radioactively labelled, usually with tritium, to high specific activity, and consequently the preparation and purification of such compounds is of particular importance. We have recently demonstrated that [^{3}H]mepyramine is a suitable ligand for the study of histamine H_1 receptors in guinea-pig small intestine (Hill, Young & Marrian, 1977) and brain (Hill & Young, 1978; Hill, Emson & Young, 1978). Similar results have been obtained by Chang, Tran & Snyder (1978) in rat brain. In this communication we describe in detail a convenient method for the preparation of [^{3}H]mepyramine. The synthetic route adopted has the advantages that it (a) starts from commercially available material, (b) involves one simple chemical reaction to produce the bromoderivative required for catalytic reduction, (c) yields a tritiated product of high specific activity with the tritium in a known position and (d) proceeds via an intermediate which has a lower affinity for the H_1 receptor than the final product [^{3}H]mepyramine. The last point, (d), is an important consideration if the problems which could arise from incomplete purification of the tritiated product are to be minimised.

The same synthetic route can be used for other non-halogenated antihistamines containing the 2-aminopyridyl residue, but for two we have examined, tripelennamine and methapyrilene, the particular advantage (d) above does not hold.

The route adopted (Scheme 1) turns on the ease with which 2-aminopyridines can be brominated to yield the corresponding 5-bromo-derivative. Thus mepyramine (I) on treatment with bromine in acetic acid yields the monobromo-derivative (II) which is easily and quantitatively converted back to the parent compound (I) by catalytic reduction in ethanolic triethylamine solution. The structure of the bromomepyramine (II) was established from the ^{1}H nmr spectrum, which clearly showed



that bromination had taken place in the 5-position of the aminopyridine ring, leaving the *p*-methoxyphenyl group intact.

The ready bromination of mepyramine (I) to give the 5-bromopyridyl derivative (II) suggested that this reaction should be applicable to other 2-aminopyridyl antihistamines. This was confirmed by the isolation of the 5-bromopyridyl-derivatives as the major reaction products from the bromination of tripelennamine (III) and methapyrilene (V). The nmr spectra of the bromo-componds (IV & VI) were again consistent with the structure assigned. Both were reduced to the parent compounds (III & V, respectively) with the consumption of the theoretical amount of hydrogen gas, but the reduction of bromomethapyrilene (VI) was slow, presumably because of the effect of the sulphur on the catalyst.

Bromomepyramine (II). Mepyramine maleate (5 g, May & Baker) was dissolved in water, treated with 5M NaOH (6·5 ml) and the free base formed extracted into ether. The ethereal layer was washed, dried over Na_2SO_4 and evaporated under vacuum. The residue in acetic acid (90 ml) was stirred at room temperature and bromine (2·0 g, i.e. equimolar quantity) in acetic acid (90 ml) added slowly. The resulting solution was evaporated and the residue taken up in a little warm water. The solid which separated on cooling was recrystallized from water (20 ml) yielding impure *N-2-(5-bromo)pyridinyl-N-[(4-methoxyphenyl)methyl]-N'N'-dimethyl-1,2-ethanediamine hydrobromide* (II) (4 g, m.p. 169–170°), which after recrystallization three times from methanol formed colourless prisms, m.p. 177–178° [Found (in material dried over P_2O_5 at room temp.): C, 45·8; H, 5·2; N,

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9·65; Br, 34·85. $C_{17}H_{22}N_3BrO.HBr$ requires C, 45·9; H, 5·2; N, 9·45; Br, 36·0%]. 100 MHz Proton nmr spectra were obtained on D_2O solutions, using t-butanol as both field-frequency lock and internal reference (δ = 1·22). H_3, H_4 and H_6 refer to protons in the 3-, 4- and 6-positions on the 2-aminopyridine ring. δ 6·48 (d, J=8 Hz, H_3) 7·46 (dd, J=2, 8 Hz, H_4) 8·05 (d, J=2 Hz, H_6)—cf for mepyramine maleate δ *c.* 6·7 (m, $H_3 + H_5$) 7·45 (ddd, J=2,7,7 Hz, H_4) 8·02 (dd, J=2,5 Hz, H_6).

Bromotripelennamine (IV) *and bromomethapyrilene* (VI). The same method as adopted for bromomepyramine (II) was used for the bromination of tripelennamine (CIBA-Geigy) and methapyrilene (Eli Lilly) yielding, respectively, N-2-(5-*bromo*)*pyridinyl*-N-(*phenylmethyl*)-N′,N′-*dimethyl*-1,2-*ethanediamine hydrobromide* (IV), m.p. 210–211° (from 0·1M HBr) [Found (in material dried under vacuum at 70°): C, 46·1; H, 5·0; N, 10·0. $C_{16}H_{20}N_3Br$. HBr requires C, 46·3; H, 5·1; N, 10·1%]; δ (D_2O) 6·58 (d, J=8 Hz, H_3) 7·56 (dd, J=2,8 Hz, H_4) 8·15 (d, J=2 Hz, H_6) and N-2-(5-*bromo*)*pyridinyl*-N-(2-*thienylmethyl*)-N′N′-*dimethyl*-1,2-*ethanediamine hydrobromide* (VI), m.p. 153–154° (from acetonitrile) [Found (in material dried under vacuum at 40°): C, 39·8; H, 4·5; N, 10·1. $C_{14}H_{18}N_3S$ Br. HBr requires C, 39·9; H, 4·55; N, 10·0%]; δ (D_2O) 6·64 (d, J=9 Hz, H_3) 7·49 (dd, J=2,9 Hz, H_4) 8·09 (d, J=2 Hz, H_6).

Catalytic reduction of the bromo-derivatives. Bromo-derivative (100 mg) in 95% ethanol (10–15 ml, distilled over Raney Ni) containing triethylamine (0·5 ml) was shaken at room temperature in an atmosphere of hydrogen gas with 10% palladium on charcoal (*ca* 100 mg) as catalyst. Hydrogen uptake was quantitative and, except for the reaction with bromomethapyrilene (VI), rapid. In each case the ultraviolet spectrum of the resulting solution was identical with that of the parent compound from which the bromo-derivative had been formed. From one reaction with bromomepyramine (II) the product, isolated as the maleate, was shown to be mepyramine maleate (I) by m.p., mixed m.p. and identity of the infrared spectrum with that of an authentic sample.

Relative antihistaminic potency of the bromo-derivatives and parent compounds. The potency of the compounds studied as antagonists at the histamine H_1 receptor was assessed from the inhibition of the contractile response of intestinal smooth muscle to histamine. Longitudinal muscle strips from guinea-pig small intestine were suspended in Krebs-Henseleit solution at 30° in a conventional 10 ml organ bath and gassed with 5% CO_2 in oxygen. Histamine, added every 3 min, was in contact with the tissue for 15–30 s and contractions were measured isotonically. The antagonists were added to the reservoir and allowed to equilibrate with the tissue for at least 30 min. Affinity constants were calculated from the extent of the shift of the log dose-response curve, using the relationship: Dose ratio = $A.K_a + 1$, where A is the antagonist concentration and K_a its affinity constant.

The effects of the antagonists were frequently difficult to reverse, a well-known problem with some antihistamines (see e.g. Rocha e Silva, Fernandes & Antonio, 1972). The bromo-derivatives were much worse than the parent compounds in this respect and the shift of the dose-response curve produced when they were applied at a concentration of 10^{-7} M could never be reversed completely, even after washing with Krebs solution for up to 2 h. In contrast the response of the tissue after exposure to methapyrilene could usually be fully restored. This problem often made it difficult to obtain more than one reliable value from a muscle strip and limited the accuracy with which the affinity constants could be determined. The values obtained are set out in Table 1.

The constants for mepyramine (I), tripelennamine (III), and methapyrilene (V) are in reasonably good agreement with those of Marshall (1955). All three bromo-compounds are potent antihistamines, but the observation of particular interest is that whereas the bromination of tripelennamine (III) or methapyrilene (V) leads to no significant loss of potency, bromomepyramine (II) has only 25% of the activity of mepyramine (I). These findings are consistent with the observations of Biel (1949) who synthesized the chloro-analogue of bromomepyamine (II) and found it to be of the same order of potency as tripelennamine (III), although it is not posible to calculate the affinity constants from his data. The chloro-analogue of bromotripelennamine (IV) was reported to be somewhat more potent than tripelennamine (III).

The relative potency of the bromo-derivatives and the corresponding non-halogenated compounds is an important consideration, since the last step in this synthesis of [^{3}H]antihistamine is removal of the bromine to give the parent compound. The higher the relative potency of the intermediate bromo-compound the more important it becomes to remove traces of the intermediate from the final product. Ideally the activity of the intermediate should be very low. Of the three pairs of compounds we have examined (Table 1) clearly bromomepyramine (II) → mepyramine (I) meets this criterion best.

Table 1. *Affinity constants of antihistamines and their brominated derivatives.*

Compound	Ka (M^{-1})
Mepyramine (I)	$1·6 \pm 0·3 \times 10^9$ (5)
Bromomepyramine (II)	$4·0 \pm 0·4 \times 10^8$ (5)
Tripelennamine (III)	$5·5 \pm 0·8 \times 10^8$ (5)
Bromotripelennamine (IV)	$4·8 \pm 0·7 \times 10^8$ (6)
Methapyrilene (V)	$3·8 \pm 0·4 \times 10^8$ (4)
Bromomethapyrilene (VI)	$4·6 \pm 1·3 \times 10^8$ (6)

Values are means ± s.e. (number of determinations).

[^3H]*Mepyramine* (N-[4-*methoxyphenyl*)*methyl*]-N-2-[5-^3H]*pyridinyl*-1,2-*ethanediamine*)-Bromomepyramine (II) (10 mg) in 95% ethanol–triethylamine (5:2 v/v) with 10% Pd/C as catalyst was stirred at room temperature in an atmosphere of tritium gas (reaction carried out by The Radiochemical Centre, Amersham). The catalyst was removed by filtration, the solvent evaporated under vacuum and the residue taken up in chloroform and extracted with 0·5M NaOH. The organic phase was washed twice with distilled water, the solvent removed under vacuum and the residue taken up in 10% maleic acid in ethanol (2 ml). The product was purified by thin-layer chromatography on silica gel G (Merck) in two stages. A preliminary purification was achieved using the solvent systems ethanol–acetic acid (9:1, v/v), chloroform–triethylamine (9:1, v/v) and light petroleum (b.p. 80–100)–ether–triethylamine (9:9:1, v/v/v) and the product from this step, stored at −10° as the maleate, further purified by running in ethanol–acetic acid–water (8:1:2, v/v/v) and, finally, chloroform–triethylamine (9:1, v/v). The final product was eluted with ethanol, acidified with acetic acid to give a 5% solution and stored at −10°. The concentration of [^{3}H]mepyramine was determined from the ultraviolet absorbance at 310 nm, using non-radioactive mepyramine as standard (the equimolar amount of maleate or the excess acetic acid does not interfere at 310 nm). The specific activity was 20 Ci mmol^{-1}.

The radiochemical purity of the product was established (a) by thin-layer chromatography (Prepared Polygram silica gel layers with fluorescent indicator, Machery & Nagel), from the coincidence of the R_F value for the single peak of radioactivity in [^{3}H]-mepyramine (run with added non-radioactive mepyramine as carrier) with that for authentic mepyramine run concurrently, in three solvent systems: ethanol–acetic acid–water (8:1:2, v/v/v), benzene–triethylamine (9:1, v/v) and chloroform–triethylamine (9:1, v/v) and (b) by high voltage electrophoresis (Shandon Southern, model L24) on Whatman No 1 paper in 0·1 M citric acid-phosphate buffer, pH 3·05, at 40 V cm^{-1} for 45 min, where the peak of radioactivity migrated towards the anode at the same rate as authentic mepyramine. The high-voltage electrophoresis achieved a better separation between mepyramine (I) and bromomepyramine (II) than any of the t.l.c. systems.

The chemical purity of the [^{3}H]mepyramine was established by bioassay, using the inhibition of the contractile response of longitudinal muscle strips from guinea-pig small intestine to histamine. The affinity constant, deduced from 3 measurements of the extent of the shift of the dose-response curve to histamine, was $1{\cdot}6 \pm 0{\cdot}4 \times 10^9$ M^{-1}, in excellent agreement with the value of $1{\cdot}6 \pm 0{\cdot}3 \times 10^9$ M^{-1} obtained for non-radioactive meypramine (Table 1).

The synthetic approach described above provides a convenient and easy route to high-specific activity [^{3}H]-mepyramine, the chemical and radiochemical purity of which is borne out by its successful use in labelling histamine H_1 receptors in guinea-pig intestine (Hill & others, 1977) and brain (Hill & Young, 1978; Hill & others, 1978).

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New hydrophilic vehicle enabling rectal and vaginal absorption of insulin, heparin, phenol red and gentamicin

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Drugs that are poorly absorbed or decomposed in the gastrointestinal tract, such as insulin, heparin and certain amino-glycoside antibiotics, require to be administered parenterally for effective systemic action. The use of the rectal or vaginal routes for these drugs has failed to accomplish significant absorption or clinical effects. Preparations of heparin are available for topical use, but there is no evidence of systemic action.

We have developed a hydrophilic vehicle from which systemic action is obtained after rectal or vaginal administration of drugs not normally active via the gastrointestinal tract. The base comprised the non-ionic surface-active agent Cetomacrogol 1000 (polyethylene glycol 1000 monocetyl ether) in combination with various concentrations of polyethylene glycols. Con-

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