J. Pharm. Pharmac., 1970, **22**, 279–283 *Received July 18*, 1969

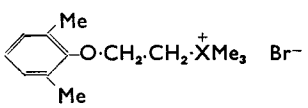
Phosphocholine 2, 6-xylyl ether bromide: proton-phosphorus coupling constants and preliminary pharmacological assessment

E. R. CLARK AND I. E. HUGHES

Department of Pharmacology, School of Medicine, Leeds LS2 9NL, U.K.

Phosphocholine 2,6-xylyl ether bromide [*PPP*-trimethyl-2-(2,6-xylyloxy)ethylphosphonium bromide], has been synthesized by reacting 2-(2,6-xylyloxy)ethyl bromide with a solution of trimethylphosphine in phenol, but ethylenebis(trimethylphosphonium bromide), 1,2-di(2,6-xylyloxy)ethane and 2,6-xylenol were the only reaction products identified when ether was used as solvent. The $^2J_{PH}$ and $^3J_{PH}$ coupling constants for these phosphonium salts have been determined. Although the phosphocholine xylyl ether blocks the Finkleman preparation in concentrations of 2–3 $\times$ 10^{-5} g/ml, this blockade does not have all the characteristics of the adrenergic neuron blockade produced by xylocholine.

The substitution of a phosphorus atom for a nitrogen atom in quaternary salts usually leads to a reduction in agonist activity. Thus Hunt & Renshaw (1925) demonstrated that tetramethylphosphonium iodide is less active than tetramethylammonium iodide in both the atropinized and non-atropinized anaesthetized cat, and Holton & Ing (1949) showed that acetylphosphocholine possesses only 8% of the potency of acetylcholine when tested on cat blood pressure, the rabbit intestine and the frog heart. A similar fall in potency on substituting a phosphorus atom for the nitrogen atom in xylocholine (I) could lend support to the postulated involvement of acetylcholine in adrenergic transmission. Phosphocholine 2,6-xylyl ether bromide [PPP-trimethyl-2-(2,6-xylyloxy)ethylphosphonium bromide] (II) has therefore been synthesized and tested for adrenergic neuron-blocking action on the Finkleman rabbit intestine preparation.



I, X = N; II, X = P

EXPERIMENTAL

Chemistry

Infrared spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer. The mass spectrum of 1,2-di(2,6-xylyloxy)ethane was recorded on an A.E.I. MS902 spectrometer and its nmr spectrum on a Varian A60 spectrometer. Nmr spectra of the quaternary phosphonium salts were recorded on a Varian HA-100D spectrometer at the Physicochemical Measurements Unit, Harwell, using sweep widths of 1000 and 100 Hz, enabling coupling constants to be measured to an accuracy of 0·1 Hz.

Preparation of trimethylphosphine. A solution of phosphorus trichloride (0·4 mol) in dry ether (1000 ml) was added slowly to a vigorously stirred solution of methylmagnesium iodide (2·5 mol) in ether (1000 ml) at −40° under nitrogen. When the addition was complete the reaction mixture was allowed to warm to room temperature and stirred for a further hour. The trimethylphosphine and ether were distilled from the reaction mixture by gentle warming and condensed at −70°. When the bulk of the ether had distilled and a thick syrup remained in the reaction vessel, distillation was terminated. The distillate was warmed to 0° and extracted with 5 × 100 ml of air-free cold 2·5N sulphuric acid.

Trimethyphosphine was isolated as an ethereal solution by neutralizing the acid extract with 20–30% aqueous potassium hydroxide solution under nitrogen, warming gently and drying the ether–trimethylphosphine vapours by passage up a potassium hydroxide tower before condensing at −70°.

When the trimethylphosphine was to be reacted subsequently in phenol, the acid extract was first heated to 70° to remove the bulk of the dissolved ether and then cooled before liberation and isolation of the trimethylphosphine as described above.

Reaction of trimethylphosphine with 2-(2,6-*xylyloxy*)*ethyl bromide.* 2-(2,6-Xylyloxy)ethyl bromide (0·25 mol) (prepared from sodium 2,6-xylyl oxide and 1,2-dibromoethane in t-butanol) was reacted with trimethylphosphine, prepared as above, either in 500 ml of dry ether or in 200 g of phenol. Both reaction mixtures were allowed to stand (protected from moisture) for 7 days at room temperature.

(*a*) In the ethereal reaction mixture, a white precipitate was produced (22·4 g) which was recrystallized from 95 pts methanol–5 pts light petroleum (60–80°) to give white crystals, m.p. above 340° ν_{max} (KCl disc—assignments are derived by comparison with spectrum of Me_4PI and are tentative): 1434 s (CH_3 def.); 1398 (CH_2 def.); 1300 s, 1295 s (CH_3-P); 1228 s; 1144 s; 980 vs (C–P); 983 s; 885 s; 783 s, 762 m (PCH_3); 688 m. Found: C, 28·15; H, 6·45; Br, 47·2; P, 17·9. Ethylenebis(trimethylphosphonium bromide, $C_8H_{22}Br_2P_2$, requires C, 28·25; H, 6·5; Br, 47·0; P, 18·2%. Extraction of the ethereal mother-liquor from the reaction mixture with 10% sodium hydroxide solution followed by acidification of the alkaline extract, extraction of the acid mixture with ether, evaporation of the ether and distillation of the residue under reduced pressure yielded a material (1·8 g) whose infrared spectrum, b.p., m.p., and mixed m.p. were identical with an authentic sample of 2,6-xylenol. Removal of the ether from the ethereal phase remaining from the alkaline extraction and distillation of the residue under reduced pressure yielded 10·1 g of unreacted starting material and 7·4 g of a colourless liquid, b.p. 158–162° at 1 mm, nmr (CCl_4, TMS): *ca* τ 3·1 (intensity *ca* 6, m, ArH), τ 5·96 (intensity *ca* 4, s, CH_2), τ 7·70 (intensity 12, s, $ArCH_3$). ν_{max} (liquid film): 1605w, 1593m, 1512m (aromatic ring); 1298 vs (aralkyl ether); 768 s (ArH). Mass spectrum (m/e values; % intensity in parentheses): 270 (80), 149 (100), 135 (19), 121 (41), 105 (98), 28 (15). Found: C, 79·65; H, 8·4. 1,2-Di(2,6-xylyloxy)ethane, $C_{18}H_{22}O_2$ requires C, 79·95; H, 8·2%.

(*b*) On pouring the phenolic reaction mixture into dry ether (1000 ml), an oil was formed which quickly gave a white solid (44·8 g) on agitation. The solid was dissolved in a small amount of dry ethanol and poured into dry ether (1000 ml). White crystals formed which were recrystallized from 98 pts dry acetone–2 pts dry ethanol, m.p. 185–187°. ν_{max} (KCl disc): 1593 w, 1478 s (aromatic ring); 1427 m (CH_3 def.); 1297 s (CH_3–P); 1200 s, 1192 vs (aralkyl ether); 976 vs (C–P); 806 s (ArH). Found:

C, 51·35; H, 7·25; Br, 26·5; P, 10·25. *PPP*-Trimethyl-2-(2,6-xylyloxy)ethylphosphonium bromide, $C_{13}H_{22}BrOP$ requires C, 51·15; H, 7·25; Br, 26·2; P, 10·15%.

Pharmacology

Rabbit intestine preparation. Short pieces of ileum or duodenum were taken from freshly killed rabbits and prepared according to the method of Finkleman (1930) using Tyrode solution (35°) gassed with 5% carbon dioxide in oxygen. Supramaximal stimulation of the periarterial nerves was carried out with rectilinear pulses (supramaximal voltage: 0·5 ms duration; 20/s for 30 s in every 7½ min) and longitudinal contractions of the preparation were recorded with a frontal writing lever on smoked paper.

RESULTS AND DISCUSSION

Chemistry

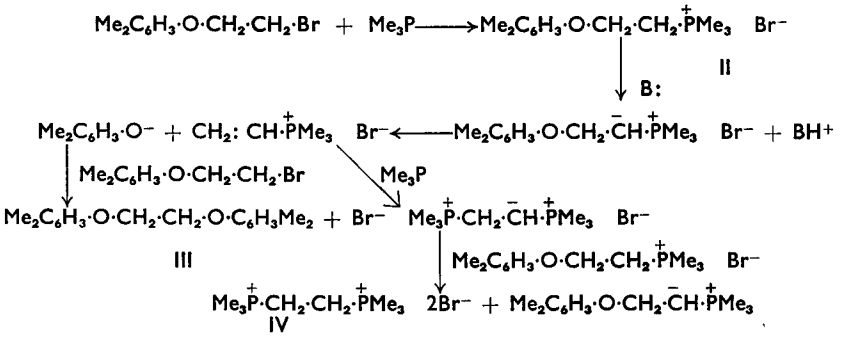
The synthesis of phosphocholine 2,6-xylyl ether bromide (II) was first attempted by the reaction of 2-(2,6-xylyloxy)ethyl bromide with trimethylphosphine in ethereal solution. Ether was chosen as the solvent because an ethereal solution of trimethylphosphine resulted from its method of preparation. With ether as the solvent, however, none of the required phosphonium salt was obtained, 1,2-di(2,6-xylyloxy)-ethane (III), ethylenebis(trimethylphosphonium bromide) (IV) and 2,6-xylenol being the only identifiable products. The same result, except for the isolation of the bis-phosphonium salt as the iodide, was obtained when 2-(2,6-xylyloxy)ethyl iodide was used instead of the analogous bromide.

The structure of the di-ether III was established by infrared, nmr, and mass spectrometry while the infrared spectrum, boiling point, melting point and mixed melting point of the isolated 2,6-xylenol were identical with those of an authentic sample.

The proton magnetic resonance spectrum of the bisphosphonium compound IV in D_2O, with TMS as external reference, is similar to that described by Carty & Harris (1967) for 1,2-bis(diphenylphosphino)ethane bismethiodide. The CH_2 resonance is a doublet around a much less intense and unresolved region of absorption, the mid-point of the signal occurring at τ 7·35. Separation of the outer lines gives $|^2J_{PH} + {}^3J_{PH}| = 6{\cdot}4$ Hz. [The coupling constant notation is essentially that of Musher & Corey (1962), as modified by Carty & Harris (1967).] The three peaks of the methyl resonance signal are in the approximate ratio 2 : 1 : 2 and are centred at τ 8·03. The separation of the outer lines gives $|^2J_{PMe} + {}^5J_{PMe}| = 14{\cdot}2$ Hz, and if ${}^5J_{PMe} = 0$ (Hendrickson, Maddox & others, 1964), $|^2J_{PMe}| = 14{\cdot}2$ Hz. This is similar to the value of $|^2J_{PMe}| = 14{\cdot}8$ Hz found for tetramethylphosphonium iodide. Carty & Harris (1967) give $^2J_{PMe} = -13{\cdot}0$ Hz for 1,2-bis(diphenylphosphino)ethane bismethiodide and $-14{\cdot}4$ Hz for tetramethylphosphonium iodide.

The formation of the bisphosphonium salt and 2,6-xylenol from the reaction of 2-(2,6-xylyloxy)ethyl bromide and trimethylphosphine in ethereal solution is analogous to the isolation of ethylenebis(triphenylphosphonium bromide) and phenol by Schweizer & Bach (1964) from reaction of triphenylphosphine and 2-phenoxyethyl bromide in non-protonic solvents. Schweizer and Bach did not isolate or identify any 1,2-diphenoxyethane. They established a mechanism involving triphenyl-vinylphosphonium bromide and excluded the participation of phenyl vinyl ether. It seems probable that an analogous mechanism will be involved in the formation of

ethylenebis(trimethylphosphonium bromide) and that the di-ether III is formed by the attack of a xylyloxy-anion on the 2-(2,6-xylyloxy)ethyl bromide.



The failure of Schweizer and Bach to identify 1,2-diphenoxyethane amongst the products of their reaction may be due to their use of vapour-phase chromatography to analyse their reaction solution and to the very high boiling point of the di-ether, rather than to the absence of this material.

The required phosphocholine 2,6-xylyl ether bromide (II) was obtained as the sole identified product by reacting 2-(2,6-xylyloxy)ethyl bromide with trimethylphosphine in phenol. The proton magnetic resonance spectrum of this monophosphonium salt dissolved in D_2O, and with TMS as external reference, showed a complex signal for the aromatic protons at approximately τ 3·0 (intensity 3), a doublet (intensity 9) at τ 8·07 due to the methyl group attached to the phosphorus atom ($^2J_{PMe}$ = 14·5 Hz), and a singlet at τ7·80 (intensity 6) due to the aryl methyl groups. The two methylene groups occur as a well-resolved doublet of triplets (intensity 2) at τ 5·93 ($^3J_{PH}$ = 17·7 Hz) assigned to the O.CH_2 protons and a less well-resolved doublet of triplets (intensity 2) centred at τ 7·28 ($^2J_{PH}$ = 13·7 Hz) assigned to the CH_2.P protons. In this latter signal the two innermost bands are almost coincident and in a lower resolution spectrum the signal appears as five peaks. The mean J_{HH} for the methylene groups is 6·25 Hz. Manatt, Juvinall & Elleman (1963) deduce that $^3J_{PH}$ is always positive and that $^2J_{PH}$ may be positive or negative. By using the $^2J_{PH}$ coupling constant for the monophosphonium compound (II), which is less likely to be influenced by the proximity of the ether linkage than is $^3J_{PH}$, and substituting into the relation $|^2J_{PH} + {}^3J_{PH}| = 6{\cdot}4$ Hz, we obtain $^3J_{PH} = +7{\cdot}3$ Hz or $+20{\cdot}1$ Hz for the bisphosphonium compound (IV). Since $^3J_{PH}$ for the monophosphonium compound is 17·7 Hz, we favour the larger of these two values. Carty & Harris (1967) suggested two values, $+11{\cdot}0$ or $+22{\cdot}2$ Hz for $^3J_{PH}$ for 1,2-bis-(diphenylphosphino)ethane bismethiodide, and favoured, from a comparison with other coupling constants, the larger value, but had no direct evidence for their choice. Our values for $^3J_{PH}$ support their belief that the larger value is correct.

Pharmacology

Phosphocholine 2,6-xylyl ether bromide in concentrations of 2–3 $\times$ 10^{-5} g/ml blocks the effects of stimulation of the periarterial adrenergic nerves in the Finkleman (1930) preparation. The blockade, which can be observed shortly after administration of the phosphonium compound, deepens slowly over 1 h, and is difficult to reverse by washing. Amphetamine sulphate (0·3–1 $\times$ 10^{-5} g/ml) does not prevent

the establishment of the blockade and has little effect on the intensity of the blockade once it is established, though these concentrations are effective in preventing and reversing the blocking action of xylocholine (Fig. 1).

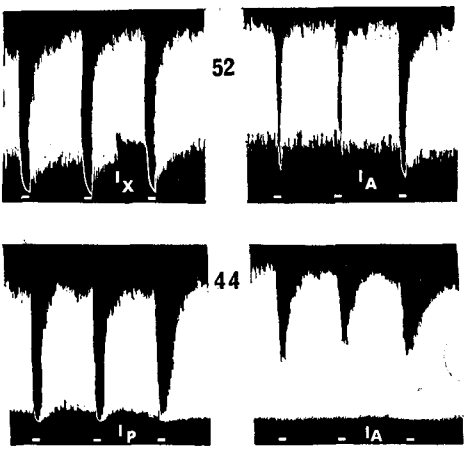


FIG. 1. Rabbit intestine suspended in Tyrode solution at 35°. Showing the effect of xylocholine (4×10^{-6} at X) and phosphocholine 2,6-xylyl ether bromide (3×10^{-5} at P) on the response to supramaximal stimulation of the periarterial nerves (40 V, 0·5 ms duration, 20/s for 30 s in every $7\frac{1}{2}$ min) at the white bars. Amphetamine sulphate (7×10^{-6}) was present in the bath from A. The figures show the time elapsed (min) between the two parts of each record and all concentrations are expressed in g/ml final bath concentration.

Although the slow onset and difficulty in reversing the blockade produced by the phosphonium compound by washing are compatible with a specific adrenergic neuron blocking action such as that possessed by xylocholine, the inability of amphetamine to reverse the blockade suggests that the blocking action of phosphocholine 2,6-xylyl ether might be attributable to mechanisms other than adrenergic neuron blockade.

Acknowledgements

We thank Professor B. Lythgoe for permission to use the Varian A 60 spectrometer, the Science Research Council for facilities at the Physico-chemical Measurements Unit and for defraying the cost of these services, and Messrs. A. Hedley and J. A. Stewart for the microanalyses.

REFERENCES

CARTY, A. J. & HARRIS, R. K. (1967). *Chem. Commun.*, 234–236.

FINKLEMAN, B. (1930). *J. Physiol., Lond.*, **70**, 145–157.

HENDRICKSON, J. B., MADDOX, M. I., SIMS, J. J., & KAESZ, H. D. (1964). *Tetrahedron*, **20**, 449–459.

HOLTON, P. & ING, H. R. (1949). *Br. J. Pharmac. Chemother.*, **4**, 190–196.

HUNT, R. & RENSHAW, R. R. (1925). *J. Pharmac. exp. Ther.*, **25**, 315–355.

MANATT, S. L., JUVINALL, G. L. & ELLEMAN, D. D. (1963). *J. Am. chem. Soc.*, **85**, 2664–2665.

MUSHER, J. I. & COREY, E. J. (1962). *Tetrahedron*, **18**, 791–809.

SCHWEIZER, E. E. & BACH, R. D. (1964). *J. org. Chem.*, **29**, 1746–1751.