

## O-Substituted Pyridine-N-oxide Derivatives

### 2. A New Type of Bisquaternary Ammonium Derivatives \*

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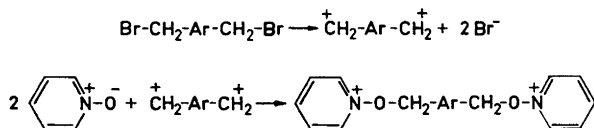
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Submitted in honour of the ninetieth birthday of Professor *Hans von Euler*

The synthesis of a series of *O*-substituted bis-pyridinium-*N*-oxides containing a *p*-xylylenyl and a *p*-phenylene-diethyl substituent, respectively, is described. These derivatives were obtained by a reaction between the *N*-oxide and *p*-xylylene dibromide or *p*-phenylene-diethyl dibromide. They are inhibitors of cholinesterases and cause ganglionic blockade.

The reaction of pyridine-*N*-oxide with alkyl or aryl halogenides occurs either in melt or in the presence of a solvent. In the case of bromocholine, pyridine-*N*-oxide reacted when heated in the absence of any solvent<sup>1</sup>. For the dimethylpyridine-*N*-oxides, on the other hand, this technique gave rise to a brownish mass of unknown composition, and therefore a solvent was used, acetonitrile giving the best result in most cases. This technique was also found to give the highest yield of *O*-substituted *N*-oxides when *p*-xylylene dibromide and *p*-phenylene- $\beta,\beta'$ -diethyl dibromide were used instead of bromocholine.

The reaction between pyridine-*N*-oxide and the dibromide probably proceeds according to the following reactions:

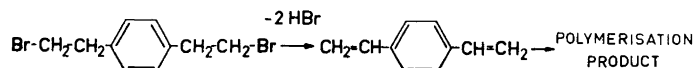


The reaction velocity of the first reaction is determined by the polarisation of the C-Br bond. This polarisation is increased by the polar *N*-oxide, the polarity of which is regulated by the inductive and hyperconjugation (Baker-Nathan) effects of the methyl groups in the ring of pyridine homologues.

It was previously reported<sup>1</sup> that 2,6-lutidine-*N*-oxide (in contradistinction to other dimethyl derivatives) did not react with bromocholine, either in aceto-

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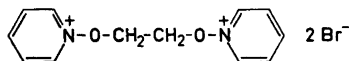
nitrile or by melting the two components together; reaction occurred only in aqueous solution. Similar observations were made with this *N*-oxide in its reaction with *p*-phenylene-diethyl dibromide. The two components, dissolved in acetonitrile, did not react when heated at 100°C. When heated in a closed tube at 120°C for 18 h the components (4.8 mmoles of the *N*-oxide and 1.2 mmoles of the dibromide) gave a dark brown melt. From this was isolated the HBr-salt of 2,6-dimethyl-pyridine-*N*-oxide (m.p. 240°C), identified by mixed melting-point with the product obtained by reacting the *N*-oxide with HBr. A possible side reaction is:



in which the HBr produced combines with the *N*-oxide.

The explanation for the fact that 2,6-dimethyl-pyridine-*N*-oxide differs from the other dimethyl derivatives is probably similar to that suggested for the reaction with bromocholine, *i.e.*, weak H-bonds block the oxygen in the *N*-oxide, and thus prevent oxygen from reacting with the carbenium ion. The higher dielectric constant of water, compared with acetonitrile, may be the reason for the fact that the reaction proceeds in water but not in acetonitrile.

By reacting pyridine-*N*-oxide with ethylene dibromide in acetonitrile, the dibromide of *N,N'*-1,2-dioxyethane-bis-pyridinium was also synthesised:



Most of the compounds synthesised have ganglion-blocking properties and cause a decrease in blood pressure, *N,N'*-*p*-xylylenoxy-bis-pyridinium dibromide (AH 4) being most effective, 4–6 times less effective than hexamethonium. *N,N'*-*p*-Xylylenoxy-bis-(2,6-dimethyl-pyridinium) dibromide (AH 15) was the only compound in this series found to produce neuromuscular block accompanied by apnoe. A full account of the pharmacological studies will be published in due course in collaboration with Drs. Lennart Albanus and Anders Sundwall.

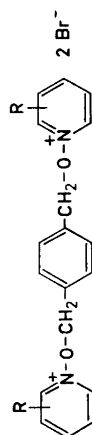
All compounds in both the *p*-xylylenoxy and *p*-phenylene-diethoxy series are inhibitors of cholinesterases. The inhibitory effect of the mono-methyl-pyridinium derivatives, except the 2-methyl derivative, is stronger towards butyrylcholinesterase (human serum;  $\text{pI}_{50}$  4–5) than towards acetylcholinesterase (electric organ;  $\text{pI}_{50} \sim 3$ ). The dimethyl-pyridinium derivatives, on the other hand, are stronger inhibitors of acetylcholinesterase ( $\text{pI}_{50}$  5.8 for AH 15 and AH 31) than of butyrylcholinesterase ( $\text{pI}_{50}$  3.5 and 4.1, respectively). This structure-activity relationship will be further elaborated in a forthcoming report.

## EXPERIMENTAL

### *N,N'*-*p*-Xylylenoxy-bispyridinium derivatives

*General techniques.* A mixture of the *N*-oxide and *p*-xylylene dibromide dissolved in acetonitrile was heated for various periods of time. After completion of the reaction, the product was isolated in crystalline form and recrystallised. All compounds synthe-

Table 1.



Derivative (R = CH <sub>3</sub> or 2 CH <sub>3</sub> )	Code	Mol. formulae	Mol. wt.	m.p. °C	% C		% H		% Br		% N		Yield	
					calc.	found	calc.	found	calc.	found	calc.	found	g	% of theor.
Unsubsti- tuted (R = H)	AH 4	C <sub>18</sub> H <sub>18</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	454.17	188 - 189	47.60	47.78	4.00	4.19	35.2	34.8	6.17	6.23	1.9	84
2-methyl	AH 14	C <sub>20</sub> H <sub>22</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	482.22	160 - 161	49.81	49.77	4.60	4.48	33.1	33.9	5.81	5.41	1.85	77
3-methyl	AH 12	C <sub>20</sub> H <sub>22</sub> Br <sub>2</sub> N <sub>2</sub> O	482.22	179 - 180	49.81	49.99	4.60	4.77	33.1	33.4	5.81	5.69	2.1	87
4-methyl	AH 13	C <sub>20</sub> H <sub>22</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	482.22	173.5 - 174.5	49.81	49.85	4.60	4.59	33.1	33.8	5.81	5.63	1.9	79
2,4-dimethyl	AH 16	C <sub>22</sub> H <sub>26</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·H <sub>2</sub> O	528.29	187 - 188	50.01	50.19	5.34	5.41	30.3	30.7	5.30	5.46	1.9	90
2,5-dimethyl	AH 19	C <sub>22</sub> H <sub>26</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·3H <sub>2</sub> O	564.32	180 - 180.5	46.82	46.83	5.72	5.91	28.3	28.8	4.97	5.12	0.96	89.5
2,6-dimethyl	AH 15	C <sub>22</sub> H <sub>26</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·2H <sub>2</sub> O	546.31	165 - 167	48.36	48.20	5.54	5.30	29.3	30.2	5.13	5.25	2.5	91.5
3,5-dimethyl	AH 17	C <sub>22</sub> H <sub>26</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·H <sub>2</sub> O	528.29	174	50.01	50.26	5.34	5.60	30.3	29.7	5.30	5.49	1.9	94.8

sised in this series, unless otherwise stated, were readily soluble in water and hot methanol, and sparingly soluble in ethanol and acetone. Table 1 summarises melting points (uncorrected), analytical data and yield.

The *N*-oxides used were either synthesised according to previously described methods or purified from commercial grade products (see Ref.<sup>1</sup> for details). Xylylene dibromide was synthesised according to a method described by Ruggli and Theilheimer<sup>2</sup>.

*N,N'*-*p*-Xylylenoxy-bis-pyridinium dibromide (AH 4). Pyridine-*N*-oxide (0.95 g, 10 mmoles) was dissolved in acetonitrile (1 ml) and mixed with a solution of *p*-xylylene dibromide (1.32 g, 5 mmoles) in acetonitrile (4 ml). The mixture was heated on a boiling water bath for 10 min; crystallisation started after about 2 min heating. After cooling, the crystals were filtered off and washed with acetonitrile and acetone. The crude product melted at 188–189°C, and was recrystallised from water-ethanol. The colourless prisms were sparingly soluble in isopropanol and ethyl acetate; other solubility properties were those mentioned above.

*N,N'*-*p*-Xylylenoxy-bis-(2-methyl-pyridinium) dibromide (AH 14). A solution of 1.3 g (12 mmoles) of 2-picoline-*N*-oxide in 5 ml acetonitrile was added to a solution of 1.32 g (5 mmoles) of *p*-xylylene dibromide in 15 ml acetonitrile. After 10 min on a boiling water bath, the clear solution was left for 24 h at room temperature, when the reaction product crystallised as small colourless rods. The crude product (m.p. 151–155°C) was filtered off and washed with acetonitrile and acetone. After recrystallising twice from a small amount of boiling ethanol, the pure compound was obtained as colourless prisms.

*N,N'*-*p*-Xylylenoxy-bis-(3-methyl-pyridinium) dibromide (AH 12). The procedure for synthesis of this compound was the same as for AH 14. The crude product (small colourless prisms), melted at 176–178°C.

*N,N'*-*p*-Xylylenoxy-bis-(4-methyl-pyridinium) dibromide (AH 13) was synthesised from 4-picoline-*N*-oxide using the same procedure as described for AH 14. The crude product melted at 170–172°C, when the crystalline mass became light brown. Recrystallisation was performed twice from methanol-ethanol (1:4) resulting in small colourless prisms which were readily soluble in methanol.

*N,N'*-*p*-Xylylenoxy-bis-(2,4-dimethyl-pyridinium) dibromide (AH 16). 2,4-Lutidine-*N*-oxide (1.97 g, 16 mmoles) dissolved in 5 ml acetonitrile was mixed with a solution of 1.06 g (4 mmoles) of *p*-xylylene dibromide in 5 ml acetonitrile, and heated on a boiling water bath for 30 min. Crystallisation began after half a minute. After cooling, the crystals were filtered off and washed with acetonitrile and acetone. The crude product (m.p. 176–179°C) was recrystallised from as small a volume as possible of boiling ethanol, resulting in large blade-like and colourless crystals, which were readily soluble in cold methanol, boiling ethanol, and slightly soluble in acetone.

*N,N'*-*p*-Xylylenoxy-bis-(2,5-dimethyl-pyridinium) dibromide (AH 19). A mixture of 5 ml acetonitrile containing 0.98 g (8 mmoles) of 2,5-lutidine-*N*-oxide and 5 ml of the same solvent containing 0.5 g (1.9 mmoles) of *p*-xylylene dibromide was heated on a boiling water bath for 30 min. After approximately 5 min the reaction product began to crystallise as colourless needles. The crystalline mass was washed with acetonitrile and acetone, resulting in a crude product which melted at 176–177°C. The pure compound was obtained from ethanol as small colourless rhomboids. The solubility properties were similar to those for AH 16.

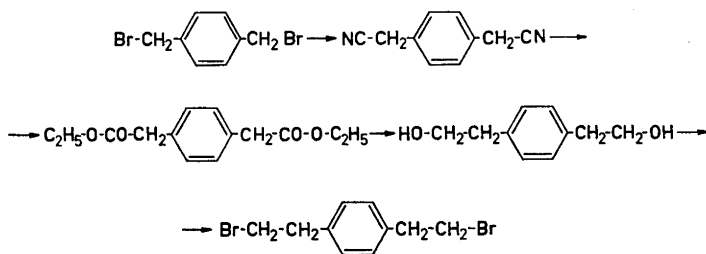
*N,N'*-*p*-Xylylenoxy-bis-(2,6-dimethyl-pyridinium) dibromide (AH 15). 2,6-Lutidine-*N*-oxide (1.76 g, 14 mmoles) and *p*-xylylene dibromide (1.32 g, 5 mmoles) were dissolved in 5 ml acetonitrile. The mixture was heated for 30 min on a boiling water bath and then left for 18 h at 35°C. After addition of 50 ml acetone, the mixture was heated to boiling and then the insoluble product filtered off while still hot. The crystals were washed with excess acetone, giving a crude product with m.p. 165–167°C. A small amount of methanol was used for recrystallisation, when the product was obtained in large prisms containing methanol. By washing with acetone or drying in air, the crystals decomposed rapidly to a crystalline powder with solubility properties similar to those for AH 16.

*N,N'*-*p*-Xylylenoxy-bis-(3,5-dimethyl-pyridinium) dibromide (AH 17). 3,5-Lutidine-*N*-oxide (2.0 g, 16 mmoles) in 5 ml acetonitrile was mixed with *p*-xylylene dibromide (1.0 g, 3.8 mmoles) in 15 ml acetonitrile and the mixture heated on a boiling water bath. Crystallisation in small prisms started after 5 min. After 45 min the crystals were filtered off and washed with acetonitrile; m.p. 170–171°C for the crude product. Recrystallisa-

tion was performed twice from ethanol and resulted in a microcrystalline powder with solubility properties similar to those of AH 16.

### *p*-Phenylene- $\beta,\beta'$ -diethyl dibromide

This compound was prepared in four steps according to the following sequences of reactions:



*p*-Phenylene diacetonitrile was synthesised according to a method described previously,<sup>3</sup> in which *p*-xylylene dibromide reacted with KCN in a boiling ethanol-water solution.

The *p*-phenylene-diacetic acid ester of ethanol was prepared by boiling the diacetonitrile with HCl-saturated ethanol<sup>4,5</sup>, in which reaction nitrogen was bound as NH<sub>4</sub>Cl.

*p*-Phenylene diethanol. Ruggli and Theilheimer<sup>2</sup> synthesised this compound by reducing the ester with metallic sodium in butanol, while increasing the temperature slowly up to 150°C. The yield reported was 60 %, but in our own experiments this yield was highly dependent on the degree of dryness of butanol. Moreover, the procedure is much time consuming. The same yield (60 %) was obtained easier and more rapidly when the reduction of the ester was performed with LiAlH<sub>4</sub> according to the following procedure. The diester (5 g, 20 mmoles) was dissolved in 40 ml water-free diethyl ether and was then added dropwise with continuous stirring to a suspension of 3 g (80 mmoles) of LiAlH<sub>4</sub> in 60 ml ether during a period of 30 min. Stirring was continued for 30 min after mixing the two components, and then ethyl acetate was added dropwise in order to destroy unreacted LiAlH<sub>4</sub>. The mixture was made acid with dilute HCl, the ether layer was separated, washed with water, dried with anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated in vacuum to dryness. The remaining crystalline mass was treated with 10 ml cold benzene, which dissolved unreacted ester. The crystals were filtered off and washed with a small volume of benzene. The final product, recrystallised from benzene, was obtained in 63 % yield (2.1 g) and had m.p. 85–86°C.

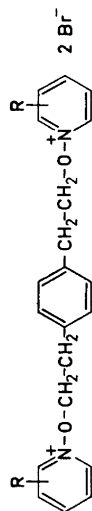
*p*-Phenylene- $\beta,\beta'$ -diethyl dibromide was prepared by heating the dialcohol and acetic acid, the latter saturated with HBr,<sup>2</sup> to 100°C in a closed tube.

### *N,N'*-*p*-Phenylene- $\beta,\beta'$ -diethoxy-bispyridinium derivatives

*General techniques.* A mixture of the *N*-oxide and *p*-phenylene-diethyl dibromide was heated, either in the absence of any solvent or dissolved in acetonitrile or water. After completion of the reaction, the product was isolated in crystalline form. All compounds in this series were soluble in water, ethanol and methanol, sparingly soluble in acetonitrile and ethyl acetate, and decomposed by NaOH to the corresponding pyridine derivative. Table 2 summarises melting points (uncorrected), analytical data and yield.

*N,N'*-*p*-Phenylene- $\beta,\beta'$ -diethoxy-bispyridinium dibromide (AH 29). *p*-Phenylene-diethyl dibromide (0.35 g, 1.2 mmoles) and pyridine-*N*-oxide (0.455 g, 4.8 mmoles) were heated on a boiling water bath for 30 min. After cooling, 10 ml acetone was added when a soft mass was produced, being converted to a micro-crystalline powder by grinding with a

Table 2.



Derivative (R = CH <sub>3</sub> or 2 CH <sub>3</sub> )	Code	Mol. formulae	Mol. wt.	m.p. °C	% C		% H		% Br		% N		Yield	
					calc.	found	calc.	found	calc.	found	calc.	found	g	% of theor.
Unsubsti- tuted (R = H)	AH 29	C <sub>20</sub> H <sub>22</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·2H <sub>2</sub> O	518.26	104—105	46.35	46.18	5.06	5.05	30.8	31.1	5.41	5.51	0.41	65.8
4-methyl	AH 30	C <sub>22</sub> H <sub>26</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub>	510.28	160—161	51.78	51.24	5.14	5.40	31.3	31.6	5.49	5.20	0.21	34.5
2,6-dimethyl	AH 31	C <sub>24</sub> H <sub>30</sub> Br <sub>2</sub> N <sub>2</sub> O <sub>2</sub> ·2H <sub>2</sub> O	574.36	130—131	50.18	50.28	5.97	5.97	27.8	27.2	4.88	5.07	0.25	43.6

glass rod. The crude product was filtered off and washed with acetone, had then m.p. 98–101°C, and was purified by recrystallising twice from acetonitrile. The pure compound crystallised as colourless prisms and gave an intense yellow colour upon treatment with 20 % NaOH; heating to 40°C resulted in a brownred colour, a precipitate and smell of pyridine.

*N,N'*-*p*-Phenylene- $\beta,\beta'$ -diethoxy-bis-(4-methyl-pyridinium) dibromide (AH 30). A mixture of *p*-phenylene-diethyl dibromide (0.35 g, 1.2 mmoles), 4-picoline-*N*-oxide (0.525 g, 4.8 mmoles) and acetonitrile (1 ml) was heated on a boiling water bath. After 15 min acetonitrile had evaporated, leaving a yellow-brown mass, which after another 20 min changed to a red-brown melting mass partly containing colourless prisms. The product was dissolved in 10 ml acetone after cooling, and the mixture was heated rapidly to boiling. Upon cooling and grinding with a glass rod, crystallisation occurred; m.p. of the crystalline powder, 154–158°C. Three recrystallisations from ethanol-acetone (1:20) gave small colourless needles arranged in ball-like aggregates. Upon treatment with 20 % NaOH, the pure compound produced a yellow-brown precipitate and smell of picoline.

*N,N'*-*p*-Phenylene- $\beta,\beta'$ -diethoxy-bis-(2,6-dimethyl-pyridinium) dibromide (AH 31). As noted above, reaction between the components did not occur either in melts or in acetonitrile, but only in water. A mixture of *p*-phenylene-diethyl dibromide (0.49 g, 2.0 mmoles), 2,6-lutidine-*N*-oxide (0.29 g, 1.0 mmole) and water (2 ml) was refluxed on a boiling water bath for 8 h. The reaction mixture was then poured into a petri dish and evaporated on the water bath. The brown-red oil thus obtained was treated three times with ethyl acetate which was decanted each time. Upon treatment with acetone, the insoluble oily product rapidly crystallised. The crystals were filtered off and washed with a large amount of acetone. The brown prisms, m.p. 122–125°C, were dissolved in as a small volume as possible of ethanol at 40°C, and then 1.5 volumes of acetone was added. After 30 min at 20°C brown amorphous flocks were filtered off, and the clear almost colourless filtrate was mixed with ethyl acetate until crystals began to form as small colourless prisms (m.p. 129–130°C). The final product was obtained in large prisms after recrystallisation twice from ethanol-ethyl acetate. Decomposition with 20 % NaOH resulted in a yellow colour and smell of lutidine.

### Reaction of pyridine-*N*-oxide with ethylene dibromide

*N,N'*1,2-dioxyethane-bis-pyridinium dibromide (AH 36). Pyridine-*N*-oxide (4.75 g, 50 mmoles), ethylene dibromide (4.70 g, 25 mmoles) and acetonitrile (5 ml) were refluxed for 2 h on a boiling water bath when the reaction product crystallised as large colourless blades. After cooling, the crystalline mass was filtered off, washed with acetonitrile and dried; m.p. 173–174°C. The pure compound was obtained as large colourless blades after recrystallisation from a small volume of ethanol, and had m.p. 175°C. Yield 51 % (4.8 g). After exposure to light for a long period of time the crystals became brown coloured on the surface. (Found: C 38.24; H 3.70; Br 42.2; N 7.19; Calc. for C<sub>12</sub>H<sub>14</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub> (378.08): C 38.12; H 3.73; Br 42.3; N 7.41).

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