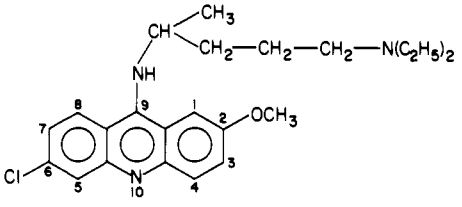
Tautomerism and Steric Effects in 1-Nitro-9-(alkylamino)acridines (Ledakrin or Nitracrine Analogues): Probing Structure–Activity Relationships at the Molecular Level

John J. Stezowski,*† Petra Kollat,† Maria Bogucka-Ledóchowska,‡ and Jenny P. Glusker*§

Contribution from the Institut für Organische Chemie, Biochemie und Isotopenforschung der Universität Stuttgart, 7000 Stuttgart 80, Federal Republic of Germany, the Department of Pharmaceutical Technology and Biochemistry, The Technical University, 80-952 Gdańsk, Poland, and The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111. *Received July 23, 1984*

Abstract: The steric hindrance that results from 1-nitro substitution in 9-aminoacridine derivatives has been investigated. Crystal structures for four derivatives of ledakrin, 9-[(3-(dimethylamino)propyl)imino]-1-nitro-9,10-dihydroacridine (C-283), have been determined, two as free bases and two as hydrochloride salts. The compounds studied are 9-[(5-(dimethylamino)pentyl)imino]-1-nitro-9,10-dihydroacridine (C-309), 9-[(3-(methylamino)propyl)imino]-1-nitro-9,10-dihydroacridine (C-849), 9-[(2-hydroxyethyl)imino]-1-nitro-9,10-dihydroacridine (C-857), and 9-[(3-carboxypropyl)imino]-1-nitro-9,10-dihydroacridine (C-921). There are strong steric interactions between the nitro group at C(1) and the side chain at C(9) which cause rotation of the nitro group and distortion of the side chain at C(9) out of the plane of the acridine ring system. In the free bases, the nitro group is tilted with a torsion angle of approximately 60° to atoms in the acridine ring; in salts this angle is decreased to approximately 34°. The combination of these steric effects and the presence of an alkyl side chain on the nitrogen atom of C(9) result in a preference for imino (rather than amino) tautomer formation for the free bases; in these the C(9)–N(9) bond is a double bond. In the salts studied, N(9) is protonated and forms intermolecular hydrogen bonds; as a result the side chain orientation is opposite of that found in the free bases. By contrast in ledakrin (C-283), with a strong base in the side chain, protonation occurs at the end of the side chain and the imino tautomer is favored. The variations of tautomeric and protonated forms in the solid state are listed for the 1-nitro-9-aminoacridines studied to date. It is suggested that the steric problem described persists through metabolic activation of the ledakrin derivative, followed by intercalation of the product in DNA; this then provides a suitable mechanism for cross-linking DNA.

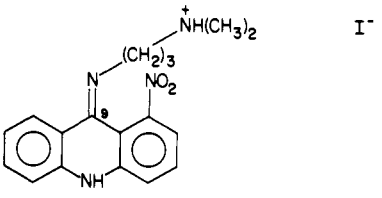
The specificity of acridines for tissues has long been known, and various derivatives have been found to display biological activity.[1] For example, a family of 9-(alkylamino)acridines derived from quinacrine, I (such as the ICR compounds ICR 191 and ICR 170), have been effective mutagens and mild antitumor agents,[1-18] some also showing carcinogenic activity. The aromatic



I

acridine groups are approximately planar and the side chains at C(9) extend from this generally in the plane of the ring system if a methylene group is bonded to N(9) and perpendicular to the plane if this methylene group is alkylated.

A second family of 9-(alkylamino)acridines, containing a 1-nitro substituent on the acridine nucleus, has attracted considerable recent interest because ledakrin, II (WHO name nitracrine), has enhanced antitumor activity and has been successfully used as an antitumor agent in humans in Poland.[9-11] The introduction of the nitro group causes considerable steric overcrowding, and the nature and results of this overcrowding are the subject of this article. Crystal structure determinations for ledakrin (9-[(3-(dimethylamino)propyl)imino]-1-nitro-9,10-dihydroacridine)[12] (C-283), for 9-[(3-(dimethyloxyamino)propyl)imino]-1-nitro-9,10-dihydroacridine[13] (C-684) (III), for 9-[(3-(dimethylamino)propyl)amino]-2-nitroacridine[14,15] (C-264)(IV), for 9-



C-283

II

[(2-(dimethylamino)-1-methylethyl)imino]-1-nitro-9,10-dihydroacridine[16] (C-829) (V), and for the analogues 1-nitro-9-

† Institut für Organische Chemie.
‡ The Technical University.
§ The Institute for Cancer Research.

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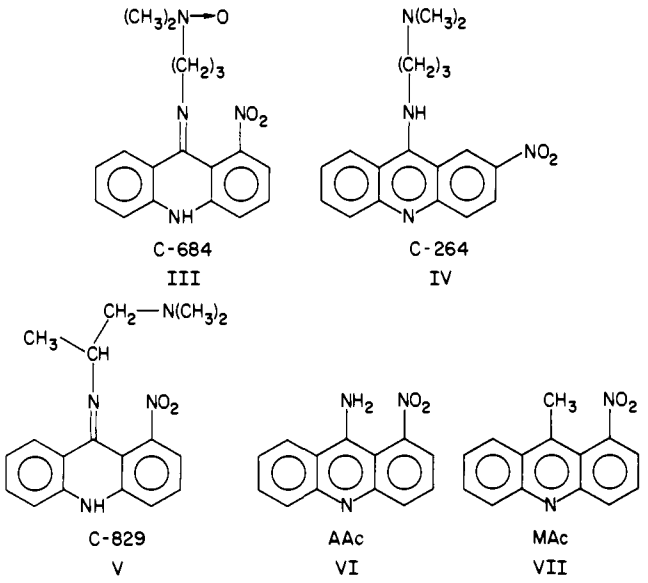
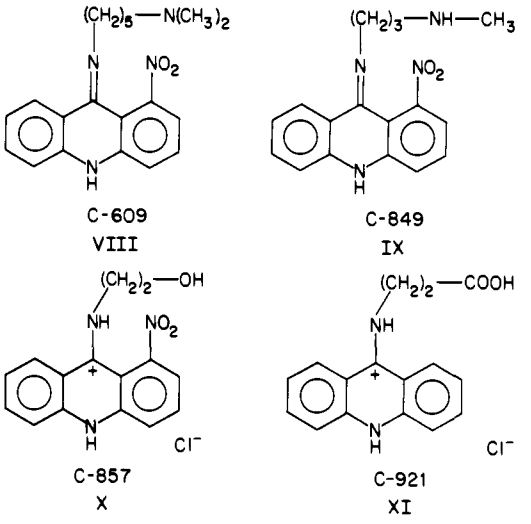
aminoacridine[17] and 1-nitro-9-methylacridine (AAc (VI) and MAc (VII), respectively) have already been reported in the literature.



Of these, the 1-nitroacridines, C-283, C-684, and C-829, are known to be active antitumor agents whereas the 2-nitroacridine, C-264, is inactive.

The antitumor activity of the 1-nitro-9-(alkylamino)acridines is believed to result from the ability of activated metabolites to cross-link DNA and induce DNA single-strand breaks; the compounds themseleves do not have this ability. 2-, 3-, and 4-Nitro-9-(alkylamino)acridines can intercalate in DNA but do not cross-link it in vivo.[18-24] In an earlier report,[16] we used computer graphics techniques to demonstrate that intercalation of C-829, V, into a dinucleoside phosphate would present a stereochemically reasonable opportunity for cross-linking of the two strands of DNA by activated sites of the ledakrin analogue. Such interactions involved nitrogen atoms bonded directly to the acridine ring system (positions 1 and 9), the common feature of the biologically active compounds. In a similar way, a covalent binding to one DNA strand may facilitate single-strand breaks. Although it is not known which effect is more important,[18] there is evidence that both cytotoxic activity in vitro and antitumor activity in vivo are correlated with DNA cross-linking potency.[19]

In an effort to better understand the interrelationships between chemical structure and biological activity of the 1-nitro-9-(alkylamino)acridines, we have analyzed the crystal structures of an additional four members of this family. The compounds in question differ from those described above in the nature of the 9-(aminoalkyl) side chain; included are an example of an extended side chain [9-[(5-(dimethylamino)pentyl)imino]-1-nitro-9,10-dihydroacridine (C-609)] (VIII), an example with a secondary amine at the chain end [9-[(3-(methylamino)propyl)imino]-1-nitro-9,10-dihydroacridine (C-849)] (IX), an example of an alcohol substituent [9-[(2-hydroxyethyl)imino]-1-nitro-9,10-dihydroacridine (C-857)] (X), and its carboxylate analogue [9-[(3-carboxypropyl)imino]-1-nitro-9,10-dihydroacridine (C-921)] (XI). Two of these (VIII and IX) were studied as free bases and two as hydrochloride salts (X and XI). We have examined these four structures with the following chemical and biochemical questions in mind;



(1) What chemical (i.e., electronic *and* steric) effects are found in 1-nitro compounds but not in 2-, 3-, or 4-nitro compounds (since only the 1-nitro compounds are active antitumor agents)?

(2) What are the possible tautomeric forms of the free base and salts; under what conditions does each occur and why?

(3) What influences the degree of folding of the acridine about the C(9)-N(10) vector?

(4) What is the geometry of any hydrogen bonding?

(5) What are the configurations of the side chains, and what are the effects of intermolecular interactions on conformation?

(6) Can any of these effects be correlated with antitumor activity?

Experimental Section

Structure Determinations. The four compounds studied here were recrystallized to give transparent yellow crystals. Crystals of the free bases contained one molecule of water per 1-nitro-9-(alkylamino)acridine; those of the acid salts were anhydrous. Crystal data are presented in Table I. X-ray data were measured with a Syntex $P1$ four circle diffractometer (monochromatized Cu Kα radiation) using an ω-scan technique. Three reference reflections were measured every 200 reflections. Reflections with $I \geq 3\sigma I$ (where σI was determined from counting statistics) were classified as observed. Data were corrected for Lorentz and polarization factors but not absorption.

The initial structure determinations were obtained by direct methods by using MULTAN[25] and developed with the XRAY version 1976 program library.[26] The atomic positions, anisotropic thermal parameters of non-hydrogen atoms, and isotropic temperature factors for hydrogen atoms were refined by a variable-block diagonal least-squares technique. The quantity minimized was $\sum w[|F_o| - |F_c|]^2$, and corrections for anomalous scattering were applied for chloride ions.[27] The final R values are listed in Table I.

Biological Data. Pawlak et al.[19] have reported estimated therapeutic effectivenesses, TE, for a number of 1-nitroacridines. Those described here can be ranked as follows: C-283 ≥ C-857 > C-849 ≫ C-921.

Data on the effectiveness of each compound studied have been reported in the literature as follows:

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Table I

Compound	C-609 (VIII)	C-849 (IX)	C-857 (X)	C-921 (XI)
CA Registry No.	56366-74-6		77280-93-4	75106-56-8
side chain on 9	$=N(CH_2)_5N(CH_3)_2$	$=N(CH_2)_3—NH·CH_3$	$—NH(CH_2)_2OH$	$—NH(CH_2)_2COOH$
nucleus	1-nitroacridine	1-nitroacridine	1-nitroacridine	1-nitroacridine
empirical formula	$C_{20}H_{24}N_4O_2·H_2O$	$C_{17}H_{18}N_4O_2·H_2O$	$C_{15}H_{13}N_3O_3·HCl$	$C_{16}H_{13}N_3O_4·HCl$
fw	370.453	328.372	319.748	347.758
space group	$P2_1/c$	$P\bar{1}$	$P2_1/n$	$Pbca$
Z	4	4	4	8
a, Å	8.446 (1)	11.0684 (4)	8.3393 (4)	8.1847 (7)
b, Å	11.475 (1)	11.4679 (4)	20.7131 (9)	16.834 (2)
c, Å	20.702 (3)	14.9652 (6)	8.9282 (3)	22.667 (2)
α, deg		90.228 (3)		
β, deg	97.475 (8)	115.806 (3)	112.862 (3)	90.000
γ, deg		77.750 (4)		
V	1989.22	1662.70	1421.04	3123.16
D_x, g·cm^{-3}	1.24	1.31	1.49	1.48
crystal size (mm)	1.10 × 0.40 × 0.01	0.30 × 0.35 × 0.30	0.20 × 0.30 × 0.50	0.60 × 0.60 × 0.01
temp, °C	24 (1)	24 (1)	24 (1)	24 (1)
R value	0.052	0.036	0.049	0.060
weighted R value	0.072	0.051	0.080	0.080
no. of contributing reflections[a]	3120	4515	2434	2425
no. of unique data	3533	5859	2532	2773
no. of obsd data	2677	3494	2373	2057

[a] Those reflections classified as observed, for which the calculated intensity was greater than the cut-off value, contributed to the refinement.

C-609 has not undergone clinical tests but preclinical and pharmacological examinations have been reported.[28-33] No TE value was determined[19] but C-609 appears to be one of the more active analogues, especially with respect to its DNA cross-linking potency. Its activity is generally similar to that of ledakrin (nitracrine).

C-849. Preliminary studies in vivo (mice) and in vitro have shown that this compound is an active antitumor agent[19] but at considerably higher doses than C-283. Their respective TE values are 0.9 and 1.5.

C-857 is one of the most active and least toxic analogues (TE = 1.3) thus far studied.[19,34-37] It is now undergoing clinical tests. It has the advantage than it does not produce digestive system upsets (in experiments with mice).

C-921. In vitro and in vivo tests[37,38] demonstrate that this compound is an active antitumor agent but that it is much less effective (TE = 0.5) than ledakrin (C-283).

Results

Fractional atomic coordinates for the four structures are listed in Table II; stereoscopic projections[39] illustrating the conformations of the molecules and molecular cations studied here are presented in Figure 1. There are two molecules in the asymmetric unit of C-849 (IX), and these are designated nonprimed (or a) and primed (or b) (see Table II).

Geometrical data (bond lengths, bond angles, and torsion angles), averaged to reflect chemical equivalence (neutral molecules separately from cations), are presented in Figure 2. Major differences between the free base and cation are found for distances for C(9)–N(9), C(9)–C(1a), C(9)–C(8a) and C(2)–C(3); one large difference is a shortening of C(9)–N(9) in the free base. The angles vary considerably in the area of steric overcrowding, i.e., C(1)–C(1a)–C(9), C(1a)–C(9)–N(9), C(8a)–C(9)–N(9), and C(9)–N(9)–C; the main effect is an angular change that pushes N(9) further from the nitro group in salts than in free bases. As a result, the torsion angles about the nitro group are lower for cations than free bases, as shown in Table III. Also the direction of the side chain (torsion angle C(1a)–C(9)–N(9)–C) is opposite in cations and free bases. This presumably reflects the location of a proton on N(9) of the salts.

Least-squares planes for the acridine moiety are listed in Table IV, which shows that the angle between the two outer rings is large (13–21°). In each structure the central ring (B) is the least planar, and in each case the angle between the outer rings is the greatest.

The hydrogen bonding in each compound is described in Table V. Each proton on N(10) takes part in a hydrogen bond to water (free base) or a chloride ion (cation). If there is a proton on N(9), as in the salts, then this proton takes part in a hydrogen bond to the chloride ion. The functional group at the end of the side chain also takes part in hydrogen bonding whenever possible.

Discussion

Tautomerism and Amphoterism. The 1-nitro-9-(alkylamino)-acridines are a family of compounds capable of adopting more than one tautomeric structure. In Figure 3a, possible tautomers of free bases and monocations (XIIa–d) are illustrated; examples of three of the four types shown here have been observed in the crystalline state. Structures XIIa and c are amino tautomers of the free bases and salt (protonated at N(10)), respectively; XIIb and d are the corresponding imino tautomers. The crystal structure determinations of 1-nitro-9-aminoacridines carried out here and of those reported earlier[12-17] can be assigned to one of the structures depicted in this figure. In addition, depending upon the nature of the substituents in the side chain, the 1-nitro-9-(alkylamino)acridines are also able to adopt a number of ionization states (amphoterism); thus the alkyl group R in Figure 3 may be positively or negatively charged or neutral.

As Table I shows, we have studied two examples of monovalent cation salts (both anhydrous), C-857 and C-921, both as hydrochlorides and two of free bases (both monohydrates). The structure XIIb has been observed for the free base analogue while structure XIIc has been found for the monovalent cations; the structure XIIa is found for C-264, the inactive 2-nitro compound. No examples of the structure XIId have yet been found in this series of compounds, possibly an indication that this is a less likely tautomeric form.

Steric and Electronic Effects on Tautomerism. There is crystallographic evidence that electronic effects and steric effects operate in a synergistic manner to determine the selection of the

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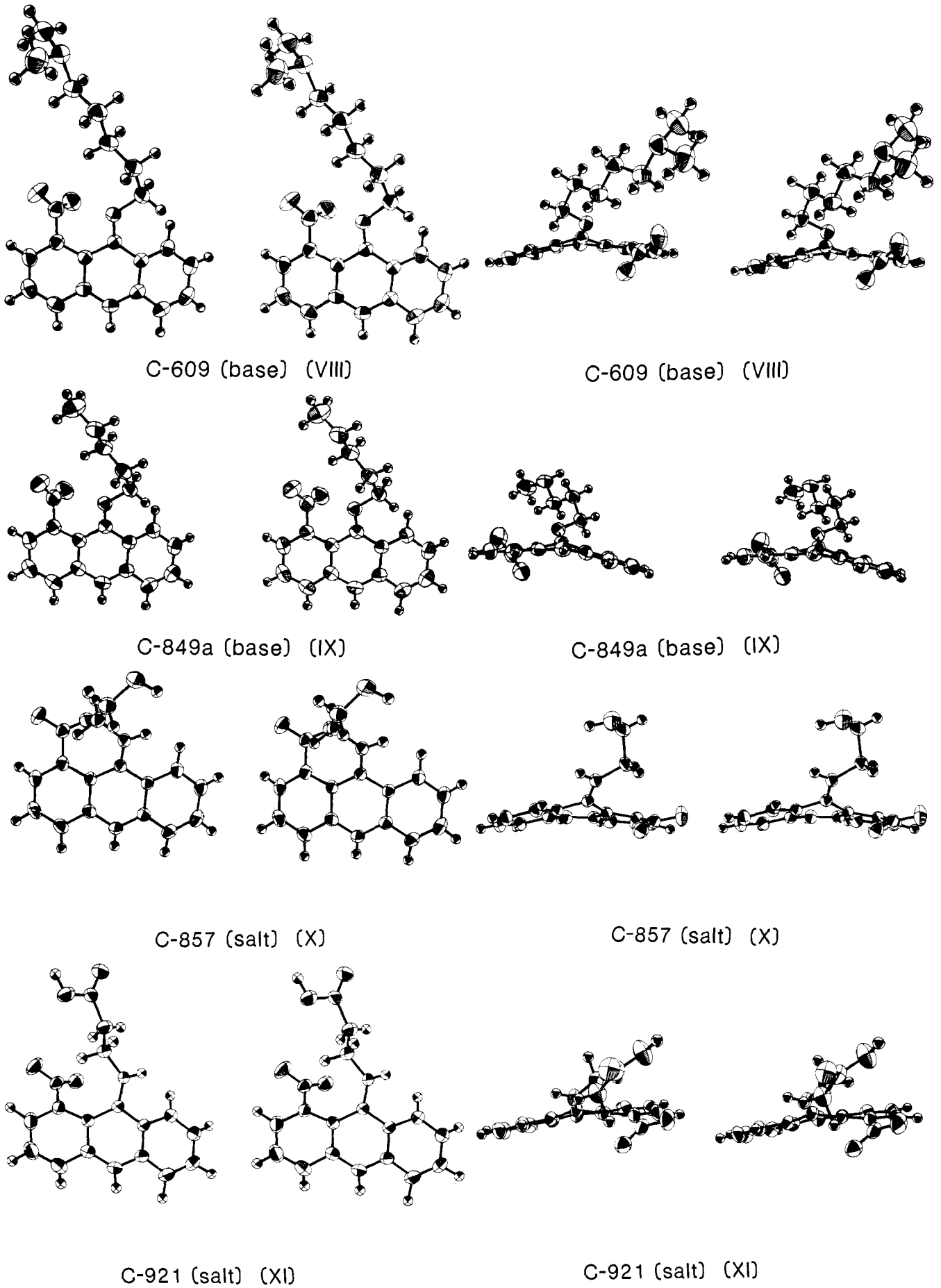

Figure 1. Stereoscopic projections illustrating[39] the conformation of each 1-nitro-9-(alkylamino)acridine analogue observed in the crystal structure determinations for C-609 and C-849 free bases (only C-849a is illustrated) and for C-857 and C-921 hydrochloride salts.

Table II. Positional and average temperature factors for (a) C-609 (VIII) (b) C-849 (IX), (c) C-857 (X), and (d) C-921 (XI)[a]

atom	*x*	*y*	*z*	*U*	atom	*x*	*y*	*z*	*U*
		(a) C-609 (VIII)					(b) C-849 (IX) (continued)		
N(1)	0.1951 (2)	0.7211 (2)	0.09584 (8)	0.059	C(8)′	1.1745 (2)	0.3066 (2)	0.5142 (1)	0.056
O(1)	0.2366 (2)	0.7773 (2)	0.05111 (7)	0.094	C(8a)′	1.2540 (2)	0.3663 (1)	0.4881 (1)	0.046
O(2)	0.1705 (2)	0.6156 (2)	0.09355 (8)	0.074	C(1a)′	1.2789 (2)	0.5510 (1)	0.4191 (1)	0.045
C(1)	0.1621 (2)	0.7842 (2)	0.15415 (9)	0.048	C(4a)″	1.3705 (2)	0.4786 (2)	0.3881 (1)	0.049
C(2)	0.0556 (2)	0.8755 (2)	0.1441 (1)	0.058	N(10)′	1.4133 (2)	0.3572 (1)	0.4168 (1)	0.056
C(3)	0.0087 (3)	0.9308 (2)	0.1977 (1)	0.064	C(9)′	1.2457 (2)	0.4969 (1)	0.4930 (1)	0.044
C(4)	0.0653 (2)	0.8959 (2)	0.2594 (1)	0.057	N(9)′	1.2250 (1)	0.5639 (1)	0.5563 (1)	0.050
C(5a)	0.3066 (2)	0.6604 (2)	0.34300 (8)	0.045	C(11)′	1.2123 (2)	0.5143 (2)	0.6409 (1)	0.055
C(5)	0.3162 (3)	0.6100 (2)	0.40484 (9)	0.057	C(12)′	1.2656 (2)	0.5857 (2)	0.7303 (1)	0.056
C(6)	0.3849 (3)	0.5015 (2)	0.4155 (1)	0.061	C(13)′	1.1788 (2)	0.7103 (2)	0.7213 (2)	0.067
C(7)	0.4460 (3)	0.4419 (2)	0.3659 (1)	0.058	N(14)′	1.1899 (2)	0.7957 (1)	0.6568 (1)	0.065
C(8)	0.4397 (2)	0.4926 (2)	0.30546 (9)	0.050	C(15)′	1.1122 (3)	0.9183 (2)	0.6568 (3)	0.086
C(8a)	0.3719 (2)	0.6039 (2)	0.29249 (8)	0.043	O(W1)	0.5456 (2)	0.2003 (1)	0.3118 (1)	0.068
C(1a)	0.2287 (2)	0.7463 (2)	0.21621 (8)	0.043	O(W2)	0.3722 (3)	0.3025 (2)	0.1214 (2)	0.096
C(4a)	0.1749 (2)	0.8028 (2)	0.26959 (8)	0.046					
N(10)	0.2302 (2)	0.7669 (1)	0.33148 (8)	0.052			(c) C-857 (X)		
C(9)	0.3632 (2)	0.6632 (2)	0.22873 (8)	0.042	Cl	0.82372 (7)	0.60824 (2)	0.40250 (5)	0.043
N(9)	0.4578 (2)	0.6585 (1)	0.18468 (7)	0.047	N(1)	0.2737 (2)	0.29893 (8)	0.3146 (2)	0.042
C(11)	0.6043 (2)	0.5893 (2)	0.1918 (1)	0.054	O(1)	0.3021 (2)	0.24421 (8)	0.3720 (2)	0.061
C(12)	0.7144 (2)	0.6380 (2)	0.1460 (1)	0.058	O(2)	0.1305 (2)	0.31784 (7)	0.2224 (2)	0.047
C(13)	0.6473 (3)	0.6257 (2)	0.0747 (1)	0.058	C(1)	0.4196 (2)	0.34438 (8)	0.3613 (2)	0.036
C(14)	0.7541 (3)	0.6773 (2)	0.0284 (1)	0.059	C(2)	0.5428 (3)	0.3377 (1)	0.5157 (2)	0.043
C(15)	0.6825 (3)	0.6651 (2)	−0.0418 (1)	0.059	C(3)	0.6761 (3)	0.3835 (1)	0.5734 (3)	0.047
N(16)	0.7861 (2)	0.7060 (2)	−0.08901 (8)	0.059	C(4)	0.6737 (3)	0.4376 (1)	0.4849 (2)	0.046
C(16)	0.7191 (4)	0.6712 (3)	−0.1548 (1)	0.079	C(5a)	0.3875 (2)	0.51887 (8)	0.1151 (2)	0.037
C(17)	0.8074 (5)	0.8321 (3)	−0.0858 (2)	0.088	C(5)	0.3601 (3)	0.58261 (9)	0.0567 (3)	0.046
O(W1)	0.9064 (2)	0.3833 (2)	0.06994 (8)	0.065	C(6)	0.2207 (3)	0.5974 (1)	−0.0814 (3)	0.054
					C(7)	0.1019 (3)	0.5495 (1)	−0.1679 (3)	0.047
		(b) C-849 (IX)			C(8)	0.1276 (3)	0.48670 (9)	−0.1146 (2)	0.041
N(1)	1.1623 (2)	0.1786 (2)	0.8975 (1)	0.077	C(8a)	0.2709 (2)	0.46980 (8)	0.0276 (2)	0.034
O(1)	1.2886 (2)	0.1592 (2)	0.9396 (2)	0.109	C(1a)	0.4167 (2)	0.39654 (8)	0.2576 (2)	0.033
O(2)	1.0971 (2)	0.1042 (1)	0.8586 (1)	0.093	C(4a)	0.5386 (2)	0.44641 (9)	0.3308 (2)	0.037
C(1)	1.0820 (2)	0.3023 (2)	0.8881 (1)	0.059	N(10)	0.5270 (2)	0.50368 (8)	0.2524 (2)	0.039
C(2)	1.1017 (2)	0.3869 (2)	0.8353 (2)	0.074	C(9)	0.3124 (2)	0.40384 (8)	0.0843 (2)	0.032
C(3)	1.0166 (3)	0.5016 (2)	0.8147 (2)	0.076	N(9)	0.2659 (2)	0.35675 (7)	−0.0219 (2)	0.033
C(4)	0.9149 (2)	0.5271 (2)	0.8447 (1)	0.062	C(11)	0.3133 (3)	0.28827 (8)	0.0068 (2)	0.040
C(5a)	0.7513 (2)	0.3759 (2)	0.9609 (1)	0.052	C(12)	0.3532 (3)	0.25823 (9)	−0.1297 (2)	0.045
C(5)	0.6218 (2)	0.3999 (2)	0.9611 (2)	0.065	O(12)	0.2051 (2)	0.25234 (8)	−0.2747 (2)	0.056
C(6)	0.5766 (2)	0.3100 (2)	0.9886 (2)	0.074					
C(7)	0.6590 (2)	0.1940 (2)	1.0168 (2)	0.070			(d) C-921 (XI)		
C(8)	0.7877 (2)	0.1696 (2)	1.0192 (1)	0.057	Cl	0.9306 (1)	0.95507 (4)	0.16779 (3)	0.048
C(8a)	0.8384 (2)	0.2593 (2)	0.9933 (1)	0.049	N(1)	0.2623 (4)	0.7780 (2)	0.4517 (1)	0.049
C(1a)	0.9829 (2)	0.3239 (2)	0.9244 (1)	0.050	O(1)	0.3748 (3)	0.7404 (1)	0.4300 (1)	0.056
C(4a)	0.8958 (2)	0.4388 (2)	0.8986 (1)	0.052	O(2)	0.2445 (4)	0.7873 (2)	0.5052 (1)	0.072
N(10)	0.7917 (2)	0.4644 (1)	0.9273 (1)	0.056	C(1)	0.1467 (4)	0.8161 (2)	0.4114 (1)	0.042
C(9)	0.9762 (2)	0.2401 (1)	0.9958 (1)	0.048	C(2)	0.0891 (5)	0.8889 (2)	0.4285 (1)	0.056
N(9)	1.0898 (2)	0.1667 (1)	1.0543 (1)	0.053	C(3)	0.0058 (5)	0.9360 (2)	0.3866 (2)	0.059
C(11)	1.0998 (2)	0.0902 (2)	1.1363 (1)	0.057	C(4)	−0.0056 (5)	0.9125 (2)	0.3299 (2)	0.056
C(12)	1.2466 (2)	0.0647 (2)	1.2191 (1)	0.056	C(5a)	0.1207 (4)	0.7509 (2)	0.2319 (1)	0.042
C(13)	1.3487 (2)	−0.0107 (2)	1.1883 (1)	0.056	C(5)	0.1308 (5)	0.7387 (2)	0.1709 (1)	0.053
N(14)	1.4921 (2)	−0.0292 (2)	1.2649 (1)	0.061	C(6)	0.1939 (5)	0.6697 (2)	0.1496 (1)	0.057
C(15)	1.5880 (3)	−0.0958 (3)	1.2291 (2)	0.087	C(7)	0.2514 (5)	0.6109 (2)	0.1880 (1)	0.052
N(1)′	1.1299 (2)	0.7532 (1)	0.4023 (1)	0.068	C(8)	0.2406 (4)	0.6214 (2)	0.2474 (1)	0.044
O(1)′	1.1565 (2)	0.8467 (1)	0.4379 (1)	0.108	C(8a)	0.1727 (4)	0.6915 (2)	0.2719 (1)	0.037
O(2)′	1.0209 (2)	0.7256 (1)	0.3789 (1)	0.085	C(1a)	0.1233 (4)	0.7842 (1)	0.3541 (1)	0.039
C(1)′	1.2367 (2)	0.6723 (2)	0.3831 (1)	0.053	C(4a)	0.0572 (4)	0.8383 (2)	0.3119 (1)	0.043
C(2)′	1.2832 (2)	0.7225 (2)	0.3251 (1)	0.067	N(10)	0.0512 (3)	0.8188 (1)	0.2538 (1)	0.047
C(3)′	1.3771 (2)	0.6490 (2)	0.2990 (1)	0.069	C(9)	0.1478 (4)	0.7032 (2)	0.3340 (1)	0.037
C(4)′	1.4194 (2)	0.5292 (2)	0.3294 (1)	0.061	N(9)	0.1378 (4)	0.6392 (1)	0.36767 (9)	0.041
C(5a)′	1.3456 (2)	0.2999 (2)	0.4547 (1)	0.048	C(11)	0.0847 (4)	0.6284 (2)	0.4286 (1)	0.042
C(5)′	1.3673 (2)	0.1746 (2)	0.4585 (1)	0.059	C(12)	0.2140 (5)	0.5832 (2)	0.4628 (1)	0.053
C(6)′	1.2900 (2)	0.1180 (2)	0.4867 (2)	0.066	C(13)	0.1628 (4)	0.5530 (2)	0.5219 (1)	0.044
C(7)′	1.1913 (2)	0.1835 (2)	0.5129 (2)	0.066	O(13)	0.2177 (3)	0.4928 (1)	0.5435 (1)	0.057
					O(14)	0.0524 (4)	0.5986 (1)	0.5486 (1)	0.065

[a] *x*, *y*, and *z* are listed as fractions of cell edges. The temperature factor has the form exp(−T), where $T = 8\pi^2 U \sin^2\theta/\lambda^2$. The estimated standard deviation of the last significant digit is given in parentheses.

imino tautomer (XIIb) for the free base. The importance of the steric effect is indicated by the observation that C-264 free base (IV), which has no steric constraints and is approximately planar, is found as the amino tautomeric form XIIa; the importance of the electronic effect (the presence of an electron-donating substituent on the nitrogen atom bonded to C(9)) is indicated by the structure for AAc (VI) with only two hydrogen atoms, no alkyl group, on N(9); it is found as tautomer XIIa, again an amino tautomer even though steric effects have caused rotation of the nitro group (see Table III). All free bases of 9-(alkylamino)-acridines with no 1-nitro substituents (i.e., no steric problems) are found as the amino tautomer XIIa.[4-8] In contrast, all free base analogues possessing both 9-(alkylamino) and 1-nitro substituents (i.e., with steric problems) are found as the imino tautomer XIIb in their crystal structures. Thus steric problems (between N(9) and the 1-nitro group) are not sufficient to cause

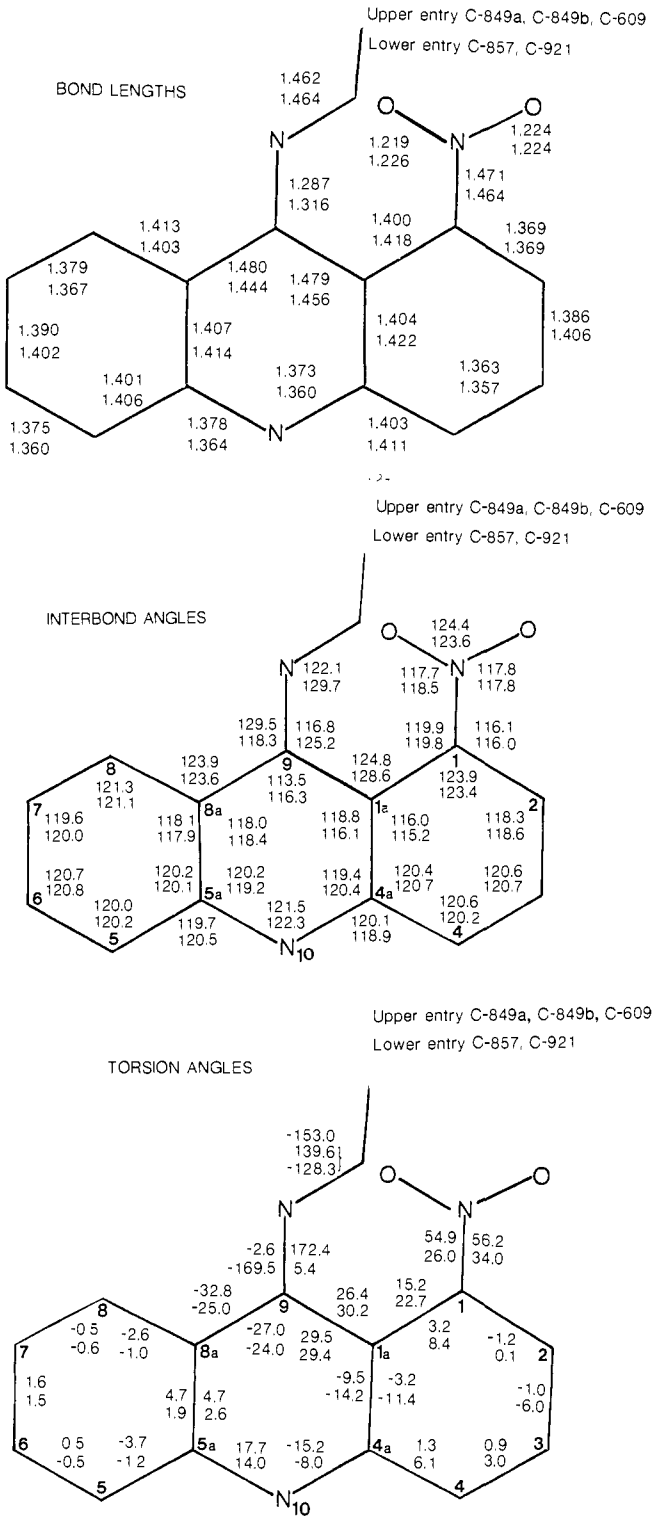

Figure 2. Bonding geometry averaged to reflect chemical equivalence. The lower values are for the hydrochloride salts (C-857 and C-921) and the upper ones for the free bases (C-609 and C-849): (a) bond distances (estimated standard deviations <0.006 Å), (b) bond angles (esds <0.2°), and (c) torsion angles (esd values <0.2°).

formation of tautomer XIIb; the additional effect of an alkyl group on N(9) is required.

In the case of salts, the influence of the nature of the substituent at the end of the alkylamino side chain on the tautomeric form adopted by the monovalent cation is also evident from the crystal structure determinations. The presence of a strong base, e.g., a dimethylamino group as in C-283, favors protonation at the end of the side chain and formation of imino tautomer XIIb, whereas the presence of an alcohol or carboxyl substituent results in the formation of the amino tautomer XIIc with the positive charge associated with the acridine ring system (formally an acridinium ion). In the divalent cation of C-283 (Ledóchowska, M., unpublished results), N(9) is also protonated to give the amino tautomer.

Conformational Characteristics of Tautomers. There are now enough crystal structure determinations of 1-nitro-9-aminoacridines to suggest that there are interrelationships between the conformation of the acridine ring system and the tautomeric form adopted (see Figure 3). For example, for tautomer XIIa, the acridine ring system is approximately planar, whereas it is distinctly nonplanar for tautomers XIIb and c (no crystallographic evidence is available to date for the other tautomer, XIId). A comparison of the various structures is given in Figure 4.

The orientation of the side chain, as illustrated by the C-(1a)–C(9)–N(9)–C torsion angles (Table III and Figures 1 and 2) also appears to be correlated with the tautomeric form. The imino tautomers, XIIb (C-609, C-849, C-684, C-829, and C-283), have values for this torsion angle near 180° whereas the amino tautomers, XIIc (C-857, C-921), have values near 0°. This may reflect the tendency for the proton on N(9) in XIIc to be involved in intermolecular hydrogen bonding (rather than intramolecular hydrogen bonding to the 1-nitro group); if no proton is found on N(9) as in the free bases, then the lone pair on N(9) points toward the nearest oxygen atom of the nitro group. However, recent studies on a divalent cation of C-283 (Ledóchowska, M., unpublished results) show that the torsion angle can also be at 180° for an amino tautomer (see Table III and Figure 3b).

The torsion angles that describe the orientation of the nitro group [C(1a)–C(1)–N(1)–O(1,2)] are also dramatically affected by the tautomeric form; they are near 60° for the imino tautomer, XIIb, and near 30° for the amino tautomer, XIIc, as shown in Table III. In Table IV deviations of atoms from the plane through C(1a), C(4a), C(5a), and C(8a) (illustrated in Figure 5) are shown. In the free bases N(1) is less distorted from this plane than in the salts since the torsion of the nitro group has relieved steric strain. In spite of these differences in torsion angle, the geometry of the nitro group (shown in Figure 2) does not vary significantly as a function of torsion angle.

There are also marked differences in the bond distances and angles involving C(9) and N(11) in the imino and amino tautomers. As expected, the imino tautomer has a significantly shorter C–N bond distance, indicative of increased double bond character. However the alkyl N(9)–C bond distance appears to be insensitive to the tautomeric form adopted. There are also dramatic differences in the bond angles associated with atom C(9) and, to a lesser extent, with atom N(9). Although electronic effects, such as the interaction of the lone-pair electrons of the imino nitrogen atoms with the nitro group, may occur, steric effects appear to be largely responsible for these differences. As shown in Figure 2 the exocyclic C–C(9)–N(9) angle is greater on the side of the C(9)–N(9) bond that is cis to the N(9)-alkyl carbon atom. Similarly the C(9)–N(9)–C angle is larger when the alkyl group is oriented toward the bulkier nitro group (in the salt) than when it is oriented toward a hydrogen atom of an acridine aromatic ring (see Figure 4). In addition, as shown in the torsion angles illustrated in Figure 2, there are considerable distortions in the central ring (B) of the acridine, and these can force N(9) out of the best plane through this ring and, in so doing, affect the side chain orientation, as shown in Figure 5.

The conformation of the acridine ring system is clearly influenced by the peri effects involving the substituents at C(1) and C(9) but does not appear to be sensitive to the nature of the tautomeric form adopted. The overall conformation is that of a butterfly with the fold along the C(9)...N(10) line. The angles between least-squares mean planes fit to each six-membered ring (Table IV) are similar for examples of the amino and imino tautomers. The one example, C-857, that differs modestly from the others is explainable on the basis of steric interactions between hydrogen atoms of the methylene group bonded to N(9) and the atoms of the NO_2 group; see Figure 1. The side chain orientation in C-857 places a hydrogen atom of this CH_2 moiety nearly

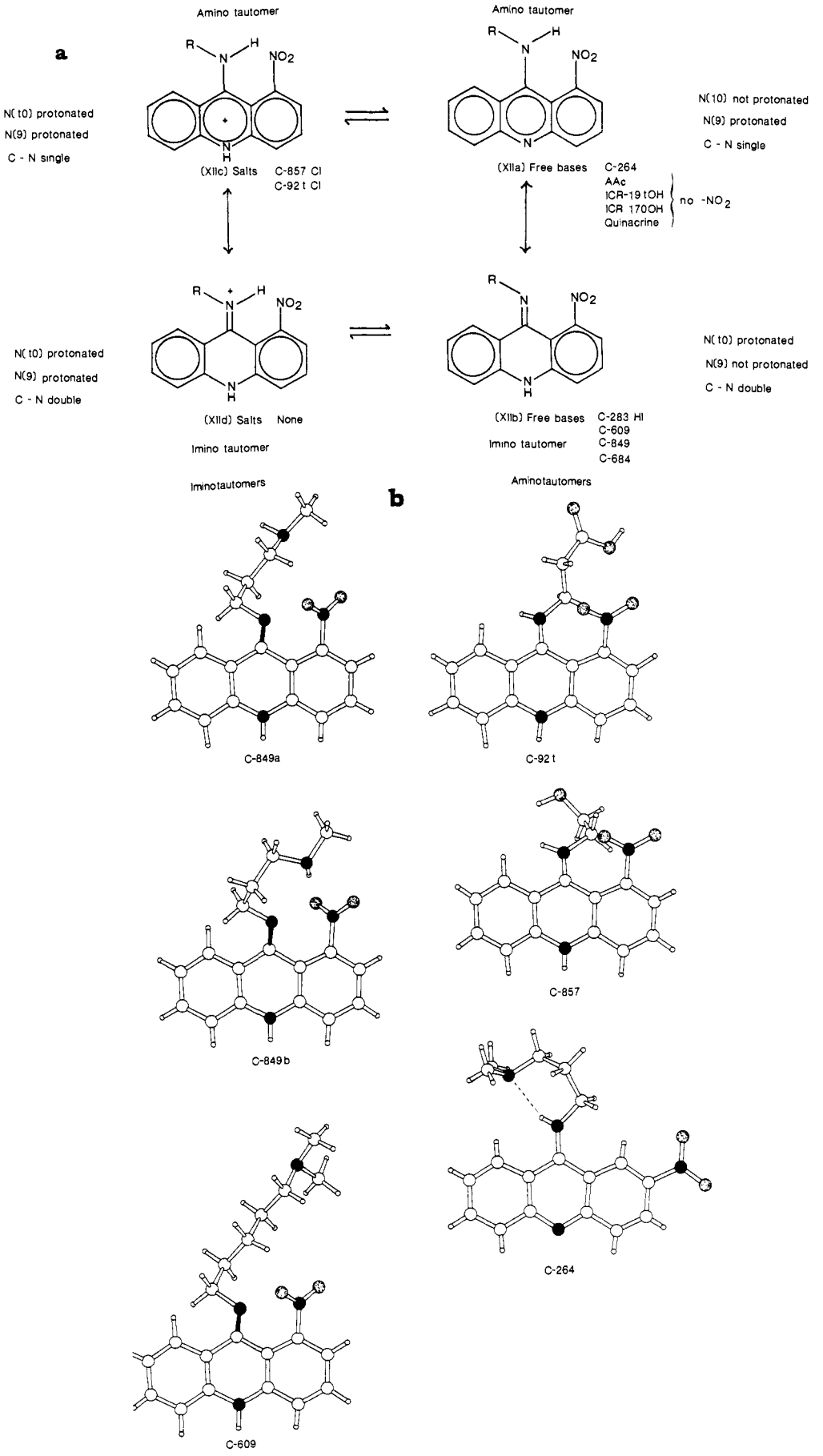

Figure 3. Chemical structures possible for 1-nitro-9-(alkylamino)acridine analogues. The depicted structures represent the results of amphoterism and tautomerism. The structures observed in crystal structures are indicated with the designation of the compound involved (e.g., C-849). (b) Diagrams of the side chain conformations in experimentally studied 1-nitroacridines. When the C(9)–N(9) bond is a double bond, it is represented in black.

Table III. Molecular Geometry of Ledakrin and Some of Its Derivatives

	bond C(9)–N(9), Å	H on N(10)	H on N(9)	torsion angles C(1a)–C(1)–N(1)–O(1) and C(8a)–C(1)–N(1)–O(2), deg	torsion angles C(1a)–C(9)–N(9)–C, deg
(a) Salts, Protonated at N(9)					
C-857[a] (X)	1.310	yes	yes, XIIc	33/26	3
C-921 (XI)	1.323	yes	yes, XIIc	35/26	8
(b) Salts, Protonated at End of Side Chain					
C-283.HI (II)	1.312	yes,	no, XIIb	65/62	175
(c) Salts, Protonated at End of Side Chain and at N(9)					
C-283.2HCl[b]	1.319	yes	yes	28/22	178,175
(d) Free Bases					
C-609 (VIII)	1.290	yes	no, XIIb	53/53	174
C-849[a] (IX)	1.286	yes	no, XIIb	61/58	172
C-849b[a] (IX)	1.284	yes	no, XIIb	54/54	171
C-829 (V)	1.285	yes	no, XIIb	56/56	176
C-684 (III)	1.272	yes	no, XIIb	62/58	174
C-283[b]	1.284	yes	no, XIIb	49/50, 63/66	178,175
(e) Inactive Analogue					
C-264 (IV)	1.331	no	yes, XIIa		12
AAc (VI)	1.341	no	yes, XIIa	51/54, 69/67 (disordered)	–

[a] Signs of torsion angles reversed to give enantiomorph of structure listed in Table II. [b] Unpublished results (M. Bogucka-Ledóchowska).

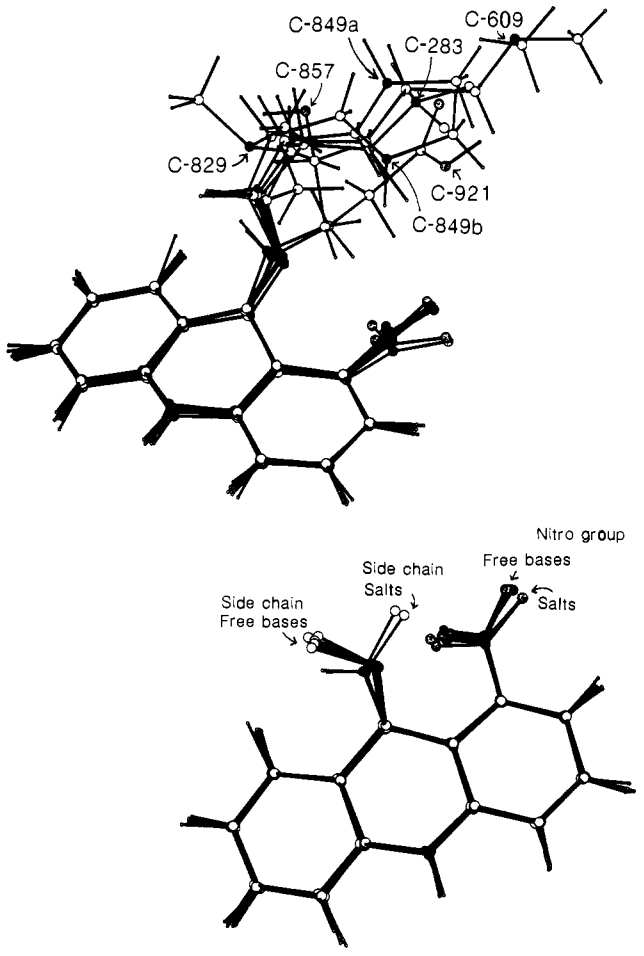

Figure 4. Least-squares fit superposition[40] diagrams illustrating the various conformations for the various 1-nitro-9-(alkylamino)acridines. Note the different ring substituent conformations of free bases and salts.

between the oxygen atoms of the nitro group, whereas the analogous hydrogen atom of C-921 is positioned almost directly over the nitrogen atom. The angle is similarly small for C-829, V, which has a more bulky side chain attached to N(9). Thus, the variation from 10° to 21° may merely represent different responses to intermolecular forces and is indicative of the flexibility of the acridine ring system.

Side Chain Conformations. The side chains, as expected, show considerably more conformational flexibility than does the ring system. These side chains differ both in the chemical nature of the functional groups at the chain end and in the number of methylene carbon atoms that separate them from atom N(9). The stereoscopic projections in Figure 1 illustrate the conformations of the side chains.

The chemical structures of C-609 and C-849 differ in that the former has two more methylene groups in its side chain and in that it contains a tertiary amine rather than a secondary amine group at the chain end. The side chain conformation of one symmetry-independent molecule of C-849 (molecule a) is very similar to that of C-609, whereas that of the second molecule (C-849b) is different beyond the second methylene group. The relevant values for the torsion angle C(11)–C(12)–C(13)–C(14),N(14) are 178°, 176°, and −74° for C-609 and C-849a and b, respectively.

The conformations of the side chains of the two salts differ markedly. One is extended (C-921), the other folded (C-857). The torsion angles characterizing their conformations are C(9)–N(9)–C(11)–C(12) = 140° and −128° and N(9)–C(11)–C(12)–O(12),C(13) = 69° and −168° for C-857 and C-921, respectively.

It seems probable that the conformations of the side chains in the free bases (imino tautomers) and the acid salts (amino tautomers) are more influenced by intermolecular rather than intramolecular interactions, particularly hydrogen-bonding interactions. This is in contrast to unhindered acridines such as C-264, ICR-170-OH, and ICR-191-OH[12,13,16] where, in the absence of a nitro group, there is internal hydrogen bonding in the side chain involving a proton on N(9).

Intermolecular Interactions. The geometry of the intermolecular hydrogen-bonding interactions is characterized in Table IV. The influence of these interactions on side chain conformation is most apparent from the crystal structures of C-849 and C-857. Figure 6 illustrates the hydrogen-bonding interactions for the crystal structures of the free base C-849. The endocyclic N(10)–H(N10) groups of each acridine donate hydrogen bonds to a water oxygen atom, (O(W2) for C-849a and O(W1) for C-849b), and both chain end amino nitrogen atoms accept hydrogen bonds from water molecule O(W1). The two water molecules are hydrogen bonded to each other (O(W1) accepts a hydrogen bond from O(W2)). The only indication of an intramolecular hydrogen bond in any of the four crystal structures examined here is found for molecule b of C-849. The amino hydrogen atom H(14) may be weakly hydrogen bonded to atom O(1′); N(14′)–H(14′) = 1.06 Å, H(14′)···O(1′) = 2.36 N(14′)···O(1′) = 3.175 Å, and N(14′)–H(14′)···O(1′) = 157°. Considering that the bonding geometry of the N(14′)···H–O(W1) hydrogen bond, with an angle of 177°, is indicative of a strong hydrogen bond, it seems likely that this latter intermolecular hydrogen bond has more influence in de-

Table IV. Least-Squares Planes for the Acridine Moiety

plane A: C(1), C(2), C(3), C(4), C(4a), C(1a)
plane B: C(1a), C(4a), N(10), C(5a), C(8a), C(9)
plane C: C(5a), C(5), C(6), C(7), C(8), C(8a)
plane D: C(1a), C(4a), C(5a), C(8a)

	C-609 (VIII)	C-849a (IX)	C-849b (IX)	C-857 (X)	C-921 (XI)
		(a) Standard Deviations from Planarity, Å			
A	0.011	0.015	0.012	0.043	0.047
B	0.136	0.132	0.133	0.102	0.130
C	0.011	0.012	0.025	0.010	0.006
		(b) Interplanar Angles, deg			
A/C	20.5	20.2	20.9	13.2	20.1
A/B	8.9	9.9	9.1	10.6	14.0
B/C	12.9	12.9	13.7	5.0	8.5

	C-283 (II)	C-684 (III)	C-829 (V)	MAc (VII)	C-264 (IV)	AAc (VI)
			(c) Additional A/C Interplanar Angles, deg			
A/C	19.8	19.2	10.2	3.0	2.1	2.0

	C-609 (VIII)	C-849a (IX)	C-849b (IX)	C-857 (X)	C-921 (XI)
		(d) Deviations from Plane D, Å			
N(1)	−0.47	−0.51	−0.38	−0.82	−0.85
O(1), O(2)	0.18, −1.34	0.21, −1.47	0.32, −1.22	−0.67, −1.32	−0.67, −1.50
N(10)	0.18	0.16	0.16	0.12	0.09
C(9)	0.33	0.33	0.35	0.35	0.27
N(9)	0.92	0.93	0.98	1.01	0.82

Table V. Hydrogen-Bonding Geometry[a]

	C-609 (VIII)	C-849 (IX) a	C-849 (IX) b	C-857 (X)	C-921 (XI)
A[b]	O(W1)	O(W2)	O(W1)	Cl^-	Cl^-
dN(10)–H(N10)	0.96 Å	0.87	0.87	0.80	0.91
dH(N10)···A	1.86 Å	1.92	2.11	2.38	2.28
dN(10)···A	2.809 Å	2.788	2.952	3.167	3.168
N(10)–H(N10)···A	169°	174°	165°	173°	164°
A				Cl^-	Cl^-
dN(9)–H(N9)				0.91 Å	0.79
dH(N9)···A				2.43 Å	2.51
dN(9)···A				3.265 Å	3.251
N(9)–H(N9)···A				152°	157°
A	N(16)	N(14)	N(14′)		
dO(W1)–H	0.96 Å	0.99	0.96		
dH···A	1.81 Å	1.85	1.80		
dO(W1)···A	2.772 Å	2.838	2.766		
O(W1)–H···A	177°	177°	177°		
X[c]:A	1:O(1),O(2)[d]	2:O(1)[d]	2:O(W1)		
dO(WX)–H	0.84 Å	0.73	1.05		
dH···A	2.59, 2.56 Å	2.41	1.69		
dO(WX)···A	3.217, 3.469 Å	3.062	2.736		
O(WX)–H···A	133° 161°	149°	175°		
X:A				12:Cl^-	14:Cl^-
dO(X)–H(OX)				0.94 Å	0.90
dH(OX)···A				2.17 Å	2.13
dO(X)···A				3.080 Å	3.018
d(X)–H(OX)···A				164°	167°

[a] Estimated standard deviations involving hydrogen atoms are ca 0.03 Å and 1°. [b] Acceptor atom in the hydrogen bond. [c] Variable subscript for the donor moiety in the hydrogen bond. [d] Probably too long to be a hydrogen bond.

termining the conformation of the side chain than does the intramolecular interaction involving O(1′). The chain end methylamino moiety of molecule a is not involved in hydrogen bonding.

The influence of hydrogen bonding on the conformation of the (hydroxyl)ethanamine side chain of C-857 can be seen from examination of Figure 7. Both side chain hydrogen bond donors (N(9)–H(N9) and O(12)–H(12) interact with the same Cl^- ion; the third hydrogen bond to the Cl^- ion involves a donor from a symmetry-related C-857 molecule.

Interrelationships between Chemical Structure Conformation and Antitumor Activity. The purpose of our study of this family of compounds is to contribute to the understanding of the interrelationships between their chemical structure, conformation and antitumor activity. It has been noted[22] that 2-, 3-, and 4-nitro analogues are ineffective antitumor agents. Since electronic effects arising from substitution at positions 1 and 3 should be similar, it appears that the steric effects that we have described above for the 1-nitro analogues are important to their antitumor activity. It has been established[18-24] that metabolic activation is required for antitumor activity in ledakrin analogues. Our earlier computer graphics-based molecular modeling[16,40,41] of intercalation of C-829

(40) Badler, N.; Stodola, R. K.; Wood, W. "DOCK" Program from the Institute for Cancer Research; The Fox Chase Cancer Center: Philadelphia, PA, 1982,

(41) Carrell, H. L. "VIEW"; Program from the Institute for Cancer Research: Philadelphia, PA, 1976.

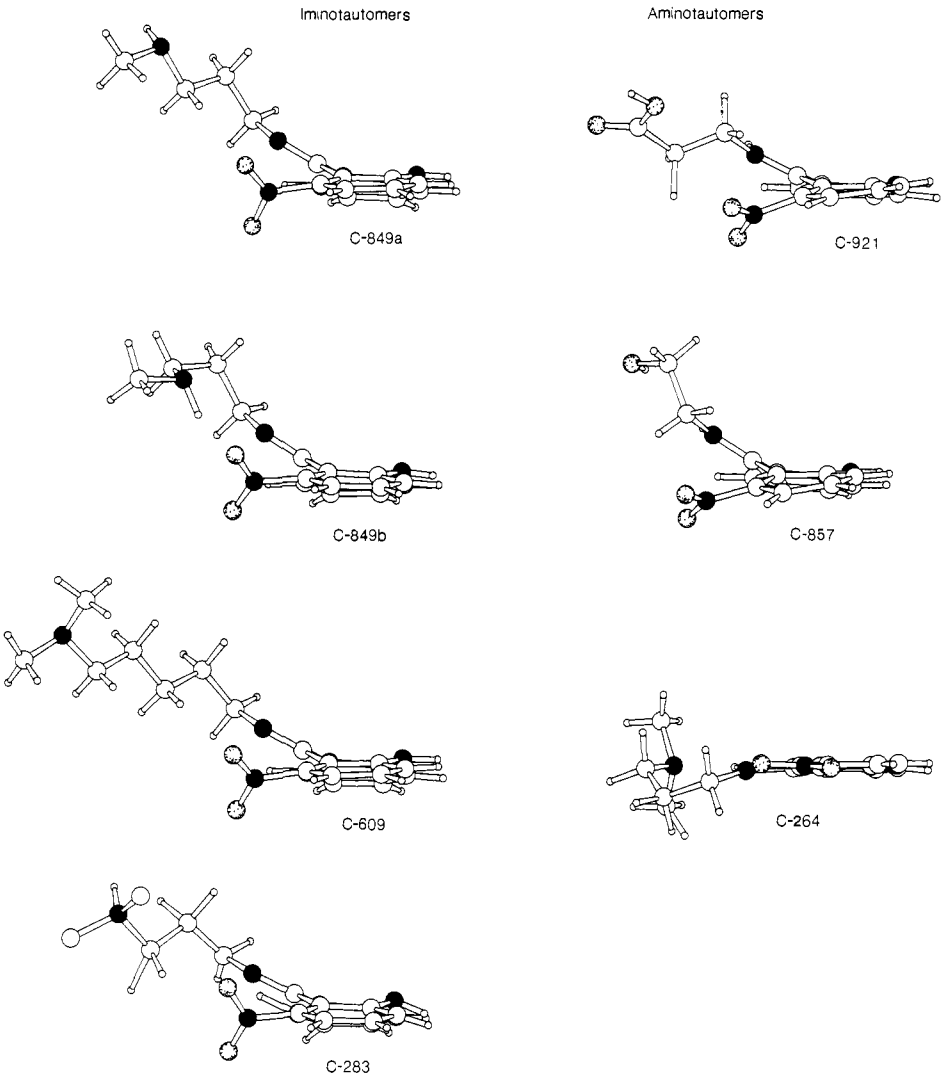

Figure 5. Side chain conformation viewed[41] with respect to the plane through C(1a), C(4a), C(5a), and C(8a).

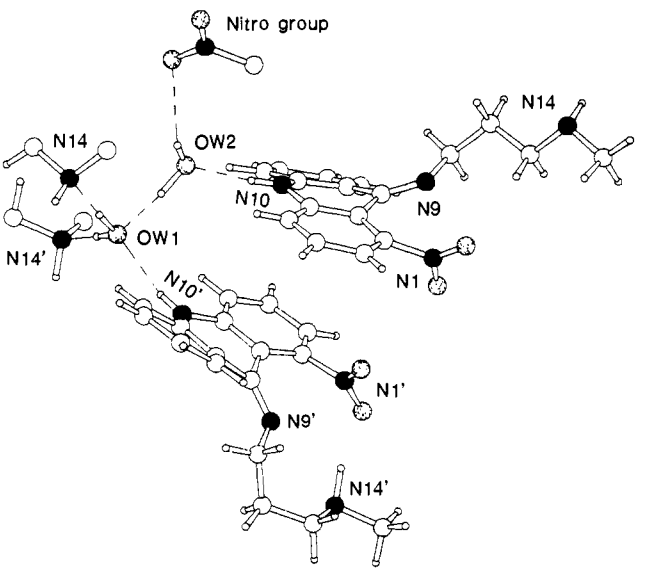

Figure 6. Illustration[41] of the hydrogen-bonding interactions for C-849. There are two molecules of this 1-nitro-9-(alkylamino)acridine analogue per asymmetric unit of the crystallographic space group.

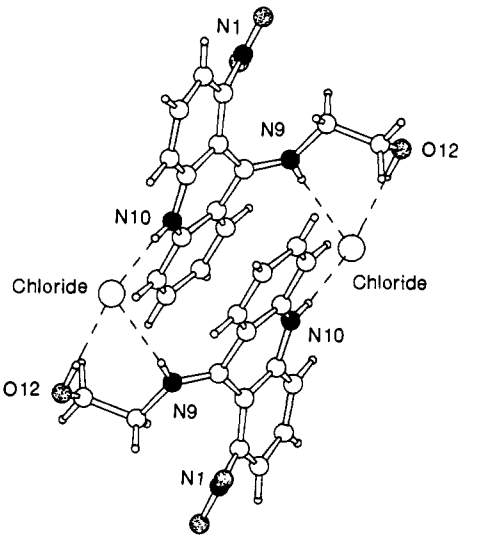

Figure 7. Illustration[41] of the hydrogen-bonding interactions in the crystals of C-857 hydrochloride. Note the interaction of two hydrogen bond donors of the alkylamino side chain with the "same" chloride ion.

into a dinucleoside phosphate revealed a number of interatomic contacts (distance less than 3.5 Å) between potentially activated atoms of the drug molecule and atoms of the nucleoside phosphate. This work has been repeated for the compounds studied here, and a representative sample is shown in Figure 8; other data are deposited. The results of this present study support the concept that such interactions may indicate a potential mechanism for DNA strand cross-linking or single-strand destabilization.

The fact that all the compounds examined here are active antitumor agents implies that the functional groups at the end

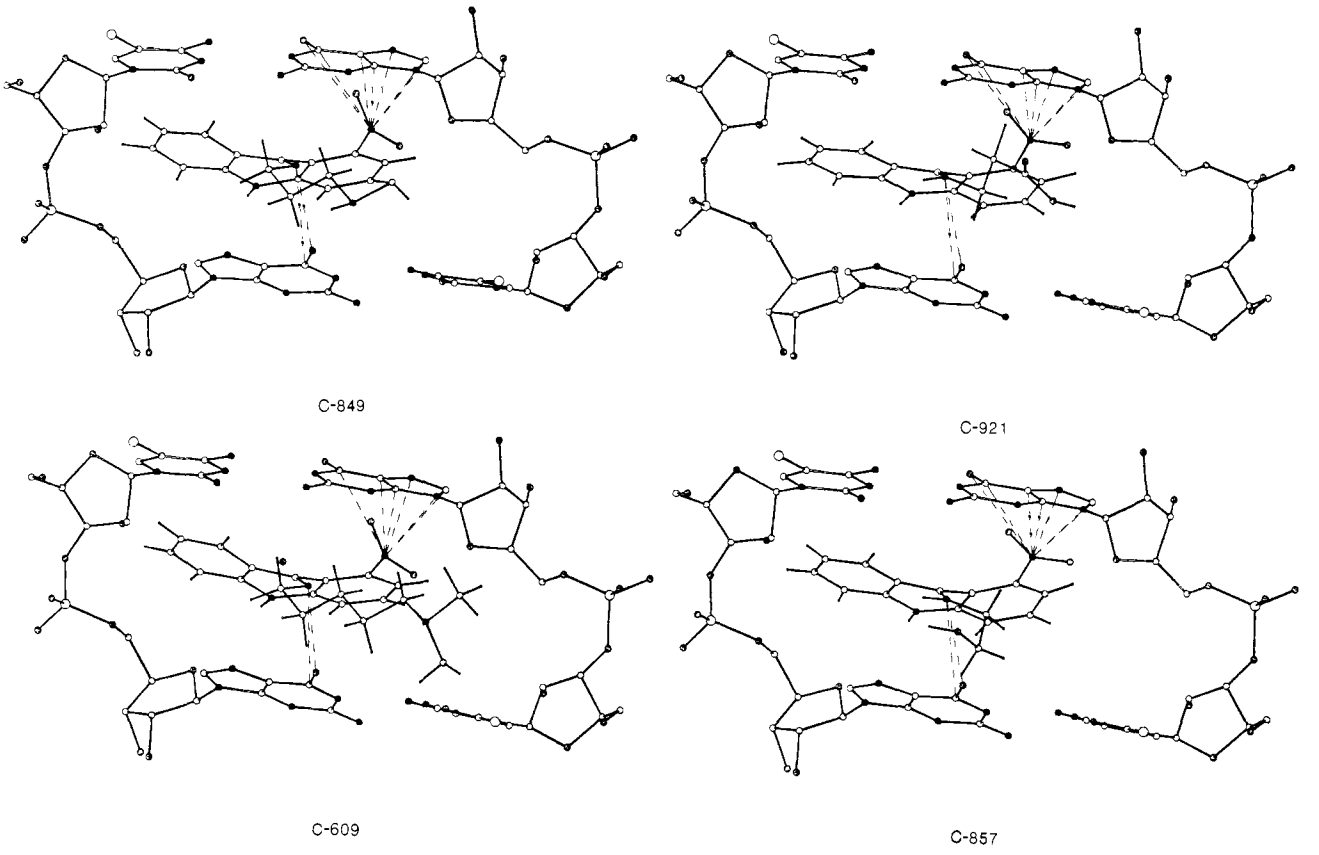

Figure 8. Adaptation of a computer-generated model[16] for the intercalation of a 1-nitro-9-aminoacridine in DNA. Intermolecular contact distances between potentially activated atoms of the drug and of DNA less than 3.5 Å are shown with dashed lines. One representative view is shown for each ledakrin analogue (the others are in the supplementary material).

of the alkyl side chain are more likely to facilitate solubility of the drug in vivo than they are to be crucial for interaction with DNA strands. This conclusion is also consistent with the observation by Pawlak et al.[19] that there are no significant differences in the ease of in vitro reduction of the 1-nitro group under anaerobic conditions among a series of 1-nitroacridine derivatives of considerably different in vivo and in vitro activity. It would seem more reasonable to consider the 1-nitro-9-aminoacridine group, activated to a suitable alkylating agent by metabolic processes, as the important portion involved in the covalent binding to DNA. Such activation has been suggested to involve formation of hydroxylamines involving the exocyclic nitrogens and/or epoxides involving the outer acridine rings.[18-24]

Epoxide formation is generally thought to be favored by localized double bond character in C–C bonds. Comparison of the C–C bond distances in the analogue studied here with those for C-264 indicates that there is more localized double bond character in the 2-nitro analogue (C-264) than in the 1-nitro analogues. This observation suggests that epoxide formation is not the primary source of activation for antitumor activity since C-264 is inactive whereas the 1-nitro analogue, C-283, is an effective antitumor agent.

The cytotoxicity of ledakrin has been ascribed to reductive metabolism to 1-hydroxylamine.[18-24] Miller and Miller[42] have pointed out that hydroxylamines, especially secondary aromatic amines, form alkylation products.[43] We have pointed out above that synergistic interaction involving alkylation of N(9) and steric effects combine to stabilize the imino tautomer for the 1-nitro-9-(alkylamino)acridines. Should these effects persist through metabolic activation of the substituents at C(1) and C(9), carbon atoms C(2) and probably C(7) could be carbonium ion centers. We find it noteworthy that both of these atoms are among the acridine ring atoms involved in close contact with dinucleoside phosphate atoms[44] in the intercalation model we have constructed (Figure 8). It is particularly interesting to note that each activated center in the ledakrin analogue "interacts" with an N(7) atom of guanine (separate strands); N(7) is considered to be the site of attack by carbonium ions derived from nitrogen mustard chemotherapeutic agents. The model described in Figure 8 is thus consistent with a possible steric mechanism for the covalent binding to DNA of analogues of the 1-nitro-9-(alkylamino)acridine family via their sterically strained area from N(1) to N(9).

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Supplementary Material Available: Observed and calculated structure factors (150 pages). Ordering information is given on any current masthead page.

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(43) Weisburger, J. H.; Weisburger, E. K. *Pharmacol. Rev.* **1973**, *25*, 1-66.
(44) Sakore, T. D.; Jain, S. C.; Tsai, C. -C.; Sobell, H. M. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 188-192.