ACTA CHEMICA SCANDINAVICA 27 (1973) 3307–3311

Choline Acetylase Inhibitors

II.* The Preparation of Some 3-Substituted Acetonyltrimethylammonium Salts

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Four 3-halogenoacetonyltrimethylammonium halides have been prepared, one of each of the halogens F, Cl, Br, and I. All except the fluorine derivative showed a strong choline acetyl transferase inhibiting activity. Acetonyl- and 3-diazoacetonyltrimethylammonium salts, which were obtained as intermediates in the synthetic routes, did not possess inhibitor activity.

In the search for a specific choline acetyl transferase inhibitor, we have synthetized some quaternary ammonium salts, structurally related to choline and mainly belonging to the general formula

$$[(CH_3)_3N-(CH_2)_n-CO-X]^+ \ Y^-$$

where $n=1$ or 2, $X=CH_3$, CH_2F, CH_2Cl, CH_2Br, CH_2I, CHN_2, CH_2OH, $CO-CH_3$, $CH_2N^+(CH_3)_3$ *etc.* and $Y=$halogen. A previous paper [1] contains a description of one of these, 3-bromoacetonyltrimethylammonium bromide, named BAT by others [2] for convenience. We have now developed an improved method for the preparation of this substance and also found paths to its fluorine, chlorine, and iodine analogues.

The method reported [1] for preparing BAT had the disadvantage of utilizing 1,3-dibromoacetone, which is not commercially available and is obtained *via* careful distillation from a complex mixture of bromination products of acetone. We therefore switched a couple of years ago to the more easily procurable monobromoacetone as starting material in the preparation of BAT. The monobromoacetone is thus reacted with an excess of trimethylamine in, for instance, acetone, and the acetonyltrimethylammonium bromide so obtained is brominated in glacial acetic acid with an equivalent of bromine. The yield exceeds 80 %.

* Part I, see Ref. 1.

The iodine analogue, 3-iodoacetonyltrimethylammonium iodide, can be obtained from BAT through a simple Finkelstein exchange, using an equivalent of sodium iodide in acetone. This method, in which initially an iodine-rich, crystalline product formed, which after decomposition in water (to give elemental iodine) and washing with carbon tetrachloride gave 3-iodoacetonyltrimethylammonium iodide, however, gives a rather low yield. To get a better yield, the 3-iodoacetonyltrimethylammonium iodide could instead be prepared from equimolecular amounts of trimethylamine and 1,3-diiodoacetone, synthetized from freshly prepared 3-oxoglutaric acid [3] according to Lederer.[4]

The chlorine analogue, 3-chloroacetonyltrimethylammonium chloride, has so far defied all our efforts to isolate it in a pure state from the reaction product of trimethylamine and 1,3-dichloroacetone. The difficulty probably lies in the poor quaternizing ability of the chlorine function, combined with the enhanced probability of condensation and elimination reactions of 1,3-dichloroacetone under the basic conditions created by the trimethylamine. Trimethylammonium chloride is a dominant component in the dark-coloured reaction products. This crude reaction product does, however, show a strong choline acetylase inhibiting activity.[5]

The preparation of reasonably pure 3-chloroacetonyltrimethylammonium chloride has been achieved in two ways. In the earlier of these two methods, 3-chlorodiazoacetone, prepared according to Arndt and Amende,[6] was reacted with trimethylamine and the resulting 3-diazoacetonyltrimethylammonium chloride was treated in a second step with hydrogen chloride in anhydrous ethanol. More recently we have obtained 3-chloroacetonyltrimethylammonium chloride in a less elaborate way by converting 3-iodoacetonyltrimethylammonium iodide with silver chloride in aqueous methanol.

The fluorine analogue, 3-fluoroacetonyltrimethylammonium bromide, finally, has been prepared from 3-bromodiazoacetone, synthetized from bromoacetyl bromide in strict analogy to the chlorine compound [6] mentioned above. A solution of the diazoketone in benzene or methylene chloride was treated with anhydrous hydrogen fluoride in heterogeneous phase, and the 1-bromo-3-fluoropropan-2-one so obtained reacted with trimethylamine, without intervening isolation.

The synthetized trimethylammonium salts have been tested for their inhibitory action on choline acetylase (acetyl-CoA:choline *O*-acetyl transferase, EC 2.3.1.6) from bovine caudate nucleus. The chloro-, bromo-, and iodoacetonyltrimethylammonium halides showed a strong inhibitory action, each giving about 90 % inhibition at a concentration of 2×10^{-5} M,[7] whereas the acetonyl-, 3-diazoacetonyl-, and 3-fluoroacetonyltrimethylammonium bromides did not inhibit the enzyme to any noticeable extent.[7]

EXPERIMENTAL

Acetonyltrimethylammonium bromide. 40.5 g of freshly distilled monobromoacetone was dissolved in 500 ml of dry acetone. Using argon as a carrier gas, 26.1 g (*i.e.* 1.5-fold molar excess) of anhydrous trimethylamine was blown into the solution, stirred and cooled on an ice-salt bath. A cream-coloured precipitate formed during addition of the

amine. The reaction mixture was kept at room temperature overnight and the precipitate was then collected on a filter, washed with dry acetone and recrystallized from anhydrous ethanol. Yield: 51.0 g (88 %) of colourless crystals melting at 187 – 188°C. (Found: C 36.6; H 7.47; Br 40.5; N 7.40. Calc. for $C_6H_{14}BrNO$: C 36.7; H 7.20; Br 40.8; N 7.14.)

3-Bromoacetonyltrimethylammonium bromide (BAT), improved method. 2.00 g of acetonyltrimethylammonium bromide was dissolved in 10 ml of glacial acetic acid. To this solution was added 1.60 g of bromine in 5 ml of glacial acetic acid. During the addition, which was performed at room temperature, dropwise and with stirring, a brick-red, crystalline precipitate was formed. This disappeared when the reaction mixture was kept at room temperature overnight. The yellow solution formed was evaporated to dryness and the cream-coloured residue was crystallized from ethanolic ether to give brilliant, white flakes melting at 129 – 130°C. Yield: 2.66 g (95 %). (Found: C 25.2; H 4.63; Br^- 28.9, Br(total) 58.1; N 4.98. Calc for $C_6H_{13}Br_2NO$: C 26.2; H 4.77; Br^- 29.1; Br(total) 58.1; N 5.09.)

3-Iodoacetonyltrimethylammonium iodide, method A. A mixture, prepared from a slurry of 0.275 g of BAT in 30 ml of dry acetone and a solution of 0.300 g of sodium iodide in 15 ml of the same solvent, was shaken overnight at room temperature. The reaction mixture was filtered as it still contained a solid phase consisting of unreacted starting material. Evaporation of the filtrate gave some dark amber granules, which on dissolution in water lost iodine. Washing with carbon tetrachloride and evaporation of the water gave a low yield of small, yellow crystals melting at 160 – 170°C (dec.). (Found: C 19.9; H 3.75; I^- 34.1; N 3.92. Calc. for $C_6H_{13}I_2NO$: C 19.5; H 3.55; I^- 34.4; N 3.80.)

Method B, improved method. A solution was prepared from 375 ml of dry ether and 9.30 g of 1,3-diiodoacetone [4] which had been freshly recrystallized from ether at −78°C. Another solution, made from 1.77 g of anhydrous trimethylamine and 30 ml of dry ether, was added while swirling the flask in a dry ice bath. The reaction mixture was allowed slowly to attain room temperature. After some days the supernatant was decanted and the precipitate was recrystallized from methanol. 4.0 g (36.1 %) of transparent, faintly yellow flakes, melting at about 170°C (dec.), were obtained. The IR spectra of the products from methods A and B were identical. (Found: C 19.6; H 3.55; I^- 33.6; I(total) 67.6; N 3.80. Calc. for $C_6H_{13}I_2NO$: C 19.5; H 3.55; I^- 34.4; I(total) 68.8; N 3.80.)

3-Diazoacetonyltrimethylammonium chloride. To a chilled solution of 0.14 g of 3-chlorodiazoacetone [6] in 5 ml of dry ether was added 0.6 g (8.5-fold molar excess) of anhydrous trimethylamine. The reaction mixture was kept at +5°C for several days, during which time an amorphous, tan to light yellow precipitate formed. Crystallisation from anhydrous ethanolic ether gave clear, faintly yellow crystals, melting at 130 – 150°C (dec.). Yield: 0.067 g (31.7 %). (Found: Cl 19.6; N 22.7. Calc. for $C_6H_{12}ClN_3O$: Cl 20.0; N 23.7.)

3-Chloroacetonyltrimethylammonium chloride, method A. 0.90 g of 3-diazoacetonyltrimethylammonium chloride was dissolved in 100 ml of cold, anhydrous ethanol, which had been saturated shortly before with dry hydrogen chloride. Having been kept at −18°C for three days the reaction mixture was evaporated and the tan, hygroscopic, syrupy residue was immediately transferred to a desiccator, where it was stored *in vacuo*. The yield was quantitative, 0.94 g. Attempts to crystallize the product were not successful. (Found: Cl^- 21. Calc. for $C_6H_{13}Cl_2NO$: Cl^- 19.)

Method B, preparation from the iodine analogue. A slurry of about 0.50 g of silver chloride in 12.5 ml of methanol was prepared by bringing together a hot solution of 0.593 g of silver nitrate in 20 ml of distilled water with a hot solution of 0.204 g of sodium chloride in 20 ml of distilled water under vigorous stirring, and then washing the precipitate several times, at first with water, later with methanol, carefully decanting off the washings so as to minimize the loss of silver chloride. To this slurry, in an ampoule, was added a solution of 0.500 g of 3-iodoacetonyltrimethylammonium iodide in 12.5 ml of distilled water. The ampoule was sealed and shaken for three days at room temperature. The reaction mixture was filtered and the solid phase was thoroughly washed with several portions of (1) ammonia, (2) water, and (3) methanol, dried and weighed. The amount of silver iodide formed was 0.6298 g (98.8 %). The filtrate was evaporated as far as possible and kept for a long time in good vacuum over phosphorus pentoxide. Under these conditions the tan, syrupy residue showed a tendency to develop lightly yellow

crystalline structures. These structures disappeared rapidly, however, when the substance was removed from the desiccator, probably due to hygroscopicity of the substance. Infrared and NMR spectrometry demonstrated the structural identity of the substance with the product prepared from 3-diazoacetonyltrimethylammonium chloride and hydrogen chloride according to method A above. The NMR spectrum from a solution of the substance in deuterium oxide showed the expected three singlets, namely the two-proton singlet from the 1-methylene protons, the two-proton singlet from the 3-methylene protons and the nine-proton singlet from the methyl protons at δ 4.70, 4.44, and 3.32, respectively, as referred to an external TMS (in chloroform) standard. Despite the vigorous measures for desiccation, it did not seem possible to obtain an anhydrous product. (Found: C 36.9; H 7.53; N 7.08. Calc. for $C_6H_{13}Cl_2NO + 1/2\ H_2O$: C 36.9; H 7.23; N 7.18.)

3-Bromodiazoacetone. A dry, pure solution of diazomethane in ether was prepared [8] and standardized [9] by established methods. To 175 ml of the solution (0.35 M with respect to diazomethane), a solution of 4.95 g of bromoacetyl bromide in 50 ml of dry ether was added slowly with efficient stirring. The molar ratio diazomethane:bromoacetyl bromide 2.5:1 was chosen as a compromise between the desire to suppress the formation of 1,3-dibromoacetone and the wish not to waste diazomethane. After the reaction mixture had been kept at -20°C for three days, the ether, the methyl bromide, and the excess of diazomethane were removed by evaporation, and the residual golden yellow oil, was distilled *in vacuo*, b.p. 52 – 54°C/0.3 torr. Further purification was achieved by repeated crystallization from rather concentrated (15 – 20 % v/v) solutions in ether at -78°C. Yield: 2.3 g (58 %) of faintly yellow crystals, melting at -1 to $+1$°C to form a golden yellow liquid, n_D^{25} 1.5734. The substance was found to be a skin irritant. Its infrared spectrum showed the typical features of an α-diazoketone with a diazo stretching frequency of 2100 cm^{-1} and a carbonyl stretching of relatively low frequency, about 1630 cm^{-1}. Its NMR spectrum (from a 5 % solution in carbon tetrachloride) showed the expected two singlets, the one-proton singlet at δ 5.84 and the two-proton singlet at δ 3.83, as referred to an internal TMS standard. (Found: C 22.3; H 1.89; Br 49.7; N 17.0. Calc. for $C_3H_3BrN_2O$: C 22.1; H 1.86; Br 49.0; N 17.2.)

3-Fluoroacetonyltrimethylammonium bromide. In a 25 ml polyethylene bottle, 1.0 g of 3-bromodiazoacetone was dissolved in 10 ml of dry benzene. The solution was cooled until part of the solvent solidified. 0.5 g of anhydrous hydrogen fluoride was added dropwise and the reaction mixture was shaken by hand for a couple of minutes, during which time nitrogen was evolved and the yellow colour of the diazoketone disappeared. The benzene phase was separated from the excessive hydrogen fluoride, treated with freshly fused potassium fluoride and concentrated by evaporation. This benzene solution of the reaction product darkened rather quickly, even at -20°C, and attempts to isolate 1-bromo-3-fluoropropan-2-one at room temperature were not successful. However, infrared and NMR spectra, registered on the crude products obtained, indicated that this ketone was the main component. In the NMR spectrum, registered on a carbon tetrachloride solution, the two-proton singlet from the bromomethyl group was found at δ 4.03, whereas the signal from the fluoromethyl group occurred as a doublet, J_{HF} 47.5 Hz centered at δ 5.03, as referred to an internal TMS standard.

To a clear, light, freshly-prepared solution of 1-bromo-3-fluoropropan-2-one in benzene was added 6.14 ml of an 1 M solution of trimethylamine in anhydrous ethanol. The reaction mixture was kept at $+5$°C for several days, during which time the bottom of the reaction vessel became covered with small, colourless crystals; these were collected on a filter and washed with and recrystallized from a mixture of isopropanol and isopropyl ether. The overall yield, as calculated on the 3-bromodiazoacetone used, was poor and did not exceed 10 %. The colourless to faintly brownish crystals melted at 140 – 145°C (dec.). (Found: C 33.1; H 6.62; Br 36.3; N 6.60. Calc. for $C_6H_{13}BrFNOC$: 33.7; H 6.12; Br 37.3; N 6.54.)

The spectroscopic data reported above have been obtained from the following instruments:

NMR spectra have been recorded on a 60 MHz Varian A 60 a nuclear magnetic resonance spectrometer, using, when possible, carbon tetrachloride solutions with an internal tetramethyl silane (TMS) standard. The salts have been studied in deuterium oxide solutions and compared with an external standard of TMS in chloroform.

Infrared spectra were recorded on a Perkin-Elmer Model 225 grating infrared spectrophotometer, whereby non-ionic substances were studied in carbon tetrachloride solutions

or neat (liquids or smears) or both and salts were studied in solid phase using KBr technique.

Elemental analyses with respect to C, H, and N have been performed on a Hewlett Packard F & M Scientific 185 Carbon Hydrogen Nitrogen Analyzer. Halogen ion has been determined *via* potentiometric titration with silver nitrate using a mercurous sulphate reference electrode. Non-ionic halogen has been transformed, when desired, into ionic form with Schöninger combustion technique.

Acknowledgements. I express my gratitude to Dr. L. Larsson for encouraging leadership and valuable discussions. My thanks are also due to Dr. J. Sollenberg, who performed the enzymological tests.

REFERENCES

1. Persson, B. O., Larsson, L., Schuberth, J. and Sörbo, B. *Acta Chem. Scand.* **21** (1967) 2883.
2. Steinberg, G. M. and Cramer, J. A. *Biochem. Pharmacol.* **19** (1970) 632.
3. Adams, R., Chiles, H. M. and Rassweiler, C. F. *Org. Syn. Coll. Vol.* **1**, 2nd Ed. (1946) 10.
4. Lederer, L. *Deutsches Reichspatent* Nr. 95440, 1897.
5. Schuberth, J. *Unpublished results*, 1966.
6. Arndt, F. and Amende, J. *Ber.* **61** (1928) 1122.
7. Sollenberg, J. *Private communication*, 1970.
8. de Boer, Th. J. and Backer, H. J. *Org. Syn. Coll. Vol.* **4** (1963) 250.
9. Arndt, F. *Org. Syn. Coll. Vol.* **2** (1943) 166.

Received June 7, 1973.