Single Crystal X-Ray Structures of Chemotherapeutic Agents. 3. Structure of 1,4-Diazabicyclo[2.2.1]heptanes,[1] a New Heterocyclic Ring System

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The crystal structure of 1,4-bis(2-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane diperchlorate (**1**) has been determined. The crystallographic study provided conclusive evidence that reaction between 2-haloethylamines and formalin can be employed to obtain 1,4-diazabicyclo[2.2.1]heptanes.

Until 1964, no reported preparation of the 1,4-diazabicyclo[2.2.1]heptane system[2] could be substantiated. Reaction between certain 2-haloethyl amines and formalin was shown, however, to provide representatives of this new heterocyclic ring system.[2,3] For example, when bis(2-chloroethyl)amine was allowed to react with formalin in EtOH solution followed by addition of $HClO_4$, the diperchlorate salt of 1,4-bis(2-chloroethyl)-1,4-diazabicyclo[2.2.1]heptane (**1**) was isolated. Based on elemental anal. data, cryoscopic formula weight determinations, and spectral data, the 1,4-diazabicyclo-[2.2.1]heptane structure was assigned.[2] As past assignments of the 1,4-diazabicyclo[2.2.1]heptane structure proved unfounded, combined with the significant activity of **1** and the corresponding bromoethyl derivative against growth of Walker 256 carcinoma,[3] the crystal structure of the perchlorate salt was examined. Evidence provided in the sequel removes any remaining uncertainty concerning the 1,4-diazabicyclo[2.2.1]heptane structural assignment.

Compound **1** was recrystallized from EtOH–H_2O and had the following lattice parameters: a = 8.37, b = 14.24, c = 13.80 Å and β = 96.35 (d_{calcd} = 1.723, d_{obsd} = 1.684 by flotation in CH_2Br_2–CCl_4). The space group was determined to be $P2_1/c$ from the systematic absences. Approximately 1000 reflections were collected on a 4-circle automatic diffractometer up to a 2θ of 40° with Mo Kα radiation using balanced filters, [θ–2θ scans up to 30° and 30–40° using the peak count method]. An additional 700 reflections were scanned above a 2θ of 40°.

The data were processed in the usual manner, and normalized structure factors (E's) were calcd using Wilson statistics and renormalized in parity classes, index sets, and ranges of sin θ. Values of $E > 1.5$ were used in deriving a set of signs by the direct method using the Syd Hall tangent refinement program.[4] The E-synthesis calcd using these signs revealed the positions of 2 perchlorate ions and 2 other Cl atoms. A difference synthesis was produced subtracting out the contributions of the perchlorate and of the Cl atoms. Bond distance and angles between the major peaks in this calculation clearly indicated the rest of the structure.

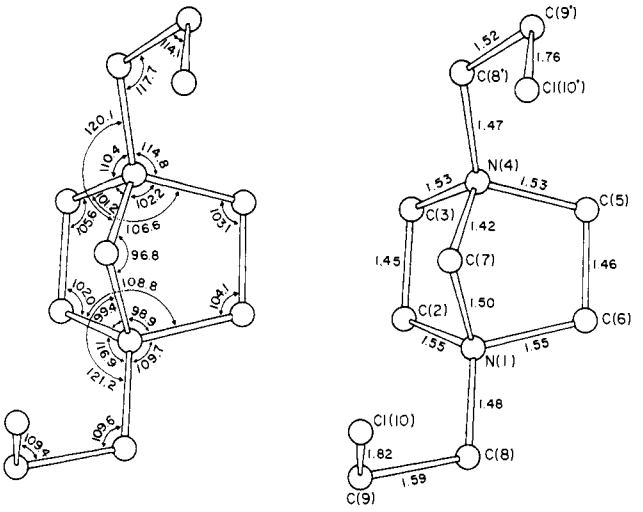

Figure 1.

TABLE I
ATOMIC COORDINATES

Atom identification[a]	X	Y	Z
Cl (10)	−0.1178	0.1965	0.3122
Cl (10′)	0.1119	0.1066	0.0088
N (1)	0.2659	0.1150	0.3356
N (4)	0.2989	0.0242	0.2117
C (2)	0.2993	0.0171	0.3832
C (3)	0.3268	−0.0404	0.3001
C (5)	0.4445	0.0896	0.2157
C (6)	0.4224	0.1514	0.2976
C (7)	0.1773	0.0848	0.2404
C (8)	0.1997	0.1883	0.3964
C (8′)	0.2690	−0.0314	0.1217
C (9)	0.0220	0.1606	0.4158
C (9′)	0.2628	0.0205	0.0253
Perchlorate 1			
Cl 1	0.1998	0.3986	0.1835
O 1	0.2726	0.4565	0.1177
O 2	0.1976	0.3027	0.1547
O 3	0.2949	0.4016	0.2751
O 4	0.0448	0.4282	0.1948
Perchlorate 2			
Cl 1′	−0.3561	0.2510	0.0496
O 1′	−0.4752	0.1957	−0.0098
O 2′	−0.3526	0.3435	0.0122
O 3′	−0.2038	0.2067	0.0438
O 4′	−0.3899	0.2517	0.1477

[a] The atom numbering corresponds to that in Figure 1 for the cationic portion of the molecule. The perchlorate ions are not shown in Figure 1 so that a better view of the bicyclo ring system is given.

(1) Part II: see D. J. Abraham, R. D. Rosenstein, and E. L. McGandy, *Tetrahedron Lett.*, **46**, 4085 (1969). This paper is Part XXVII of the series "Antineoplastic Agents;" for Part XXVI refer to G. R. Pettit, J. L. Hartwell, and H. B. Wood, *Cancer Chemother. Rep.*, in press. We gratefully acknowledge support of this investigation by Contract Number PH 43-67-1186 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Bethesda, Md.

(2) G. R. Pettit and J. A. Settepani, *Chem. Ind. (London)*, 1805 (1964); G. R. Pettit, D. C. Fessler, and J. A. Settepani, *J. Org. Chem.*, **34**, 2978 (1969).

(3) D. C. Fessler, G. R. Pettit, and J. A. Settepani, *J. Med. Chem.*, **12**, 542 (1969).

(4) S. R. Hall (1967), adapted to the IBM 7090 by H. Berman (1969), revised by R. Shiono, University of Pittsburgh, unpublished report (1970).

The results showed the 1,4-diazabicyclo[2.2.1]heptane structure proposed by Pettit.[2,3]

Two cycles of isotropic least squares for all atoms reduced the R factor to 18% and one cycle of full-matrix anisotropic least squares gave an R value of 14.6%. Bond distances and angles are given in Figure 1 (a second set of data taken with Ni-filtered Cu radiation gave an R factor of 13.1% after one cycle of anisotropic least squares). A list of atomic coordinates for this structure is given in Table I.

New Compounds

Synthesis of Enantiomeric Chloroacetylcarnitine Chlorides

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Convincing experimental evidence exists for the role of (*R*)-(−)-carnitine in enzyme-mediated transport of activated acyl groups across mitochondrial and possibly other membranes.[1-6] Further, the structural similarity of carnitine (**1**) and acetylcarnitine (**2**) to choline and acetylcholine, respectively, the possible biotransformation of **1** and **2** to β-methylcholine,[7] and the use of **1** in the clinic,[8] point to possible therapeutic potential[9] and/or pharmacologic utility of these types of biological molecules or related derivatives. We report a convenient synthesis of (*R*)-(−)-, (*S*)-(+)-, and racemic chloroacetylcarnitine chlorides (**3**),[10] which have been investigated for cholinergic activity,[12,13] and in tissue culture.[14]

$$(CH_3)_3N^+CH_2CHO{-}X$$
$$\qquad\qquad\quad |$$
$$\qquad\qquad CH_2COO^-$$

1, X = H
2, X = $COCH_3$
3, HCl, X = $COCH_2Cl$

(1) G. Wolf, Ed., "Recent Research on Carnitine," M.I.T. Press, Cambridge, Mass., 1965, Parts II and III, and ref cited therein.

(2) I. B. Fritz and N. R. Marquis, *Proc. Nat. Acad. Sci. U. S.*, **54**, 1226 (1965).

(3) K. R. Norum and J. Brenner, *J. Biol. Chem.*, **242**, 407 (1967).

(4) J. F. A. Chase, *Biochem. J.*, **104**, 510 (1967).

(5) A. M. Snoswell and G. D. Henderson, *ibid.*, **119**, 59 (1970).

(6) B. Wittels and P. Hochstein, *J. Biol. Chem.*, **242**, 126 (1967).

(7) E. A. Khairallah and G. Wolf, *ibid.*, **242**, 32 (1967).

(8) E. Gravina and G. Gravina-Sanvitale, *Clin. Chim. Acta*, **23**, 376 (1969).

(9) E. A. Hosein, S. J. Booth, I. Gasoi, and G. Kato, *J. Pharmacol. Exp. Ther.*, **156**, 565 (1967).

(10) (*R*)-(−)-Bromoacetylcarnitine has been synthesized,[11] but the yield was not reported and purity was ascertained only by chemical assay and by tlc. This derivative in the presence of CoA has been demonstrated to be a reversible inhibitor of acetyl-CoA:L-carnitine *O*-acetyltransferase [E.C. 2.3.1.7]; in the absence of CoA it is an irreversible inhibitor, which is postulated to act by an active-site-directed mechanism.[11]

(11) J. F. A. Chase and P. K. Tubbs, *Biochem. J.*, **116**, 713 (1970).

(12) R. T. Louis-Ferdinand, K. R. Cutroneo, D. C. Kosegarten, R. C. Vasavada, J. G. Turcotte, and D. R. DeFanti, *J. Pharm. Pharmacol.*, **22**, 704 (1970).

(13) K. R. Cutroneo, R. T. Louis-Ferdinand, R. C. Vasavada, J. G. Turcotte, and D. R. DeFanti, *ibid.*, **22**, 940 (1970).

(14) (*RS*)-**3**, (*R*)-(−)-**3**, and (*S*)-(+)-**3** showed modest and comparable inhibition of murine leukemic lymphoblast (L5178Y) growth in culture, indicating that these quaternary ammonium salts may cross the plasma membrane; radioactive **1** has been shown to be progressively taken up by intact Ehrlich ascites tumor cells.[15]

(15) A. A. Spector, *Arch. Biochem. Biophys.*, **122**, 55 (1967).

Experimental Section[16]

(*R*)-(−)-**Chloroacetylcarnitine Chloride** (**3**).—A mixt of 1.0 g (0.005 mole) of (*R*)-(−)-carnitine chloride, 0.9 g (0.005 mole) of chloroacetaic anhydride, and 0.1 g of *p*-TsOH was stirred at 70–75° for 75 min. The syrupy reaction mixt then was cooled to 25°, washed with Et_2O (3 × 5 ml), and taken up in 3.5 ml of *i*-PrOH. After standing 2–3 hr at 25°, and overnight at 5°, 0.85 g (62%)[18] of a cryst product was obtd. One recrystn from EtOH–*i*-PrOH afforded white crystals: mp 186–188°; $[\alpha]^{22}D$ −27.7° (*c* 8.03, H_2O); tlc (silica) R_f 0.07, CH_3CN–CH_3OH–NH_3 (10:5:2); ir (μ, Nujol) 5.69 (C=O, ester), 5.89 (C=O, acid); pmr (δ, D_2O) 2.91 (2, d), 3.2 (9, s), 3.81 (2, m), 4.32 (2, s), 5.67 (1, m). *Anal.* ($C_9H_{17}Cl_2NO_4$), C, H, N, Cl.

(*S*)-(+)-**Chloroacetylcarnitine chloride** (**3**) was obtained in 62% yield:[18] mp 186–188°; $[\alpha]^{22}D$ +29.8° (*c* 8.89, H_2O). *Anal.* C, H, N, Cl.

(*RS*)-**Chloroacetylcarnitine chloride** (**3**) with *p*-TsOH·H_2O as catalyst was obtained in 66% yield,[18] mp 179°. *Anal.* C, H, N, Cl.

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(16) (*R*)-(−)-Carnitine chloride, $[\alpha]^{22}D$ −21.7° (lit.[17] −23.7°), (*S*)-(+)-carnitine chloride, $[\alpha]^{22}D$ +23.1° (lit.[17] +23.6°), and (*RS*)-carnitine chloride were obtd from Nutritional Biochemicals Co., Cleveland, Ohio 44128. (*R*)-(−)-**3**, (*S*)-(+)-**3**, and (*RS*)-**3** were prepd using the same method and only details of the synthesis of (*R*)-(−)-**3** are given. Optically active precursors and products were found to be extremely hygroscopic, and it was necessary to use *anhyd* *p*-TsOH as catalyst and to scrupulously exclude moisture in order to obtain cryst products—operations requiring moisture-free conditions were carried out in a Labconco controlled atm glove box. The ir and pmr spectra of each compd were consistent with the expected structure and are reported for (*R*)-(−)-**3**. Melting points were detd with a Thomas-Hoover Uni-Melt capillary melting point apparatus and are uncorrected. Where analyses are indicated only by the symbols of the elements, anal. results obtained for those elements were within ±0.4% of the theor values; analyses by Micro-Analysis, Inc., Marshallton, Wilmington, Del.

(17) G. Kato and E. A. Hosein, *Can. J. Chem.*, **47**, 1177 (1969).

(18) Yields by this method approach those reported[19] using three different methods designed to improve yields of O-acylation of (*RS*)-carnitine chloride.

(19) H. J. Ziegler, P. Bruckner, and F. Binon, *J. Org. Chem.*, **32**, 3989 (1967).

3-Amino-4-hydroxy-L(−)-butyramide Hydrochloride[1]

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In the current investigation of compounds related to asparagine for antitumor activity, 3-amino-4-hydroxy-

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