quement trois sites équivalents par cet axe. Il ne paraît pas intervenir dans la cohésion du cristal, et ne sert qu'à assurer la neutralité électrique du composé.

La cohésion de l'édifice cristallin est assurée dans les trois directions de l'espace par des chaînes des liaisons Ca–O–Ca–O··· qui sont doublées par quelques liaisons hydrogène. Les autres liaisons hydrogène servent à tenir le nitrate de type III dans la cavité délimitée par les molécules d'eau.

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The Conformation of Acetylcholine and the Crystal Structure of 2,2-Dimethylbutyl 3,5-Dinitrobenzoate

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The 2,2-dimethylbutyl ester is the neutral, isoelectronic analogue of the choline ester. The choline analogue assumes a variety of conformations, none of which exhibit the close intramolecular contact between the ester O and a terminal methyl observed in acetylcholine. These observations substantiate theoretical quantum chemical calculations predicting that there are multiple minima with low energy differences for the analogue structure and they also support the concept that the positive charge associated with the quaternary N in choline stabilizes the *gauche* N⁺–C–C–O conformation and its resultant N–methyl–O close contact. The title compound crystallizes in space group $P\bar{1}$ with unit-cell dimensions $a = 11 \cdot 347$ (5), $b = 11 \cdot 906$ (5), $c = 13 \cdot 198$ (5) Å, $\alpha = 95 \cdot 04$ (5), $\beta = 111 \cdot 32$ (5) and $\gamma = 109 \cdot 05$ (5)°. The cell parameters and the 5044 intensity data were measured on a four-circle diffractometer using monocromated Cu radiation, $\lambda = 1 \cdot 54178$ Å. The structure was solved by direct methods and refined by full-matrix least squares to an $R = 0 \cdot 11$ for all reflections and $0 \cdot 08$ for the 2968 reflections considered observed.

Introduction

Single crystals of 2,2-dimethylbutyl 3,5 dinitrobenzoate (DMDNB) were grown at $-20°C$ from an acetone solution by evaporation. A prismatic specimen was selected for the structure determination. Its length was 0·36 mm, and its cross-section approximated a diamond-shaped figure with edges 0·19 mm long. The cell constants and the intensities were measured on a Nonius CAD4 diffractometer equipped with a monochromator. The reflections were scanned in the θ–2θ mode. The scan speed was variable. Background counts were taken for a quarter of the scan time on

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each side of the reflection. Three standard reflections were monitored after every sixty intensity measurements. The data were scaled to compensate for fluctuations in the standards, which remained within ±6% of their initial values throughout the data collection. Corrections were not made for absorption or extinction. Of the 5044 reflections that were measured 2076 were considered as unobserved [$\leq 2 \cdot 0\sigma(I)$ based on counting statistics].

Although a variety of well known and widely used programs for the application of the direct method were employed in efforts to solve the structure, all were unsuccessful until the negative quartet relationship derived by Hauptman (1974) was applied to the problem. The negative quartets were computed with a

program written by Blank (1974). Two negative quartets which entered into many sigma 2 relationships and generated many signs in *MULTAN* (Germain, Main & Woolfson, 1971) were chosen as a starting set; two additional reflections were chosen to aid in generating the phases. The rationale for including the negative signs in the starting set was to avoid a trivial solution. Of several phase sets computed by *MULTAN* we chose the only one which had no discrepancies with the negative quartet relationships of high probability. The E map calculated from this set could be interpreted to provide 30 of the 42 heavy-atom

Table 1. *Fractional coordinates and thermal parameters* $(\times 10^4)$ *for DMDNB*

Thermal parameters are for the expression $\exp[-(\beta_{11}h^2 + \ldots + 2\beta_{12}hk + \ldots)]$. The estimated standard deviations are given in parentheses.

(a) Nonhydrogen atoms*

	x	y	z	β_{11}	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
N(1)A	3738 (4)	56 (4)	6841 (3)	180 (5)	146 (5)	117 (3)	76 (4)	61 (3)	10 (3)
N(2)A	1836 (4)	−4379 (3)	5100 (3)	179 (5)	127 (4)	101 (3)	56 (3)	52 (3)	46 (3)
O(1)A	2443 (3)	−2360 (3)	2091 (2)	233 (5)	139 (3)	88 (2)	44 (3)	72 (3)	34 (2)
O(2)A	3390 (3)	−304 (3)	2824 (3)	214 (5)	147 (3)	129 (3)	62 (3)	80 (3)	66 (3)
O(3)A	4059 (4)	1101 (3)	6723 (3)	291 (6)	120 (3)	158 (4)	99 (4)	79 (4)	21 (3)
O(4)A	3832 (4)	−200 (3)	7725 (3)	303 (7)	175 (4)	112 (3)	60 (4)	109 (4)	13 (3)
O(5)A	1574 (4)	−4601 (3)	5893 (3)	274 (6)	153 (3)	120 (3)	52 (3)	104 (3)	59 (3)
O(6)A	1727 (4)	−5171 (3)	4368 (3)	330 (7)	123 (3)	109 (3)	87 (4)	80 (3)	38 (2)
C(1)A	2845 (4)	−1669 (4)	3950 (3)	129 (4)	128 (4)	87 (3)	56 (3)	44 (3)	37 (3)
C(2)A	3274 (4)	−709 (4)	4871 (3)	134 (5)	103 (4)	107 (3)	47 (3)	47 (3)	25 (3)
C(3)A	3234 (4)	−974 (3)	5848 (3)	136 (4)	115 (4)	98 (3)	59 (3)	53 (3)	24 (3)
C(4)A	2762 (4)	−2164 (4)	5949 (3)	136 (5)	145 (5)	87 (3)	64 (3)	54 (3)	36 (3)
C(5)A	2333 (4)	−3096 (3)	5009 (3)	133 (4)	111 (3)	86 (3)	55 (3)	48 (3)	27 (2)
C(6)A	2382 (4)	−2872 (4)	4030 (3)	128 (4)	119 (4)	75 (3)	51 (3)	37 (3)	21 (2)
C(7)A	2929 (4)	−1350 (4)	2902 (3)	150 (5)	152 (5)	92 (3)	60 (4)	52 (3)	45 (3)
C(8)A	2501 (7)	−2178 (5)	1030 (4)	238 (8)	172 (6)	84 (3)	47 (6)	68 (4)	46 (3)
C(9)A	1839 (7)	−3468 (5)	253 (4)	262 (9)	175 (6)	97 (4)	56 (6)	81 (5)	50 (4)
C(10)A	1781 (6)	−3539 (5)	−939 (4)	245 (8)	197 (6)	94 (4)	83 (6)	89 (4)	59 (4)
C(11)A	1000 (12)	−4942 (8)	−1623 (7)	593 (24)	224 (10)	154 (7)	25 (12)	170 (11)	1 (7)
C(12)A	951 (12)	−2905 (11)	−1586 (6)	525 (22)	495 (20)	124 (6)	353 (18)	80 (9)	107 (9)
C(13)A	3202 (8)	−3006 (13)	−885 (6)	220 (10)	694 (27)	136 (7)	91 (13)	96 (7)	64 (11)
N(1)B	5308 (5)	4390 (4)	7376 (3)	223 (7)	157 (5)	98 (3)	57 (5)	44 (4)	47 (3)
N(2)B	419 (5)	1688 (5)	4911 (5)	160 (6)	243 (7)	145 (5)	92 (5)	70 (4)	56 (5)
O(1)B	2850 (3)	2152 (3)	2362 (3)	189 (4)	161 (4)	85 (2)	30 (3)	32 (3)	27 (3)
O(2)B	5093 (3)	3344 (3)	3468 (3)	156 (4)	197 (4)	101 (3)	70 (3)	46 (3)	47 (3)
O(3)B	6493 (4)	4873 (4)	7475 (3)	187 (5)	197 (5)	116 (3)	57 (4)	27 (3)	28 (3)
O(4)B	4975 (5)	4492 (4)	8145 (3)	288 (7)	244 (6)	92 (3)	21 (5)	73 (4)	25 (3)
O(5)B	176 (5)	1851 (6)	5733 (4)	213 (6)	420 (11)	157 (5)	120 (6)	105 (5)	98 (6)
O(6)B	−444 (4)	1056 (5)	3983 (5)	152 (5)	326 (8)	185 (5)	37 (5)	71 (4)	−25 (5)
C(1)B	3502 (4)	2859 (4)	4284 (3)	139 (5)	115 (4)	93 (3)	55 (3)	34 (3)	38 (3)
C(2)B	4543 (5)	3585 (4)	5355 (3)	149 (5)	113 (4)	96 (3)	57 (3)	44 (3)	40 (3)
C(3)B	4184 (5)	3640 (4)	6235 (3)	164 (6)	122 (4)	84 (3)	58 (4)	38 (3)	31 (3)
C(4)B	2854 (5)	3060 (4)	6128 (4)	180 (6)	148 (5)	97 (4)	84 (4)	59 (4)	47 (3)
C(5)B	1872 (5)	2362 (4)	5070 (4)	154 (6)	144 (5)	122 (4)	74 (4)	61 (4)	51 (3)
C(6)B	2149 (5)	2224 (4)	4133 (4)	145 (5)	122 (4)	110 (4)	59 (4)	37 (3)	42 (3)
C(7)B	3907 (4)	2813 (4)	3337 (3)	147 (5)	133 (4)	89 (3)	58 (4)	38 (3)	43 (3)
C(8)B	3175 (7)	2045 (6)	1402 (4)	289 (11)	208 (8)	79 (4)	29 (7)	62 (5)	22 (4)
C(9)B	1758 (8)	1820 (9)	409 (6)	164 (10)	197 (11)	87 (5)	89 (8)	55 (6)	21 (5)
C(9)D	2335 (24)	1050 (19)	316 (15)	318 (34)	206 (24)	95 (14)	38 (23)	55 (17)	26 (14)
C(10)B	1751 (5)	1589 (5)	−690 (4)	170 (6)	170 (6)	113 (4)	71 (5)	68 (4)	51 (4)
C(11)B	296 (12)	1341 (13)	−1687 (9)	226 (14)	278 (20)	125 (8)	117 (15)	68 (8)	74 (11)
C(11)D	493 (25)	705 (19)	−1630 (18)	221 (30)	161 (23)	95 (15)	57 (19)	29 (16)	14 (14)
C(12)B	2888 (8)	2528 (8)	−855 (7)	262 (11)	316 (13)	175 (8)	46 (9)	112 (8)	119 (8)
C(13)B	1912 (14)	312 (11)	−897 (10)	456 (25)	234 (14)	180 (11)	211 (16)	155 (14)	44 (10)
C(13)D	1224 (25)	2364 (25)	123 (21)	237 (35)	285 (35)	187 (24)	113 (27)	130 (25)	−9 (22)

(b) Hydrogen atoms*

Fractional coordinates $\times 10^3$; isotropic thermal parameters $\times 10$

	x	y	z	B		x	y	z	B
H(C2)A	358 (5)	−5 (4)	482 (4)	49 (11)	H(C4)A	269 (4)	−234 (4)	654 (4)	61 (10)
H(C6)A	204 (5)	−354 (4)	347 (4)	62 (11)	H1(C9)A	233 (6)	−408 (5)	67 (5)	112 (15)
H1(C8)A	364 (7)	−178 (6)	124 (5)	121 (16)	H2(C9)A	84 (7)	−390 (6)	10 (5)	130 (18)
H2(C8)A	200 (7)	−152 (6)	67 (6)	158 (20)	H(C2)B	550 (5)	402 (4)	556 (3)	51 (9)
H(C4)B	254 (4)	311 (4)	670 (4)	55 (9)	H(C6)B	140 (5)	169 (4)	344 (4)	61 (9)

* The terminal designations A, B and D indicate molecule A, molecule B and the disordered atoms of molecule B respectively.

positions in the two independent molecules. A series of structure factor and difference Fourier syntheses yielded the entire structure including ten of the H positions and four disordered C atoms in the terminus of the side chain for molecule B. The relative occupancies of the disordered atoms were estimated from the difference map and were not varied in the least-squares refinement. Least-squares refinement of a model containing anisotropic thermal parameters for heavy atoms

and isotropic temperature factors for the H atoms gave $R = 0.08$ for the observed data and 0.11 for all the data.* The computations were made with programs written by Shiono (1971). The final positional and thermal parameters are listed in Table 1. The interatomic distances and angles are shown in Fig. 1.

Discussion

The conformational properties of acetylcholine and of its agonists and inhibitors have been studied extensively both experimentally and theoretically in order to ascertain which steric features are essential for promoting and for blocking cholinergic neurotransmission. The description of acetylcholine that emerges is one of a flexible molecule whose conformational energy surface exhibits a global minimum and a number of local minima, all falling within a narrow range of energies. Depending upon the theoretical methods employed in the computations, the estimates of the range vary from 0.7 to about 5 kcal mole^{-1} (Ajò, Bossa, Damiani, Fedenzi, Gigli & Ramunni, 1972; Beveridge, Radna & Guth, 1972; Pullman & Courrière, 1972). According to the PCILO method, the range is 3 kcal mole^{-1} (Pullman & Courrière, 1972). The PCILO method agrees with experiment in that the conformation of the global minimum and those of experimentally observed structures occupy the same trough of the energy map. The PCILO global minimum occurs when τ_1, τ_3, and τ_4 are 180° and when τ_2 is 60°. The experimentally observed conformations coming closest to the theoretical global minimum are those seen in the crystal structures of the chloride and perchlorate salts of acetylcholine (Table 2). In acetylcholine bromide (Table 2) the torsion angle τ_3 at 79°, deviates greatly from the one in the global minimum. In all observed forms of the molecule the configuration of atoms N$^+$–C–C–O, defining τ_2, is in the *gauche*
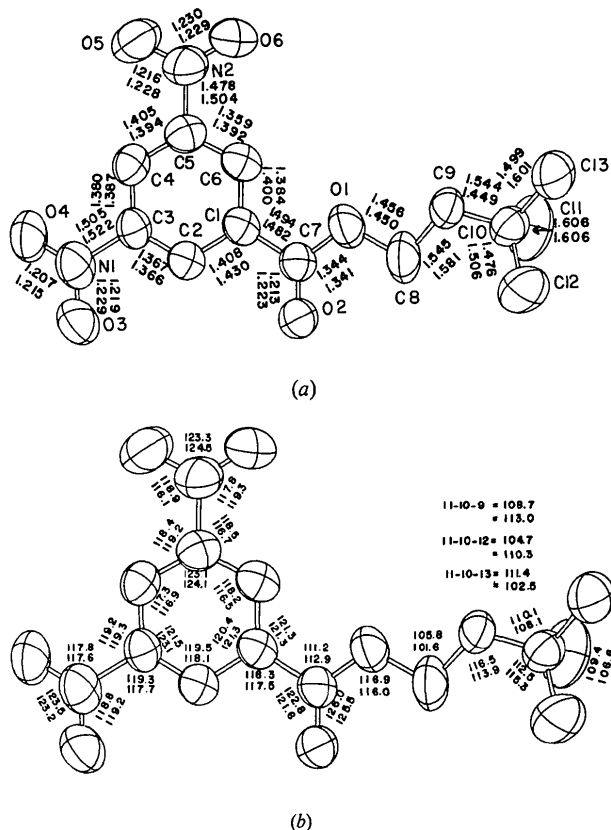

(a)

(b)

Fig. 1. *ORTEP* representation of DMDNB, molecule A, showing (a) the atomic numbering scheme and the interatomic distances (Å) and (b) the valency angles (°). The upper number for each pair is the value for molecule A; the lower is the value for the major constituent of molecule B.

Table 2. *Comparison of torsion angles for 'acetylcholine' side chain*

Me   O
   \ τ_1 τ_2 τ_3 τ_4 //
Me—R—CH$_2$—CH$_2$—O—C
   /   \
Me

$R = N^+$ in choline derivatives or C in DMDNB

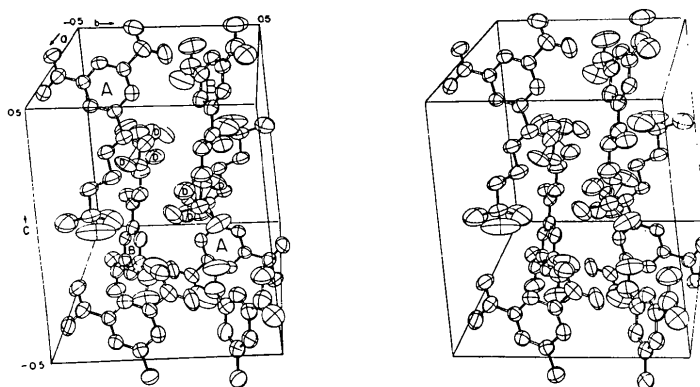| | DMDNB | | | Acetylcholine* | |
	Molecule A	Molecule B(67%)	Molecule B(33%)	bromide	chloride	perchlorate
τ_1	-177.4	179.1	-154.6	175.5	171.4	168.3
τ_2	-179.5	-175.8	114.6	78.4	84.7	73.7
τ_3	-178.1	-154.0	158.0	78.9	-166.9	179.8
τ_4	1.3	1.8	1.8	4.1	5.2	0.8

* Svinning & Sørum (1975)

Fig. 2. A stereo *ORTEP* packing diagram of DMDNB. The two independent molecules are labeled *A* and *B*. The low occupancy disordered atoms of molecule *B* are connected with single line bonds and are labeled *D*.

form, just as it is in a variety of other chemically similar and biologically important molecules, where it is usually observed to have a torsion angle of $\pm(60 \pm 15°)$ (Sundaralingam, 1972). The theoretical studies indicate that the greater stability of the *gauche* over the *trans* conformation is due mostly to electrostatic interaction between the cationic head and the ester O atom. This conclusion is substantiated experimentally by the work of Craven & Hite (1973) on homocholine.

In a theoretical analysis of the forces stabilizing the N^+–C–C–O conformation, Beveridge *et al.* (1972) using INDO molecular orbital calculations compared the total energies of norcholine* and choline. Multiple minima occur in both cases, but the range of the minima is much narrower (≤ 1 kcal mole^{-1}) in the absence of the formal positive charge on the N atom. Furthermore, computations by classical empirical methods (Ajò *et al.*, 1972) indicate that the *trans* conformation for the acetylcholine chain is favored slightly if the electrostatic energy is neglected in the mathematical treatment. One would expect on the basis of these theoretical results to witness a more flexible molecule with a slight preference for the *trans* form, if the positively charged quaternary N atom in acetylcholine were to be replaced by neutral and isoelectronic C. As a test of this proposition, we undertook the crystal structure analysis of DMDNB. The structure analysis reveals that the two independent molecules in the asymmetric unit assume three distinct conformations (Fig. 2). Molecule *A* is exclusively *trans* but *B* is disordered between two conformational states differing from that of *A*. The torsional angles and relative abundance of the two forms of molecule *B* are given in Table 2. The occurrence of three conformations in a single crystal structure does confirm rather dramatically the prediction that DMDNB is a very flexible molecule, more so than a choline ester. Our analysis further shows that crystal-

lization of multiple conformations in the asymmetric unit can be an important vehicle for decreasing the lattice free energy of a crystal composed of flexible molecules.

Finally, it should be noted that contrary to the situation in acetylcholine close contacts do not occur in DMDNB between the ester O and a terminal methyl group. The short 2·95 (3) Å distance between a terminal methyl and the ester O must not be regarded as significant, for it involves atoms in molecule *B* that are in sites with the lowest occupancy factors. In a CPK model of the molecule with conformational angles identical to those in the low occupancy form of molecule *B*, the ester O and methyl groups are not contiguous.

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* Norcholine, $\begin{matrix} CH_3 \\ \diagdown \\ CH_3 \diagup \end{matrix}$ N–CH$_2$–CH$_2$–O–C=O, contains a tertiary N and is formally neutral in contrast to the positive quaternary N in acetylcholine. (with CH$_3$ on the C=O carbon)