# QUATERNARY AMMONIUM SALTS WITH LABILE $N^+ - C$ BONDS AS DRUG PRECURSORS

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Recently there has been significant interest in the reversible chemical modification of drug preparations to form what are called precursors (prodrugs), which, upon introduction into an organism, cleave to produce the original preparation in unchanged form [1-3].

Interest in precursors is explained by the fact that, with their help, and without changing the structure of the active starting material, it is possible to influence the fate of the drug in the organism, to modify its distribution in tissues, to localize it in a particular organ, and to change the rate of absorption and removal from the organism. In short, precursors make possible the attainment of the goal of changing the pharmacokinetics of drugs [4].

Reversible chemical modification usually is brought about by means of groups available in the drug; OH, COOH, CO, $NH_2$, NH [5, 6]. However, in some cases, the desired result is not attainable, either as a result of high stability of the formed bond, or because of side reactions. Moreover, the indicated functional groups are not present in many drug substances. In connection with this great interest, the modification of drugs through changes in a tertiary amino group could be a possibility. This primarily refers to psychotropic substances, analgesics, cholinolytics, and other materials not carrying another functional group apart from the tertiary amino group. Examples of the preparation of drug precursors by means of the tertiary amino group are absent from the literature.

One method of modifying such compounds is by alkylation of the tertiary amino group to give the quaternary ammonium salt (QS), in which the newly formed $-C-\overset{+}{N}$ bond may be sufficiently labile.

It is known that QS carrying alkyl or aryl radicals are stable and dealkylatable only under strenuous conditions, while salts carrying the alkoxymethyl radical cleave under more gentle conditions (alcoholic medium, 60-80°C) [7]. According to [7], it is necessary that the $QS-\overset{+}{N}-CH_2O-$ bond be very highly reactive to give cleavage to the initial tertiary amine. The behavior of these QS in aqueous medium has not been studied.

On the basis of the literature data, we selected as alkylating agents halomethylated esters and ethers of the general formula $HlgCH_2OY$, where Y = Alk, CoAlk, and CoPh. The tertiary amines used were substances of a variety of chemical structures, representing different groups of pharmacologically active compounds: cholinolytics (diferidin, etc.), cholinomimetics (aceclidine), neuroleptics (chlorpromazine, amitryptyline), anticholinesterases (galanthamine), antihistamines (dimedrol), analgesics (codeine), etc., as well as some model substances of related structure (Table 1).

Alkylation with halomethyl ethers proceeded quantitatively in nonpolar aprotic solvents at 5-20°C in 1 h to form the QS with the following structure:

$$(R_1R_2R_3\overset{+}{N}CH_2OR)\ \overline{X},$$

where $R_1R_2R_3$ are substituents on the quaternary nitrogen atom of the starting drug (see Table 1).

$$R = CH_3,\ [CH(CH_3)CH_2CH_2OCH_2NR_1R_2R_3]X,\ [(CH_2)_3OCH_2\overset{+}{N}R_1R_2R_3]\ \overline{X}.$$

As expected, the halomethyl esters showed a lower reaction rate. They alkylated at 25-60°C in polar aprotic solvents. The length of time for alkylation varied from several hours to several days. An increase in the reaction temperature above 60°C resulted in the partial thermal decomposition of the $\alpha$-haloester and produced HCl, which markedly decreased the yield of QS.

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TABLE 1. Properties of Quaternary Salts $(X\text{-}CH_2OR)^+hal^-$

| Compound | X | $CH_2OR$ | Solvent | Yield, % | mp, °C | Found, % Cl (Br) | Empirical formula | Calculated, % |
|---|---|---|---|---|---|---|---|---|
| I | 4-Hydroxymethyl-pyridine | $CH_2OCH_3$ | Acetone | 97 | 98-100 (Anhydrous alcohol + ether) | 34,40 | $C_8H_{12}NBrO_2$ | 34,20 |
| II | 4-Hydroxymethyl-pyridine | $CH_2OCOCH_3$ | » | 71 | 120-2 (Anhydrous alcohol + ether) | 31,08 | $C_9H_{12}NBrO_3$ | 30,51 |
| III | Benzatsin | $CH_2OCH_3$ | » | 95 | 135—7 | 18,95 | $C_{20}H_{26}NBrO_4$ | 18,87 |
| IV | » | $CH_2OCH_2—CH=CH_2$ | » | 86,2 | 121—2 | 8,84 | $C_{22}H_{28}NClO_4$ | 8,74 |
| V | » | $CH_2OCH(CH_3)CH_2OCH_2$ | » | 69 | Gygroscopic | 9,12 | $C_{42}H_{53}N_2Cl_2O$ | 9,03 |
| VI | » | $CH_2OCOCH_3$ | » | 68 | 141—2 | 17,40 | $C_{21}H_{26}HBrO_5$ | 17,67 |
| VII | » | $CH_2OCO—C(CH_3)=CH_2$ | » | 87,3 | 155—6 | 8,63 | $C_{23}H_{28}NClO_5$ | 8,80 |
| VIII | » | $CH_2OCOC_6H_5NO_2$ (n) | » | 50 | 163—4 | 6,72 | $C_{26}H_{27}N_2ClO_7$ | 6,89 |
| IX | » | $CH_2OCOC_6H_4$ (n)$COCH_2$ | » | 60 | 149(decomp.) | 7,81 | $C_{46}H_{50}N_2Cl_2O_{10}$ | 8,06 |
| X | Tsiklozil | $CH_2OCOCH_3$ | $CHCl_3$ | 61 | 136—9,5 | 17,82 | $C_{20}H_{30}HBrO_5$ | 18,03 |
| XI | » | $CH_2OCO—C(CH_3)=CH_2$ | Acetone | 60 | 160 | 8,52 | $C_{22}H_{32}NClO_5$ | 8,33 |
| XII | » | $CH_2OCOC_6H_5$ | » | 40 | 142—3 | 8,12 | $C_{25}H_{32}NClO_5$ | 7,68 |
| XIII | » | $CH_2OCOC_6H_5COOCH_2$ | HMPT | 70 | 102—4 | 7,97 | $C_{44}H_{58}N_2Cl_2O_{10}$ | 8,38 |
| XIV | » | $CH_2OCOC(CH_3)_3$ | Acetone | 48 | 155,5—8 | 8,17 | $C_{23}H_{36}NClO_5$ | 8,03 |
| XV | Azulon | $CH_2O(CH_2)_3OCH_2$ | $CHCl_3$ | 20 | 160—3 | 9,08 | $C_{15}H_{52}N_2Cl_2O_4$ | 9,39 |
| XVI | » | $CH_2OCOCH_3$ | $CHCl_3$ | 81 | 142—3 | 18,10 | $C_{23}H_{26}NBrO_3$ | 17,98 |
| XVII | » | $CH_2OCOC(CH_3)_3$ | | 62 | 139—40 | 8,14 | $C_{26}H_{33}NClO_3$ | 8,04 |
| XVIII | » | $CH_2OCOC(CH_3)=CH_2$ | | 72,9 | 133—4 | 16,61 | $C_{25}H_{23}NBrO_3$ | 16,97 |
| XIX | Diferidin | $CH_2OCOC_6H_5$ | HMPT | 59,3 | 104—7 | 7,38 | $C_{29}H_{30}NClO_2$ | 7,45 |
| XX | Aceclidine | $CH_2OCOC(CH_3)=CH_2$ | Acetone | 68,5 | 169—71 | 11,47 | $C_{14}H_{22}HClO_4$ | 11,72 |
| XXI | » | $CH_2OCOC_6H_5$ | » | 70 | 187—9 | 10,48 | $C_{17}H_{22}NClO_4$ | 10,46 |
| XXII | Chlorpromazine | $CH_2OCOC_6H_5$ | » | 81,5 | 163—70 | 7,12 | $C_{25}H_{26}N_2Cl_2Cl_2O_2S$ | 7,25 |
| XXIII | » | $CH_2OCOC(CH_3)=CH_2$ | » | 79 | 99—102 | 8,32 | $C_{22}H_{26}N_2Cl_2O_2S$ | 7,92 |
| XXIV | Codeine | $CH_2OCOC(CH_3)=CH_2$ | 73 | 174—6 | 8,02 | $C_{26}N_{28}NClO_5$ | 7,57 |
| XXV | Butiroksan | $CH_2OCOC(CH)_3=CH_2$ | Acetone | 40 | 121 | 7,09 | $C_{27}C_{31}NClO_5$ | 7,39 |
| XXVI | Galanthamine | $CH_2OCOC(CH_3)=CH_2$ | $CHCl_3$ | 95 | 158—60 | 8,0 | $C_{22}H_{28}NClO_5$ | 8,42 |
| XXVII | Amitryptyline | $CH_2OCOC(CH_3)=CH_2$ | $CHCl_3$ | 87 | 80—2 | 8,30 | $C_{28}H_{30}NClO_5$ | 7,98 |
| XXVIII | Dimedrol | $CH_2OCOC(CH_3)=CH_2$ | Acetone | 80 | 160—70 | 9,08 | $C_{22}H_{28}NClO_3$ | 9,15 |

NOTE. Names of the drugs given in the table are taken from [16]. HMPT = hexamethylphosphotrimide, $[(CH_3)_2N]_3PO$.

TABLE 2. Aqueous Solubility of Starting Materials and Their Quaternary Salts

| Compound | Temp., °C | Solubility, % | | |
|---|---|---|---|---|
| | | pH 4,6 | pH 6,7 | pH 8,1 |
| Tsiklozil | 20 | 5,80 | 2,68 | 0,46 |
| QS XI | 20 | Unlimited | | |
| Diferidin | 100 | — | 10* | — |
| QS XIX | 20 | Unlimited | | |

* Data from the "Farmakon" plant, Leningrad.

The haloester alkylation was significantly influenced by the basicity of the tertiary amino group and its steric accessibility. Preparation of QS from amines with low basicity (nikethamide, $pK_a$ 3.7; caffeine, $pK_a$ 0.61) is not possible. This reaction does not take place with sterically hindered amines, yet the iodomethylates of such compounds have been described [8].

All of the synthesized QS are colorless crystalline substances, easily soluble in water, alcohol, DMSO, DMF, and poorly soluble in acetone, acetonitrile, and ether. Compounds I, III, V, and XV containing the alkoxymethyl radical on the nitrogen are very hygroscopic, and upon storage in a dessicator decomposed fairly rapidly. Salts containing the acyloxymethyl radical were usually not hygroscopic and were stable on storage under the usual conditions.

A distinctive property of QS containing the acyloxymethyl radicals is their easy solubility in water, significantly exceeding that of the starting compounds with tertiary nitrogen atoms (Table 2).

The structures of most of the synthesized QS were confirmed by PMR spectral data, taken in $D_2O$, DMSO-$d_6$, and $CD_3OD$. The PMR spectra of these compounds showed a singlet in the 5.1-5.4 ppm region corresponding to protons of the $-\overset{+}{N}-CH_2O-$ group which is characteristic of the PMR of these compounds. For example, the most characteristic signals for the chemical shifts (δ, ppm) for compounds VII and XI are:

| Group | $-C-CH_3$ | $\overset{+}{N}(CH_3)_2$ | $\overset{\alpha}{O}CH_2\overset{\beta}{C}H_2\overset{+}{N}$ | $\overset{+}{N}-CH_2O$ | $=CH_2$ | $C_6H_5$ |
|---|---|---|---|---|---|---|
| δ, ppm | 1,95— 2,08 s | 3,10— 3,20 s | α 3,7— 3,9 m; β 4,3— 4,5 m | 5,1— 5,4 s | 5,8— 6,1 s | 7,4— 7,6 m |

For preliminary evaluation of the possibility of the use of these QS as drug precursors, their behavior was studied in aqueous solution under conditions close to physiological. Considering their structure, it might be suggested that hydrolytic decay of the QS might take place by the following scheme:

$$\overline{X}R_1R_2R_3\overset{+}{N}CH_2OR + H_2O \rightarrow R_1R_2R_3N + CH_2O + HX + ROH,$$

where $R_1R_2R_3N$ = drug or model compound with a tertiary nitrogen atom. R = Alk, COAlk, COPh; X = Cl, Br (see Table 1).

For example, for compounds III, V, XV, it was shown that in the pH range of 5.0-12.0, QS containing the alkoxymethyl radical (derived from α-haloethers) form very stable solutions.

The formation of the initial starting amine with simultaneous isolation of formaldehyde arises only by heating these QS in concentrated (20%) alkaline solution at 60-80°C. This indicates that in the alkoxymethyl salts the $\overset{+}{N}-CH_2O-$ bond is hydrolytically extremely stable. From this it follows that the alkylation of drugs containing tertiary nitrogen atoms with halomethyl ethers does not produce compounds suitable for use as precursors.

A pharmacological test of compound III, carried out on mice (arecoline model [9]), compared with unmodified benzatsin, showed that the QS did not give central cholinolytic activity, a characteristic of the orig-

TABLE 3. Hydrolysis Conditions for Quaternary Salts

| Compound | Temp., °C | pH | Hydrolysis time, min* | |
|---|---|---|---|---|
| | | | 30 % | 50 % |
| VI | 21 | 8,25 | 30 | 190 |
| XI | 21 | 9,6 | 8 | 20 |
| | 21 | 8,2 | 175 | 425 |
| | 21 | 5,1 | No change in 48 h | |
| | 37 | 8,20 | 30 | 70 |
| | 37 | 7,16 | 80 | 140 |
| XII | 21 | 9,6 | 90 | 21 |
| XIV | 21 | 8,2 | 350† | — |
| | 21 | 8,8 | 160 | 400 |
| | 37 | 8,2 | 160 | 340 |
| | 37 | 8,8 | 30 | 80 |
| XVIII | 37 | 8,25 | 80 | 130 |
| | 37 | 7,70 | 200 | 350 |
| XIX | 37 | 8,2 | 70 | 180 |
| | 37 | 8,8 | — | 40 |
| XXII | 21 | 8,2 | 100 | — |
| | 21 | 8,8 | 32 | 65 |
| XXIII | 21 | 8,8 | 40 | 85 |
| | 37 | 5,0 | No change in 48 h | |

*Each result was obtained from the average of the data from five to seven experiments.
†Time for 20% hydrolysis of the QS.

inal material.* This implies that the organism is able to dealkylate the QS III at a negligible rate or not at all. The others also behave as quaternary ammonium salts containing acyloxymethyl radicals (compounds II, XIV, XV-XXIX, XXXI). A study of the behavior of these QS in buffered solution of pH 7.0-10.0 by PMR spectroscopy, titration of the acid formed, determination of formaldehyde (with chromotropic acid or the Hema reagent), and preparative isolation of the reaction products showed that these QS indeed disintegrated at the bonds of the $\overset{+}{N}CH_2OCOR$ group to form the initial tertiary amine, formaldehyde, and the corresponding carboxylic and hydrohalogen acids.

Changes in the PMR spectra of solutions of compounds VI, VII, X, XI in a deuterobase buffer of pH 8.3-8.7 are evidence that the cleavage of these QS proceeds by the proposed scheme. These spectra show an increase with time of the proton signals of the methyl group of the tertiary nitrogen ($\delta$ = 2.8-3.0 ppm), simultaneously with a decrease in the intensity of the signals for the methyl protons of the quaternary nitrogen atom ($\delta$ = 3.10-3.20 ppm). A decrease with time also was observed for the methylene signals of the $\overset{+}{N}CH_2OCO$ group ($\delta$ = 5.1-5.4 ppm).

The difference method was used for quantitative evaluation of the rate of hydrolysis of the QS. In some cases (compounds VI, VII, X), the rate of hydrolysis was estimated by determining the evaluation of formaldehyde by a modification of our earlier method [10]. This method is not useful in cases in which the evolved amine (chlorpromazine, trifluoperazine) or the organic acid (benzoic) gives an intense color with chromotropic acid.

The most convenient and reliable methods for kinetic study of the cleavage of the QS is titration in a pH meter of the acid formed, and quantitative determination of the tertiary amine by GLC.

In Table 3 are given the data for the hydrolysis of acyloxymethyl QS, obtained by the indicated method. The rate of hydrolytic cleavage, as Table 3 shows, is strongly dependent on the pH of the solution. At pH 10.0, the cleavage of QS rises instantaneously, but the rate gradually decreases over the pH interval 9.8–7.1. Thus, at 21°C compound XI is stable at pH 5.0 for many days, but decomposes 50% in 425 min at pH 8.2, and in 20 min at pH 9.6. A similar dependence is observed for the other compounds. The rate of cleavage of the QS depends on the structure of the acyloxymethyl radical, also. Salt VI, an acetoxymethyl derivative, hydrolyzes

* Pharmacological studies on the indicated compounds were carried out at the Institute of Toxicology of the Ministry of Public Health of the USSR, in the Division of Pharmacology headed by S. N. Golikov, Academician of the Academy of Medical Sciences of the USSR.

TABLE 4. Comparative Long-Term Activity of Tsiklozil and Its Precursor (rats, per os)

| Compound | Dose, mg/kg | Therapeutic effect (prevention of tremors from arecoline) | | | | |
|---|---|---|---|---|---|---|
| | | 1 h | 1½ h | 4 h | 5 h | 6 h |
| Tsiklozil (hydrochloride) | 100 | — | — | — | + | + |
| XI | 100 | — | — | — | — | — |

Note. + = tremors present, − = tremors absent.

30% at 21°C and pH 8.2 in 80 min, while compound XI, containing the methacryloyl radical, is 30% decomposed under the same conditions in 175 min. Compound XIX, containing the pivaloyloxymethyl radical, is 20% hydrolyzed in 350 min.

Consequently, by variation of the structure of the acyloxymethyl group in the QS, it is possible to change the hydrolysis rate over wide limits. This, in turn, gives the main opportunity for regulation of the rate of evolution of drugs from precursors under the conditions in an organism.

The hydrolysis of the QS in vitro, however, is only a distant model of that which takes place in the organism. Therefore, it is important that the pharmacological behavior of such precursors be studied. Table 4 gives a comparison of the long-term activity of tsiklozil and its precursor QS XI on rats (arecoline induction [9]).

Table 4 shows that QS XI is indeed transformed by the organism into tsiklozil, in the sense that otherwise this substance, not having a noticeable capacity to penetrate the blood–brain barrier, would not show central activity, as did not QS III and the chloroethylate of tsiklozil (khlorozil) [11]. Again, QS XI, compared to tsiklozil, acts noticeably longer. Thus, the anti-arecoline activity of tsiklozil, at a dose of 100 mg/kg, ceased after 4-5 h after introduction, but modification of the tsiklozil to compound XI extended its activity at the same dose to the termination of the experiment (6 h).

Preliminary studies of the toxicity of these compounds in mice by peroral introduction of the preparations showed that the modified compounds were less toxic than tsiklozil: for tsiklozil the $LD_{50}$ was 3 g/kg, and for its methacryloyloxy derivative (XI), it was 7.5 g/kg.

The results presented are a chemical study of the properties of acyloxymethylated QS of drugs, and a preliminary pharmacological investigation confirming the possibility of extending the activity of drugs by the method considered.

## EXPERIMENTAL

PMR spectra of the compounds were studied on an RYa 2310, 60 mHz instrument. The hydrolyses of the QS were carried out with a pH meter autotitrator of the type SBR-2a/ABPIB/TTA3 "radiometer" (Denmark), at constant ionic strength, 0.1 M in KCl. The titrant was 0.05 N NaOH in the pH range of 6.5-10.0. GLC studies of the hydrolytic cleavage were carried out on a Hewlett-Packard (USA) chromatograph: column OV-17. 3%; 1.8 m; $T_c$ = 245°C; flame-ionization detector.

The determination of formaldehyde according to [10] was carried out on an SF-4A instrument at 580 nm.

Solvents used in the syntheses of the QS were carefully dried and purified by generally known methods [12].

α-Haloethers and esters were synthesized by methods described in the literature [13-15].

Methoxymethyl Bromide Salt of N,N-Dimethylaminoethyl Benzilate (QS III). A solution of 1 g (0.0033 mole) of the aminoester free base in 15 ml of acetone was cooled to 5°C and stirred while adding dropwise 0.5 g (0.035 mole) of methyl bromomethyl ether in 5 ml of acetone. After 3 h the precipitate was washed with ether and dried in a vacuum desiccator. After careful (with exposure to as little water vapor as possible) crystallization from a mixture of ethyl ether and alcohol, 1.3 g of product was obtained as colorless crystals of salt III, mp 135-137°C (see Table 1).

Methacrylcyclomethyl Chloride Salt of N,N-Dimethylaminoethyl (1-Hydroxycyclopentane-1-yl)phenylacetate (QS XI). A mixture of 0.9 g (0.003 mole) of tsiklozil free base and 0.43 g (0.0032 mole) of chloromethyl methacrylate in 25 ml of anhydrous, peroxide-free acetone was kept for 75 h in a closed vessel. The precipitate was filtered off, washed with acetone (2×5 ml) and dry ether in a vacuum desiccator to give 0.9 g (70%) of QS XI as colorless crystals, mp 160°C, easily soluble in water, alcohol DMSO, and poorly soluble in acetone, acetonitrile, and ether.

All of the compounds whose properties are described in Table 1 were prepared by the above methods, with variations in solvent and time of reaction. Completion of the alkylation reactions was determined by TLC on Silufol UV-254 by the disappearance of the basic spot, using the free base of the initial drug as standard.

Preparative Hydrolysis of QS XI. A solution of 1.5 g of QS in pH 9.0 borate buffer was kept for 1 h and the separated oil was extracted with ether (4× 10 ml). The base was converted into the hydrochloride by addition to alcoholic hydrogen chloride. The precipitate was filtered and washed with anhydrous ether (3 × 5 ml) to give 1.5 g (91%), mp 185-187°C. The melting point of a mixture with tsiklozil hydrochloride was not depressed.

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