Tetrahedron Vol 33, pp 1227 to 1234 Pergamon Press 1977 Printed in Great Britain

ROTATIONAL ISOMERISM—XXI[1]

THE CONFORMATION OF 2-AMINO-3-FLUOROPROPANOIC ACID (2-AFP) AND 2-FLUORO-3-AMINOPROPANOIC ACID (3-AFP) AS THE ZWITTERION, CATION AND ANION, AN NMR AND MO STUDY

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(*Received in UK 9 September 1976; Accepted for publication 11 October 1976*)

Abstract—The NMR spectra of 2-AFP And 3-AFP in neutral, acid and alkaline solutions have been obtained and completely analysed to give the ^1H, ^{19}F and the ^{13}C chemical shifts and the HH, HF and CF coupling constants. In the analysis the use of FT spectra with inversion recovery sequences enabled an unambiguous assignment of the complex proton region to be made in certain cases due to the different relaxation times of the protons. The rotamer populations and hence relative energies have been obtained from the $^3J_{HH}$ couplings, using calculated rotamer couplings which explicitly include the orientation dependence of electronegative substituents. A MO investigation of the rotamer energies of the zwitterions, anions and cations is given. The inclusion of the counter-ion into the CNDO wave function gives calculated rotamer energies in complete agreement with those observed for 2-AFP. In 3-AFP, the counter-ion method cannot fully compensate for the larger interactions between the charged groups. Calculations on related molecules including β-alanine show this is a general effect, possibly due to the solvated water molecules.

The determination of the precise conformation of biologically active molecules in solution, and the relationship between conformation and biological activity are amongst the major problems of medicinal chemistry.[2] Two powerful techniques for the study of these problems are NMR and MO methods. The NMR technique and in particular the use of vicinal $^3J_{HH}$ couplings is one of the most reliable and general methods for conformational studies in any media and has been widely used recently.[3,4] Also much effort has been expended to attempt to utilise the advanced MO techniques now extant to predict molecular energies in solution.[2,5] The major difficulty with this method is to simulate the effect of the solvent water molecules around the solute and this is particularly important for charged species. One promising method of overcoming this problem is the super-molecule approach of Pullman *et al.*, in which the co-ordinated water molecules are explicitly considered in the MO calculations.[6,7] An alternative complementary method is to include the counter-ion in such calculations and this method gave promising results in the case of histamine.[8] It was thus of some interest to see whether such an approach would be valid for a more complex molecule, and two molecules providing a comprehensive test of both the NMR and MO methods are 2-amino-3-fluoropropanoic acid (2-AFP)† and 2-fluoro-3-amino propanoic acid (3-AFP).‡ 2-AFP is a potentially useful active antibacterial agent[9] which has only recently been prepared in large quantities;[10] 3-AFP, a metabolite of the anti-tumour drug 5-fluoro uracil[11] produced marked behavioural changes in mammals in a recent study.[12]

We present here the complete analysis of the NMR spectra of these compounds in acid, neutral and alkaline media, and these show in particular the usefulness in the analysis of complex spectra of the Inversion-Recovery pulse technique. Also a simple method for the determination of $^3J_{HH}$ couplings in multi-functional ethanes is given which enables the rotamer populations to be estimated, and these rotamer populations and therefore relative energies are compared with an MO treatment of the molecules, with and without the counter-ions. A preliminary account of the MO calculations has been given.[13]

EXPERIMENTAL AND SPECTRAL ANALYSIS

A commercial sample of 3-AFP (Koch-Light Labs) was dissolved in D_2O soln (100 mg in 2 ml, i.e. 0.5 M, pH 5.5) and conc HCl and powdered NaOH added successively to achieve acid (pH 1.5) and alkaline (pH 11.8) solns without excessive dilution.

The ^1H spectra were obtained on a Varian HA-100 spectrometer using DSS as internal reference for the acid and neutral solns and t-butanol for the alkaline soln. 94.1 MHz ^{19}F spectra referred to external CF_3CCl_3 and 25.2 MHz ^{13}C spectra using internal t-butanol reference were obtained on a Varian XL-100-15 spectrometer. The ^{13}C spectra were run on the FT mode with proton noise decoupling, sweep 5000 Hz, pulse width 25 μs and AT 0.4 sec. In addition expanded spectra were obtained (SW 200 Hz, PW 80 μs, AT 10 sec). The FID's were transformed into 2048 real data points, giving a digitisation accuracy of ±0.1 Hz (200 Hz SW) and ±0.1 ppm (5000 Hz SW). Sample temperatures on both spectrometers are 29°C (undecoupled) and 37° (XL-100) with noise decoupling.

Similar procedures were used on a 20 mg sample of 2-AFP obtained as a gift (Hoffmann-La Roche, Basle). ^1H and ^{13}C spectra were obtained on a Varian XL-100-15 spectrometer in pulsed FT mode, 5 mm tube, 0.4 ml soln at pH 1, 7 and 10.

To avoid overlap of the relatively strong HOD peak with the AB spectrum of the CH_2F protons, the solvent peak was removed by the Inversion-Recovery technique, applying a suitable (180-τ-90-T)$_n$ pulse sequence. For solutions of pH ≈ 1 (DCl/D_2O), it was noticed that the longer relaxation time of the α-proton relative to the $-CH_2F$ protons allowed for selective removal of the peaks due to this proton (Fig. 1), using a similar pulse sequence with τ suitably reduced. This allows hidden bands due to the CH_2F proton pattern to be resolved, and the analysis to be completed. This technique, previously used for T_1 measurement in carbohydrates,[14] can be applied to any complex spin system in which

†Often described as β-fluoro-α-alanine or 3-fluoro alanine.

‡Often described as fluoro-β-alanine. In order to avoid any ambiguity, we shall use the systematic nomenclature here.

Fig. 1. The proton magnetic spectrum of 2-amino-3-fluoropropanoic acid (2-AFP) in DCl./D_2O, pH ~ 1.0, (a) normal FT spectrum with pulse width 25 μsecs (52° flip angle) showing the large residual HDO peak; (b) HDO peak removed by $(180\text{-}\tau\text{-}90\text{-}T)_n$ pulse sequence (τ = 8.2 sec); (c) H_c removed by $(180\text{-}\tau\text{-}90\text{-}T)_n$ pulse sequence (τ = 5.0 sec) HDO peak inverted.

*possible impurity peaks.

one of the nuclei has a significantly longer relaxation time than the others. The difficulty experienced in the analysis of the spectra of 2-AFP due to the closely-coupled nature of the methylene spectrum at all pH's (δ_{AB} ~ 3–5 Hz, J_{AB} ~ 10 Hz), was considerably emphasised by the presence of the relatively large residual solvent peak, and techniques involving selective removal of one nucleus relative to another aid the analysis significantly.

The ^1H and ^{19}F spectra of both molecules are formally ABCX (X = F) spin systems.[11] In 3-AFP the C_2 proton was always sufficiently removed from the C_3 methylenes so as to commence the analysis as an ABMX system. From this the complete ABCX iteration using LAOCN3 could be immediately performed.[11] The analysis identifies the C_2 proton (H_c) but does not of course distinguish the methylene protons. The ^{13}C [^1H] spectra were first-order. Attempts to observe the ^{13}C [^{19}F] spectra were unsuccessful, probably due to the long relaxation times of the carbons.

The results from these analyses are collected in Tables 1 and 2, together with the probable and r.m.s. errors (LAOCN3). These are typically *ca.* 0.03 and 0.08 Hz respectively for 3-AFP suggesting that the couplings are in general accurate to *ca.* 0.1 Hz, except for the HF couplings (0.2 Hz in some cases). In 2-AFP although the r.m.s. errors are about the same, the probable errors, particularly for D_2O soln, are much higher (0.15 Hz) reflecting the very closely coupled nature of the spectrum. The acid and alkaline solution spectra gave, however, reasonable r.m.s and probable errors.

Table 1. Chemical shifts (ppm)[a] for 3-AFP and 2-AFP in neutral, acid and alkaline solutions

	H_A	H_B	H_C	F_X	C_2	C_3	CO
3-AFP							
neutral	3.530	3.384	5.081	188.45	87.9	42.2	173.7
acid	3.603	3.480	5.247	189.83	86.4	41.5	170.9
alkaline	3.039[b]	2.954[b]	4.808[b]	188.13	92.4	43.7	176.4
2-AFP							
neutral	4.87	4.84	4.06		55.8	82.9	171.1
acid	5.23	5.15	4.64		54.5	82.1	169.6
alkaline	4.89	4.85	3.78		57.0	87.4	179.2

[a]Proton shifts (δ_H) downfield from DSS; fluorine shifts (ϕ^*) upfield from $CFCl_3$ (external), and carbon chemical shifts (δ_C) from tBuOH (δ, $\underline{C}H_3$ 31.9), or dioxan (δ, $\underline{C}H_2$ 67.4).

[b]From tBuOH using δ_H (Me) 1.232.

The rotamer populations. Inspection of Table 2 shows that the coupling constants for the two amino-acids are quite different, even though the same substituents are present in the two molecules, and also the couplings often change considerably with pH. These changes may reasonably be ascribed to varying proportions of the rotamer populations in the different media (e.g. the effect of pH on the vicinal $^3J_{HH}$ coupling of α-alanine, in which no rotational isomerism is possible, is insignificant[16]). Intrinsic solvent and pH dependency has been reported for $^3J_{HH}$ couplings[17] and for HF couplings[18] but we shall be concerned largely with the $^3J_{HH}$ couplings. There are three non-equivalent rotamers for both compounds (Fig. 2), thus in order to obtain the rotamer populations from the observed couplings it is necessary to estimate the couplings in the individual rotamers. In such calculations

Table 2. NMR coupling constants (Hz) for 3-AFP and 2-AFP in neutral, acid and alkaline media

	HH				HF			CF		
	$^2J_{AB}$	$^3J_{AC}$	$^3J_{BC}$	$^2J_{CX}$	$^3J_{AX}$	$^3J_{BX}$	r.m.s.	$^1J_{CF}$	$^2J_{CF}$	$^3J_{CF}$
3-AFP										
neutral	−13.98	3.15	8.68	50.58	28.11	17.73	0.08	184	21.3	21.3
	(0.03)	(0.04)	(0.04)	(0.03)	(0.05)	(0.05)				
acid	−14.18	3.12	8.23	49.92	27.77	18.54	0.07	184	22.5	21.1
	(0.03)	(0.04)	(0.04)	(0.03)	(0.04)	(0.04)				
alkaline	14.34	3.12	6.73	51.33	26.56	25.32	0.08	181	21.0	21.4
	(0.03)	(0.06)	(0.06)	(0.03)	(0.05)	(0.05)				
2-AFP				$^2J_{AX}$	$^3J_{BX}$	$^3J_{CX}$		^1J	^2J	^3J
neutral	−10.71	5.42	2.13	45.98	47.40	29.55	0.07	169	20.0	6.3
	(0.04)	(0.15)	(0.15)	(0.12)	(0.11)	(0.04)				
acid	10.97	4.23	2.59	45.51	47.25	30.10	0.06	171	20.0	5.2
	(0.03)	(0.03)	(0.04)	(0.05)	(0.05)	(0.04)				
alkaline	−9.56	4.95	3.22	46.81	47.49	29.94	0.02	166	19.4	7.6
	(0.02)	(0.02)	(0.2)	(0.04)	(0.05)	(0.01)				

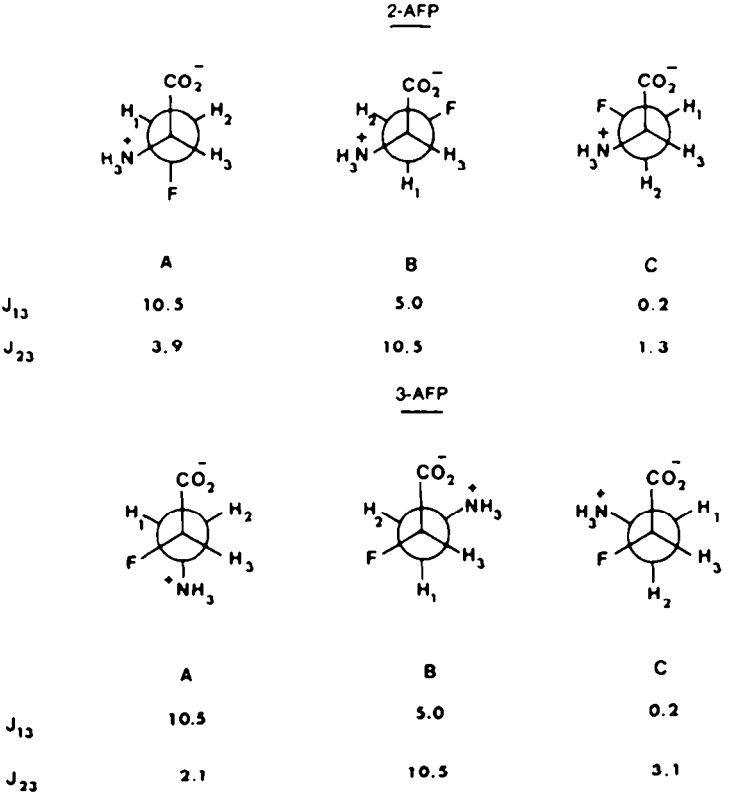


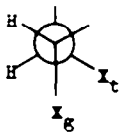
Fig. 2. The possible rotamers and the calculated $^3J_{HH}$ couplings (see text) for 3-AFP and 2-AFP.

it is crucial that the substituent orientation of such electronegative substituents as fluorine (and oxygen) with respect to the coupling protons be considered explicitly. However, we make the simplifying assumption that the rotamer couplings depend only on the substituent oreintation and electronegativity. Thus for the trans coupling (J_t) as all orientations of the substituents with respect to the couplings protons are identical (see Fig. 2) and both molecules have identical substituents we take only one value of J_t for both. Ison *et al.* in a similar study of the conformations of catecholamines $Ph{\cdot}CH(OH){\cdot}CH_2{\cdot}\dot{N}H_2R$ in aqueous solution obtained a value of J_t of 11.2 Hz from cyclic model compounds (morpholines) with the same substituents.[19] Using the equations of Abraham and Gatti[20] to correct for the difference between oxygen and fluorine gives J_t for the $C{\cdot}CHF{\cdot}CH_2\dot{N}H_2$ fragment as 10.5 Hz.

The problem of calculating the *gauche* oriented couplings is more difficult, as the orientation dependence of the couplings on the substituents can be pronounced, and the couplings are also dependent on the actual value of the dihedral angle between the coupling protons thus making the use of cyclic analogues more questionable. For the case of 1,2-disubstituted ethanes $XCH_2{\cdot}CH_2Y$ Abraham and Gatti obtained a series of equations relating the various couplings to the substituent electronegativity and orientation and these have been widely and successfully used in conformational studies.[2-4,19]

However, they are not applicable as given for multisubstituted fragments. A simple scheme based on these equations for predicting *gauche* couplings in multisubstituted ethanes is as follows.

Consider any *gauche* coupling (Scheme A). There are



only two different positions with respect to the coupling protons of any substituent X; X_t where the substituent is *trans*(*anti*) to one of the coupled protons and X_g where it is *gauche* to one of the coupled protons. To a first approximation we may write an additive relationship (eq^{n} 1).

$$J^{HH} = J_0 + \sum (X_g + X_t). \qquad (1)$$

Using the data of Ref. 20 with eqn (1) gives immediately the substituent parameters of Table 3. These then give consequently the rotamer couplings of Fig. 2. It is, however, important to note that these substituent constants are subject to the same limitations as the equations of Ref. 20, in particular the assumptions of additivity of substituent effects and the neglect of dihedral angle variations are both possible sources of error. However, these at least do take account of substituent orientation, which has often been neglected previously.[16] The effects of substituent orientation shown in the calculated couplings of Fig. 2 are clearly large, the *gauche* couplings vary from 0.2 to 5.0 Hz.

With these rotamer couplings, the populations of the rotamers may be calculated from the observed $^3J_{HH}$ couplings (Table 2) from the standard eq^{n} (2)

$$J_{13}(obs) = n_A J^A_{13} + n_B J^B_{13} + n_C J^C_{13}$$
$$J_{23}(obs) = n_A J^A_{23} + n_B J^B_{23} + n_C J^C_{23} \qquad (2)$$

where $1 = n_A + n_B + n_C$.

However, it is first necessary to assign the signals of the methylene protons and for this qualitative considerations of the rotamer populations are required. In 3-AFP zwitterion the observed couplings of *ca.* 8.7 and 3.2 Hz are characteristic of a *trans* oriented and *gauche* oriented coupling. This implies a preponderance of either rotamer A or B (Fig. 2). In β-alanine zwitterion ($H_3\dot{N}{\cdot}CH_2CH_2CO_2^-$) in D_2O solution the energy difference between the *trans* and *gauche* rotamers has been determined as 0.0 kcal/mole (see later). Electrostatic considerations alone would thus suggest that as the negatively charged F atom in 3-AFP would be expected to strongly prefer the *gauche* orientation with the NH_3^+ group, rotamer A is the preferred rotamer. This qualitative argument, which will be shown to be fully supported by the MO calculations, immediately assigns H_1 as H_B (Table 2) and H_2 as H_A. This is also supported by the $^3J_{HF}$ couplings, as in rotamer A J_{1F}, which is a *gauche* oriented coupling, should be much less than J_{2F}, a *trans* oriented coupling, as is observed. If rotamer B was preferred there would be two $^3J_{HF}$ *gauche* couplings which are not observed.

In 2-AFP there is again one larger (*ca.* 5.4 Hz) and one smaller (*ca.* 2 Hz) coupling and the assignment again follows from the relative stabilities of rotamers A and B, as in C there is no *trans* coupling. Reasoning on similar lines to 3-AFP, rotamer A, with a *gauche* $F{-}NH_3^+$ interaction would be expected to be more stable than B with a *gauche* $F{-}CO_2^-$ interaction. This immediately assigns H_1 to H_A (Table 2) and H_2 to H_B.

The application of eq^{n} 2 to the observed couplings in any solution gives three equations in the three unknowns $n_{A,B,C}$ and thus the rotamer populations can be obtained immediately. These are given in Table 4. The relative rotamer free energies can be obtained directly from these in the normal manner and making the usual assumption that the rotamer entropies are all equal these free energies then become internal energies and are given in Table 5. These will be considered together with the results of the MO calculations (next section). It is pertinent to note here that for a reasonably populated rotamer an error of 5% in the estimated population would give an energy change of 0.2 kcal/mole, and this is a reasonable estimate of the uncertainty in these values.

Table 3. Substituent parameters for J_g^{HH} (Hz)

$$J_g^{HH} = 4.0 + \sum (X_g + X_t)$$

	H	C	Br	N	Cl	O	F
X_g	0.0	0.2	0.5	0.5	0.7	0.9	1.1
X_t	0.0	−0.6	−1.1	−1.4	−1.6	−1.8	−2.6

Table 4. Rotamer populations of 3-AFP and 2-AFP as the zwitterion, cation and anion

	3-AFP			2-AFP		
	n_A	n_B	n_C	n_A	n_B	n_C
zwitterion	0.77	0.11	0.12	0.50	0.0	0.50
cation	0.73	0.10	0.17	0.38	0.03	0.59
anion	0.60	0.08	0.32	0.42	0.09	0.49

Table 5. Observed and calculated rotamer energies[a] (kcal/mole) for 2-AFP And 3-AFP

	E_A	E_B	E_C	E_B-E_C calc.	E_B-E_C obs.	E_C-E_A calc.	E_C-E_A obs.
2-AFP							
zwitterion[c]	48.8	-40.4	-46.2	5.8	>2	2.6	0.0
(+Counter-ions)[c]	169.7	-164.6	-169.5	4.9		0.2	
cation[b]	-47.7	-43.1	-47.7	4.6	1.7	0.0	-0.3
(+counter-ion)	-198.3[b]	-195.1[b]	-198.5[d]	3.4		-0.2	
Anion[b,f]	-25.2	-21.5	-21.9	0.4	1.0	3.3	-0.1
(+counter-ion)	-92.2	-90.9	-91.5	0.6		0.1	
3-AFP							
zwitterion	-27.6[b]	-40.2[e]	-45.3[e]	5.1	0.0	-17.7	1.2
(+counter-ions)	167.5[b]	168.6[e]	172.4[e]	3.8		4.9	
cation	-46.8[d]	-46.2[e]	-51.4[e]	5.2	0.3	-4.6	0.9
(+counter-ion)	-199.0[d]	197.3[d]	200.8[e]	3.5		-1.8	
anion[b,g]	-28.6	-30.5	30.9	0.4	0.8	-2.3	0.4
(+counter-ion)	-101.7	-104.3	-104.9	0.6		-3.2	

[a]The binding energy (CNDO) is the tabulated energy - 3000 kcal/mole $w_2 = 90°$(b); 120°(c); -90°(d); 0°(e).
[f]$NH_{11}H_{12}$. [g]$NH_{12}H_{13}$.

For the less populated rotamers the energy differences are less accurate and in particular E_B-E_C in 3-AFP is an energy difference between two minor constituents and therefore is much more dependent on the assumptions made in the analysis. For example if J^A_{23} was increased from 2.1 to 3.0 Hz, then the populations in the zwitterion become 0.82 (A); 0.02 (B) and 0.18 (C), giving E_B-E_C and E_C-E_A values of 1.4 and 1.0 kcal/mole respectively (*ca.* 0.0 and 1.2 in Table 5).

With these rotamer populations the observed values of $^3J_{HF}$ can be used to further check the assignments made earlier. Assuming merely one value of J_g and J_t then values of 16.0 (J_g) and 32.0 (J_t) combined with the populations of Table 4 give for 3-AFP in neutral, acid and alkaline solution values of J_{1F} of 17.9, 18.7 and 21.2 and of J_{2F} 28.3, 27.7 and 25.5 in good agreement with the observed values.

For 2-AFP using the same J_g and J_t values, the calculated values of J_{1F} are 24.0, 25.6 and 23.8 Hz all somewhat less than the observed values (29.6, 30.2 and 29.9) but with the correct trend. In view of the assumption of one J_g and one J_t value, these results support the assignments of the methylene protons made earlier.

Rotamer energy calculations. We now wish to calculate the rotamer energies and in particular to note the effect of introducing the counter-ions in such calculations. We use, as previously, the CINDO programme of Pople *et al.*,[21] which has been widely used in similar calculations, and this is used in the CNDO approximation.[22] One difficulty with such MO programmes in these calculations is that they underestimate the repulsive interactions between atoms, and as a consequence of this the minimum energy separation of two oppositely charged ions is much closer than is observed experimentally.[23,24] Thus standardised, not minimised bond lengths and distances must be used. Also the problem of determining the minimum energy conformation for each of the possible rotamers with and without the counter-ions is a complex one. There are three dihedral angles which can in principle be varied, the $C_3C_1C_2M_4$ (M = F or N) angle (W_1), the $C_2C_1C_3O_8$ angle (W_2) and the dihedral angle about the CN bond (Fig. 3). Furthermore the orientation of the proton on the carboxyl group in the cation has to be defined as well as the conformation of the amino group in the anion. Finally, the positions of the counter-ions need to be defined. Thus in

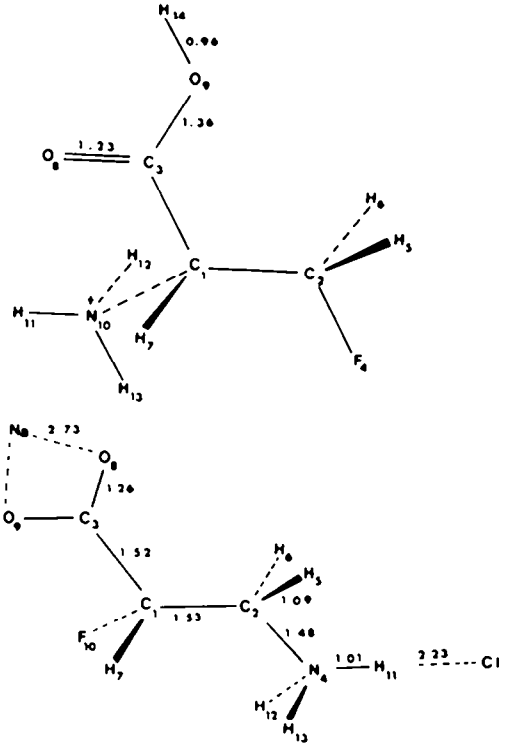


Fig. 3. 2-amino-3-fluoro propanoic acid (2-AFP) cation and 2-Fluoro-3-Amino propanoic acid (3-AFP) zwitterion, with the nomenclature and geometry used in the calculations. W_1 is the $C_3C_1C_2M_4$ dihedral angle and W_2 in $C_2C_1C_3O_8$ dihedral.

order to curtail the calculations it is necessary to make some simplifying assumptions concerning the molecular geometries. The importance of using the correct geometry in MO calculations has been repeatedly emphasised, and this is also the case here as far as the absolute energies are concerned. However, as we shall show later reasonable modifications of the geometry have very little effect on the *relative* rotamer energies, which are the parameters under study. Thus our approach was to use initially standard geometries in order to investigate the effects of the counter-ions in these calculations and subsequently to

test the validity of these standardised energies by particular calculations with more refined geometries.

The standard bond lengths[24] used are shown in Fig. 3, and the bond angles were taken as tetrahedral or 120°. The positions of the counter-ions, in this case Na and Cl, were obtained from the known ionic radii of Na, Cl, NH_4 and O[25] and as previously[6] the counter-ions were placed in positions dictated by symmetry and the absence of steric effects (Fig. 3).

There still remains the problem of the three dihedral angles, and the conformations of the CO_2H and NH_2 groups. It has been shown repeatedly that groups with 3-fold symmetry such as CH_3 and NH_3 can be placed in the classical staggered position in such calculations without serious error and this procedure was followed here for the NH_3 group. Furthermore, although this is not such a good assumption, this procedure was adopted for the $C_1C_2C_3M_4$ dihedral (W_3) and the rotamer energies were calculated for the classical values of this angle, 60, 180 and 300. This is not too serious an approximation in those cases in which the rotamer energies are similar in magnitude, as the hindering potential will then be to a good approximation a 3-fold potential having minima at the staggered positions. For those cases in which there is a large energy difference between the rotamers due to major interactions between the substituent groups, for example between the NH_3 and CO_2 in 3-AFP zwitterion, this approximation is more open to question. However, there is no other procedure, because in such a case searching for the minimum energy conformation by CNDO will result in an incorrect geometry due to the approximations in the wave function mentioned earlier. Fortunately, the cases for which the calculated energies are least accurate are also those for which the experimental energy differences are least accurate, due to the small amounts of one rotamer being present. This will be considered subsequently.

Finally, the orientations of the carboxyl and amino (NH_2) groups were obtained by searching for the minimum energy conformations, with the restriction that the amino group is tetrahedral and retains the staggered orientation about the CN bond. This, of course, is not the case for the carboxyl as the intrinsic barrier to rotation for the symmetric CO_2 group as in CH_3CO_2 will be 6-fold and therefore very small, for example in CH_3NO_2 which is iso-electronic V_6 equals 6.0 cal/mole as compared to methylamine 1.976 kcal/mole. As the molecules now give different results it is necessary to consider them separately.

2-Amino-3-fluoro propanoic acid (*2-AFP*). In practice the molecular energy was calculated for various values of W_2 and for the different conformations of the amino group specified by any two of H_{11}, H_{12} and H_{13} (Fig. 3). Table 5 gives the resultant conformations and minimum energies for the three rotamers together with the calculated and observed rotamer energy differences E_B-E_C and E_C-E_A. We note that these energy differences may be considered to be the $F\cdots NH_3$ and $F\cdots CO_2$ (*gauche* to *trans*) interactions in this molecule (Fig. 2).

In the zwitterion the minimum energy conformation is for $W_2 = 120°$ and this is the preferred conformation of many α-amino acid zwitterions in the crystal.[28] Rotamer B is of much higher energy than the other rotamers, both in the free molecule and counter-ion calculations, as observed.

Interestingly although rotamer C is also of much higher energy than rotamer A in the free molecule calculations, implying repulsion between the fluorine and carboxyl substituents; in the calculations including the counter-ions rotamers C and A have identical energies, and this is also observed experimentally. This shows that even when the carboxyl group has a formal negative charge, in solution there is little repulsive interaction between the fluorine and the carboxyl group. This may be considered as another example of the very interesting interaction between electronegative substituents, in which for example in 1,2-disubstituted ethanes, electronegative substituents show a marked preference for the *gauche* conformation, even though considerations based solely on charge distribution would indicate otherwise.[29] On the other hand both calculations and observation show the marked preference of the fluorine for the *gauche* conformation with respect ot the NH_3 group, and this is very likely due to electrostatic attraction.

In the cation a similar situation prevails though both calculations show that rotamer B is comparatively much more stable than in the zwitterion. In this case, however, the calculated energy difference (E_B-E_C) is still considerably larger than the observed value, and this is the only serious disagreement of the calculated and experimental data for this molecule. In contrast the energy difference (E_C-E_A) calculated by either method is the same and in complete agreement with the observed value. Comparison of the calculated energy differences (E_C-E_A) for the zwitterion and cation show very clearly the influence of the counter-ion, in that in the isolated molecule case the zwitterion calculations are *ca.* 3 kcal/mole in error, due to the $F\cdots CO_2$ interaction. Both by adding the counter-ion, and by protonation, (as in the cation), this spurious excess interaction is removed giving the correct energy difference.

In the anion rotamer B is slightly more stable, comparatively than in the cation, and the calculated energy differences (E_B-E_C for both calculations) agree very closely with the observed value. Once again the free molecule calculation of E_C-E_A gives too high a value, as again we have the uncompensated $F\cdots CO_2$ interaction.

Introducing the counter-ion gives good agreement in this case also. Note that the preferred conformation of the amino group is always $NH_{11}H_{12}$ (Fig. 3), i.e. with the nitrogen lone-pair in a *trans* (*anti*) orientation to the carboxyl group. In this conformation the orientation of the F atom in rotamer C with respect to the amino group is analogous to that of the chlorine w.r.t. the OH in 2-chloroethanol.[30] In this compound although the preference for a *gauche* conformation was originally ascribed to H-bonding, more recent studies have indicated that specific H-bonding is a relatively minor interaction and the cause of the preference for the *gauche* conformation is probably simply electrostatic interaction.[30] Thus there does not appear to be any strong specific intramolecular hydrogen bonds in 2-AFP, and certainly there is no indication that H-bonding makes any major contribution to the rotamer energies.

2-Fluoro-3-amino propanoic acid (3-AFP). The MO calculations for 3-AFP are also given in Table 5 together with the observed relative energies E_B-E_C and E_C-E_A, which now may be considered to represent the $F\cdots NH_3$ and the $NH_3\cdots CO_2$ (*trans* to *gauche*) interactions (Fig. 2).

In the zwitterion the free molecule calculations show as expected the large attraction between the two charged groups, giving rotamer A considerable excess energy.

This excess energy is much diminished in the counter-ion, but even so the calculations do not predict that A is the most stable rotamer, which is the experimental result. This is not too surprising as we are attempting to compensate for a very large electrostatic attraction by the counter-ion technique (see later). Somewhat more surprising is the result for E_B-E_C. Here the calculations predict, as expected, a sizeable attraction between the fluorine and $\dot{N}H_3$ groups, as indeed was observed in 2-AFP (calc. 4.9 kcal/mole; obs. >2). In 3-AFP in contrast the observed value of E_B-E_C is *ca.* 0. It has been noted that this ΔE is less accurate than the other values. Even so, this discrepancy is too large to be accounted for by experimental error.

Interestingly, the same phenomenon is seen in the results for the cation. The calculated value of E_C-E_A decreases markedly, again as expected, the value of E_C-E_A in the free molecule cation being the same as for the zwitterion plus counter-ions. This was also the case for 2-AFP demonstrating again that the effect of the counter-ion is very similar to protonation of the carboxyl, i.e. primarily a removal of negative charge. Also the calculated values of E_B-E_C are unchanged in going from zwitterion to cation, as weould be expected if this is simply an $F\cdots\dot{N}H_3$ interaction. Once more the experimental value is much less than the calculated one.

The same general pattern is seen in the anion in that again rotamer A has a calculated energy much higher than observed, the calculated value of E_C-E_A again being of the wrong sign. In this case, however, the relative energies of rotamers B and C are well reproduced, both the free molecule and counter-ion calculations giving similar results. The values for this energy difference in the 3-AFP anion compare very well with the corresponding values in the 2-AFP anion, suggesting that the interaction of an amino group and fluorine atom is roughly independent of the other groups present and is *ca.* 0.8–1.0 kcal/mole in favour of the *gauche* orientation.

DISCUSSION

The general picture emerging from the calculation is that the counter-ion model gives calculated rotamer energies to almost experimental accuracy for 2-AFP, but fails to reproduce even the order of the rotamer energies for 3-AFP.

An obvious reason for this, mentioned above, is that in 2-AFP the interactions are between uncharged (F, H atoms) and the charged amino-acid moiety, whereas in 3-AFP we are attempting to remove the much larger interactions between two charged groups.

It could be argued, however, that as the precise molecular geometry used in MO calculations is so important the agreement obtained here for 2-AFP is fortuitous. Thus we have repeated some of these calculations using the more refined nontetrahedral geometry recently given for amino-acid zwitterions and cations.[28] In the zwitterion the major difference from the standardised geometry of Fig. 3 is for the carboxyl group, in that the C.C.O. angles are 117.0° and O.C.O. angle 126°; in the cation the carboxyl geometry is C.C. = O 122.0°; C.O.H. 112.0°. These new geometries gave calculated rotamer energy differences in precise agreement with those of Table 5, although of course the actual molecular energies are very different. For example in 2-AFP zwitterion (plus counter-ions) the calculated rotamer energies were −180.3 (A); −175.0 (B) and −180.1 (C) kcal/mole giving E_B-E_C and E_C-E_A values of 5.1 (cf. 4.9) and 0.2 (cf. 0.2) kcal/mole. A similar calculation for 2-AFP cation gave rotamer energy differences of 3.7 (cf. 3.4) and −0.4 (cf. −0.2) kcal/mole. Also varying the positions of the counter-ions has little effect on the rotamer energies, decreasing the $\dot{N}H\cdots Cl$ distance from 2.23 to 1.6 Å (the minimum energy position from CNDO) gives identical rotamer energies for the zwitterion, though the calculated molecular energies are 40 kcal/mole more stable. Thus we may safely conclude that any reasonable change in the molecular geometry will not alter the agreement obtained between the calculated and observed rotamer energies.

A related question is whether the much larger interactions between the charged groups in 3-AFP would also be unaffected by changes in the molecular geometry. To examine this and simultaneously to test the counter-ion method in a related molecule we considered β-alanine. A recent precise determination of the proton couplings has been recorded, giving $N(J+J')$ equal 13.55 Hz.[33] Using this with the values of N_g and N_t for this fragment given previously of 11.67 and 17.37 Hz gives immediately the rotamer populations and hence E_g-E_t of 0.0 kcal/mole (the calculated value of N_{AV}, the value for 'free rotation' is 13.57 Hz). Note that this method avoids any ambiguity over the assignment of the proton couplings.

Using the same method and standardised geometry as for 3-AFP, the calculated energy difference (E_g-E_t) for β-alanine zwitterion was −20.1 kcal/mole in the free molecule, decreasing to −8.2 kcal/mole on adding the counter-ions. Incorporating the more refined geometry for the CO_2^- group given earlier and with C.C.C. and C.C.N. angles of 111.0° only alters these values to −18.0 and −7.3 kcal/mole. Thus we may conclude that the 3-AFP result (for E_C-E_A) is general and that any reasonable geometry will not remove the large spurious calculated stabilisation of *ca.* 6 kcal/mole favouring the *gauche* conformation of the NH_3 and CO_2^- groups in these molecules.

It is of some interest to consider whether other methods based on similar approximate wave functions could resolve this discrepancy or whether it is the approximate nature of the wave function which is really responsible.

The super-molecule approach of Pullman *et al.* has recently been applied to the related case of GABA (γ-amino butyric acid).[34] In the isolated molecule, the folded *gauche–gauche* conformation is much more stable than any other form, but in the super-molecule in which three water molecules were attached to both the CO_2^- and NH_3^+ groups, the calculations showed that there were many conformations of similar energies, in agreement with experiment. If these results can be extrapolated to β-alanine they suggest that the free rotation in β-alanine is due to the compensation of the electrostatic attraction of the charged groups by repulsions involving the attached water molecules, i.e. the effective 'size' of the charged groups has been increased by hydration.

The alternative possibility is that the CNDO approximation under-estimates the steric effects present in these molecules, as it is well known that the inter-atomic repulsions calculated by CNDO are usually too low. A simple test of the extent of this discrepancy is to consider sterically similar but uncharged molecules for example, to replace $\dot{N}H_3$ by the isoelectronic Me group in the calculations. We have therefore calculated the *gauche–trans* energies, again using the same methods and geometry for similar molecules. In n-butane and pro-

panoic acid, the calculated values of E_g-E_t are −0.3 and −1.0 kcal/mole, *both cq.* 1 kcal/mole lower than the observed values (0.6 and 0.4 kcal/mole). In sodium propanoate, the discrepancy is larger (calc. −3.3, obs. 0.3 kcal/mole), but of course this calculated value is obtained from the counter-ion method.

In conclusion these considerations suggest that only a small part of the discrepancy found in the calculated interaction of the two charged groups is due to the wave function used. The effects of the solvated water molecules would appear to be the most probable answer. In contrast the approximations in the wave function could well account for much of the discrepancy in the value of E_g-E_c found for 3-AFP (Table 5). In this case there are two adjacent *gauche–gauche* interactions, i.e. rotamer C is much more sterically crowded than A or B. In this the calculated CNDO energy for C would be expected to be much lower than the true energy, as found. Support for this explanation is found in the fact that in the anion, this discrepancy disappears, and this is consistent with the smaller effective size of the amino group. Furthermore, this anomaly is not found in the analogous 2-AFP rotamer C, as here the much smaller F atom has little steric effect even in this case.

Acknowledgements—R.J.A. and P.L. acknowledge S.R.C. Grants towards the purchase of the Varian XL-100 and HA-100 spectrometers and an S.R.C. maintenance award (to P.L.).

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