SYNTHESIS OF 2,2,6,6-TETRAMETHYL-4-DIAMINOALKYLPIPERIDIN-1-OXYLS

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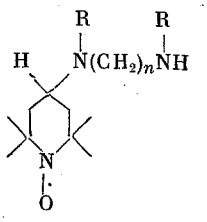
The set of spin labels in which the chain length between the nitroxyl and the group through which they can combine with a macromolecule varies within wide limits is of interest for the investigation of the structure and function of biopolymers. This was demonstrated during investigation of the active centers of anhydrase B and antibodies by means of spin-labeled sulfamides and haptenes, in which the sulfamide and nitrophenyl groups are separated from the nitroxyl by various numbers of methylene groups [1, 2, 3]. A series of benzofluorosulfones with spin labels of the pyrroline, pyrrolidine, and piperidine series and with the fluorosulfonyl group at various positions in the benzene ring were synthesized in order to investigate the active centers of trypsin and chymotrypsin [4, 5]. The synthesis of pyrrolidine nitroxyls with aminoalkyl side chains of various lengths was described in [6]. In the above-mentioned compounds the spin-labeled fragment was attached to the aliphatic part through the amide or ester bond. Nitroxyls in which the aminoalkyl fragment is attached to the ring through a C—C or a C—N bond have been described significantly less [3, 7-9].

In the present work we developed a convenient method for the synthesis of 2,2,6,6-tetramethylpiperidin-1-oxyls with aminoalkyl substituents of various lengths at position 4 of the ring and the general formula:



where R = H, MeCO, n = 2, 4, 7.

The synthesis was realized according to Scheme 1.

This method makes it possible to obtain the nitroxyls of the piperidine series with aminoalkyl fragments containing almost any number of methylene groups. The reductive condensation of the ketone group of the triacetoneamine (I) with the amine group of the monoacetylalkyldiamine (II-IV) takes place under mild conditions at active platinum dioxide. If the reaction is carried out in benzene or toluene without a catalyst, it is possible to isolate the intermediate ketimine by distilling the released water with benzene or toluene. The monoacetyl derivatives (VI, VII) are thick colorless liquids, and (V) is a white crystalline substance. The structures of the products were confirmed by the PMR spectra. In the IR spectra of the products there are bands at 1660 and 1520 cm^{-1}, characteristic of monosubstituted amides.

During the production of the nitroxyls the secondary amino group of the substituent was protected by acetylation with acetic anhydride in order to prevent oxidation. The diacetyl derivatives (VIII, IX) are white crystalline substances, and (X) is an oil. In the PMR spectra of (VIII-X) in heavy water at ∿20°C the signal for the methyl of the disubstituted acetamide group is split into a doublet, which changes into a singlet on heating above 50°C. This is evidently due to restricted amide rotation. In the IR spectra of (VIII-X) there is an amide band at 1630 cm^{-1}.

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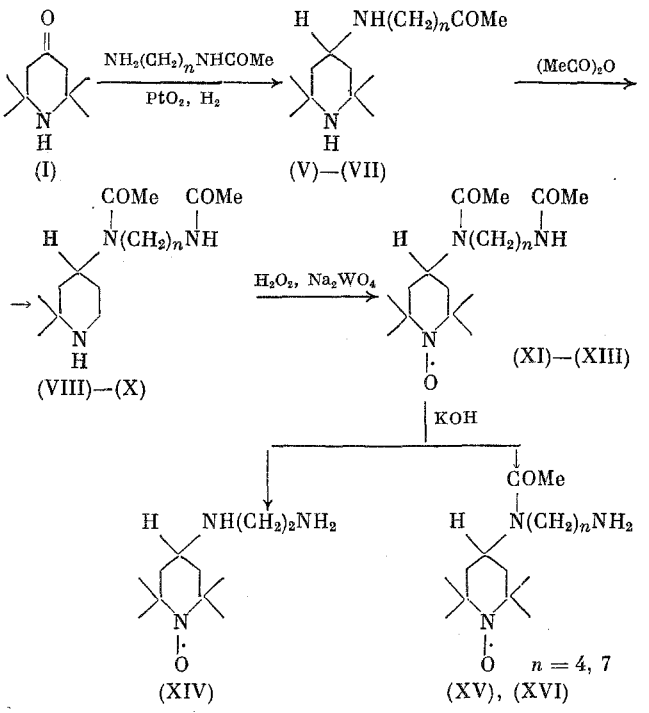
Scheme 1



Potentiometric titration of the monoacetyl derivatives (V, VI) and diacetyl derivatives (VIII, IX) with hydrochloric acid showed that (V, VI) absorb two equivalents of HCl, and the titration curve has two inflections, while (VIII) and (IX) absorb one equivalent of HCl.

Compounds (VIII-X) are readily oxidized by dilute hydrogen peroxide to the nitroxyls (XI-XIII); compounds (XI) and (XII) are colorless crystalline substances with distinct melting points, while (XIII) is a dark-red thick oil.

In the UV spectra of (XI-XIII) there is a band at 240 nm which is absent in compounds (VIII-X). The PMR spectra of the nitroxyls (XI-XIII) after reduction with phenylhydrazine hardly differ at all from the spectra of (VIII-X).

After removal of the protecting acetyl groups by alkaline hydrolysis in compounds (XI-XIII) it was found that the acetyl of the terminal amino group is fully eliminated under the employed conditions, whereas that at the secondary amino group is eliminated only in (XI) and remains practically unchanged in (XII) and (XIII). This is demonstrated by the PMR spectra of the hydrolysis products treated with phenylhydrazine. In the UV spectra the absorption maximum at about 240 nm, characteristic of nitroxyls, remains.

In the IR spectra of (XV) and (XVI) the bands at 1660 and 1520 cm^{-1} of the monosubstituted amide disappear, and the peak of the disubstituted amide at 1620 cm^{-1} remains, whereas all the bands at 1500-1660 cm^{-1} practically disappear in the IR spectrum of (XIV).

The retention of the acetyl group at the nitrogen atom attached to the piperidine ring is very convenient for the subsequent chemical transformations, since it protects the secondary amino group from modification with the introduction into the primary amino group of a fragment which combines it with the macromolecule for the investigation of the structure and function of the latter.

EXPERIMENTAL

The PMR spectra were recorded on a Tesla BS-487C spectrometer at 80 MHz. The IR spectra were recorded on a Specord IR-71 spectrometer for 10% solutions in chloroform with a layer thickness of 0.1 mm. The UV spectra were obtained on a Specord UV-VIS instrument. The ESR spectra were obtained on the previously described nonmodulation spectrometer [10]. The purity with respect to the spin label was determined from the ratio of the concentration of the paramagnetic, measured on the spectrometer, to the calculated value in the solution; it was not lower than 95%. The triacetoneamine (I) was synthesized according to data in [11].

Monoacetylethylenediamine (II). The compound was obtained by the method in [12]; bp 159-160°C (15 mm Hg). PMR spectrum (deuterochloroform, 10% solution, δ, ppm): 1.91 s ($COCH_3$), 2.64-2.79 t ($NH_2\underline{CH_2}$), 3.09-3.31 q ($\underline{CH_2}NHCO$).

N-Acetyl-1,4-diaminobutane (III). A mixture of 1400 ml of 1,4-diaminobutane and 455 ml of ethyl acetate was kept for 16 days. After removing the excess of the diamine, we distilled the residue under vacuum. We obtained 850 g of (III); bp 162-163°C (3 mm Hg). PMR spectrum (deuterochloroform, 20% solution, δ, ppm): 1.77 s ($COCH_3$), 1.3 m ($CH_2(\underline{CH_2})_2CH_2$), 2.5 m ($NH_2CH_2$), 3.0 m ($CH_2NHCO$).

N-Acetyl-1,6-diaminoheptane (IV). A mixture of 100 g of 1,7-diaminoheptane and 25 ml of ethyl acetate was kept for 14 days. After removing the excess of the diamine under vacuum [84-85°C (0.5 mm Hg)], we obtained 32 g of (IV); bp 146-150°C (0.5 mm Hg). PMR spectrum (deuterochloroform, 10% solution, δ, ppm): 1.89 s ($COCH_3$), 1.6 m ($CH_2(\underline{CH_2})_5CH_2$), 2.5-2.66 t ($NH_2CH_2$), 3.0-3.22 q ($CH_2NHCO$).

2,2,6,6-Tetramethyl-4-(2-acetylaminoethylamino)piperidine (V). A mixture of 25 g of (I), 13.5 g of (II), and 16 ml of glacial acetic acid in 225 ml of absolute ethanol was hydrogenated at 30°C and atmospheric pressure in the presence of 0.3 g of platinum dioxide until the absorption of hydrogen had stopped. After removing the solvent, we dissolved the residue in the smallest amount of water. The solution was saturated with sodium hydroxide or potassium carbonate. After removing the solvent we distilled the residue under vacuum. We obtained 27.5 g of (V); bp 174-176°C (1 mm Hg). After several days in the refrigerator the product crystallized; mp 71-72°C (from carbon tetrachloride). It was titrated with 0.01 N hydrochloric acid. Found, %: N 11.35. $C_{13}H_{27}N_3O$. Calculated, %: N 1170. PMR spectrum (deuterochloroform, 10% solution, δ, ppm): 0.94-1.04 d [$C(CH_3)_2$], 1.92 s ($COCH_3$), 2.8 m ($NHCH_2$), 3.4 m (CH_2NHCO), 1.7 m (CH_2 of ring). IR spectrum (ν, cm^{-1}): 3430, 3322 (NH), 1662 (C=O).

2,2,6,6-Tetramethyl-4-(4-acetylaminobutylamino)piperidine (VI). Similarly to (V), from 10 g of (I), 7.6 g of (III), and 9 ml of glacial acetic acid in 90 ml of absolute ethanol we obtained 14.5 g of (VI) in the form of a thick colorless liquid; bp 196-197°C (1 mm Hg). Found, %: C 66.94; H 11.91. $C_{15}H_{31}N_3O$. Calculated, %: C 66.87; H 11.60. The product was titrated with 0.01 N hydrochloric acid. Found, %: N 10.12. $C_{15}H_{31}N_3O$. Calculated, %: N 10.40. PMR spectrum (deuterochloroform, 10% solution, δ, ppm): 1.05-1.14 d [$C(CH_3)_2$], 1.45-1.74 m [CH_2 of ring and $CH_2(\underline{CH_2})_2CH_2$], 1.90 s ($COCH_3$), 2.54-2.60 t ($CH_2NH$), 3.04-3.28 q ($CH_2NHCO$). IR spectrum (ν, cm^{-1}): 3470, 3325, 1512, (NH), 1665 (C=O).

2,2,6,6-Tetramethyl-4-(7-acetylaminoheptyamino)piperidine (VII). The hydrogenation of a mixture of 17.6 g of (I), 17.3 g of (IV), and 15 ml of glacial acetic in 160 ml of absolute ethanol was realized as in the production of (V). After removing the catalyst and the solvent, we washed the white precipitate with acetone. The yield of the diacetate (VII) was 38.5 g; mp 141-142°C. Found, %: C 60.84; H 10.41. $C_{18}H_{37}N_3O\cdot(CH_3COOH)_2$. Calculated, %: C 61.22; H 10.51. A 13.8 g sample of the diacetate (VII) was dissolved in 100 ml of water, and sodium hydroxide was then added until the organic layer separated. The organic layer was extracted with chloroform and dried over potassium carbonate. After removing the solvent, we treated the residue under vacuum. We obtained 9 g of a thick colorless oil (VII).

2,2,6,6-Tetramethyl-4-[acetyl(2-acetylaminoethyl)amino]piperidine (VIII). To a solution of 32 g of (V) in 100 ml of absolute ether we added 25 g of acetic anhydride. After 2 days the white precipitate was filtered off, washed with ether, dissolved in 100 ml of water, and treated with sodium hydroxide until the organic layer separated. The product was extracted with chloroform and dried over potassium carbonate. When the solvent had been removed, we obtained 30 g of (VIII). After recrystallization from carbon tetrachloride, mp 129-130°C. Found, %: C 63.13; H 10.35; N 14.62. $C_{15}H_{29}N_3O_2$. Calculated, %: C 63.57; H 10.31; N 14.83. The product was titrated with 0.01 N hydrochloric acid. Found, %: N 4.94. $C_{15}H_{29}N_3O_2$. Calculated, %: N 4.94. PMR spectrum (D_2O, 10% solution, δ, ppm): 0.91-1.01 d [$C(CH_3)_2$], 1.30-1.56 m (CH_2 of ring), 1.76 s ($NHCOCH_3$), 1.96-2.0 d ($NCOCH_3$), 3.14-3.19 d [$(CH_2)_2$]. IR spectrum (ν, cm^{-1}): 3430, 3325 (NH), 1658 (NHC=O), 1615 (NC=O).

2,2,6,6-Tetramethyl-4-[acetyl(4-acetylaminobutyl)amino]piperidine (IX). Similarly to (VIII), from 14.8 g of (VI) in 50 ml of absolute ether and 11.3 g of acetic anhydride we isolated 16.5 g of (IX); mp 116-117°C (from carbon tetrachloride). Found, %: C 65.21; H 10.85. $C_{17}H_{33}N_3O_2$. Calculated, %: C 65.55; H 10.68. PMR spectrum (deuterochloroform, 10% solution, δ, ppm): 1.09-1.18 d [$C(CH_3)_2$], 1.32-1.61 m [CH_2 of ring and $CH_2(\underline{CH_2})_2CH_2$], 1.90 s ($NHCOCH_3$), 2.01-2.09 d ($NCOCH_3$), 2.92-3.32 m ($CH_2NCO$).

2,2,6,6-Tetramethyl-4-[acetyl(7-acetylaminoheptyl)amino]piperidine (X). Similarly to (VIII), from 9 g of (VII) in 75 ml of absolute ether and 10 ml of acetic anhydride we obtained 8.7 g of (X) in the form of a thick colorless oil. PMR spectrum (deuterochloroform, 10% solution, δ, ppm): 1.08-1.10 d [$C(CH_3)_2$], 1.19-1.55 m [CH_2 of ring and $CH_2(\underline{CH_2})_5CH_2$], 1.90 s ($NHCOCH_3$), 2.02-2.08 d ($NCOCH_3$), 3.0-3.2 m ($CONCH_2$).

2,2,6,6-Tetramethyl-4-[acetyl(2-acetylaminoethyl)amino]piperidine-1-oxyl (XI). To 30 g of (VIII) in 130 ml of water we added 44 ml of 27% hydrogen peroxide in 130 ml of water, 1.7 g of Trilon B, and 1.7 g of $Na_2WO_4 \cdot 2H_2O$. The mixture was kept in the dark at ∼20°C for 5 days and was then saturated with potassium carbonate until the organic layer separated. The red oil was extracted with chloroform and dried over potassium carbonate. After removing the solvent and rubbing the residue in absolute ether we obtained 25.5 g of the red crystalline product (XI): mp 157-158°C (from absolute benzene). Found, %: C 60.20; H 9.51. $C_{15}H_{28}N_3O_3$. Calculated, %: C 60.37; H 9.47. TLC on Silufol: R_f 0.64 (ethanol–hexane, 1:1). PMR spectrum (deuterochloroform, 10% solution, 0.1 ml of phenylhydrazine, δ, ppm): 1.08 s [$C(CH_3)_2$], 1.38-1.65 m (CH_2 of ring), 1.80 s ($NHCOCH_3$), 2.05 s ($NCOCH_3$), 3.14-3.25 d [$(CH_2)_2$]. IR spectrum (ν, cm^{-1}): 3442, 3340, 1516 (NH), 1662 (NHC=O), 1628 (NC=O). UV spectrum (water, λ_{max}, nm, ε, liter/mole·cm): 434(14), 242 sh (2200), 202 (12,900).

2,2,6,6,6-Tetramethyl-4-[acetyl(4-acetylaminobutyl)amino]piperidin-1-oxyl (XII). Similarly to (XI) from 15.3 g of (IX) in 60 ml of water, 16.8 ml of 22% hydrogen peroxide in 60 ml of water, 0.56 g of Trilon B, and 0.56 g of $Na_2WO_4 \cdot 2H_2O$ we obtained 14 g of (XII); mp 119-120°C (from absolute benzene). Found %: C 62.68; H 10.06. $C_{17}H_{32}N_3O_3$. Calculated, %: C 62.54; H 9.88. TLC on Silufol: R_f 0.50 (ethanol–hexane, 1:1). PMR spectrum (deuterochloroform, 10% solution, 0.1 ml of phenylhydrazine, δ, ppm): 1.12 s [$C(CH_3)_2$], 1.44 m [CH_2 of ring and $CH_2(\underline{CH_2})_2CH_2$], 1.84 s ($NHCOCH_3$), 1.97-2.02 d ($NCOCH_3$), 3.1 m ($CH_2N$). IR spectrum (ν, cm^{-1}) 3470, 3356, 1522 (NH), 1663 (NHC=O), 1628 (NC=O). UV spectrum (water, λ_{max}, nm, ε, liter/mole·cm): 434(12), 240 sh (2250), 202 (13,800).

2,2,6,6-Tetramethyl-4-[acetyl(7-acetylaminoheptyl)amino]piperidin-1-oxyl (XIII). Similarly to (XI), from 8 g of (X) in 30 ml of water, 8.1 ml of 20% hydrogen peroxide in 14 ml of water, 0.25 g of Trilon B, and 0.25 g of $NaWO_4 \cdot 2H_2O$ we obtained 7 g of (XIII) in the form of a thick red-orange oil. A 0.7 g sample of the crude compound (XIII) was purified on a column with silica gel L 100/60 (column height 10 cm, diameter 3.2 cm, 1:1 ethanol–hexane). We isolated 0.58 g of the pure (XIII). During TLC on Silufol the product gave one spot with R_f 0.75 in the 1:1 ethanol–hexane system. Found, %: C 65.41; H 10.66. $H_{20}H_{38}N_3O_3$. Calculated, %: C 65.18; H 10.39. PMR spectrum (deuterochloroform, 10% solution, 0.1 ml of phenylhydrazine, δ, ppm): 1.12 poorly resolved d [$C(CH_3)_2$], 1.24-1.46 m [CH_2 of ring and $CH_2(\underline{CH_2})_5CH_2$], 1.85 s ($NHCOCH_3$), 1.98-2.11 d ($NCOCH_3$), 2.90-3.32 m ($CH_2NCO$). IR spectrum (ν, cm^{-1}); 3448, 3345, 1521 (NH), 1663 (NHC=O), 1623 ($\rangle NC(=O)$). UV spectrum (water, λ_{max}, nm, ε, liter/mole·cm): 435(12), 242 sh (2300), 202 (14,500).

2,2,6,6-Tetramethyl-4-(2-aminoethylamino)piperidin-1-oxyl (XIV). A mixture of 25.5 g of (XI) and 85 ml of a 15% solution of potassium hydroxide in 50% ethanol was boiled under a reflux condenser until the spot with R_f 0.64 disappeared on the TLC on Silufol in the 1:1 ethanol–hexane system. The mixture was then saturated with potassium hydroxide and extracted with chloroform. After drying over potassium carbonate and removal of the solvent the remaining red oil was distilled under vacuum. We isolated 13.4 g of (XIV); bp 128-132°C (0.9 mm Hg). The product crystallized after prolonged storage in the refrigerator. Found, %: C 60.19; H 11.14. $C_{11}H_{24}N_3O$. Calculated, %: C 60.90; H 10.22. PMR spectrum (deuterochloroform, 10% solution, 0.1 ml of phenylhydrazine, δ, ppm): 1.05-1.08 q [$C(CH_3)_2$], 15 m (CH_2 of ring), 2.55-2.65 q [$NH(CH_2)_2NH_2$]. IR spectrum (ν, cm^{-1}): 3425, 3285 (NH_2, NH). UV spectrum (water, λ_{max}, nm, ε, liter/mole·cm): 424 (12), 242 (350).

2,2,6,6-Tetramethyl-4-[acetyl(4-aminobutyl)amino]piperidin-1-oxyl (XV). Similarly to (XIV), from 6.2 g of (XII) and 30 ml of 15% potassium hydroxide solution in 50% ethanol we obtained 5 g of a thick red oil, which we treated with 300 ml of absolute ether in two portions. After removing the ether, we obtained 4 g of (XV) in the form of a thick dark-red oil. PMR spectrum (deuterochloroform, 10% solution, 0.1 ml of phenylhydrazine, δ, ppm): 1.06-1.09 d [$C(CH_3)_2$], 1.44 m [CH_2 of ring and $CH_2(\underline{CH_2})_2CH_2$], 1.96-2.01 d ($NCOCH_3$), 2.56 m ($CH_2NH_2$), 3.08 m ($CH_2NCO$). IR spectrum (ν, cm^{-1}): 3370 (NH_2), 1625 (NC=O). UV spectrum (water, λ_{max}, nm, ε, liter/mole·cm): 431(12), 246 sh (1300), 203(6700).

2,2,6,6-Tetramethyl-4-[acetyl(7-aminoheptyl)amino]piperidin-1-oxyl (XVI). The hydrolysis was realized similarly to (XIV). From 7 g of (XIII) in 80 ml of a 15% solution of potassium

hydroxide in 50% ethanol we isolated 5.6 g of (XVI). The product was purified as in the case of (XV). PMR spectrum (deuterochloroform, 10% solution, 0.1 ml of phenylhydrazine, δ, ppm): 1.07-1.11 d [$C(CH_3)_2$], 1.18-1.55 m [CH_2 of ring and $CH_2(\underline{CH_2})_5CH_2$], 1.98-2.01 m ($NCOCH_3$), 2.58 m ($CH_2NH_2$), 3.0 m ($CH_2NCO$). IR spectrum (ν, cm^{-1}): 3380 ($\overline{NH_2}$), 1622 (NC=O). UV spectrum (water, λ_{max}, nm, ε, liter/mole·cm): 433(11), 242(1470), 204(7500).

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CONCLUSIONS

A convenient method was developed for the production of 2,2,6,6-tetramethylpiperidin-1-oxyls with aminoalkyl substituents at position 4 of the piperidine ring. They can be used in the synthesis of spin labels with various lengths in the linking bridge for the modification of macromolecules.

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