0.5 mL of anhydrous CH_3CH_2OH containing $CHCl_3$ was heated at 75 °C for 7 min. The tube was cooled and opened, and the volatile material was transferred under vacuum to a dry ice–acetone cooled trap. GC analysis of the volatile part with column D showed the complete absence of **10**. NMR analysis of the nonvolatile residue showed a quantitative formation (100%) of **4** *p*-toluenesulfonate. This was repeated with **10**:HOTs in a 1.00:0.12 mol ratio. In this case, the final ratio of **10**:**4** was found to be 0.88:0.12.

Ethanolysis H–D Exchange with 4 Perchlorate. A solution of 16 mg (0.075 mmol) of **4** perchlorate in 0.3 mL of CD_3CD_2OD under N_2 in a sealed NMR tube was heated at 75 °C. The ensuing reaction was tracked by NMR at selected time intervals as described above. The signals at δ 1.45 (t-C_4H_9, s), 2.20 (CH_3, d), and 8.35 (methine C–H, q) for **4** decreased as signals for **7** (δ 1.30 (CH_3, d) 4.80 (methine C–H, q) and **8** (δ 1.38 (t-C_4H_9, s)) appeared. In addition, the δ 2.20 and 8.35 signals of **4** decreased in intensity relative to the δ 1.45 peak with time as both signals gradually broadened and lost resolution. Also, the two signals decreased in relative intensity at different rates, indicating that the two sites undergo H–D exchange at different rates. The δ 4.80 signal of **7** was observed as a multiplet instead of a quartet.

Equilibration between 4, CD_3CD_2OD, 7, and 8. A mixture of 40 mg (0.34 mmol) of **7** and 60 mg (0.32 mmol) of **8** in 0.35 mL of $CD_3CD_2OD/CDCl_3$ mixed solvent (2:1 v/v) was heated at 75 °C. Appearance of **4**, decreases of **7** and **8**, and H–D exchanges were followed by NMR. Changes in concentrations of **4**, **7**, and **8** appeared to cease after ca. 6 h.

Ethanolysis of 3 with Added Lutidine Base in $CDCl_3$. A solution consisting of 21 mg (0.078 mmol) of **3** and 37 mg (0.35 mmol) of 2,6-lutidine in 0.74 mL of $CD_3CD_2OD/CDCl_3$ mixed solvent (2:1 v/v) was sealed under N_2 in an NMR tube and heated in a constant-temperature bath. Kinetic measurements were made by the above-described ^1H NMR method. Rate constant values at various temperatures are listed in Table I.

After completion of ethanolysis (ca. 10 half-lives), NMR samples were used for product identification and yield determination. The NMR signals at δ 4.70 (2 H, m, vinyl H) of **5**, δ 3.85 (2 H, q, $-CH_2-$) of *trans*-**10**, and δ 1.30 (18 H, s, t-C_4H_9) of **11** were free of interference from other peaks. Integration of these signals showed yields of **5**, **10**, and **11** to be 13%, 55%, and 45%, respectively. The NMR tube was opened, and the volatile part was transferred under vacuum into a dry ice–acetone cooled trap. When a sample of the volatile material was shaken with 70% aqueous $HClO_4$, **10** with δ 1.20 (9 H, s, t-C_4H_9) was converted to **4** with δ 1.45 (9 H, s, t-C_4H_9). Integration of the remaining δ 1.20 (9 H, s, t-C_4H_9) signal belonging to **6** and the δ 1.45 signal of **4** corresponded to 40% and 55% yields of **6** and **10**, respectively. Preparative GC with column B afforded **6** and *trans*-**10** and with column A gave **7**. The structures were confirmed by GC retention times and NMR spectral comparisons with authentic samples. GC analyses with columns C and E showed a 38–43% yield of **6** and a 25–35% yield of **7**, respectively. Control experiments with the above GC analyses indicated that diethyl ether (**9**) is not a reaction product. The NMR spectrum ($CDCl_3$) of the nonvolatile residue remaining from vacuum transfer showed the presence of lutidinium perchlorate and one other salt with δ 1.35 (18 H, s, t-C_4H_9), which appeared to be **11** perchlorate. Shaking the $CDCl_3$ salt solution with 40% KOH caused the *tert*-butyl singlet to shift to δ 1.05, which is the same chemical shift as authentic, 1,2-*tert*-butylhydrazine. The freed hydrazine solution was allowed to stand in air (O_2) for several days. After this treatment, the *tert*-butyl singlet had shifted to δ 1.20, which is the exact chemical shift of authentic 2,2′-azobis(isobutane) (300-MHz NMR). The GC retention times of the product from **11** perchlorate and known 2,2′-azobis(isobutane) were identical.

During the above NMR kinetic study, each spectrum was monitored carefully in the δ 4.9 region for evidence of H–D exchange in the $-CH_2-$ moiety of **3**. No changes in integration ratios or signal shape were observed.

Stability of Products 7 and 11 in Ethanol/Lutidine ($CDCl_3$). A solution of 10.0 mg (0.032 mmol) of **11** *p*-toluenesulfonate, 4.0 mg (0.033 mmol) of **7**, 11 mg (0.01 mmol) of lutidine, and Me_4Si in 0.4 mL of $CD_3CD_2OD/CDCl_3$ (2:1 v/v) was sealed under N_2 in an NMR tube and heated at 75 °C for 4 h (ca. 10 ethanolysis half-lives of **3**). The various ^1H NMR signals for **7** and **11** had the correct initial integration values, and there were no change in these values or the spectrum during the time period.

A Search for Iminium Cation 14. During the NMR kinetic studies of the thermolysis, ethanolysis, and ethanolysis with lutidine reactions of **3**, the NMR spectra were scrutinized for the appearance of **14**. No signals were ever observed that could be taken as evidence for **14**.

Acknowledgment. We express appreciation to the National Science Foundation for support of this work with Grant CHE-7913781. We also acknowledge NIH Biomedical Research Support Grant No. RR07092 for providing a part of the funds used in the purchase of the Hewlett-Packard 5880A gas chromatograph used in this work.

Registry No. **3**, 82571-40-2; **4** *p*-toluenesulfonate, 82571-42-4; **10**, 65444-37-3; **12**, isomer 1, 82571-43-5; **12**, isomer 2, 82571-45-7; 1-*tert*-butyl-2-ethylhydrazine, 82571-44-6.

Analysis of the Atomic Environment of Quaternary Ammonium Groups in Crystal Structures, Using Computerized Data Retrieval and Interactive Graphics: Modeling Acetylcholine–Receptor Interactions

Richard E. Rosenfield, Jr.,*[1a] and Peter Murray-Rust*[1b]

Contribution from the Biographics Laboratory, Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, and the Department of Chemistry, University of Stirling, Stirling FK9 4LA, Scotland. *Received August 10, 1981*

Abstract: The atomic environment of the trimethylammonium methyl cationic group (**1**) has been reexamined in 34 reported crystal structures containing simple halides (Cl, Br, I) as counterions and in nine structures containing oxyanionic groups (carboxylate, picrate, phosphate, perchlorate). For the halides (X), three-dimensional scatter plots of N···X vectors <5.65 Å about a reference $RCH_2NMe_3^+$ group clearly show clustering in the N···X approach directions. Clustering occurs close to the centers of the three sterically unhindered faces of the tetrahedron formed by the carbon atoms of the quaternary ammonium group, allowing minimization of d(N···X). For the oxyanion structures, a similar scatter plot of intra- and intermolecular N···O vectors <5.0 Å shows a much more even distribution over **1**'s entire surface. Some oxyanions coordinate to **1** via two or three oxygen atoms. The preferences observed for the N···X directions suggest that the binding of acetylcholine to its receptor may involve a preferred orientation between the quaternary ammonium group and anionic groups in the receptor. This work is an example of computer-assisted retrieval, visualization, and analysis of data in the Cambridge Crystallographic Data Files, performed on a PDP 11/40 minicomputer equipped with a Vector General series 3 graphics display.

The trimethylammonium methyl cationic group (**1**, Figure 1) is an important biochemical fragment, occurring in choline, acetylcholine, choline phospholipids, and related compounds. Unlike most other organic cations, it cannot form N–H···R hy-

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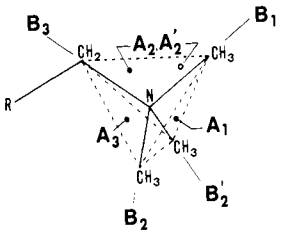

Figure 1. Labeling of the faces and vertices of the N–C4 tetrahedron in an idealized trimethylammonium methyl cationic group.

drogen bonds. Therefore the forces causing it to bind to an enzyme or receptor, or controlling its packing in a crystal, must be largely a function of the distribution of charge within the entire cation. By studying the environment[2] of this group in crystal structures, we and others[3] have been able to get clues to the nature of these forces and their importance relative to other packing constraints.

Computerized Retrieval and Display of Crystallographic Data

The use of the Cambridge Crystallographic Data Files and associated programs[4] greatly facilitates the analysis[5] of a large number of structures. In the Biographics Laboratory, these computer files and programs have been adapted[6] to a PDP 11/40 minicomputer equipped with interactive graphics.[7] The Bibliographic (BIB), Chemical Connectivity (CON), and Crystal Data (DAT) files are searched directly from magnetic tape (with subfiles stored in disk memory for later use), using modified versions[6] of the Cambridge programs BIBSER, CONNSER, RETRIEVE, and GEOM78. Dynamic stereoscopic images[7b] of crystal structures retrieved from the DAT file can be immediately composed and displayed on the graphics screen by using the program PACK.[8a] The use of interactive graphics, via PACK and other programs,[8b] makes the inspection and analysis of crystal structures relatively easy and elucidates intermolecular geometry.

Results

We searched the CON file (using CONNSER[4]) for occurrences of **1**, matching atom and bond types in the file to those in our search question. The atomic coordinates and literature references of 55 unique structures were subsequently retrieved (with RETRIEVE) from the DAT and BIB files[9] (supplementary material). Some of these were excluded from the analysis because of structural disorder (three structures), the presence of counterions with transition elements (five structures), or the predominance of AgI (four structures). This left 43 crystal structures, of which 27 were choline derivatives.[10a] The counterions were either simple halides (34 structures)[10b] or oxyanions (nine structures);[10c] these two groups were treated separately.

For the halide structures, we calculated (using GEOM) distances between the nitrogen in **1** and the halide counterions around it up to a maximum of $r(X) + 3.5$ Å, where $r(X)$ is the respective van der Waals radius of the halogen (Cl 1.8, Br 1.95, I 2.15 Å[11]). Beyond this distance the density of N···X^- contacts falls rapidly, so that it represents the limit of **1**'s first coordination sphere with halides. A total of 40 crystallographically independent quaternary ammonium cations were examined, and 34 Cl, 59 Br, and 95 I unique N···X^- distances were found.

A scatter plot of the location of halide ions around a reference $RCH_2NMe_3^+$ cation shows striking concentrations of contacts in certain directions (Figure 2a). The highest densities occur near the centers of the three sterically unhindered faces of the N–C4 tetrahedron (A_1, A_2, A'_2 in Figure 1), while on the fourth face (A_3), partly hidden by the R group, there is a much greater spread in the N···X^- directions. Some directional clustering also occurs along the three N–Me vectors (B_1, B_2, B'_2), mainly in the iodide structures. Average values for the minimum distances observed along these approach directions are $r(X) + 2.33$ Å for A and $r(X) + 3.12$ Å for B, with standard deviations of 0.10 and 0.05 Å, respectively. These values were obtained from histograms of $d(N\cdots X) - r(X)$ for N···X vectors lying within 20° of the back side (for A) or front side (for B) of one of the four N–C bond directions.[13]

For the oxyanion structures, distances up to 5 Å were calculated between the nitrogen in **1** and every oxygen. Intramolecular N···O distances were included, except those to the choline oxygens. A total of 11 crystallographically independent cations were examined, and 119 unique N···O distances were found. Because each ox-

(1) (a) Texas A&M University. (b) University of Stirling. Present address: Glaxo Laboratories Ltd., Greenford Road, Greenford, Middlesex UB6 0HE, England.

(2) Previous studies of the crystal environment of chemical groups includes the following: (a) Sulfides: Rosenfield, R. E., Jr.; Parthasarathy, R.; Dunitz, J. D. *J. Am. Chem. Soc.* **1977**, *99*, 4860–4862. Guru Row, T. N.; Parthasarathy, R. *J. Am. Chem. Soc.* **1981**, *103*, 477–479. (b) Sulfonium ions: Britton, D.; Dunitz, J. D. *Helv. Chim. Acta* **1980**, *63*, 1068–1073. (c) Carbon–halogen bonds: Murray-Rust, P.; Motherwell, W. D. S. *J. Am. Chem. Soc.* **1979**, *101*, 4374–4376. (d) Amides and carboxylic acids: Leiserowitz, L.; Schmidt, G. M. J. *J. Chem. Soc. A.* **1969**, 2372–2382. Leiserowitz, L. *Acta Crystallogr., Sect. B* **1976**, *32B*, 775–802. Berkovitch-Yellin, Z.; Leiserowitz, L. *J. Am. Chem. Soc.* **1980**, *102*, 7677–7690 and references cited therein. (e) Calcium–water and –carboxylate interactions: Einspahr, H.; Bugg, C. E. *Acta Crystallogr., Sect. B* **1980**, *36B*, 264–271; **1981**, *37B*, 1044–1052.

(3) A concurrent study of the crystal structures of cholinergic agonists (including eight original structures) has come to our attention. The spatial distribution of counterions about the quaternary ammonium group is examined in a manner similar to that reported here, with similar results. The authors go on to relate nonbonded distances and directions to observed pharmacological activity: (a) Kokkinidis, M. Doctoral Dissertation, Technische Universität, Munich, 1981. We gratefully acknowledge prepublication access to this extensive work. (b) Gieren, A.; Kokkinidis, M. *Naturwissenschaften* **1981**, *68*, 482–483.

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(5) (a) Murray-Rust, P.; Motherwell, W. D. S. *Acta Crystallogr., Sect. B* **1978**, *34B*, 2518–2526, 2534–2546. (b) Allen, F. H. *Acta Crystallogr., Sect. B* **1980**, *36B*, 81–96; **1981**, *37B*, 890–900. (c) Wilson, S. R.; Huffman, J. C. *J. Org. Chem.* **1980**, *45*, 560–566.

(6) Rosenfield, R. E., Jr.; Swanson, S. M. Abstracts of the Sixth European Crystallography Meeting (Barcelona), 1980, p 129. Rosenfield, R. E., Jr. Abstracts of the American Crystallographic Association (Winter Meeting, Texas), 1981, p 26. The DAT file has been condensed in order to save space and speed processing, and the algorithm in CONNSER and GEOM that searches for chemical fragments has been improved. Data-dependent errors found in BIBSER, CONNSER, and GEOM that would cause some entries to be improperly hit or missed, depending on the search question, were corrected and reported to the Cambridge Crystallographic Data Centre. All programs require less than 64K bytes of memory. Copies may be obtained from the Crystallograhic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England, or from the Biographics Laboratory.

(7) (a) The computer hardware consists of a Digital Equipment (DEC) PDP 11/40 minicomputer with 224K bytes of core memory, a DEC TU10 magnetic tape drive (36 000 characters/s), and 17.5 megabytes of disk memory. The computer operates under DEC's RSX11M multi-user system, version 3.1, with memory management. (b) The graphics hardware consists of a Vector General series 3 controller with ten analogue dials. Two-dimensional projections of objects, showing left- and right-eye views, are drawn about 30 times a second, just like successive frames in a motion picture. Graphics programs give the user the potential to change the view (rotation, scale, etc.) at each frame, thus giving the illusion of movement in three dimensions.

(8) (a) Swanson, S. M.; Rosenfield, R. E., Jr.; Meyer, E. F., Jr. *J. Appl. Crystallogr.* **1982**, *15*, 439–442. (b) Meyer, E. F., Jr.; Cole, G. M.; Presta, L. G.; Rosenfield, R. E., Jr.; Swanson, S. M. "Structure of Complexes between Biopolymers and Low Molecular-Weight Compounds"; Fickert, R., Ed.; Heyden & Sons: London, 1982; in press.

(9) The March 1979 update of the Cambridge Files was used; it contained 22 398 bibliographic references.

(10) (a) β- (but not α-) substituted choline derivatives were included. (b) Eight chloride, twelve bromide, and fourteen iodide structures were found. (c) The oxyanions found were β-resorcylate, picrate, phosphate (zwitterionic), tartrate, and perchlorate.

(11) Pauling, L. "The Nature of the Chemical Bond", 3rd ed.; Cornell University Press: Ithaca, NY, 1960; p 260.

(12) For those crystal structures where the choline chain is extended (i.e., ω(NCCO) is about 180°), the distribution of halide ions is symmetric. For those structures displaying a gauche conformation, a slight asymmetry is observed, most pronounced in the A_3 region. Making the entire distribution of contacts exactly symmetric across the mirror plane of the $RCH_2NMe_3^+$ cation, therefore, does not affect the overall picture seriously.

(13) (a) About 80% of the total number of halide contacts lie within these limits. Histograms of $d(N\cdots X) - r(X)$ show major peaks, containing 95 and 27 distances, respectively, for the A and B directions. Two very short B-type distances, lying outside the B peak, were not used in calculating the average: $d(N\cdots Br) = r(Br) + 2.79$ Å (ref 13b) and $d(N\cdots I) = r(I) + 2.92$ Å (ref 13c). (b) Barrans, Y. *Acta Crystallogr., Sect. B* **1972**, *28B*, 651–653. (c) Jensen, B. *Acta Chem. Scand.* **1970**, *24*, 2517–2524.

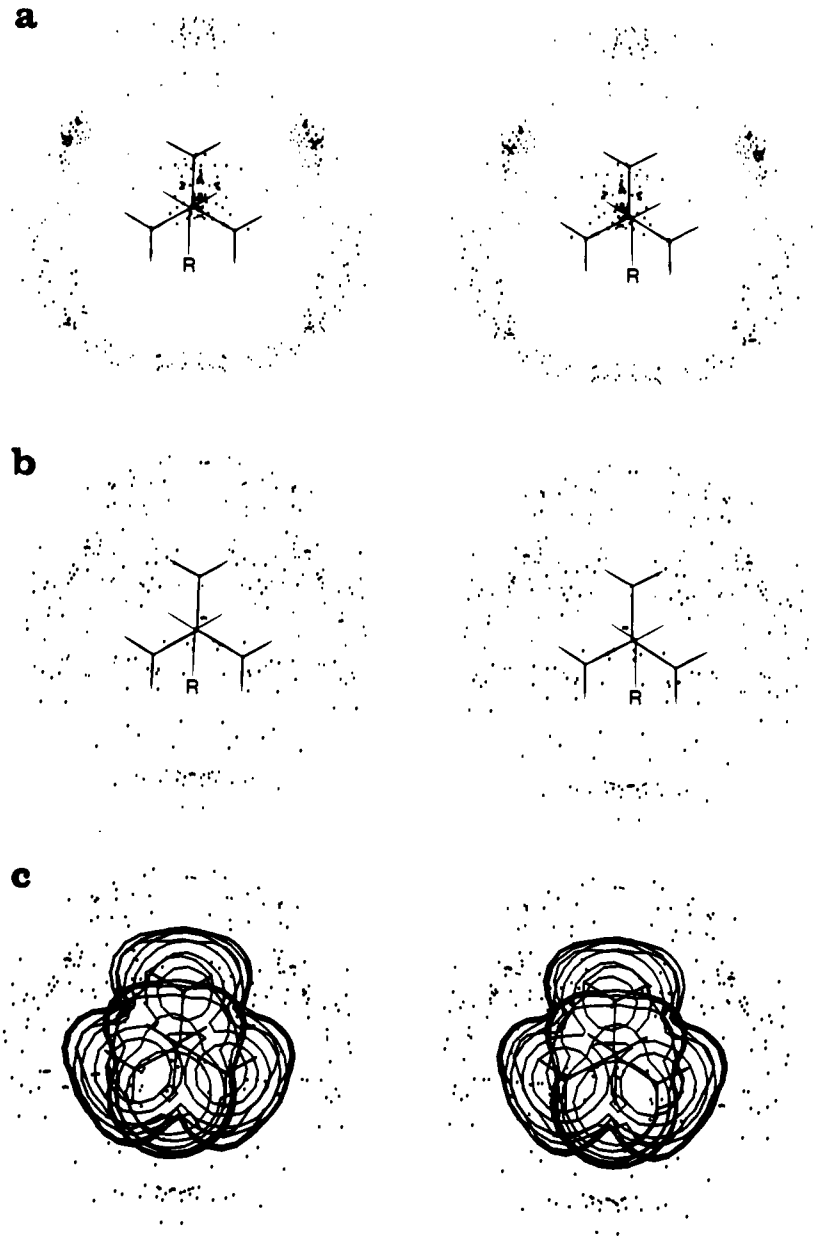

Figure 2. Stereoscopic scatter plots, drawn with interactive graphics,[8b] showing the distribution (a) of halide anions and (b and c) of oxygen atoms about a reference $RCH_2NMe_3^+$ cation. Both distributions have been symmetrized about the cation's plane.[12] The A_1 face of the cation (Figure 1) is toward the viewer. In (c) the van der Waals envelope of the $RCH_2NMe_3^+$ cation is shown, using the atomic radii N 1.5, C 1.7, and H 1.1 Å.

yanionic species contains several oxygen atoms, bi- and tridentate coordination to **1** occurs (Figure 3a), and there is no clear delineation to **1**'s first coordination sphere with oxygen.

A scatter plot of the location of oxygen atoms about a reference $RCH_2NMe_3^+$ cation is shown in Figure 2b. Directional preferences are not distinctly observed. Instead, the oxygen positions are nearly evenly distributed over the surface of the cation, modeled explicitly in Figure 2c. Although charged groups are found in the A sites, these sites are also occupied by formally uncharged oxygen-containing groups (e.g., OH, RCO_2R').

Discussion

We can understand the high local symmetry of the halide contacts (approximately T_d) by recalling the structure type of $NMe_4^+X^-$. This is a slightly distorted CsCl-type structure (Figure 3b), presumably determined almost entirely by ionic and geometric factors, with four A-type and four longer B-type N···X^- distances. This packing motif seems to dominate the crystal structures of $RCH_2NMe_3^+$ halides, even when the R group is bulky or irregularly shaped. In many structures, moreover, other molecular fragments (e.g., carboxyl, hydroxyl, water) enter the cation's coordination sphere. Still, the halide counterions tend to pack as closely as possible around the tetrahedrally shaped cation. This is best achieved by their approach toward the center of the faces of the tetrahedron. Therefore, the clustering observed in Figure 2a should not be interpreted as implying directional C–H···X^- bonding. Although the additional presence of halides along the N–Me bonds in Figure 2a is not unexpected (Figure 3b), we cannot explain the disproportionately large number of N···I^- contacts in these directions.[15]

In its biological role as neurotransmitter, acetylcholine binds to receptor protein in postsynaptic membranes. The trimethylammonium group, essential for binding,[16] presumably coordinates to oxyanionic groups (e.g., RCO_2^-, $RPO_4R'^-$) in the receptor.[17]

(14) (a) Jensen, B. *Acta Chem. Scand. Ser. B*, **1976**, *30*, 643–650. (b) Wyckoff, R. W. G. "The Structure of Crystals"; Chemical Catalog Co.: New York, 1931; pp 365–366, 369.

(15) About 30% of the N···I^- distances are directed toward the B sites, as compared with about 20% of the N···Cl^- and N···Br^- distances.

(16) Michelson, M. J.; Zeimal, E. V. "Acetylcholine, An approach to the molecular mechanism of action", translated from Russian by E. Lesser and M. Lesser; Pergamon Press: Oxford, 1973; pp 74–83.

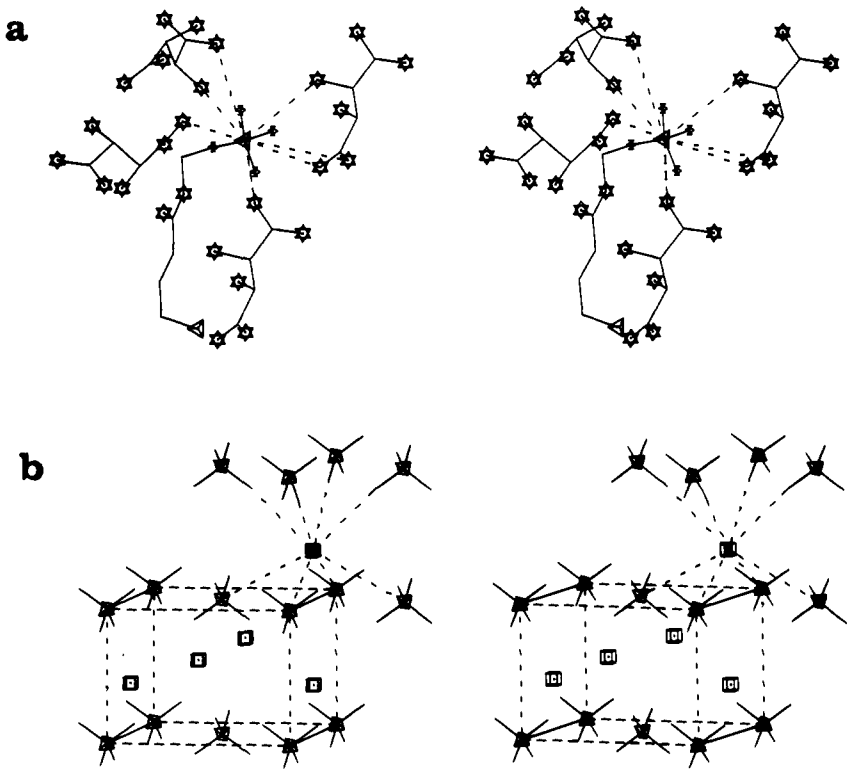

Figure 3. Stereodiagrams, drawn by program PACK,[8a] showing the environment of the quaternary ammonium group in two crystal structures. Atoms N, O, and Br are distinguished by different marks; there are no hydrogen coordinates. (a) γ-Aminobutyric acid choline ester tartrate.[14a] Four tartrate anions surround the quaternary ammonium group: N···O distances lie between 3.7 and 4.3 Å. (b) Tetramethylammonium bromide.[14b] Crystallographic unit cell axes are drawn with full (*a* axis), long-dashed (*b* axis), and short-dashed (*c* axis) lines. N···Br^- coordination distances to the A and B sites of the quaternary ammonium group are 4.39 and 5.21 Å, respectively.

Therefore, the oxyanion-containing structures are of special interest. We might expect, on the basis of the halide structures, that short N···O^- distances directed toward the tetrahedral faces of $-CH_2NMe_3^+$ are important. However, the results of the oxyanion structures do not clearly support any preferred orientation for cation binding to receptor-site oxygen atoms, and longer N···O distances directed elsewhere on the cation's surface (Figure 2c) may also play a role in transmitter–receptor recognition and binding.[18]

The pictures derived from the halide crystal structures do suggest that in acetylcholine–receptor binding, the approach of receptor anions to the quaternary ammonium cation might be directed preferentially toward the A sites.[3] Beckett et al.[19] previously showed that muscarinic activity of acetylcholine greatly exceeds that of its α(*R*)- and α(*S*)-methyl derivatives. Although this may be due to changes in preferred molecular conformation or in charge distribution,[20] our halide results would support their earlier proposal[19] that steric hindrance to cation binding of the α-methyl derivatives is likely, since α substituents will block anion approach to the cation's A_2 and A'_2 faces.

Conclusion

There is now a remarkably large amount of structural information in the Cambridge Crystallographic Data Files that can be used for examining the environments of common molecular fragments. We have used these files to illustrate the distribution of counterions about the $RCH_2NMe_3^+$ group. For simple halide counterions directional preferences are clearly seen. Interactive graphics has been an important tool for these studies, permitting the rapid evaluation of three-dimensional chemical data.

Acknowledgment. We thank Dr. S. M. Swanson and G. M. Cole for their assistance with computer programs, Dr. R. J. Radna for helpful discussions, and the National Institutes of Health (Grant GM-25469 to Dr. E. F. Meyer, Jr.) for financial support.

Supplementary Material Available: A listing of the names, registry numbers, and the bibliographic references of the crystal structures used in this study (8 pages). Ordering information is given on any current masthead page.

(17) Triggle, D. J.; Triggle, C. R. "Chemical Pharmacology of the Synapse"; Academic Press: New York, 1976; p 293.

(18) In phospholipid bilayers, intermolecular association between methyl protons of **1** and phosphate groups has been indicated by exploiting the $^{31}P\{^1H\}$ nuclear Overhauser effect in ^{31}P NMR spectra: Yeagle, P. L.; Hutton, W. C.; Huang, C.; Martin, R. B. *Biochemistry* **1977**, *16*, 4344–4349. Seelig, J. *Biochim. Biophys. Acta* **1978**, *515*, 105–140.

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