

## Pyridyl-carbinol Acetates

KLAS-BERTIL AUGUSTINSSON and HANS HASSELQUIST

*Institute of Biochemistry, University of Stockholm, Sweden*

In connection with studies on the reaction mechanism of cholinesterases, a series of acetylcholine related acetates of pyridyl-carbinols and *N*-alkylpyridinium-carbinols have been prepared and some of their properties described.

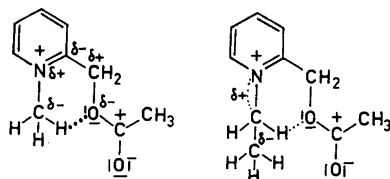
A great number of esters related to acetylcholine and other choline esters have been tested as substrates for cholinesterases. Those esters which contain a quaternary nitrogen atom or another positively charged atom (*e.g.*, sulphur) are of particular interest. Pyridine and pyridinium derivatives have been used frequently in inhibition studies on these enzymes,<sup>1</sup> and pyridinium-oximes have recently gained great importance as reactivators of phosphorylated cholinesterases.<sup>2</sup> The closely related esters of *N*-methyl-pyridinium-carbinols have not been described and consequently have not been studied as substrates for cholinesterases. The syntheses of these esters are described in the present report and the enzymatic studies in a subsequent one.

Most of the pyridine- and *N*-methyl-pyridinium-carbinols used for the synthesis of acetates were previously described, except the *N*-methylpyridinium-3-carbinol, the three isomers of *N*-ethyl-pyridinium, *N*-propylpyridinium-2-carbinol, and the *N*-methyl-pyridinium-2,6-dicarbinol. These *N*-alkylated derivatives were synthesised from the corresponding pyridylcarbinols by incubation with alkyl iodide in the dark, the time of incubation varying from a few hours (*N*-methyl derivatives) to four days (*N*-propyl derivatives). The *N*-methylation of the 2,6-dicarbinol was carried out at a higher temperature and in the presence of acetonitrile.

The acetylation of the carbinols was carried out with acetic anhydride, except in the case of the monoacetate of pyridyl-2,6-dicarbinol, which was prepared from the dicarbinol and acetyl chloride in tetrahydrofuran solution. The *N*-alkyl-pyridinium-carbinol acetates were synthesised either by *N*-alkylation of the pyridyl-carbinol acetate or acetylation of *N*-alkylpyridinium-carbinol with acetic anhydride.

The melting points of the *N*-alkyl-2-carbinols are higher than those of the *N*-alkyl-4-carbinols, the difference being 54°C for both the *N*-methyl and *N*-ethyl derivatives. This difference is probably due to stronger hydrogen

bonding between the  $\text{CH}_3$  group and the carbinol oxygen for the 2-carbinol than for the 4-carbinol. The melting points of the acetates of the *N*-methyl-monocarbinols are higher than those of the corresponding carbinols. In the series of the *N*-ethyl derivatives the melting point of the 2-carbinol ester is lower than that of the carbinol, and this relationship also holds for the 2,6-dicarbinol relative to its di-acetate. The hydrogen bond in these cases may be strengthened by the carboxyl group in the ester. On the other hand, the carbon atom of the ethyl group adjacent to the pyridine nitrogen atom is more positive in comparison with that of its methyl homologue, which in its turn lowers the strength of the hydrogen bond. In the free carbinol this lowering in bond strength is not as great as in the ester due to the absence of a  $>\overset{+}{\text{C}}-\overset{-}{\text{O}}$ -group. These views considering the structural differences between *N*-methyl and *N*-ethyl derivatives of pyridyl- and pyridinium-carbinol acetates are further elaborated in connection with the enzyme studies described in a subsequent report.<sup>3</sup>



As far as the solubility properties are concerned, the *N*-methyl carbinols and their acetates are more soluble in acetone than their *N*-ethyl homologues. The *N*-propyl-monocarbinol acetate is sparingly soluble in acetone in contrast to the ready solubility of the corresponding carbinol in this solvent.

During the studies on the enzymic hydrolysis of these esters it was shown that only one of the two ester linkages in the dicarbinol acetate was hydrolysed. The pyridyl-2,6-dicarbinol monoacetate (as hydrochloride) was therefore included in the series of esters synthesised. During the synthesis of this compound, thin layer chromatography was employed to separate and identify the carbinol mono- and diester in a mixture.

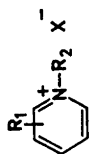
These new pyridine derivatives have been used in studies<sup>3</sup> on a comparison of acetylcholinesterase and butyrylcholinesterase concerning the reaction mechanism, and particularly the nature of active esterase sites.

## EXPERIMENTAL

### *N*-Alkyl-pyridinium-monocarbinol iodides

Pyridine-monocarbinols (commercial products of high purity) were *N*-alkylated by incubating a mixture of the carbinol (1 mole) and alkyl iodide, (3 moles) unless otherwise stated, in the dark for various periods of time. Both the *N*-methyl and *N*-ethyl derivatives were obtained as yellow rods or prisms, except *N*-ethyl-pyridinium-2-carbinol iodide which was obtained as colourless plates. All derivatives were readily soluble in water. Table 1 gives melting points (uncorrected) and elemental analysis for nitrogen and halogen.

Table 1.



Compound No.	R <sub>1</sub>	R <sub>2</sub>	X	Mol. formulae	Mol. wt.	M.p. °C*	% N calc. found	% X calc. found
AH 51	2-CH <sub>2</sub> OH	CH <sub>3</sub>	I	C <sub>7</sub> H <sub>10</sub> INO	251.08	151–152 (153) <sup>4</sup>	—	50.5 49.9
AH 49	3-CH <sub>2</sub> OH	CH <sub>3</sub>	I	C <sub>7</sub> H <sub>10</sub> INO	251.08	82.5–84	5.58 5.32	50.5 49.7
AH 50	4-CH <sub>2</sub> OH	CH <sub>3</sub>	I	C <sub>7</sub> H <sub>10</sub> INO	251.08	97–98 (96–98) <sup>5</sup>	—	50.5 49.3
AH 58	2-CH <sub>2</sub> OH	C <sub>2</sub> H <sub>5</sub>	I	C <sub>8</sub> H <sub>12</sub> INO	265.10	138–139	5.29 5.19	47.9 47.8
AH 59	3-CH <sub>2</sub> OH	C <sub>2</sub> H <sub>5</sub>	I	C <sub>8</sub> H <sub>12</sub> INO	265.10	51–52	5.29 4.98	47.9 47.4
AH 55	4-CH <sub>2</sub> OH	C <sub>2</sub> H <sub>5</sub>	I	C <sub>8</sub> H <sub>12</sub> INO	265.10	84–85	5.29 4.88	47.9 47.6
AH 60	2-CH <sub>2</sub> OH	n-C <sub>3</sub> H <sub>7</sub>	I	C <sub>8</sub> H <sub>14</sub> INO	279.13	102.5–103	5.02 4.90	45.5 46.3
AH 62	2,6-(CH <sub>2</sub> OH)	CH <sub>3</sub>	I	C <sub>8</sub> H <sub>12</sub> INO <sub>2</sub>	281.10	164–165	—	45.2 44.9
AH 57	2-CH <sub>2</sub> OCOCH <sub>3</sub>	H	Cl	C <sub>8</sub> H <sub>10</sub> ClNO <sub>2</sub>	187.63	94–95	—	18.9 18.6
AH 44	3-CH <sub>2</sub> OCOCH <sub>3</sub>	H	Cl	C <sub>8</sub> H <sub>10</sub> ClNO <sub>2</sub>	187.63	96–98	—	18.9 19.6
AH 45	4-CH <sub>2</sub> OCOCH <sub>3</sub>	H	Cl	C <sub>8</sub> H <sub>10</sub> ClNO <sub>2</sub> · H <sub>2</sub> O	205.64	154–156	—	17.2 17.1
AH 52	2-CH <sub>2</sub> OCOCH <sub>3</sub>	CH <sub>3</sub>	I	C <sub>9</sub> H <sub>12</sub> INO <sub>2</sub>	293.11	160–161	4.78 4.20	43.3 43.6
AH 46	3-CH <sub>2</sub> OCOCH <sub>3</sub>	CH <sub>3</sub>	I	C <sub>9</sub> H <sub>12</sub> INO <sub>2</sub>	293.11	130–131	4.78 4.59	43.3 43.8
AH 47	4-CH <sub>2</sub> OCOCH <sub>3</sub>	CH <sub>3</sub>	I	C <sub>9</sub> H <sub>12</sub> INO <sub>2</sub>	293.11	142–143	4.78 4.39	43.3 43.3
AH 48b	2-CH <sub>2</sub> OCOCH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	I	C <sub>10</sub> H <sub>14</sub> INO <sub>2</sub>	307.14	115–116	4.56 4.20	41.3 41.0
AH 56	4-CH <sub>2</sub> OCOCH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	I	C <sub>10</sub> H <sub>14</sub> INO <sub>2</sub>	307.14	113–114	4.56 4.26	41.3 41.0
AH 61	2-CH <sub>2</sub> OCOCH <sub>3</sub>	n-C <sub>3</sub> H <sub>7</sub>	I	C <sub>11</sub> H <sub>16</sub> INO <sub>2</sub>	321.17	126–127	4.36 4.42	39.5 39.0
AH 65	2,6-(CH <sub>2</sub> OCOCH <sub>3</sub> ) <sub>2</sub>	H	Cl	C <sub>11</sub> H <sub>14</sub> ClNO <sub>4</sub>	259.69	132–133	5.39 5.44	13.65 13.7
AH 68	2-CH <sub>2</sub> OCOCH <sub>3</sub>	H	Cl	C <sub>9</sub> H <sub>10</sub> ClNO <sub>3</sub>	217.65	135–137	6.44 6.20	16.3 16.95
AH 63	6-CH <sub>2</sub> OH	CH <sub>3</sub>	I	C <sub>12</sub> H <sub>16</sub> INO <sub>4</sub>	365.18	149–150	3.84 3.87	34.8 36.0

\* Values reported by other authors, if available, are in brackets.

*N-Methyl-pyridinium-monocarbinol iodides* (AH 51, 49, 50). The 2- and 4-carbinol derivatives have been previously described.<sup>4,5</sup> All three isomers were synthesised by a direct reaction between the carbinol and methyl iodide at 12°C (tap-water cooling) in the dark. After 2 h the crystalline product was treated with an equal volume of acetonitrile, filtered off and washed with a little of this solvent. Recrystallisation was carried out from acetonitrile (2-derivative) or ethanol (3- and 4-derivatives). Yield 75–85 %. (Found for *N*-methyl-pyridinium-3-carbinol iodide (AH 49); C 33.81; H 4.32. Calc. for C<sub>7</sub>H<sub>10</sub>INO: C 33.48; H 4.02).

*N-Ethyl-pyridinium-monocarbinol iodides* (AH 58, 59, 55). A mixture of the carbinol and ethyl iodide (no solvent) was left for 24 h at 20°C in the dark. The upper layer of the two layers formed contained the reaction mixture, a brownish-yellow viscous oil, which crystallised on rubbing with a glass rod. The crystalline mass was treated with an equal volume of ethyl acetate and the crystals filtered off and washed with ethyl acetate. The 2-carbinol (AH 58) was recrystallised from glacial acetic acid, the 3-carbinol (AH 59) from acetic acid-ethyl acetate, and the 4-carbinol (AH 55) from isopropanol. Yield 50–60 %.

*N-Propyl-pyridinium-monocarbinol iodide* (AH 60). A mixture of pyridyl-2-carbinol (1 mole) and propyl iodide (1.5 moles) was left in a closed vessel for four days at 20°C in the dark. The reaction product in the form of colourless prisms was filtered off and washed with a little isopropanol. Two recrystallisations from the same solvent gave the pure compound as large colourless prisms, readily soluble in acetone and hot acetonitrile; as will be seen below these solubility properties are the reverse for the corresponding acetate. Yield 55 %, which could be increased to 70 % when the reaction mixture was left for another four days at 20°C. (Found: C 38.72; H 5.17. Calc. for C<sub>9</sub>H<sub>14</sub>INO: C 38.85; H 5.20. See Table 1 for N and I).

### Pyridyl-monocarbinol acetates

These esters, previously described,<sup>6-9</sup> were synthesised in this laboratory by the reaction of the carbinol with acetic anhydride and subsequent fractional distillation of the reaction product under reduced pressure. Yield 85–90 %. B.p.: 2-derivative, 103–104°C/15 mm (115–118°C/22 mm);<sup>6</sup> 3-derivative, 123–124°C/20 mm (118°C/12 mm);<sup>7</sup> 4-derivative, 126°C/20 mm (53–55°C/0.3 mm);<sup>8,9</sup> values in brackets refer to those reported by other authors.

The hydrochlorides (AH 57, 44, 45) were prepared by dissolving the carbinol acetate (1 vol.) in dry diethyl ether (50 vol.), followed by the bubbling of dry HCl gas through the mixture until the precipitation of the hydrochloride was complete (approx. 20 min). The salt was filtered off, washed rapidly with diethyl ether and then transferred without delay to a desiccator which was then evacuated. After drying, the salts were recrystallised from glacial acetic acid. The pure salts which crystallised as colourless rods or prisms, were highly hygroscopic and readily soluble in acetic acid and acetone, except the 4-carbinol acetate hydrochloride which was slightly soluble in acetone; they are sparingly soluble in diethyl ether. Yield 90–96 %. Melting points and analytical data are given in Table 1.

### *N*-Alkyl-pyridinium-monocarbinol-acetate iodides

The synthesis of these compounds was carried out by two alternative procedures: alkylation of the acetate (Procedure *a*) and acetylation of the *N*-alkyl carbinol (Procedure *b*). For the *N*-methyl-monocarbinol derivatives the two methods gave the same yield (80–90 %).

### *N*-Methyl-pyridinium-monocarbinol-acetate iodides (AH 52, 46, 47)

*Procedure a.* The carbinol acetate (1 mole) was mixed with methyl iodide (3 moles) and the mixture left at 12°C for 2 h. The resulting crystalline mass was treated with an equal volume of glacial acetic acid, filtered off, washed with a little acetone and dried.

*Procedure b.* The *N*-methyl-pyridinium-monocarbinol iodide was boiled for 1–5 min with ten times its weight of acetic anhydride and a catalytic amount of concentrated sulphuric acid. After cooling, the crystalline mass was filtered off, washed with acetone, and dried.

All three isomers could be obtained by either method. The two methods gave in each case identical compounds, proved by mixed melting point determination. Recrystallisation was carried out from glacial acetic acid. The pure compounds were obtained as colourless prisms (2-), light yellow prisms (3-) and light pink rods (4-), and were readily soluble in water and acetonitrile; the 3-carbinol acetate was soluble in acetone, the 2- and 4-isomers were sparingly soluble in this solvent. The solution of the 2-carbinol acetate in methanol was brownish-red. The solution of the 4-carbinol acetate was light sensitive, the surface turning rapidly yellow-brown, especially in the presence of a small amount of ethyl acetate.

### *N*-Ethyl-pyridinium-monocarbinol-acetate iodides (AH 48a, 48b, 56)

*Procedure a.* The reaction mixture of the carbinol acetate (1 mole) and ethyl iodide (3 moles) was left for five days at 20°C in the dark in a closed vessel. The reaction product of the 2-carbinol acetate had crystallised after this period, those of the two isomers gave oily products. The crystalline product (AH 48a) from the 2-derivative had a higher melting point (242°C) than the product obtained by Procedure *b*. It was recrystallised from ethanol and was slightly soluble in acetone and acetic acid. Yield 2%. (Found: C 38.56; H 4.17; I 41.3; N 4.31. Calc. for C<sub>10</sub>H<sub>14</sub>INO<sub>2</sub>: see next paragraph).

The filtrate obtained after filtering off the crystals was evaporated under reduced pressure, and the resulting oil crystallised on freezing. A little glacial acetic acid was added to the crystal line mass, which was isolated by filtration and recrystallised from acetic acid to yield yellow cubes or parallelepipeds. This compound, yield 50%, was shown (by analyses and mixed melting point) to be identical with the compound obtained by procedure *b*.

The oily homogeneous mixtures obtained with the 3- and 4-carbinol acetates were treated with a large volume of ethyl acetate, and the reaction products separated as yellow oils. The 3-isomer could not be obtained in the crystalline form. The 4-isomer (AH 56) crystallised when the mixture was cooled down and rubbed with a glass rod. The crystals were filtered off, washed with ethyl acetate and recrystallised from isopropanol to give brown-violet rods. Yield 50%. (Found: C 38.87; H 4.78. Calc. for C<sub>10</sub>H<sub>10</sub>INO<sub>2</sub>: C 39.10; H 4.60. See Table I for N and I).

*Produce b.* The carbinol ethyl iodide mixed with ten times its weight of acetic anhydride and a little concentrated sulphuric acid was boiled for 5 min. The 2-isomer (AH 48b) crystallised on cooling and was then recrystallised from glacial acetic acid: The pure compound had a lower melting point (115–116°C) than that found for the compound (AH 48a) obtained in low yield by Procedure *a*. Yield 70%. (Found: C 39.39; H 4.47. Calc. for C<sub>10</sub>H<sub>14</sub>INO<sub>2</sub> (307.14): C 39.10; H 4.60). The 3- and 4-isomers could not be synthesised by this procedure.

*N*-Propyl-pyridinium-monocarbinol-acetate iodide (AH 61) was prepared by Procedure *b*. *N*-propyl-pyridyl-monocarbinol iodide (AH 60) was boiled for 3 min with ten times the amount of acetic anhydride, giving a clear solution. After cooling and adding an equal volume of benzene, a light-yellow oil was formed which on rubbing with a glass rod crystallised as yellow prisms. These were filtered off, washed with benzene and dried. Two recrystallisations from acetic acid-benzene yielded the pure compound (82% of theor.) which was sparingly soluble in acetone and readily soluble in acetonitrile (cf., AH 60 above). (Found: C 41.11; H 4.92. Calc. for C<sub>11</sub>H<sub>16</sub>INO<sub>2</sub>: C 41.13; H 5.02. See Table I for further analyses).

### Pyridyl-dicarbinol-acetates

*Pyridyl-2,6-dicarbinol diacetate* (AH 64). Pyridyl-2,6-dicarbinol (13.9 g, 0.10 moles) and acetic anhydride (20.0 g, 0.20 moles) were heated until reaction occurred. When

the mixture had cooled to 40°C it was left for 30 min on a boiling water bath. After cooling, the mixture was poured with stirring into ice water, and after 20 min solid  $\text{Na}_2\text{CO}_3$  was added until the solution was weakly alkaline. The solution was then extracted with chloroform ( $3 \times 50$  ml). The combined extracts were dried over anhydrous  $\text{Na}_2\text{SO}_4$ , filtered and fractionally distilled under reduced pressure. The ester, previously described,<sup>9</sup> was obtained as a light yellow liquid, b.p. 187–190°C/20 mm (135–139°C/0.3 mm), readily soluble in most of the organic solvents tested. Yield 49 %.

The hydrochloride (AH 65) was prepared by dissolving AH 64 (1.4 g, 6.3 moles) in anhydrous diethyl ether (50 ml) and introducing dry HCl gas into the solution. The salt was obtained as small colourless needles which were filtered off, washed with diethyl ether and dried in a desiccator over solid KOH. Two recrystallisations from acetonitrile yielded the pure compound (73 % of theor.). (Found: C 50.64; H 5.33. Calc. for  $\text{C}_{11}\text{H}_{14}\text{ClNO}_4$ : C 50.87; H 5.31).

*Pyridyl-2,6-dicarbinol-monoacetate* (AH 68). Pyridyl-2,6-dicarbinol (1.39 g, 10 mmoles) was dissolved in tetrahydrofuran (50 ml) by heating. After cooling to 20°C, acetyl chloride (0.39 g, 5 mmoles; N. B. the mole ratio for the dicarbinol/acetyl chloride, 2/1) was added slowly with stirring over a period of 10 min. The mixture was heated at 60°C for 20 min after which time the dicarbinol hydrochloride had partially crystallised. After cooling, twice the volume of diethyl ether was added and after 30 min at 20°C the dicarbinol hydrochloride was filtered off; the identity of this compound was confirmed by thin layer chromatography (see below). The filtrate was evaporated under reduced pressure at 20°C, and the resulting oil containing some crystals was treated with diethyl ether (50 ml). The mixture was filtered, and the filtrate evaporated to dryness under reduced pressure. After repeating this procedure, the oily product (the monoacetate) was dissolved in diethyl ether (100 ml), and the solution saturated with HCl gas. The mixture was left for 2 h at 20°C, and the light yellow-brown crystalline product was filtered off, washed with diethyl ether and then with acetone, and dried in vacuum at 20°C.

The yield of the crude product was 350 mg with m.p. 95–125°C; in addition to the monoester, both the diester and the dicarbinol were identified on a thin layer chromatogram. The product was recrystallised three times from propanol-benzene; in this mixture the dicarbinol and its diacetate are more soluble than the monoester. After each recrystallisation the product was analysed by thin layer chromatography. The pure compound, yield 42 mg (3.9 %), had a melting point only slightly higher than that of the diacetate (see Table 1). The monoester was readily soluble in methanol and ethanol, and slightly soluble in acetone, in which solvent the diester is readily soluble. (Found: C 49.66; H 5.56. Calc. for  $\text{C}_9\text{H}_{12}\text{ClNO}_3$ : C 49.83; H 5.67).

### N - M e t h y l - p y r i d i n i u m - d i c a r b i n o l - d i a c e t a t e

Of the two procedures tested for the synthesis (see above) only Procedure *b* was successful for the preparation of the 2,6-dicarbinol derivative.

*N-Methyl-pyridinium-2,6-dicarbinol iodide* (AH 62). A mixture of pyridyl-2,6-dicarbinol (7.0 g, 50 mmoles), methyl iodide (10.0 g, 70 mmoles) and acetonitrile (40.0 ml) was refluxed at 50°C for 24 h. After cooling, the crystalline reaction product was filtered off, washed with ethanol, and dried. Recrystallisation from methanol gave the pure compound as large light-brown prisms, m.p. 164–165°C Yield 6.2 g (44 %).

*N-Methyl-pyridinium-2,6-dicarbinol-diacetate iodide* (AH 63). The dicarbinol (AH 62) was boiled for 5 min with five times its weight of acetic anhydride. While still warm, the yellow-brown solution was mixed with five times its volume of ethyl acetate after which the ester crystallised as yellow plates. These were filtered off, washed with ethyl acetate and recrystallised twice from acetonitrile-ethyl acetate. Yield 45 %. (Found: C 40.01; H 4.52. Calc. for  $\text{C}_{12}\text{H}_{16}\text{INO}_4$ : C 39.47; H 4.42).

### T h i n l a y e r c h r o m a t o g r a p h y

This technique was used to identify the di- and monoacetates during the syntheses and in the reaction mixtures after enzymic ester hydrolysis. It is very useful for the separation of all pyridyl-carbinols and their acetates mentioned in this paper. Silica gel (Kieselgel

G "Merck") was used for the absorbent layers (0.25 mm thick, size 200 mm × 200 mm). One  $\mu$ l containing 1–5  $\mu$ g of the compound(s) to be analysed was applied to the plate 3.5 cm from the edge. After drying at room temperature for 15 min the chromatograms were developed with chloroform: 96 % ethanol (14:1) for 30 min, when the solvent had moved approx. 7 cm from the starting line. After drying for 30 min at room temperature the chromatograms were sprayed with a modified Dragendorff-reagent<sup>10</sup> when the pyridine derivatives appeared as orange-red spots on a yellow background; the colour of the spots faded in about 2 h.  $R_F$  values obtained with HCl salts: pyridyl-2,6-dicarbinol, 0.17; monoacetate, 0.50; diacetate, 0.93

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