several types of overall conformation are possible depending on sequence.

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(16) Note that the interpretation of the relative magnitudes of solvent shifts of N-H resonances is complicated by the fact that the peak positions are also dependent upon temperature in each solvent. Hence, the size of the observed shift is not simply related to accessibility. The finding of large dependences on temperature and a significant downfield shift between CD_2Cl_2 and Me_2SO-d_6 solutions for the D-Ala N-H resonance indicates that this N-H is exposed to solvent.
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(23) Models may be constructed with either a type I or a type II Pro-Ser β turn, though the former appears to be more likely. Theoretical predictions[3] and findings with *cyclo*-(X-Pro-Y)$_2$ cyclic hexapeptides[11] also suggest that type I would be favored for the Pro-Ser sequence.
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Proton, Carbon-13, and Nitrogen-15 Nuclear Magnetic Resonance and CNDO/2 Studies on the Tautomerism and Conformation of Amiloride, a Novel Acylguanidine

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Abstract: The favored ground-state structures were determined for the novel acylguanidine diuretic, amiloride (**2a**·HCl), and its free base form (**2a**) using natural-abundance ^1H, ^{13}C, and ^{15}N NMR techniques and CNDO/2 theoretical calculations. Amiloride was found to exist primarily in the acylamino tautomer form as planar conformer F1, whereas free base **2a** was shown to prefer the acylimino tautomer form as planar conformer A1 (and/or A4). The conformational preference (i.e., as conformer A1 or as conformer A4) of **2a** was not established. The dynamic mechanism(s) for the experimentally observed rapid equilibration of the terminal amino groups in **2a** and **2a**·HCl and, when N-substituted, their substituents were explored by the CNDO/2 method. Of the six possible pathways considered for effecting N-10–N-11 interconversion in **2a**, a novel mechanism involving a synchronous rotation around ϕ_2 and ϕ_3 (path F) was calculated to have the lowest barrier to interconversion. Experimental verification of this novel mechanism was attempted, but not found, by preparation of an appropriate model, **11**, and subsequent determination of the $\Delta G^\ddagger$ values (14.7–14.8 kcal/mol) for **11** and pyrazine analogues **4e** and **4e**·HCl using the dynamic ^{13}C NMR technique in Me_2SO-d_6–CD_3OD. Based on the results of these studies, it is concluded that free base **2a** is likely to undergo N-10–N-11 interconversion via simple ϕ_3 rotation and/or ϕ_2 rotation plus inversion. Accordingly, amiloride (**2a**·HCl) must equilibrate by a ϕ_3 rotation mechanism.

The noteworthy discovery[2] that certain acylguanidines such as compound **1** display saluretic–diuretic activity, i.e., promote loss of sodium chloride and water, while repressing potassium excretion in experimental animals provided impetus

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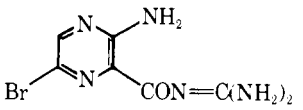
Table I. ^{13}C Chemical Shifts[a,b] for C-6 Variants

compd	C-2	C-3	C-5	C-6	C-7	C-9
2a	119.2	154.8	151.2	117.7	174.0	162.3[c]
	(109.3)	(155.9)	(154.3)	(119.8)	(165.2)	(155.3)
2b	117.5	155.9	155.9	120.8	174.6	161.6
	(109.7)	(154.7)	(155.1)	(122.8)	(165.9)	(155.7)
2c	120.6	154.9	152.2	108.9	173.8	162.3
	(110.7)	(155.8)	(155.1)	(110.6)	(165.2)	(155.2)
2d	122.8	154.8	154.2	83.4	173.2	162.0
	(113.3)	(155.6)	(156.9)	(87.9)	(168.3)	(155.3)

[a] Chemical shifts in parts per million relative to Me_4Si. [b] Values within parentheses are for the corresponding monohydrochloride salts. [c] Determined by ^{13}C enrichment (see Experimental Section).

for an extensive synthetic program based on this novel class of structures.[2,3] Of the more than 300 compounds which were synthesized and evaluated biologically following this discovery, amiloride (**2a**·HCl)[4a] proved to elicit an optimal electrolyte



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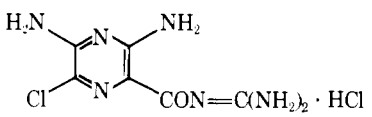
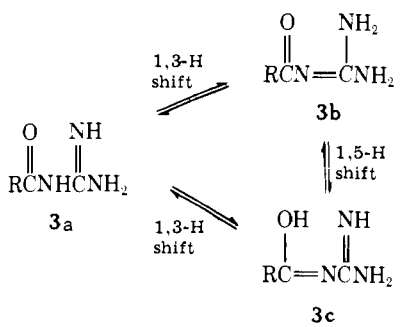


2a · HCl

excretion pattern[4b] and, subsequently, was shown to be a clinically effective potassium-sparing diuretic.[5] Although the pharmacological basis upon which amiloride manifests its diuretic activity appears to be well established,[6,7] elucidation of its mechanism of action at the molecular level ultimately awaits the determination of its active in vivo conformation(s) as well as the isolation and structural characterization of the appropriate renal receptor(s).[8] Ideally, attainment of these challenging objectives would contribute to a better understanding of the tautomeric and conformational dynamics of acylguanidines and, simultaneously, facilitate the rational design of future diuretic agents with improved therapeutic properties.

Acylguanidines devoid of nitrogen substituents can exist in three likely (i.e., low energy) tautomeric forms, the acylamino **3a**, acylimino **3b**, and isoimino **3c** types, which can be inter-



R = alkyl, aryl

converted formally via 1,3- and 1,5-prototropic shifts. Likewise, the existence and interconversion of their conjugate acids (i.e., RCONHC (=N^+H_2)NH_2, etc.) is possible.

The tautomeric preferences of acylguanidines, in turn, play an integral role in determining the scope of conformational possibilities for the parent structures. Previous studies by Matsumoto and Rapoport[9] have shown that correlation of ultraviolet spectral behavior with pH and relative hydrolytic stability can be used to distinguish between tautomer types **3a** and **3b**, and, by inference, **3c**. As anticipated from resonance considerations, these workers observed that pK_a increased in parallel with the ease of amide bond hydrolysis in going from acylguanidines of the acylimino **3b** type to those of the acylamino **3a** type.

The pK_a (8.7)[10] of amiloride (**2a**·HCl) implies that the drug is most likely to exist as a cation in the physiological pH range, particularly within the kidney nephron. However, in view of amiloride's complex structure, its chemical stability (in vitro and in vivo) in aqueous milieu, and the limited literature currently available on the tautomeric and conformational dynamics of acylguanidines, investigation of both the protonated and free-base drug forms was deemed essential. The limited solubilities of compound **2a** and its protonated species in useful solvents at necessary pHs precluded application of the UV spectral technique in this instance. These experimental difficulties were circumvented by the use of NMR and theoretical (CNDO/2) techniques,[11] the latter technique serving to guide the experimental approach. Herein we wish to report the results of our studies on the tautomerism and conformation of amiloride.

Experimental Section

Materials. With the exception of acylguanidines [^{13}C]-**2a** and **11**, the syntheses of the compounds described in this paper have been reported.[2,3a-g] Creatinine (**6**) was purchased from Nutritional Biochemicals Corp. and used as received. Clonidine (**7**), the generic name for 2-(2,6-dichlorobenzeneamino)-2-imidazoline, was synthesized by a published procedure.[12] Carbon-13-enriched guanidinium nitrate (90 atom % ^{13}C), used in the preparation of [^{13}C]-**2a** described below, was obtained from Merck Sharp & Dohme, Canada.

3,5-Diamino-*N*-[bis(amino)-[^{13}C]methylidenyl]-6-chloropyrazinecarboxamide (2a). Using ^{13}C-enriched guanidinium nitrate (10 mmol, 90 atom % ^{13}C) in the general procedure described by Shepard et al.,[13] [^{13}C]-**2a** was obtained in 61% yield as a pale yellow solid, mp 241 °C dec, ^{13}C NMR (see Table I).

2-Amino-*N*-[bis(methylamino)methylidenyl]-5-chlorobenzenecarboxamide (11). To a freshly prepared solution of $NaOCH_3$ (1.3 g, 0.024 mol) in anhydrous CH_3OH (20 mL) maintained under N_2 was added 1,2-dimethylguanidine hydriodide (5.4 g, 0.025 mol) and methyl 5-chloroanthranilate (0.93 g, 5 mmol). The resulting reaction solution was heated at reflux for 18 h and evaporated in vacuo to provide a residual solid which was partitioned between ether and water. The ethereal layer was washed with water and then extracted with 2 N hydrochloric acid. Upon addition of 2 N NaOH (excess) to the aqueous extract, **11** was deposited as a white solid (0.16 g, 13%) which crystallized from toluene as colorless needles: mp 138 °C; ^1H NMR 2.92 (d, 6, J = 4.5 Hz, $NHCH_3$), 6.73 (d, 1, $J_{3,4}$ = 8.5 Hz, H-3), 7.09 (m, 1, $J_{3,4}$ = 8.5, $J_{4,6}$ = 2 Hz, H-4), and 8.09 ppm (d, 1, $J_{4,6}$ = 2 Hz, H-6); ^{13}C NMR 28.1 ($NHCH_3$), 118.6 (C-3), 121.6 (C-5), 131.2, 131.6 (C-4, C-6), 150.2 (C-2), 162.3 [-N=$C(NHCH_3)_2$], and 177.2 ppm (C=O).

Anal. ($C_{10}H_{13}ClN_4O$) C, H, N: calcd, 23.27; found, 22.17.

NMR Spectroscopy. ^1H and ^{13}C NMR spectra were determined in Me_2SO-d_6, unless otherwise noted, on Varian T-60A and CFT-20 spectrometers, respectively. The latter instrument was operated using a 4000-Hz spectral width, a data length of 8192, and a 5-7-μs (20-30°) pulse width. ^1H and ^{13}C chemical shifts are expressed as δ (ppm) values relative to Me_4Si as internal standard. Both coupled and decoupled ^{15}N NMR spectra[14] were measured in Me_2SO-d_6 at the natural abundance level on a Bruker WH-180 spectrometer operating at 18.23 MHz with a 10 000-Hz spectral width, a data length of 16 384, a 30-μs (35°) pulse width, and a 2-s time delay. ^{15}N chemical shifts are referenced to $H^{15}NO_3/D_2O$ as external standard. Protonation shift ($\Delta\delta$) experiments were effected by measuring ^{13}C δ values as a function of stepwise addition of 12 N hydrochloric acid (1 molar equiv = 20-80 μL) to 0.15-0.45 M solutions (vol = ca. 1.2 mL) of the acylguanidine free base in Me_2SO-d_6. The chemical shifts of the monoprotonated products generated in situ were compared with those determined for the authentic acylguanidinium chlorides in Me_2SO-d_6. The determination of coalescence temperatures (T_c's) for the conformational equilibration of the guanidino $NHCH_3$ groups in com-

Table II. ^{13}C Chemical Shifts[a,b] for Nitrogen-Substituted Derivatives

compd	C-2	C-3	C-5	C-6	C-7	C-9	R
4a	117.6	154.9	149.7	118.4	174.0	162.3	14.5, 35.2
	(108.1)	(155.8)	(152.0)	(120.4)	(165.3)	(155.3)	(14.1, 35.8)
4b	118.5	155.2	151.5	117.8	173.8	164.4	41.2
	(109.0)	(155.9)	(154.2)	(119.8)	(163.6)	(155.3)	(42.5)
4c	117.0	154.9	151.7	117.4	172.0	164.9	39.4
	(110.0)	(155.9)	(154.0)	(119.2)	(163.6)	(156.1)	(40.7)
4d	120.9	153.1	152.7	119.5	173.6	162.5	40.3
	(110.2)	(153.8)	(154.2)	(119.4)	(165.1)	(155.2)	(40.7)
4e	119.6	155.8	152.6	120.4	174.1	162.2	28.1

[a] Chemical shifts in parts per million relative to Me_4Si. [b] Values within parentheses are for the corresponding monohydrochloride salts.

Table III. ^{13}C Chemical Shifts[a] for Pyrazinecarboxylic Acid Derivatives

compd	C-2	C-3	C-5	C-6	C-7	R	R_1
5a	109.6	155.8	153.2	119.3	165.8	51.1	
5b	108.3	155.7	150.7	119.7	166.0	51.1	21.8,[c] 45.2[d]
5c	110.2	155.9[b]	153.2[b]	118.7	167.2		
5d	112.7	153.8	152.3	117.1	167.9		

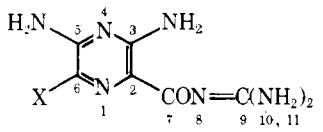
[a] Chemical shifts in parts per million relative to Me_4Si. [b] Individual assignments cannot be made. [c] Resonance for CH_3 groups. [d] Resonance for CH.

pounds **4a**, **4e**·HCl, and **11** required the use of Me_2SO-d_6-CD_3OD (3:2 v/v) as solvent.

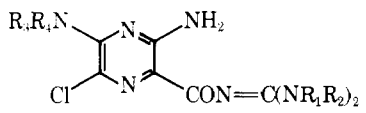
Computational Details. The molecular orbital calculations were carried out using the CNDO/2 semiempirical method,[15a] including d orbitals on chlorine. Conformations were generated and examined with an interactive computer graphics system[16] using standard bond lengths and angles (see Figure 1).

Results and Discussion

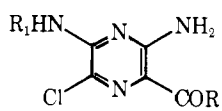
Spectral Assignments. ^{13}C NMR spectra were measured for amiloride (**2a**·HCl) and its free base (**2a**) form, for two series of closely related structures involving variations at position 6 (**2b–d**) and changes in nitrogen substituents (**4a–e**), for a series of simple pyrazinecarboxylic acid derivatives (**5a–d**), and for guanidine, creatinine (**6**), and clonidine (**7**). The ^{13}C chemical shifts for the free bases and, where appropriate, their monohydrochloride salts are presented in Tables I–IV. Resonance assignments[17] were established as follows.



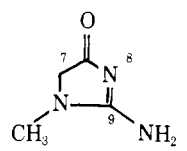
2a, X = Cl
b, X = H
c, X = Br
d, X = I



4a, $R_1 = R_2 = R_3 = H$; $R_4 = C_2H_5$
b, $R_1 = R_3 = R_4 = H$; $R_2 = -C_2H_4-$
c, $R_1 = R_2 = CH_3$; $R_3 = R_4 = H$
d, $R_1 = R_2 = H$; $R_3 = R_4 = CH_3$
e, $R_1 = CH_3$; $R_2 = R_3 = R_4 = H$



5a, R = OCH_3; R_1 = H
b, R = OCH_3; $R_1 = CH(CH_3)_2$
c, R = OH; R_1 = H
d, R = NH_2; R_1 = H



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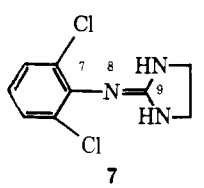

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Table IV. ^{13}C Chemical Shifts[a,b] for Model Compounds

compd	*o*	*m*	*p*	C-7	C-9	R
guanidine				160.0		
				(158.3)		
6				188.8	169.5	56.4,[c] 30.2[d]
				(172.0)	(156.7)	(54.1, 31.2)
7	128.9	127.9	121.6	146.1	157.1	41.8[e]
	(133.9)	(129.1)	(130.9)	(130.1)	(157.9)	(42.7)

[a] Chemical shifts in parts per million relative to Me_4Si. [b] Values within parentheses are for the corresponding monohydrochloride salts. [c] Resonance for ring CH_2. [d] Resonance for CH_3. [e] Resonance for ring CH_2CH_2.

The ^{13}C NMR spectrum of free base **2a** consists of resonances which appear at 174, 162, 155, 151, 119, and 117 ppm. The two upfield signals (117 and 119 ppm) were assigned to C-6 and C-2 and the two peaks centered about 153 ppm to C-3 and C-5.[18a] Specific assignment of the six resonances was accomplished by determining ^{13}C NMR protonation shifts (i.e., $\Delta\delta$ values). Three of the six peaks, 174, 162, and 119 ppm, experienced large upfield shifts upon in situ generation of **2a**·HCl. Hence, C-7, C-9, and C-2, which a priori should be markedly influenced by monoprotonation,[10] best accounted for these peaks. Therefore, the observed $\Delta\delta$'s served to distinguish C-2 from C-6.

Comparison of the spectral data for free base **2a** with that recorded in Table II for its 5-ethylamino derivative, **4a**, reveals that N-alkylation results in a shift of one of the two resonance signals assigned to C-3 and C-5; accordingly, C-5 must give rise to the 151-ppm signal in **2a**. The foregoing assignments for **2a** were verified readily by inspection of the data presented in Table I for the C-6 series, **2a–d**. Independent differentiation between the carbon resonances of the pyrazine ring was effected employing known substituent effects.[18a] In each case, C-2, C-7, and C-9 experienced large chemical shift changes upon protonation whereas C-3, C-5, and C-6 were less strongly affected. Further, it is interesting to note that the $\Delta\delta$ values were consistently more positive (or less negative) for C-5 than

Table V. ^{15}N Chemical Shifts[a,b]

compd	1	3-NH_2	4	5-NH_2	8	10	11
2a	47.3	292.4	135.9	295.1	197.4 (s)	290.4 (t)	290.4 (t)
2a–**2a**·HCl[c] (1:1)		291.9		289.6			
2a·HCl	62.3	291.5	140.9	283.8	257.1 (d)	287.4 (t)	287.4 (t)
4c	48.9	294.3[c]	135.0	295.9[d]	170.6	303.4	303.4
4d	40.5	292.2	120.3	312.9	196.8	290.7	290.7

[a] Chemical shifts in parts per million relative to $H^{15}NO_3/D_2O$. [b] Significant signal multiplicities are indicated in parentheses. [c] Prepared by admixture of equimolar quantities of **2a** and **2a**·HCl. [d] Tentative assignments.

for C-3. Differentiation between C-7 and C-9 was achieved unequivocally by synthesis and examination of **2a** enriched with ^{13}C at position 9.

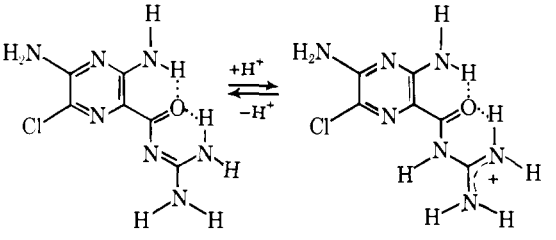
Assignments for the pyrazinecarboxylic acid derivatives **5a–d** (see Table III) were made by comparing the systematic chemical shift changes observed within this series with those of the N-substituted series (**4a–e**) and free base **2a.** The assignments shown for guanidine, creatinine (**6**), and clonidine (**7**) in Table IV were straightforward.

The ^{15}N NMR spectral results obtained for amiloride (**2a**·HCl), its free base (**2a**) form, and the terminally substituted tetramethyl (**4c**) and 5-dimethylamino (**4d**) derivatives of the latter are tabulated and codified in Table V. The coupled spectrum of **2a** contains six clearly defined resonances: singlets at 47, 135, and 197 ppm and triplets at 290 (broad), 292, and 295 ppm. An amino group substituted on a carbon α to an aromatic nitrogen causes an upfield shift[19] whereas introduction of either a chloro or aryl substituent in an α position has little effect. Hence, the singlet at 47 ppm was assigned to N-1 and that at 135 ppm to N-4. The remaining singlet (197 ppm) was delegated to N-8. Comparison of the data for **2a** with that of the 5-dimethylamino derivative (**4d**) served to distinguish between the triplets at 292 and 295 ppm which, accordingly, were assigned to the 3- and 5-amino groups, respectively. N-10 and N-11 accounted for the remaining triplet at 290 ppm. Assignment of the resonances recorded for amiloride (**2a**·HCl) was achieved in a similar manner. Correlation of the various nitrogens in **2a** with their counterparts in **2a**·HCl was accomplished by measuring the ^{15}N chemical shifts for the half-neutralized species (i.e., **2a**·0.5HCl) and, subsequently, plotting $\Delta\delta$ vs. percent neutralization.

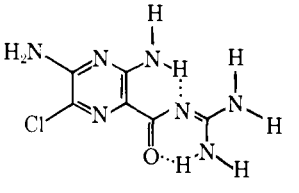
Ground-State Tautomer Form. Several lines of experimental and theoretical evidence lead to the conclusion that, in those solvents studied, the free base (**2a**) of amiloride exists primarily in the acylimino tautomer form, $RCON{=}C(NH_2)_2$, whereas amiloride (**2a**·HCl) prefers to assume the acylamino tautomer form, $RCONHC(=N^+H_2)NH_2$, where R is the 3,5-diamino-6-chloropyrazinyl moiety. First, the striking similarities in ^{13}C chemical shifts and $\Delta\delta$ values (magnitude and direction of shift) upon protonation observed for **2a,** its terminally substituted tetramethyl derivative (**4c**), and creatinine (**6**) suggest that **2a, 4c,** and **6** exist in a common tautomeric form. As is obvious, tetramethyl derivative **4c,** by virtue of substituent-imposed constraints, must assume the acylimino form. Previous spectral (UV, NMR) studies[9] have verified the assignment of this tautomer form to creatinine (**6**). Additional support for these tautomer assignments stems from the ^{15}N NMR spectral data presented in Table V. As noted earlier, the coupled ^{15}N NMR spectrum of free base **2a** consists of a broad triplet resulting from two NH_2 (N-10, N-11) groups (vide infra) and a singlet arising from a single nitrogen atom (N-8), in addition to four peaks due to the nitrogen atoms incorporated in and appended to the pyrazine ring. This spectrum is only compatible with the assignment of an acylimino tautomer form to free base **2a.** Similarly, analysis of the relevant data in Table V serves to establish an acylamino structure as the preferred tautomer form for amiloride (**2a**·HCl). Other experimental observations which corroborate these judgments are (a) the presence of a broad singlet at δ 10, attributable to an acylamino proton, in the ^1H NMR spectrum of amiloride (**2a**·HCl) and (b) the absence of an acylamino proton in the ^1H NMR spectrum of free base **2a**.

Calculations. Tautomers of free base **2a** with an internal C=N (A) and with an external C=N (B) were examined by theoretical calculations at the CNDO/2 level of approximation as a function of rotation about the dihedral angles defined by ϕ_1–ϕ_4 in Figure 1. Likewise, tautomers of **2a** possessing a ring imino (at C-3 or C-5) group (C), bearing an sp-hybridized nitrogen (D), and resulting from enolization of B (E) were examined briefly. Finally, the protonated species F was considered. The key binding energies and relative energies calculated are given in Table VI; sketches of the various configurations and conformations considered are appended as Figure 3 in the microfilm edition. In view of the size of the molecules considered and the inherent limitations of CNDO/2 theory, with one exception, no attempt was made to minimize energy as a function of total geometry. As a result, the calculated absolute binding energies are unreliable; nonetheless, the relative energies obtained by such "rigid rotations" have proven useful in our hands for gaining insights into the complexities of these systems.

The acylimino tautomer of conformation A1 (see Table VI) was calculated to be substantially lower in relative energy than all acylamino tautomers (B) examined. Two low-energy conformations (A1 and A4) of A proved to be within 0.9 kcal/mol



A1 F1



A4

of each other with A1 being the favored conformation. However, the limited magnitude of this energy difference suggests that both A1 and A4 may occur in solution with solvent effects possibly governing their relative concentrations. Based upon their calculated dipole moments, A4 (6.22 D) would be predicted to predominate over A1 (4.19 D) in polar solvents. Further, the calculated barrier to interconversion (6 kcal/mol via rotation about ϕ_1) implies that equilibration of A1 with A4 should proceed rapidly.

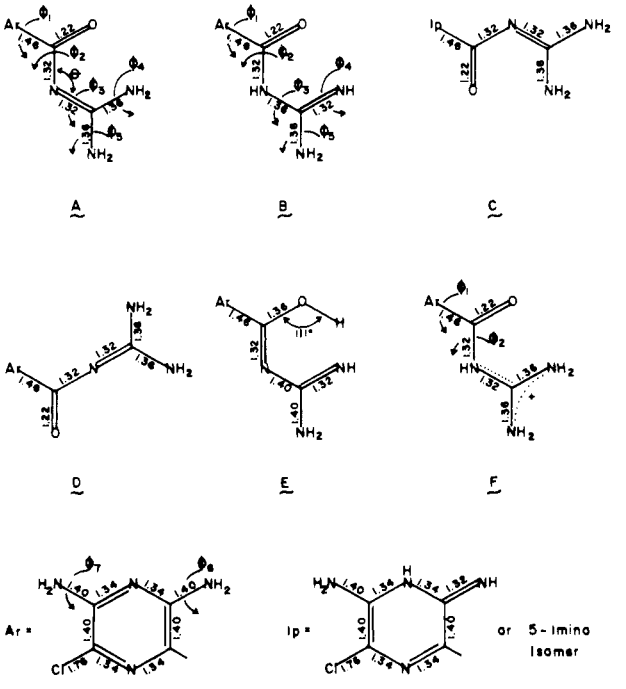


Figure 1. Standard geometries chosen for tautomers of **2a** and **2a**·HCl. All angles are 120° escept θ (120 or 180°) in A, the C-N=C angle (180°) in D, and the C-O-H angle (111°) in E.

Both conformations are stabilized by two hydrogen bonds,[20a] two N-H···O=C in A1 and N-H···O=C and N-H···NH=C in A4, as revealed by the CNDO/2 overlap populations[15b] of 0.05 -0.10 calculated for these atoms. In addition, a weak hydrogen bond exists between the 6-chloro and 5-amino substituents as indicated by an overlap population of 0.015. The hydrogen bond strengths were estimated by double rotations. In conformation A1 ϕ_1 rotation required 6 kcal/mol whereas simultaneous rotation of ϕ_1 and ϕ_6 necessitated 1.3 kcal/mol. Hence, the carbonyl-3-amino group hydrogen bond strength was estimated to be 4.7 kcal/mol. Similarly, the carbonyl-guanidine amino group (N-10) hydrogen bond strength was estimated to be 4.9 kcal/mol and that between the 6-chloro and 5-amino substituents 2.2 kcal/mol. Using the same method for conformation A4, the N-H···NH=C interaction was assigned a value of 4.9 kcal/mol and that of the N-H···O=C 6.1 kcal/mol. These estimated hydrogen bond strengths obviously depend on the geometry assumed and, therefore, should be viewed with caution. Nevertheless, they are consistent with those reported recently for a series of ortho-substituted phenols.[20b]

All examined conformations of acylamino tautomer B are substantially higher in energy than A1 and A4; for example, the most stable conformation (B3) is 10.9 kcal/mol less stable than A1. Likewise, the iminopyrazine structures (C1, C2) were calculated to be considerably higher in energy than A1 and were not considered further.

Protonation of the free base (**2a**) form of amiloride is predicted to occur at N-8 since this is the position calculated to bear the highest electronic charge in both favored conformations (A1 and A4) of **2a**. Structure F1 (F1 corresponds to F in Figure 1) was calculated to be the most stable protonated species of those examined and, accordingly, represents the theoretically preferred ground-state conformation of amiloride (**2a**·HCl).

Ground-State Conformation. Having established the preferred tautomer forms for both amiloride (**2a**·HCl) and its free base (**2a**) by experimental and theoretical methods, we next sought to determine the ground-state conformations for free base **2a** and for its conjugate acid (**2a**·HCl).

A priori, free base **2a** (i.e., tautomer A) can have two side-chain conformational sets which maintain maximal π overlap. One set involves orientation of the plane containing the guanidine π system, i.e., the (N-8)-(C-9)-(N-10)-(N-11) atom array, coplanar with the pyrazinoyl moiety. Alternatively, the two π systems can be positioned orthogonally to one another to generate the other set. The two conformational sets differ only with respect to rotation about ϕ_2, the (C-7)-(N-8) bond. Clonidine (**7**) is known to exist in an orthogonal conformation[21] analogous to the second set. A possible interpretation of the NMR results which was considered during the course of this investigation required that **2a** exist in a conformation within this second set, either invoking the presence of an sp-hybridized nitrogen (N-8) in the guanidine moiety (e.g., as in structure **D**) or requiring a 90° rotation about ϕ_2 as reflected in conformations A3, A6, and A18. Although such a conformation readily explained both the observed NMR spectral similarities between **2a** and **4d** and the observed ^{15}N NMR equivalence of N-10 and N-11, the lowest energy conformation of this type was calculated to be 8.5 kcal/mol higher in energy than conformation A1.

This observation prompted further experimental work which strongly suggests that the "coplanar" set, specifically conformation A1 (and/or A4), best represents free base **2a** on the following grounds. The ^{13}C NMR protonation shifts of **2a** more closely resemble those of creatinine (**6**), which must be planar owing to geometrical constraints, than those of either guanidine or clonidine (**7**). Furthermore, the unusual downfield shift of C-7 in **2a** also suggests that the guanidine and carbonyl group π systems are coplanar (vide infra).

Amiloride (**2a**·HCl) most likely exists in a coplanar conformation (e.g., F1). While rotation about ϕ_2 in free base **2a** does not preclude a conjugative interaction between the carbonyl and guanidine moieties, the same rotation in conjugate acid **2a**·HCl effectively prevents such an interaction. Accordingly, based on simple energy considerations (vide supra), **2a**·HCl would be predicted to exist in a coplanar conformation in agreement with the theoretical calculations noted earlier. On first glance, the experimental data do not eliminate the possibility that **2a**·HCl can exist in an orthogonal conformation since free bases **2a** and **4c** as well as their conjugate acids display similarities in their respective ^{13}C chemical shifts and protonation effects (i.e., $\Delta\delta$'s). This observation, in turn, implies that **2a** and **4c** as well as their conjugate acids must be conformationally similar. However, this apparent inconsistency can be rectified by inspection and manipulation of molecular models. Minor rotations around the (C-7)-(N-8), (N-8)-(C-9), (C-9)-(N-10), and (N-10)-CH_3 bonds in tetramethyl derivative **4c** (and **4c**·HCl) should effectively relieve or at least attenuate steric repulsion between the N-CH_3 groups and the carbonyl oxygen atom and, thereby, allow coplanarity and maximum π interaction to be approached.

The evidence in support of coplanar conformations for amiloride (**2a**·HCl) and its free base (**2a**) emerges on examination of the chemical shifts determined for the various guanidine carbons (C-9). These ^{13}C δ values fall into two groups: (a) free base **2a** and creatinine (**6**) and (b) amiloride (**2a**·HCl), creatinine (**6**) hydrochloride, the free bases and hydrochloride salts of guanidine and clonidine (**7**), and sulfaguanidine.[22] The carbonyl and guanidino moieties in creatinine (**6**) must be coplanar and directly conjugated. Analysis of the second group reveals that any conformational or structural change which disrupts this direct conjugation, e.g., rotation of one π system out of the plane of the other as in clonidine (**7**) or protonation of the amide nitrogen (N-8) as in amiloride (**2a**·HCl) and creatinine (**6**) hydrochloride, results in an upfield shift of the guanidine carbon resonance position. This type of shift is also observed in systems such as guanidine and sulfaguanidine[22] wherein direct conjugation is impossible. Therefore, amiloride

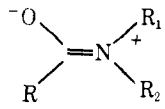
Table VI. Calculated Energy Values for Various Tautomer Conformations

structure[a]	ϕ_1	ϕ_2	ϕ_3	ϕ_4	ϕ_5	ϕ_6	ϕ_7	θ	binding energy, hartrees	rel energy, kcal/mol
A1	180	180	180	0	0	0	0	120	−11.0484	0
A2	0	0	180	0	0	0	0	120	(253.79)[b]	large
A3	0	90	180	0	0	0	0	120	−11.0281	12.74
A4	0	180	180	0	0	0	0	120	−11.0470	0.88
A5	180	0	180	0	0	0	0	120	(150.18)[b]	large
A6	180	90	180	0	0	0	0	120	−11.0348	8.53
A7	90	180	180	0	0	0	0	120	−11.0388	6.02
A8	180	180	90	0	0	0	0	120	−11.0160	20.33
A9	180	180	180	90	0	0	0	120	−11.0135	21.90
A10	180	180	180	0	90	0	0	120	−11.0218	16.69
A11	180	180	180	0	0	90	0	120	−11.0225	16.25
A12	180	180	180	0	0	0	90	120	−11.0275	13.11
A18	180	90	90	0	0	0	0	120	−10.9957	33.07
A22	90	0	90	0	0	0	0	120	−11.0003	30.18
A23	180	0	90	0	0	0	0	120	−11.0232	15.81
A30	0	180	90	0	0	0	0	120	−11.0144	21.32
B1	180	180	180	180	0	0	0	120	−11.0256	14.31
B2	180	180	180	0	0	0	0	120	−11.0205	17.51
B3	180	180	0	0	0	0	0	120	−11.0310	10.92
B4	180	180	90	0	0	0	0	120	−11.0160	20.33
C1	0	180	180	0	0	0	0	120	−10.9531	59.80
C2	0	180	180	0	0	0	0	120	−10.9433	65.95
D1	0	(180)[c]		0	0	0	0	180	−10.9043	90.42
D2	0	(90)[c]		0	0	0	0	180	−11.0047	27.42
D3	180	(180)[c]		0	0	0	0	180	−10.9947	33.70
D4	180	(90)[c]		0	0	0	0	180	−11.0146	21.21
E1	180	180	180	0	0	0	0	120	−11.0791	−19.3[d]
E2	180	180	90	0	0	0	0	120	−11.0612	−8.0[d]
F1	180	180	180	0	0	0	0	120	−10.9838	0[e]
F2	180	0	90	0	0	0	0	120	−10.9579	16.2
F3	0	90	180	0	0	0	0	120	−10.9489	21.9

[a] The dihedral angles are defined in Figure 1; all structures are shown in the Appendix (supplementary material). [b] Energy failed to converge. [c] ϕ_{1245} for Ar^1-$C^2(=O)$-N^3=C^4-$N^5(-N)$ wherein N^3 is sp hybridized. [d] Value believed to be too low (see text). [e] Standard for relative energies of cations F.

(**2a**·HCl) and clonidine (**7**) hydrochloride can be assigned to different conformational sets despite similarities in chemical shifts.

The ground-state structure, A1 (and/or A4), of free base **2a** suggests a plausible explanation for the atypical downfield resonance position displayed by C-7 in **2a** relative to C-7 in pyrazinecarboxamide **5d** and amide carbonyl carbon resonances in general.[18d,23] The upfield resonance position of amides relative to ketones has been attributed to contributions from charge-separated resonance species[18e] such as

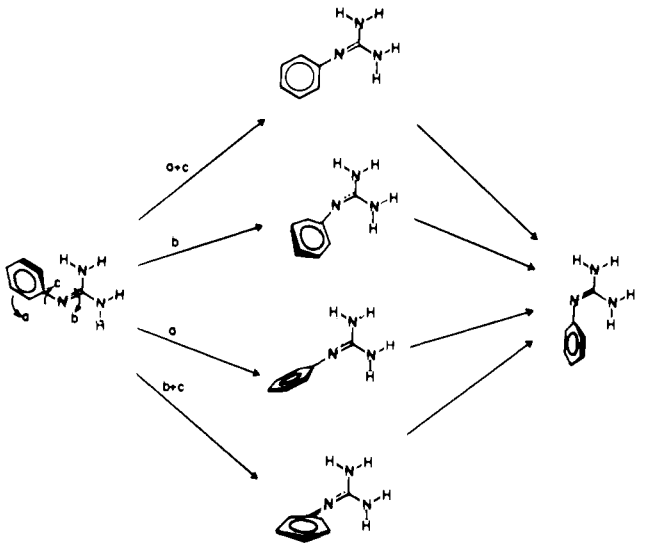


The latter are known to be less structurally important in vinylogous amides than in normal amides; cf. the similar carbonyl carbon resonances reported for 3-acetyl-$\Delta^{2,3}$-piperidine and 3-acetyl-$\Delta^{3,4}$-piperidine.[24] Effects recognized to elicit upfield shifts of the carbonyl carbon resonance are (a) increases in the electronegativity of the α carbon, e.g., an upfield shift results from α-chlorination,[18f] and (b) conjugation and cross conjugation.[18g] Both effects are operative for C-7 in free base **2a.** The amide nitrogen (N-8) is certainly more electronegative than a similarly substituted carbon atom and is masked with regard to normal carbonyl–amide nitrogen interactions; C-2 is strongly electron withdrawing and the carbonyl group is cross conjugated. Thus, the observed ^{13}C NMR behavior of C-7 in free base **2a** is more like that of a strongly shielded ketone than that of a typical amide and, therefore, truly reflects the acylimino tautomer form displayed by **2a.**

Interestingly, this analysis suggests an explanation for the unusual upfield shift of C-7 which accompanies protonation of free base **2a.** Changes in electron density are predicted to be minimal by CNDO/2 calculations and, in any case, should result in a downfield shift. An electric field effect[25] emanating from the guanidinium cation, although predicting the direction of the observed change in chemical shift, alone will not account for the magnitude of the observed shift. While contributions from changes in the average excitation energy term of the paramagnetic shielding equation similar to those observed upon protonation of pyridine[18b] cannot be excluded, the most likely explanation involves the loss of extended conjugation, i.e., the demasking of the amide nitrogen accompanying protonation, which results in a shift of the carbonyl carbon to a region more characteristic of amide carbons. Similar reasoning serves to explain the upfield resonance shifts displayed by C-2 and C-9 upon protonation of **2a**.

Dynamic Conformation. As previously noted, equivalence of the terminal amino groups (N-10, N-11) was observed in the ^{15}N NMR spectra recorded for amiloride (**2a**·HCl) and its free base (**2a**). Likewise, in each terminally substituted **2a** derivative studied (e.g., **4c** and **4e**) and their hydrochloride salts, the terminal amino groups and their substituents displayed ^1H, ^{13}C, and ^{15}N NMR equivalence.[26] These observations imply that N-10 and N-11 and, when substituted, their substituents are interconverting rapidly on the NMR time scale, i.e., a dynamic process (or processes) is operative. The simplest mechanism for equilibrating N-10 and N-11 in free base **2a** would involve a simple 180° rotation about the C=N bond, ϕ_3. However, since a relatively large barrier (20.3 kcal/mol) was calculated for this process and the subject of

Scheme I



imine conformational dynamics is currently of great interest,[27] an in-depth investigation of possible equilibration mechanisms was initiated.

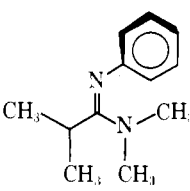
Inversion of configuration at nitrogen can occur by one of two limiting mechanisms: (a) the planar inversion[27a] or lateral shift[28] mechanism, which involves a transition state bearing an sp-hybridized, linear nitrogen, and (b) the rotation[27,28] or torsion[29] mechanism, which involves passage through a transition state in which the N substituent is rotated 90° with respect to the imine carbon substituents. These possibilities have been scrutinized extensively by theoretical methods.[27a] According to both CNDO/2 calculations (with geometry optimization)[29] and ab initio results,[30] methylenimine (CH_2=NH) isomerizes preferentially by in-plane inversion. Likewise, in-plane inversion is favored in CH_2=NOH based on CNDO/2 results.[29] On the other hand, in guanidine, CNDO/2 calculations suggest that the rotation mechanism (E_a = 28.4[29] or 30.4 kcal/mol[31]) is favored over the inversion process (E_a = 36.1 kcal/mol[29]). Presumably, the calculated preference stems from the ability of the guanidine amino groups to stabilize the positive charge on carbon in the charge-separated, rotated transition state.[32] Protonated iminium and guanidinium cations must isomerize by rotation; in-plane inversion is not possible for tetravalent nitrogen.

The calculated rotation barrier is higher for CH_2=N^+H_2 than for CH_2=NH owing to more localized charge in the twisted immonium transition state,[32b] whereas the rotation barrier calculated for the guanidinium cation (21.9 kcal/mol by CNDO/2,[31] 20.1 kcal/mol by ab initio STO-3G,[32a] 14.1 kcal/mol by ab initio 4-31G,[32a] 8.9 kcal/mol by MINDO/3[33]) is lower than that (30.4 kcal/mol by CNDO/2[31]) calculated for the double bond in guanidine. This observation is consistent with reduced C=N double bond character resulting from guanidine protonation.[34] Experimentally, the free energy of activation, $\Delta G^{\ddagger}$, for bond rotation in the guanidinium cation was determined to be ≤13 kcal/mol by NMR techniques in anisotropic liquid crystalline nematic solution.[33] The latter value is in good agreement with that (13.6 ± 1.9 kcal/mol) recently determined experimentally for guanidinium bond rotation in creatine.[31]

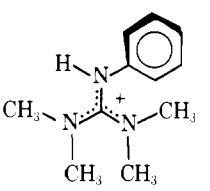
Introduction of substituents on guanidine greatly complicates the mechanistic possibilities for achieving dynamic conformational interconversion. Therefore, prior to investigating the dynamics of amiloride (**2a**·HCl) in detail, we reinvestigated the conformational dynamics of several model systems, the first being phenylguanidine, which has been studied extensively.[27,35] Energies were calculated by the CNDO/2 method for various mechanisms of conformational equilibration in this system. The calculated ground-state geometry exhibited a 60° angle between the planes of the guanidino and phenyl moieties; however, "wobble" of the angle between ca. 45° and 135° required negligible activation energy (≤1 kcal/mol). Coupled rotations about both bonds directed to the internal nitrogen were considered; possible mechanisms and calculated rotation barriers are shown in Scheme I.

Inversion with rotation (a + c) generated the highest energy transition state (38.6 kcal/mol); note that in the transition state, the sp-hybridized nitrogen bears an unfavorable doubly occupied, unconjugated in-plane p orbital. Rotation (b) about the C=N gave the next highest energy pathway (31.5 kcal/mol) which proceeds via a transition state in which a singly occupied in-plane nitrogen p orbital has been torsionally decoupled from the singly occupied guanidine carbon p orbital. Inversion (a) produced a transition state (26.7 kcal/mol) in which one singly occupied nitrogen p orbital has been Y-delocalized[36] and the other doubly occupied p orbital conjugated with the phenyl ring. The lowest energy transition state (24.8 kcal/mol), formed by a double rotation (b + c), possesses an sp^2-hybridized nitrogen with a singly occupied in-plane p orbital conjugated to the phenyl ring and a doubly occupied out of plane sp^2-hybridized orbital which has been partially delocalized into the guanidine π system. Since the lowest energy mechanisms (b + c and a) are favored by π electron conjugating aryl substituents and disfavored by alkyl moieties, we must conclude that isomerization of phenylguanidine can proceed by both inversion and rotation mechanisms.

Experimentally in nonpolar solvents, *N*-[bis(dimethylamino)methylidenyl]benzenamine (**8**) exhibits a barrier to isomerization of 12.1 kcal/mol;[35] this low value must reflect a relatively high ground-state energy which is elevated owing



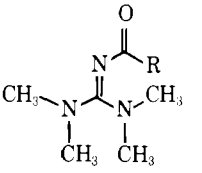
8



9

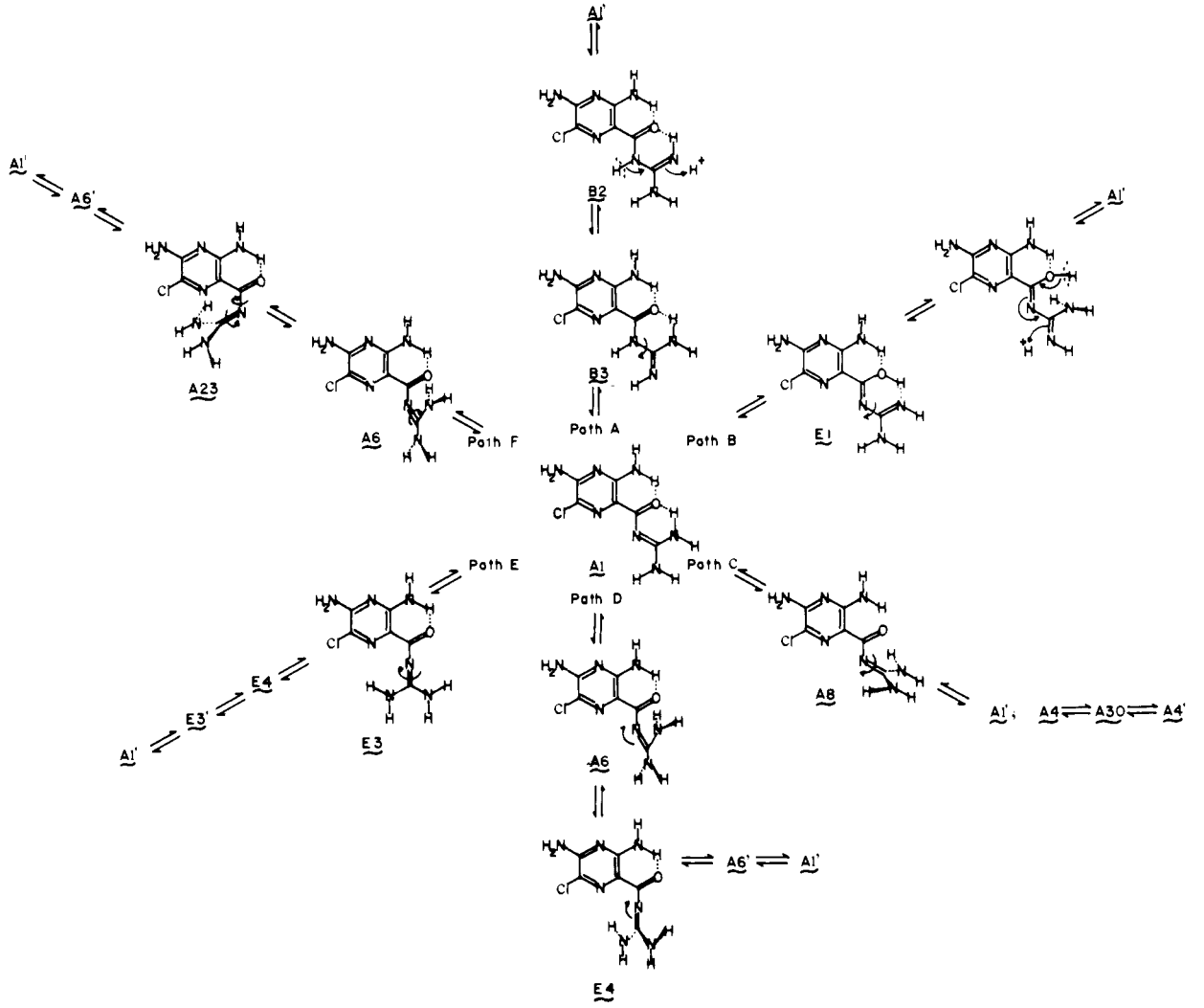
to steric repulsion between the methyl and phenyl substituents. The corresponding protonated cation (**9**) must isomerize by a rotation mechanism[27b,35] which, presumably, is analogous to the "b + c" pathway depicted in Scheme I; the experimental barrier to rotation is 12.5 kcal/mol.[35] Substituent and solvent effects, which differ depending on the state of protonation (i.e., free base vs. protonated cation), were interpreted to support the in-plane inversion mechanism for free base **8**.[27b,35] However, our calculations (vide ante) suggest that these results can also be interpreted in terms of the rotation mechanism for free base **8**.[28,37]

Aroylguanidines, e.g., benzoylguanidine and its derivatives, and the subject pyrazinoylguanidines belong to the same general structural class (i.e., acylguanidines). Accordingly, the former should constitute better models for the latter than do structures such as **8**. Although acylguanidines are yet to be studied by theoretical methods, experimentally, they appear to have low barriers to C=N rotation as exemplified by the



10a, R = C_6H_5
b, R = CH_3

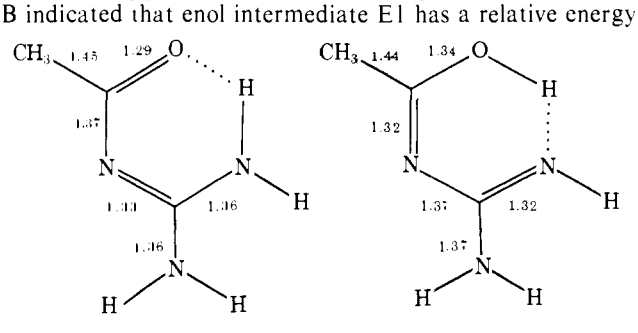
Scheme II



values, 8.6 and 8.95 kcal/mol, determined for acylguanidines **10a** and **10b**, respectively.[38] Substituent effects suggested that isomerization of structure **10a** occurs by an in-plane inversion mechanism.[38]

At least six distinct pathways can be envisioned for equilibrating the terminal amino groups (N-10, N-11) in the free base (**2a**) of amiloride as summarized in Scheme II. To achieve equilibration by a pathway involving tautomerism (paths A and B), the rate of proton exchange must exceed or at least equal that for overall equilibration. Such a proton exchange rate is inconsistent[39] with the N–H coupling (vide ante) observed in the ^{15}N NMR spectra recorded for **2a** and **2a**·HCl. Hence, paths A and B can be excluded as major processes for effecting N-10–N-11 interconversion in both **2a** and **2a**·HCl.

Interestingly, calculations on the energetics of paths A and B indicated that enol intermediate E1 has a relative energy



calculated to be 19 kcal/mol below that of conformation A1. Having established experimentally that A1 is the ground-state conformation for free base **2a**, this apparent anomaly was investigated further. Since several bond lengths were changed in going from A1 to E1 and relative energy can vary markedly as a result of such changes, the bond lengths in model structures G1 and G2 were minimized as a function of CNDO/2 binding energy. In this instance, the acylimino form (G1) was found to be favored by 0.9 kcal/mol over the isoimino form (G2). Simple bond-strength considerations also suggest that A1 should be more stable than its enol (E1) in agreement with the spectral data. If acylimino tautomer A1 predominates over enol E1 as our data suggest, this result may arise from solvent effects rather than from relative in vacuo energy differences in accord with the known influence of solvation on protomeric equilibria.[40]

Rotation about ϕ_3 (path C) is the simplest mechanism for external nitrogen interconversion; however, the calculated rotation barriers (20.3 kcal/mol for A1, 20.4 kcal/mol for A4) imply that this dynamic mode should be "frozen out" on the NMR time scale at room temperature. Hence, either this mechanism is not applicable, solvation is involved, or the calculations overestimate the rotation barriers. This point will be discussed below.

Inversion alone cannot equilibrate the terminal amino groups in **2a**, whereas rotation around ϕ_2 and subsequent inversion (path D) can serve this purpose. The overall energy required for this process is computed to be slightly higher (21.2 kcal/mol from A1, 27.4 kcal/mol from A4) than that for path

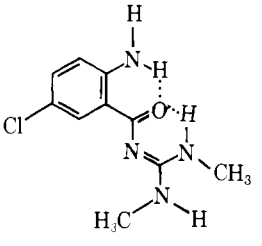
C. Similarly, inversion followed by rotation about ϕ_3 (path E) is even higher in energy (33.7 kcal/mol from A1, 90.4 kcal/mol from A4) than that for path D.

While searching for combinations of rotations capable of effecting the desired interconversion, a synchronized rotation around ϕ_2 and ϕ_3 (path F) was calculated to have the lowest energy transition state. Beginning with A1, rotation about ϕ_2 up to 90° is relatively facile (8.5 kcal/mol) as observed in path D. Continued rotation about ϕ_2 *alone* leads to an unfavorable steric interaction between the guanidino and pyrazine ring moieties, whereas synchronized rotation around ϕ_3 and ϕ_2 results in transition state A23, which is relatively low in energy (15.8 kcal/mol). An attractive nonbonded interaction between the lone pair of electrons on the pyrazine nitrogen (N-1) and the electrophilic guanidine carbon (C-9) could stabilize conformation A23. Indeed, a positive, but admittedly weak, overlap population (0.047) between N-1 and C-9 emerged from the CNDO/2 calculations in agreement with this hypothesis. It should be noted that no comparable mechanism is available to conformer A4.

The unique nature of path F prompted a detailed examination of the conformational energy surface which is presented in Figure 2. Path D (Scheme II) corresponds to traveling along the abscissa of Figure 2 from A to A′; in accomplishing exchange of the terminal amino groups, this path must cross the 20 kcal/mol energy plateau. The lower energy path, F, is delineated by the dashed line in Figure 2. Essentially pure rotation about τ_1 occurs to a value of 120°; then the ensuing rotation of 60° about τ_1 is synchronized with a 90° rotation around τ_2. The calculations infer the presence of a local depression in the energy surface when a terminal amino group (N-10 or N-11) approaches the ring nitrogen (N-1). This may be because the CNDO/2 calculations overemphasize lone pair–lone pair nonbonded attractive interactions.[41] In any case, the local depression only appears to perturb, rather than fundamentally alter, the energy surface. Once the transition state (B ≡ A23) is reached, an energetically downhill, mirror-image pathway leads to point A′ (top of Figure 2) which coincides with completion of the interconversion process.

Examination of the transition state (A23) for free base **2a** in path F suggests that the large energy required to deconjugate the imine nitrogen (N-8) from the "Y-delocalized"[36] π system can be partially offset by the favorable nonbonded interaction between the nucleophilic pyrazine nitrogen (N-1) and the electrophilic guanidine carbon (C-9).

While the calculations favor the synchronized rotation mechanism, they cannot and, indeed, do not exclude either the tautomerism or simple ϕ_3 rotation mechanisms. Furthermore, *if* path F represents the "true" equilibration mechanism for **2a** as predicted by CNDO/2, removal of the stabilizing nonbonded interaction in **2a** or an appropriate derivative by isosteric substitution at N-1 (e.g., by replacement of nitrogen by carbon) should elevate the energy of the synchronized rotation mechanism and perhaps allow the nitrogen equilibration to be "frozen out". To test this hypothesis, aroylguanidine **11** was



11

synthesized and, along with pyrazinoylguanidines **4c, 4e,** and **4e**·HCl, studied by the dynamic ^{13}C NMR technique.[42a,b]

The free energy of activation, $\Delta G^{\ddagger}$, at the coalescence

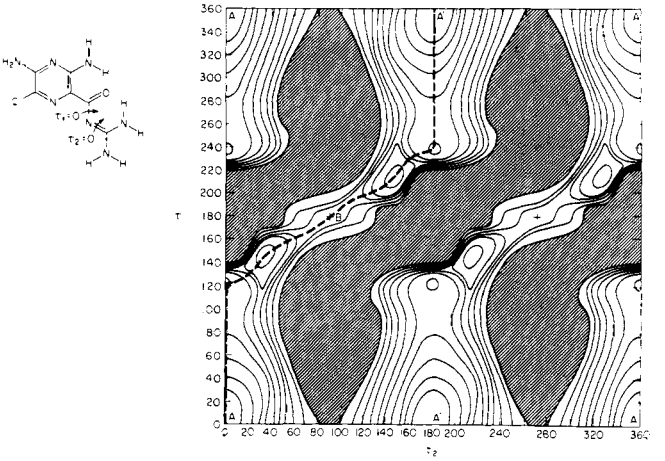


Figure 2. CNDO/2 calculated conformational energy surface for amiloride free base (**2a**) as a function of rotation about bonds τ_1 and τ_2. The dihedral angles in question are defined as 0° with respect to the view shown in the figure, which corresponds to A, the global minimum. Contours are 2.0 kcal/mol apart, up to 20 kcal/mol; higher energy regions are not shown (hashed area). The surface may be folded into a cylinder along either the x or y axis (but not along both axes simultaneously). Thus, the dashed line from A to A′ through transition state B (corresponding to path F of Scheme II) arrives at the same point as a higher energy path along the x axis from A to A′ (corresponding to path C of Scheme II). B represents a saddle point on the energy surface, and thus is a transition state, not an intermediate. "In-plane inversion at nitrogen" modes are not considered but are computed to be higher in energy (see text). Mechanisms involving tautomerism are not accommodated on this energy surface.

temperature, T_c, was calculated for compounds **4e, 4e**·HCl, and **11** using the equation

$$\Delta G^{\ddagger} = -RT_c \ln \frac{h}{kT_c} \frac{\pi(\upsilon_a - \upsilon_b)}{\sqrt{2}} \qquad (1)$$

where k = Boltzmann's constant, h = Planck's constant, and υ_a and υ_b are the resonance frequencies of the two signals.[18g] In each case, upon reaching a temperature ca. 1 °C below the T_c, the decoupled ^{13}C signal (singlet for $NHCH_3$ groups) gave way to a pair of singlets centered about the original resonance position and separated by 14 Hz. In contrast, the $N(CH_3)_2$ ^{13}C signal for tetramethyl derivative **4c** remained a singlet throughout the temperature range studied (lower limit = −60 °C). Accordingly, **4c** must have a $T_c < -60$ °C and a $\Delta G^{\ddagger}$ value < 10 kcal/mol. The resulting experimental values for the barriers to terminal nitrogen group interconversion are recorded in Table VII along with those calculated by the CNDO/2 method.

Analysis of the data in Table VII indicates that, under the conditions studied, model compound **11** undergoes N-10–N-11 equilibration at the same rate (within experimental error) as pyrazinoylguanidine **4e** and its hydrochloride salt, whereas, as anticipated, interconversion of N-10 and N-11 is more facile for tetramethyl derivative **4c** than for dimethyl derivative **4e** and model compound **11.** Furthermore, in each case, rotation about the (C-9)–(N-10) and (C-9)–(N-11) bonds must be extremely rapid. The experimental value (14.7 kcal/mol) for the barrier to $NHCH_3$ equilibration determined for **11** is obviously inconsistent with that (51.3 kcal/mol) calculated for the synchronized mechanism and, since the latter is calculated to be 31 kcal/mol higher in energy than the simple ϕ_3 rotation mechanism in **11,** these results suggest that the CNDO/2 calculations overestimated the barrier to synchronized rotation[43] in **11** and/or overemphasized the magnitude of the nonbonded attraction[41] in transition state A23. A choice between the remaining mechanistic pathways (i.e., paths C and D) cannot be made for model compound **11** on the basis of these results and, accordingly, it must be concluded that **11** may achieve $NHCH_3$ group interconversion by simple ϕ_3

Table VII. Calculated and Experimental Values for Barriers to Terminal Nitrogen Group Interconversion in Some Acylguanidines

compd	CNDO/2 calcd interconversion barriers for various mechanisms, kcal/mol						expt	
	A	B	C	D	E	F	T_c, °C	$\Delta G^{\ddagger}$, kcal/mol
2a	20.3	>12[a]	20.3	21.2	33.7	15.8		
2a·HCl	*b*	*b*	21.9	*b*	*b*	16.2		
4e							11	14.7
4e·HCl							12	14.8
11	~20.3[c]		20.3	21.2[c]	>33.7[c]	51.3	11	14.7
4c	*d*	*d*	<20[e]	<21[e]	~33[e]	<15[e]	<−60[f]	<10[f]

[a] Estimated by extrapolation from the variable bond length model (G1, G2) discussed in the text. [b] Value not calculated but expected to be high in energy. [c] Value determined by extrapolation from the corresponding value for **2a**. [d] Tautomerism mechanisms not possible for **4c**. [e] Value not calculated but should be less than the corresponding value for **2a** on steric grounds (i.e., **4c** has higher ground-state energy than **2a** owing to steric hindrance). [f] Failed to observe ^{13}C signal splitting (i.e., singlet → pair of singlets) down to −60 °C.

rotation and/or ϕ_2 rotation plus inversion. Although, by analogy, free bases **2a** and **4a** are likely to equilibrate in a manner similar to that of **11,** these results do not exclude the possibility that synchronous rotation (path F) may represent a competing mechanism for equilibration of **2a** and **4e,** particularly in nonpolar media, in vacuo, or within a biological "active site". In these situations, the predicted stability gained in effect by "internally solvating" the guanidino moiety as in conformer A23 could render path F especially attractive.

The CNDO/2 calculations suggest that the barrier to simple rotation about ϕ_3 is slightly higher in energy for amiloride (**2a**·HCl) (21.9 kcal/mol) than for its free base, **2a** (20.3 kcal/mol). Likewise, the calculated barrier to synchronized rotation is slightly larger for **2a**·HCl (16.2 kcal/mol) than that for free base **2a** (15.8 kcal/mol). Based on the theoretical calculations, path F is favored over path C for **2a**·HCl ($E_F - E_C$ = 5.7 kcal/mol) by a larger energy difference than for free base **2a** ($E_F - E_C$ = 4.5 kcal/mol). However, since solvation effects are undoubtedly more important for cation **2a**·HCl than for its free base (**2a**), the energy values for **2a**·HCl are less reliable than those for **2a.** Thus, we must conclude that amiloride (**2a**·HCl) may equilibrate by the simple ϕ_3 rotation and/or synchronized rotation mechanisms since, in this instance, an inversion mechanism is forbidden.

Summary and Conclusions

The preferred ground-state structures, conformation F1 for amiloride (**2a**·HCl) and conformation A1 (and/or A4) for free base **2a,** were determined experimentally via natural-abundance ^1H, ^{13}C, and ^{15}N NMR techniques. The concomitant use of CNDO/2 calculations, despite their known limitations,[15,41,43] proved helpful in suggesting the nature and sequence of structural questions to be answered experimentally. The collective theoretical and experimental results do not allow a preference between conformers A1 and A4 to be determined for free base **2a.** The inherent coplanarity of conformers A1, A4, and F1 with their attendant maximum π overlap between the pyrazinoyl and guanidino (guanidinium in F1) moieties and optimal intramolecular hydrogen bonding serves to explain the atypical downfield ^{13}C resonance position displayed by the carbonyl carbon (C-7) in free base **2a** as well as the upfield ^{13}C chemical shift of C-7 which occurs on protonation of **2a.** The observed NMR behavior of C-7 in **2a** results from the preference of **2a** to exist as an acylimino tautomer, and closely resembles that of a strongly shielded ketone, whereas protonation at N-8 (i.e., formation of **2a**·HCl) serves to unmask its latent amide character. These observations are, in turn, consistent with the chemical stability exhibited by compound **2a** and its conjugate acid (**2a**·HCl).

The ^{15}N NMR equivalence displayed by the terminal amino groups (N-10, N-11) in **2a** and **2a**·HCl and their N substituents in derivatives **4c** and **4e,** which implies that N-10 and N-11 are interconverting rapidly on the NMR time scale, prompted a thorough investigation of possible equilibration mechanisms by the CNDO/2 method. Subsequent verification of the calculational results was sought by the dynamic ^{13}C NMR technique. The theoretical calculations suggested the exploration of a novel synchronized rotation mechanism (path F) involving transition state A23, which was calculated to derive considerable stability via an attractive nonbonded interaction between N-1 and C-9. The $\Delta G^{\ddagger}$ value of model compound **11,** prepared to test the validity of the theoretical prediction, and those of pyrazine analogues **4e** and **4e**·HCl were determined experimentally at their coalescence temperatures and proved to be virtually identical ($\Delta G^{\ddagger}$ = 14.7–14.8 kcal/mol). Experimentally, this mechanism was shown not to be the major interconversion pathway, at least for model compound **11** under the conditions studied. Based on the results of these studies, a choice cannot be made between the simple ϕ_3 rotation and ϕ_2 rotation plus inversion pathways for effecting dynamic equilibration of N-10 and N-11 in free base **2a.** Since the inversion mechanism is obviously precluded for tetravalent nitrogen, the only dynamic pathway open to amiloride (**2a**·HCl) is ϕ_3 rotation.

In conclusion, the simultaneous use of NMR and CNDO/2 methods has proven to be a fruitful means for gaining insights into the tautomerism and conformation of amiloride (**2a**·HCl) and its free base (**2a**), compounds rendered challenging for study to theoretician and spectroscopist alike by virtue of their structural complexities and physical properties. Utilization of the information gained from these studies in the design of potential therapeutic agents will be the subject of future publications from these laboratories.

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Supplementary Material Available: Figure 3 (sketches of all tautomer configurations and conformations of **2a** and **2a**·HCl examined by the CNDO/2 method) (4 pages). Ordering information is given on any current masthead page.

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