where the five least active compounds have activities less than that predicted by the regression equation. Both of these trends suggest that a linear model is inadequate to completely account for the systematic variation in activity with structure.

Relationship to Metabolism. The data indicate that positions 2, 6, and 17 are particularly significant to biological activity. Since one of the ways to increase the activity of a molecule is to prevent its degradation, it is interesting to compare these results to the metabolism of cortisol.

In the main metabolic pathway of cortisol, the molecule is first transformed at the Δ^4 double bond to the 11β,-17α,21-trihydroxy-5α- and -5β-pregnane-3,20-diones. These two products are in turn metabolized at the C_3 carbonyl to 3α- and 3β-hydroxyl groups. The third step in this path is at C_{20} where again a carbonyl is reduced to a hydroxyl. Other initial transformations of cortisol are to 6β-hydroxycortisol, 2α-hydroxycortisol, and the 20α- and 20β-tetroles.[7] Carbons 2 and 6 are sites of alternate pathways. Carbon 6 is also adjacent to the first degradation step in the main pathway, while 2 and 17 are adjacent to the second and third steps, respectively.

Carbon 6 is adjacent to the double bond that is opened to the 5α- or 5β-hydrogen. If the increase in activity is related to prevention of bond opening, then it appears that the most effective blockage is created by moving the methyl substituent from the α position to a position between α and β, created by the 6,7 double bond. The bond itself (more properly hydrogen and the bond) has the most negative coefficient, indicating that it is not the presence of the double bond that increases activity but the spatially directing effect which it has that is important. At position 2, the position controlling the greatest variance, a methyl group promotes activity while hydrogen inhibits activity. This could be a size effect but more substituents are needed before any conclusions can be drawn.

Conclusion

A set of 44 glucocorticoids has been analyzed for structure–activity relationships by the Fujita–Ban approach. Seven different positions on the molecule were analyzed for their contribution to the variation in biological activity. Positions 2α, 17, and 6α accounted for 60% of the total variance. The three positions accounting for the greatest variance were adjacent to metabolic points in the molecular framework.

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Molecular Structure of 1-(2-Chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea

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The three-dimensional structure of 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea (MeCCNU, NSC-95441), an effective antitumor agent, has been determined by single-crystal x-ray diffraction. MeCCNU crystallizes in monoclinic space group $P2_1/c$, with cell dimensions a = 12.387, b = 10.810, and c = 10.198 Å, β = 102.62°, and Z = four molecules per unit cell. The structure was solved by direct phasing procedures and refinement by anisotropic least squares converged at a discrepancy index R = 0.065. The cyclohexyl ring is in the chair conformation with the plane of the nitrosourea moiety twisted approximately 90° from the cyclohexyl ring. The carbon–nitrogen bonds of the urea group are significantly asymmetric.

One of the most promising groups of compounds to be developed by the Chemotherapy Program of the National Cancer Institute is the 1-(2-chloroethyl)-3-alkyl-1-nitrosoureas.[1,2] The nitrosoureas are effective antitumor agents in the treatment of lymphomas[3] and solid tumors[4] in man. They are highly lipid soluble, cross the blood–brain barrier rapidly,[5,6] and exhibit unusual delayed bone marrow toxicity.[3]

The nitrosoureas are chemically reactive compounds that decompose nonenzymatically at relatively rapid rates under physiological conditions. Studies of their decomposition products reveal the formation of a highly reactive 2-chloroethyldiazene hydroxide and isocyanates,[7] with the former intermediate becoming an alkylating species, possibly in part as a 2-chloroethyl carbonium ion.[8] The mechanism of antitumor action of these compounds is not fully understood, but it is believed to involve passive diffusion of the intact nitrosourea and possibly the isocyanate across the cell membrane.[9] The isocyanates are capable of interacting with amino acids and proteins,[10–12]

Chart I. Chemical Structures of Clinically Useful Nitrosoureas

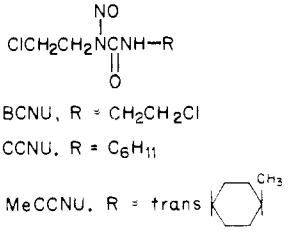

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Table I. Crystallographic Data for MeCCNU

Mol formula	$C_{10}H_{18}ClN_3O_2$
Mol weight	247.2
Color	Colorless
Habit	Tabular
Size	0.72 × 0.45 × 0.12 mm
Space group	$P2_1/c$
a	12.387 (2) A
b	10.810 (3) A
c	10.198 (2) A
β	102.62 (2)°
Volume	1332.7 A^3
Z	4
Density	1.235 g cm^{-1}
Radiation	Mo Kα
Wavelength	0.71069 A
Linear absorption coeff	2.8 cm^{-1}

while the chloroethyl carbonium ion reacts with nucleic acids by alkylating and cross-linking.[13] Some evidence suggests that the isocyanates may be associated with toxic side effects,[14,15] while the antitumor action may be caused by the carbonium ion.[16,17] However, a recent structure–activity analysis of chloroethylnitrosoureas did not find a significant correlation between carbamoylating activity and toxicity in mice.[18]

Three nitrosoureas (Chart I), bis(chloroethyl)nitrosourea (BCNU), cyclohexylchloroethylnitrosourea (CCNU), and *trans*-4-methylcyclohexylchloroethylnitrosourea (MeCCNU), have undergone extensive preclinical and clinical testing and are currently widely used in cancer treatment. In animal studies using subcutaneously implanted Lewis lung carcinoma, MeCCNU effected cures at nontoxic does, while CCNU caused only moderate tumor regression, and BCNU was hardly active.[19] This result suggested a conformational dependence for biological activity, which has been further tested by comparison of cis–trans pairs of CCNU derivatives against leukemia L1210 and Lewis lung carcinoma in mice. In the former system almost all CCNU derivatives, including several cis–trans pairs, displayed high activity,[20] although *trans*-MeCCNU was more active (and more toxic) than its cis isomer. Against Lewis lung carcinoma only the trans compounds were effective.[21] Other clinical studies have not demonstrated marked superiority of one nitrosourea over the others for some human tumors.[22] We report here the first determination of the three-dimensional molecular structure of a nitrosourea compound with anticancer activity, MeCCNU. The results are of interest in showing geometrical and stereochemical relationships in this important class of antitumor agents. Since this is the first compound of its type to be crystallographically determined, comparisons of its conformation with other agents are not yet possible. However, there is much interest in the stereochemistry of the nitrosourea alkylating agents, of which MeCCNU is a prototype, on the part of medicinal chemists, theoretical chemists, and pharmacologists. We are therefore presenting the stereochemical parameters for MeCCNU in great detail for the benefit of all possible users of the data.

Experimental Section

Crystals of MeCCNU were obtained by slow evaporation of an ether solution. Cell dimensions and space group were determined from x-ray photographs and diffractometer measurements. Crystal and cell data are listed in Table I. An automated four-circle diffractometer with Nb-filtered Mo radiation was used to measure the intensity of all independent reflections having a 2θ (Mo Kα) < 50°, corresponding to a minimum interplanar spacing of 0.84 Å. The θ–2θ scan method was employed, with stationary backgrounds measured on both sides of each scan.

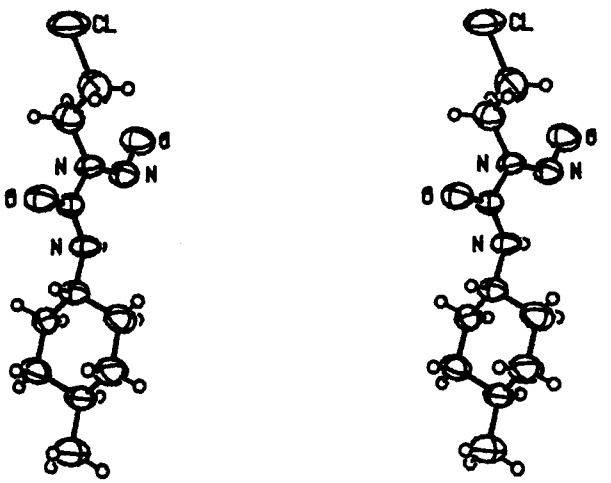

Figure 1. Stereoscopic drawing of MeCCNU showing the molecular conformation and relative sizes and shapes of the thermal motion ellipsoids. Carbon and hydrogen atoms are not labeled.

Of the 2346 reflections in the range recorded, 1670 had intensities greater than twice their standard deviations and were used in the structure refinement. An empirical absorption correction based on the variation of intensity as a function of ϕ at $\chi = 90°$ was applied. The relative transmission factors ranged from 1.0 to 0.92. The standard geometrical corrections were applied to the data set, but no extinction or dispersion corrections were made. Normalized structure amplitudes $|E|$ were obtained using the Wilson plot method.

Structure Determination. The structure was solved using the multiple-solution tangent formula program, MULTAN.[23] The 149 normalized structure factors with $|E| > 1.6$ were employed in the solution. Three origin specifying reflections and one additional reflection led to two-phase sets obtained by application of the Sayre relationship. The E map calculated from the phase set with the highest figure of merit (1.11) and the lowest residual (17.1%) revealed all 16 of the nonhydrogen atoms in the molecule. A structure–factor calculation based on this starting model gave a discrepancy factor $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ equal to 0.35.

The atomic positional and thermal parameters were refined initially by block-diagonal least-squares procedures, with the final refinement being full matrix. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where unit weights were used initially and statistical weights, $w = 1/\sigma_F^2$, were used for final refinement. Atomic scattering factors for hydrogen[24] and other atoms[25] were taken from the literature. Computations were done with the XRAY system.[26] Several cycles of refinement on nonhydrogen atom positions and anisotropic thermal parameters, followed by computation of a difference Fourier map, enabled coordinates to be assigned to all 18 hydrogen atoms in the structure. A final cycle of least-squares refinement of all atom positions, anisotropic thermal parameters for heavy atoms, and fixed isotropic thermal parameters for the hydrogens resulted in a final R of 0.065 [omitting reflections with $I < 2\sigma(I)$]. R for the entire set of reflections was 0.081. The final atomic coordinates are listed in Table II. (See paragraph at end of paper regarding Supplementary Material.)

Results and Discussion

Figure 1 is a stereoscopic diagram showing the three-dimensional structure of MeCCNU. The molecule is in an extended, chain-like configuration with the cyclohexyl ring in the chair conformation. The trans substituents, the nitrosourea and the 4-methyl group, are both equatorial on the ring as predicted.[27] The nitrosourea moiety and the carbon atoms to which it is bonded, C(2) and C(4), form a nearly planar group (all atoms are within 0.17 Å of the mean plane). This plane forms a dihedral angle of 74° with the average plane through the six ring carbons. Within this central group of eight atoms, there are two four-atom groups which are more precisely planar. The

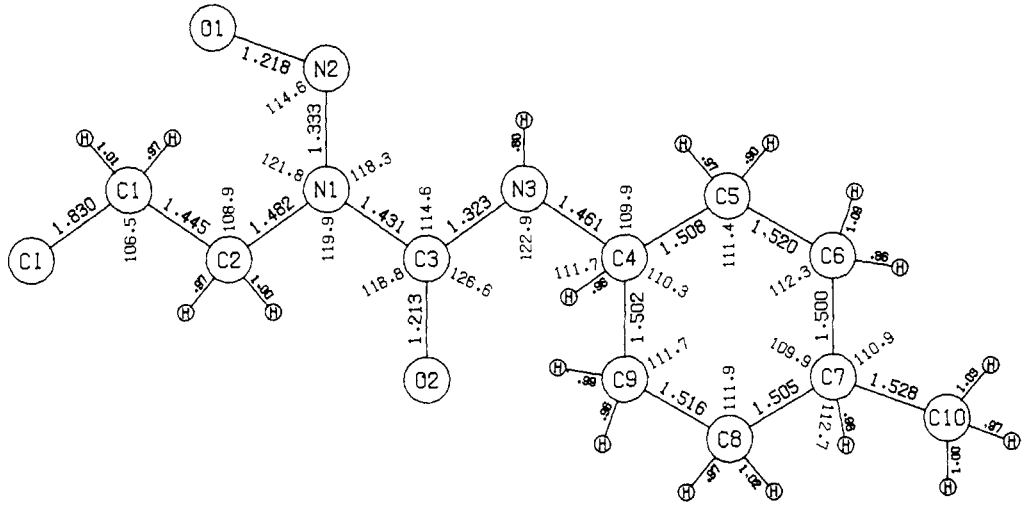

Figure 2. Bond lengths (Å) and selected bond angles (deg) in MeCCNU. Estimated standard deviations are 0.006 Å for "heavy" atom bonds and 0.03 Å for bonds involving hydrogens. Estimated standard deviations are 0.4° for the bond angles shown.

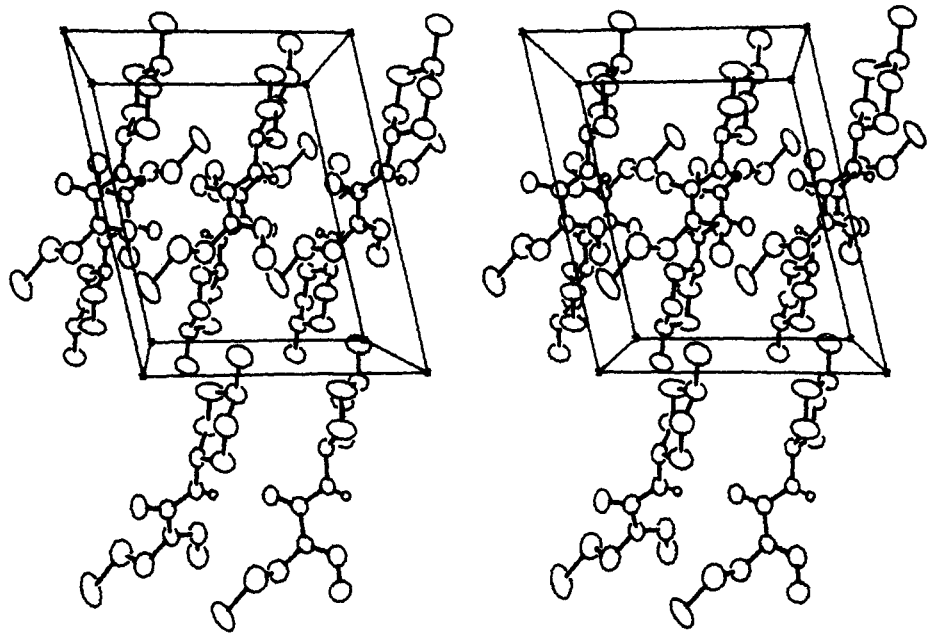

Figure 3. Stereoscopic diagram of the molecular packing. The origin is at the bottom left-hand corner with *b* pointing toward the viewer, *a* up, and *c* to the right. The only hydrogen atom shown forms a hydrogen bond.

plane of C(3) and the atoms bonded to it (maximum deviation = 0.003 Å) forms a dihedral angle of 13.0° with the plane of N(1) and the atoms bonded to it (maximum deviation = 0.008 Å). C(4) is displaced 0.16 Å from the four-atom plane to which it is adjacent, and similarly O(1) is displaced 0.06 Å from the N(1), N(2), C(2), C(3) plane. The plane of the chloroethyl group and the nitrogen to which it is attached, N(1) (maximum deviation = 0.03 Å), forms an 88° dihedral angle with the plane of the eight-atom nitrosourea fragment.

Bond lengths and angles are shown in Figure 2. The carbon–nitrogen bonds of the urea group are significantly asymmetric, with the C(3)–N(1) bond adjacent to the nitroso group lengthened to 1.431 Å compared to 1.323 Å for the C(3)–N(3) bond adjacent to the cyclohexyl ring. The shorter of these distances is in agreement with that expected (1.322 Å) for a partial double C–N bond in the N—C=O configuration.[28] The N(1)–N(2) distance of 1.333 (4) Å is not significantly greater than those found for similar bonds in studies of monomeric and dimeric nitroso compounds. A recent survey of nitroso dimers[29] found the N–N bond lengths to be in the range 1.304–1.321 Å. The two independent molecules of 2-(*N*-nitroso)methylaminoacetamide,[30] a monomeric nitroso compound, have N–N distances of 1.308 (3) and 1.328 (4) Å, which fall within the range found for the dimeric compounds. The N(2)–O(1) bond length, 1.218 (4) Å, is shorter than that found for the group of dimeric nitroso compounds, which have a range of 1.261–1.279 Å. It is in good agreement with the shorter of the two N–O bond lengths found in the nitroso monomer mentioned above, where the bond lengths were 1.237 (4) and 1.223 (3) Å. The N(1)–C(3) distance of 1.431 (4) Å is also in good agreement with the C–N distances ($C–N_{av}$ = 1.442 Å) of the nitroso monomer, while the C(2)–N(1) is a typical C–N single bond.[28] The other bond distances in MeCCNU are normal.

The spatial packing of the molecules is displayed in Figure 3. There is a relatively weak intermolecular hydrogen bond in the MeCCNU crystal structure between the N(3) hydrogen and the keto oxygen O(2) of the neighboring molecule related by the *c* glide. The H(N(3))···O(2) distance is 2.29 Å, the N···O distance is 3.03 Å, and the N–H···O angle is 155°. All other intermolecular contacts correspond to normal van der Waals distances.

Table II. Fractional Atomic Coordinates for MeCCNU[a]

	x	y	z
Cl	7477 (1)	1179 (1)	9182 (1)
O(1)	6593 (2)	592 (3)	4778 (2)
O(2)	4314 (2)	3004 (2)	6390 (2)
N(1)	5567 (2)	1868 (3)	5567 (3)
N(2)	5723 (3)	1159 (3)	4555 (3)
N(3)	3940 (2)	2542 (3)	4169 (2)
C(1)	6328 (4)	1011 (5)	7721 (5)
C(2)	6430 (4)	2012 (4)	6816 (4)
C(3)	4543 (3)	2522 (3)	5412 (3)
C(4)	2813 (3)	3031 (4)	3829 (3)
C(5)	2022 (3)	2031 (4)	3190 (5)
C(6)	844 (3)	2516 (4)	2784 (5)
C(7)	754 (3)	3615 (4)	1869 (4)
C(8)	1546 (3)	4608 (4)	2514 (4)
C(9)	2724 (3)	4132 (4)	2912 (4)
C(10)	−440 (4)	4071 (5)	1472 (5)
H(N)	418 (2)	224 (3)	358 (3)
H(1, 1)	561 (3)	99 (3)	804 (3)
H(1, 2)	635 (3)	23 (3)	726 (3)
H(2, 1)	720 (3)	204 (3)	679 (3)
H(2, 2)	640 (2)	282 (3)	730 (3)
H(4)	261 (2)	330 (3)	464 (3)
H(5, 1)	227 (3)	174 (3)	241 (3)
H(5, 2)	206 (3)	141 (3)	378 (3)
H(6, 1)	62 (3)	288 (3)	369 (3)
H(6, 2)	45 (3)	193 (3)	237 (3)
H(7)	98 (2)	332 (3)	108 (3)
H(8, 1)	128 (2)	483 (3)	337 (3)
H(8, 2)	150 (2)	530 (3)	190 (3)
H(9, 1)	319 (2)	478 (3)	338 (3)
H(9, 2)	302 (2)	387 (3)	213 (3)
H(10, 1)	−68 (3)	427 (3)	236 (3)
H(10, 2)	−47 (3)	480 (3)	91 (3)
H(10, 3)	−93 (3)	341 (3)	98 (3)

[a] The fractional coordinates have been multiplied by 10^4 for "heavy" atoms and 10^3 for hydrogen atoms.

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Supplementary Material Available: A listing of structure-factor amplitudes and of atomic thermal parameters (11 pages). Ordering information is given on any current masthead page.

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Benzomorphans. Structure of a Position Isomer

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May's benzomorphan synthesis leads not only to the α or cis isomer and the β or trans isomer but also to a position isomer hereinafter called the γ isomer. The structure and synthesis of this isomer are described. Biological activities of the α and γ isomers are compared.

In the benzomorphan synthesis developed by May and co-workers, a benzyl Grignard reagent is added to a pyridinium salt and the resulting dihydropyridine reduced and cyclized to give a benzomorphan.[1]

In their initial work May and Fry cyclized about 0.02 mol of tetrahydropyridine to obtain **1a** as the sole isolated

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