23 mg of lanostan-3-one-*30,30,30,31,31,31-d*$_6$ (LXIV); recrystallization gave mp 123–124° (lit.[23] mp 127–128° for the unlabeled compound); ir 1695 cm^{-1}; nmr δ 0.81 + 0.90 + 1.09 (CH_3-18, 19, 21, 26, 27, and 32); mass spectrum (rel intensity) 434 (10, M^+), 307 (11), 294 (68), 279 (100), 251 (61), 238 (26), 210 (63).

Lanostane-*30,30,30,31,31,31-d*$_6$ (LXV). Lanostan-3-one-*30,30,30,31,31,31-d*$_6$ (LXIV, 10 mg) was reduced under Wolff–Kishner[11] conditions. Purification[43] gave 4 mg of lanostane-*30,30,30,31,31,31-d*$_6$ (LXV) [see Table I for isotopic composition].

Lanostan-7-one (LXVI). Lithium–ammonia reduction of lanost-8-en-7-one (XV, 100 mg) in the manner described for the preparation of XI gave 92 mg of lanostan-7-one (LXVI); recrystallization gave mp 116–118°; ir 1695 cm^{-1}; nmr δ 0.78 (CH_3-18, 0.82 + 0.89 + 0.93 + 0.97, (CH_3-21, 26, 27, 30, and 31) 1.10 (CH_3-32), 1.18 (CH_3-19), 2.15–3.0 (complex, 4, CH_2-6, CH-8β, CH-15);[44] mass spectrum (rel intensity) *m/e* 428 (50, M^+), 413 (11, M − CH_3), 315 (16), 304 (5), 288 (22), 275 (12), 219 (21), 206 (100), 164 (38).

Lanostan-7-one-*6,6,8β-d*$_3$ (LXVII). Lanostan-7-one (LXVI, 40 mg) was converted to lanostan-7-one-*6,6,8β-d*$_3$ (LXVII) quantitatively in the manner described for XXVI; mass spectrum 431 (M^+).

Lanostane-*6,6,8β-d*$_3$ (LXVIII). Lanostan-7-one-*6,6,8β-d*$_3$ (LXVII, 30 mg) was reduced with lithium aluminum hydride, then the crude alcohol-*d*$_3$ was directly converted to its TMPDA derivative and reductively cleaved (see XLIX and LIII). Purification[43] gave 17 mg of lanostane-*6,6,8β-d*$_3$ (LXVIII) [for isotopic purity see Table I].

Lanostane-*1,1-d*$_2$ (LXX). Lanostan-1-one[38] (LXIX, 23 mg) was treated with lithium aluminum deuteride and the TMPDA derivative of the resulting alcohol reductively cleaved (see preparation of XLIX). After separation from olefin, the product amounted to 6 mg of lanostane-*1,1-d*$_2$ (LXX) [see Table I for isotopic purity].

Carbon-13 Nuclear Magnetic Resonance Titration Shifts in Amino Acids

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Abstract: Nmr titration curves for the individual carbon atoms in a series of representative amino acids have been determined. A computer model involving multiple ionizations has been used to obtain ionization constants and titration shifts, even when ionizations overlap. CNDO/2 molecular orbital calculations suggest a rationalization of the observed changes in ^{13}C shielding on deprotonation. For carbon atoms near the site of ionization a decrease in excitation energy dominates the chemical shift expression, resulting in deshielding despite an increase in electron density; for more distant carbon atoms changes in electron density dominate, yielding shifts in either direction.

Fundamental to the application of ^{13}C nmr to peptides and proteins is knowledge of the characteristic behavior of the ^{13}C chemical shifts of the amino acids as a function of pH.[2-6] We report the result of a ^{13}C nmr study of the titration of a number of representative difunctional and trifunctional amino acids and amino acid derivatives. Computer curve fitting[7] has been used to obtain both accurate pK values and a quantitative estimate of the contribution of each ionization to the observed ^{13}C shifts in the overall titration. CNDO/2 molecular orbital calculations[8] have been carried out for each major ionic species existing during the titration sequence in an attempt to rationalize the observed shifts on deprotonation.

Results and Discussion

In the typical titration curves in Figures 1 and 2,[9] the ^{13}C chemical shift of each carbon is sensitive to more than one titration.[2-6] The solid lines in the figures indicate the final least-squares fit to the observed ^{13}C nmr titration values. The number of ionizations in each model equation (see Experimental Section) is the minimum necessary to reproduce the shape of the experimental curve; in general this is equal to the number of ionizable groups in the molecule. The dashed lines in the figures represent the best fit with the number of ionizations reduced by one. It is clear that even remote ionizations can be monitored *via* the ^{13}C chemical shifts. The value of computer curve fitting is particularly obvious when, as in the glutamic acid titration (Figure 2), there are overlapping ionizations whose separate pK values cannot be obtained by inspection.

The computer calculated pK and ^{13}C nmr titration shift values, together with the standard deviations, are given in Table I. Estimates of the pK of the same

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(9) Throughout this paper, the imidazole ring of histidine and its derivatives is labeled according to IUPAC nomenclature rather than the common biochemical nomenclature (K. Hoffman, "Imidazole and Its Derivatives," Part I, Interscience, New York, N. Y., 1953, p 188), in which N(1) and N(3) as well as C(4) and C(5) are reversed. Complete ^{13}C titration curves for histidine are given in ref 6.

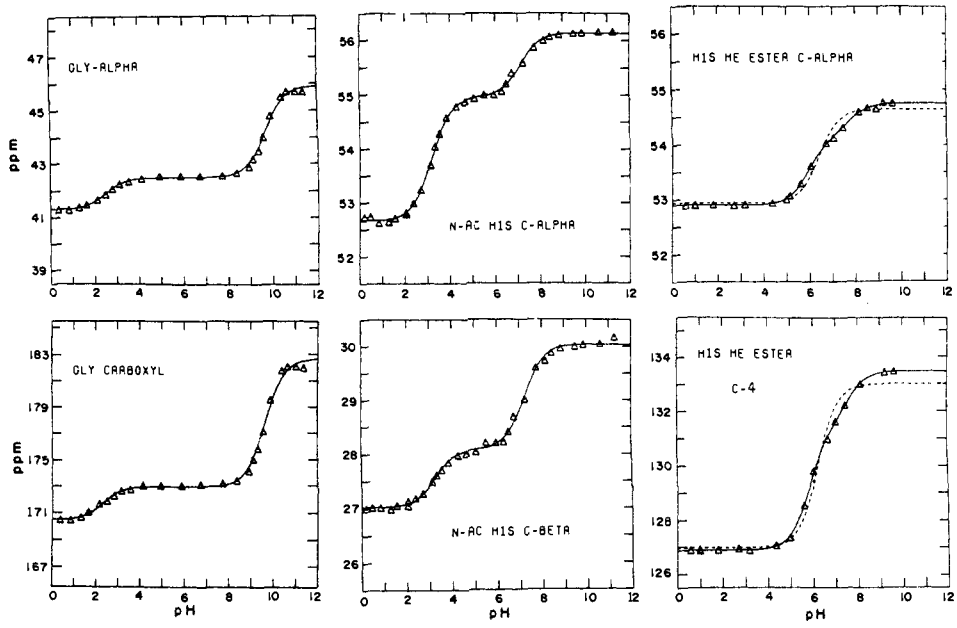

Figure 1. ^{13}C chemical shifts as a function of pH for selected carbon atoms of typical difunctional amino acids and amino acid derivatives: glycine C_0, C_α; *N*-acetylhistidine C_α, C_β; histidine methyl ester C_α, C_4. (Δ) Measured chemical shift downfield from TMS; (—) titration curves representing the best computer fit using a two-ionization model equation; (---) best computer fit for one ionization.

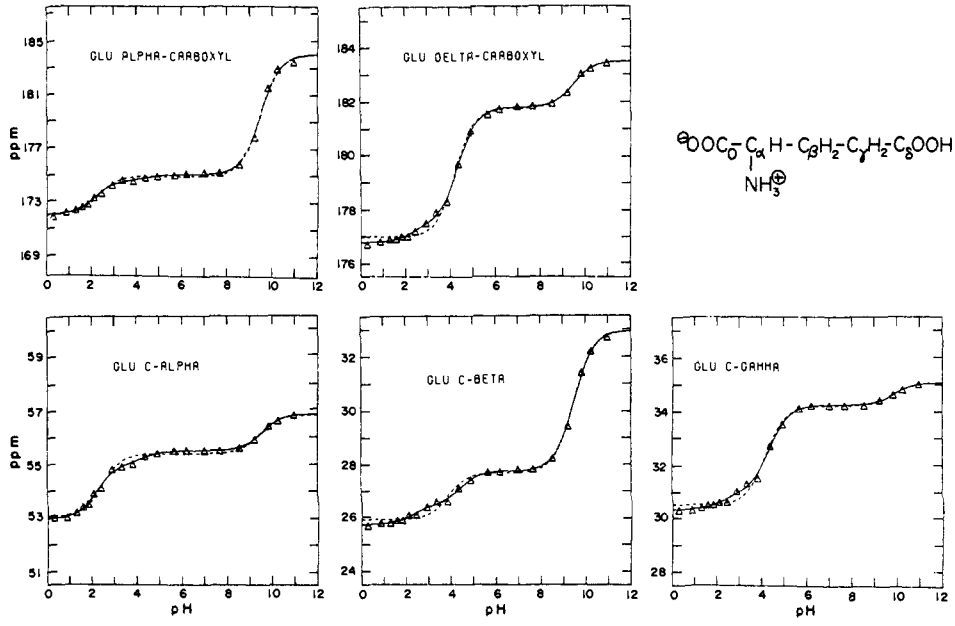

Figure 2. ^{13}C chemical shifts as a function of pH for the five carbon atoms of L-glutamic acid: (Δ) as in Figure 1; (—) and (---) as in Figure 1 but using three and two ionizations, respectively.

titrating group from different carbon atoms agree well with each other and with literature values,[10] with the exception of C_0 of histidine methyl ester. The appearance of additional small peaks in the spectra of this compound at high pH suggests the occurrence of base catalyzed ester hydrolysis, which perhaps is responsible for the discrepancy. In Table II our computer-calculated titration shifts for C_0, C_α, and C_β carbons are supplemented by literature values[3-5] for several other amino acids. With the exception of glycine and C_β of valine, the effects of both amino and carboxyl titrations on the ^{13}C shifts of these carbons are nearly independent of the nature of the side chain even when the side chain contains an ionizable group.

Several features of this pattern of ^{13}C nmr titration shifts are striking and not easily explained. In both titrations the shielding of all three carbon atoms decreases. The amino titration affects both the carbonyl carbon and the β carbon more strongly than the nearby α carbon. In contrast, in the carboxyl titration the shifts of the α and carboxyl carbons are similar and much smaller. It is interesting that in histidine methyl ester, whose carboxyl group is not ionized, the amino titration shifts of both C_0 and C_α are smaller and quite similar to each other. The blocked amino group of *N*-

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Table I. Least-Squares ^{13}C Titration Shifts and pK Values

Amino acid		α-Carboxyl titration pK	α-Carboxyl titration Δ[a]	α-Amino titration pK	α-Amino titration Δ	Side chain titration pK	Side chain titration Δ
Glycine	C_0	2.30 ± 0.15	2.52 ± 0.19	9.66 ± 0.03	9.68 ± 0.18		
	C_α	2.52 ± 0.09	1.22 ± 0.06	9.68 ± 0.03	3.38 ± 0.06		
	pK[b]	2.44 (2.35)		9.67 (9.78)			
Alanine	C_0	2.19 ± 0.11	3.15 ± 0.17	9.58 ± 0.04	9.70 ± 0.21		
	C_α	2.29 ± 0.03	1.84 ± 0.03	9.65 ± 0.07	0.85 ± 0.03		
	C_β	2.15 ± 0.20	1.05 ± 0.10	9.59 ± 0.05	4.81 ± 0.13		
	pK	2.21 (2.34)		9.61 (9.87)			
Phenylalanine	C_0	2.26 ± 0.45	2.46 ± 0.50	9.40 ± 0.11	8.76 ± 0.50		
	C_α	2.39 ± 0.07	1.95 ± 0.07	9.27 ± 0.08	1.46 ± 0.07		
	C_β	2.29 ± 0.47	0.78 ± 0.10	9.30 ± 0.07	4.90 ± 0.18		
	C_1	2.35 ± 0.22	1.22 ± 0.16	9.25 ± 0.07	3.36 ± 0.15		
	$C_{2,6}$[c]		−0.07		−0.09		
	$C_{3,5}$[c]		−0.26		−0.49		
	C_4	2.18 ± 0.22	−0.34 ± 0.05	9.25 ± 0.06	−1.16 ± 0.04		
	pK	2.33 (2.16)		9.28 (9.15)			
Glutamic acid	C_0	2.14 ± 0.15	2.57 ± 0.23	9.49 ± 0.04	8.98 ± 0.20	4.45 ± 0.84	0.46 ± 0.23
	C_α	2.24 ± 0.07	2.04 ± 0.09	9.57 ± 0.09	1.35 ± 0.07	4.32 ± 0.28	0.50 ± 0.09
	C_β	2.29 ± 0.22	0.74 ± 0.10	9.50 ± 0.03	5.23 ± 0.08	4.45 ± 0.13	1.25 ± 0.11
	C_γ	2.27 ± 0.28	0.61 ± 0.11	9.89 ± 0.20	0.85 ± 0.11	4.36 ± 0.05	3.29 ± 0.12
	C_δ[d]	2.26 ± 0.22	0.68 ± 0.10	9.50 ± 0.08	1.72 ± 0.08	4.35 ± 0.04	4.31 ± 0.10
	pK	2.23 (2.19)		9.53 (9.94)		4.37 (4.32)	
Histidine[e]	C_0	1.76 ± 0.12	2.33 ± 0.16	9.19 ± 0.03	8.60 ± 0.17	6.10 ± 0.25	1.28 ± 0.16
	C_α	1.79 ± 0.08	1.64 ± 0.07	9.20 ± 0.11	1.45 ± 0.08	6.20 ± 0.13	1.14 ± 0.08
	C_β	1.76 ± 0.18	0.79 ± 0.08	9.20 ± 0.03	4.35 ± 0.09	6.21 ± 0.07	2.20 ± 0.09
	C_2	1.35 ± 0.41	−0.32 ± 0.08	9.25 ± 0.50	−0.29 ± 0.08	6.19 ± 0.06	2.31 ± 0.07
	C_4	1.72 ± 0.15	1.11 ± 0.09	9.38 ± 0.13	1.55 ± 0.07	6.30 ± 0.04	4.74 ± 0.09
	C_5	1.98 ± 0.46	−0.56 ± 0.14	8.93 ± 0.23	1.79 ± 0.26	6.87 ± 0.41	−0.95 ± 0.26
	pK	1.74 (1.80)		9.20 (9.33)		6.25 (6.04)	
Histidine methyl ester[e]	C_0			8.47 ± 0.16	3.71 ± 0.31	6.24 ± 0.10	4.55 ± 0.28
	C_α			7.70 ± 0.10	0.62 ± 0.04	5.92 ± 0.04	1.23 ± 0.04
	C_β			7.56 ± 0.04	3.13 ± 0.10	5.89 ± 0.03	3.13 ± 0.10
	C_2			7.53 ± 0.06	0.18 ± 0.14	5.84 ± 0.05	1.35 ± 0.15
	C_4			7.52 ± 0.11	2.04 ± 0.17	5.84 ± 0.04	4.51 ± 0.18
	C_5[c]						−1.3
	pK			7.65 (7.33)		5.91 (5.38)	
N-Acetyl-histidine[e]	C_0	3.06 ± 0.03	3.25 ± 0.05			7.36 ± 0.08	1.58 ± 0.05
	C_α	3.16 ± 0.02	2.30 ± 0.02			7.14 ± 0.05	1.17 ± 0.02
	C_β	3.22 ± 0.06	1.07 ± 0.03			7.24 ± 0.04	1.95 ± 0.03
	C_2	3.06 ± 0.32	−0.26 ± 0.04			7.36 ± 0.04	2.58 ± 0.04
	C_4	3.20 ± 0.06	1.06 ± 0.03			7.20 ± 0.02	3.50 ± 0.03
	C_5	3.15 ± 0.08	−0.46 ± 0.02			7.39 ± 0.05	0.92 ± 0.02
	pK	3.14 (*f*)				7.26 (7.08)	

[a] Δ values are chemical shift changes on deprotonation (in ppm) with a positive value corresponding to deshielding. Other sources of error not reflected in the computer fit may contribute nearly ±0.1 ppm. [b] Mean of individual carbon values with each weighted inversely by its standard error. Comparison values in parentheses are from ref 10. [c] The computer program could not converge to an adequate fit. Approximate Δ values were obtained by inspection. [d] Carboxyl carbon. [e] See ref 9. [f] No value available.

Table II. ^{13}C Nmr Titration Shifts[a] for C_0, C_α, and C_β of Several Amino Acids

Compound	α-Carboxyl titration C_0	α-Carboxyl titration C_α	α-Carboxyl titration C_β	α-Amino titration C_0	α-Amino titration C_α	α-Amino titration C_β
Glycine	2.5	1.2		9.7	3.4	
Alanine	3.2	1.8	1.0	8.6	0.7	4.2
Valine[b]	2.5	2.1	0.1	8.8	1.4	2.5
Leucine[b]	2.9	2.2	1.1	8.8	1.0	4.5
Phenylalanine	2.5	2.0	0.8	8.8	1.5	4.9
Glutamine[c]	2.5	2.3	0.7	8.6	1.5	5.0
Cysteine[c]	2.6	2.1	0.9	6.3[d]	1.5[d]	4.1[d]
Glutamic acid	2.6	2.0	0.7	9.0	1.4	5.2
Histidine	2.3	1.6	0.8	8.6	1.4	4.4
Serine[b]	2.1	1.9	0.9	8.3	1.0	4.5
Methionine[e]	2.6	1.9	0.9	8.4	1.3	4.4
Mean[f]	2.6 ± 0.3	1.9 ± 0.2	0.85 ± 0.15	8.6 ± 0.2	1.2 ± 0.3	4.7 ± 0.4

[a] All shifts represent deshielding in the indicated deprotonation step. [b] Data taken from ref 3. [c] Data taken from titration curves in ref 4. [d] Values approximate. Overlapping titration curves not separated by curve fitting. [e] Data from ref 5. [f] Omitting all glycine, valine C_β, and cysteine amino titration results.

acetylhistidine has relatively little effect on the carboxyl titration shifts.

In an attempt to explain the observed nmr titration shifts we have carried out CNDO/2 molecular orbital calculations[8] on alanine, phenylalanine, histidine, and glutamic acid, which were chosen as representative di-

Table III. Calculated Changes[a] in Charge Density,[b] Average Excitation Energy,[c] and ^{13}C Chemical Shift[d] on Titration

		Overall titration				α-Carboxyl titration				α-Amino titration				Side chain titration			
		Δ_q[b]	Δ_1[e]	Δ_2[f]	Δ_0[g]	Δ_q	Δ_1	Δ_2	Δ_0	Δ_q	Δ_1	Δ_2	Δ_0	Δ_q	Δ_1	Δ_2	Δ_0
Alanine	C_0	−24	20.1	3.2	12.9	−25	20.4	0.6	3.2	1	−0.3	2.6	9.7				
	C_α	−27	11.7	−2.2	2.7	−43	15.7	10.8	1.8	16	−4.0	−13.0	0.9				
	C_β	33	16.0	4.6	5.9	17	15.4	1.5	1.1	16	0.6	3.1	4.8				
	$\Delta(\Delta E)$[c]	−0.41				−0.44				+0.03							
Phenyl-alanine	C_0	−26	18.0	2.5	11.3	−26	13.6	0.2	2.5	0	4.4	2.3	8.8				
	C_α	−20	10.2	−2.8	3.5	−39	9.6	9.6	2.0	19	0.6	−12.4	1.5				
	C_β	16	13.6	2.5	5.7	10	9.8	−0.2	0.8	6	3.8	2.7	4.9				
	C_1	42	4.2	6.4	4.6	18	1.6	2.3	1.2	24	2.6	4.1	3.4				
	$C_{2,6}$	−8	−0.6	−0.5	−0.2	−3	−0.2	−0.2	−0.1	−5	−0.4	−0.3	−0.1				
	$C_{3,5}$	−13	−1.3	−1.2	−0.8	−7	−0.6	−0.5	−0.3	−6	−0.7	−0.7	−0.5				
	C_4	−37	−3.0	−2.8	−1.5	−17	−1.4	−1.3	−0.3	−20	−1.6	−1.5	−1.2				
	$\Delta(\Delta E)$	−0.38				−0.30				−0.08							
Histidine	C_0	−18	16.3	3.8	12.2	−28	13.8	0.2	2.3	0	2.3	2.3	8.6	10	0.2	1.3	1.3
	C_α	−19	9.8	−0.4	4.2	−36	11.0	11.8	1.6	20	−1.0	−12.6	1.5	−3.	−0.2	0.4	1.1
	C_β	21	12.4	3.6	7.4	8	11.4	2.5	0.8	7	1.7	1.9	4.4	6	−0.7	−0.8	2.2
	C_2	−98	−0.7	−17.4	1.7	−22	−1.7	−1.1	−0.3	−17	−1.4	−1.3	−0.3	−59	2.4	−15.0	2.3
	C_4	0	7.5	−1.5	7.4	21	1.7	3.4	1.1	24	1.6	2.6	1.6	−45	4.2	−7.5	4.7
	C_5	−64	−1.5	−8.8	0.2	−19	−1.2	−1.3	−0.6	−17	−1.1	−1.7	1.8	−28	0.8	−5.8	−1.0
	$\Delta(\Delta E)$	−0.33				−0.32				−0.03				0.02			
Glutamic acid	C_0	−20	18.7	6.4	13.1	−32	10.4	3.6	2.6	17	−3.4	1.7	9.0	−5	11.7	1.1	0.5
	C_α	−35	9.6	−5.4	3.9	−42	6.9	10.6	2.0	−3	−6.4	−18.5	1.4	10	9.1	2.5	0.5
	C_β	30	15.2	3.6	7.2	13	8.2	0.4	0.7	13	−3.7	−0.4	5.2	4	10.7	3.6	1.3
	C_γ	−16	12.6	2.7	4.8	2	8.0	0.1	0.6	3	−4.2	−2.5	0.9	−21	8.8	5.1	3.3
	C_δ[h]	−48	20.1	7.6	6.7	−7	9.4	0.7	0.7	−7	−4.2	1.3	1.7	−34	14.9	5.6	4.3
	$\Delta(\Delta E)$	−0.39				−0.24				−0.25				+0.10			

[a] Changes on the indicated deprotonation step(s) calculated by the CNDO/2 method as described in text. [b] Based on total valence electron density (× 10^3). A negative sign indicates increased electron density. [c] Change in molecular average excitation energy in eV. [d] Change in chemical shift in ppm with deshielding taken as positive. [e] Chemical shift changes calculated using the average energy approximation. [f] Chemical shift changes calculated using the sum-over-states approximation. [g] Observed chemical shift changes from Table I. [h] Carboxyl carbon.

functional and trifunctional amino acids. The CNDO/2 program[11] was modified[12] to directly calculate ^{13}C chemical shifts. The calculated carbon charge densities and ^{13}C nmr chemical shifts are given in Table III. The diamagnetic contribution (σ_d) to the ^{13}C shifts, which is proportional to electron density,[13] was found to be relatively small in all cases. The dominant paramagnetic contribution (σ_p), which depends on both electron density and the degree of mixing of excited states,[13] was calculated using two different approximations. The first approximation is based on the average energy method of Pugmire and Grant,[14] in which a single average excitation energy (ΔE) for the molecule is obtained from the difference between the mean energies of filled and unfilled CNDO/2 molecular orbitals. This procedure has the disadvantage that a change in ΔE originating at a particular site in the molecule tends to have too strong an influence on the chemical shift of remote carbons. In the "average energy method" calculations shown in Table III, we have therefore used the ΔE values of protonated and unprotonated imidazole in calculating the ring carbon shifts for histidine,[15] and the ΔE of benzene for phenylalanine ring carbons.[12] For all other carbons the appropriate molecular ΔE was used, and all results were scaled relative to 10 eV for the ΔE of benzene. In the second approximation, the sum-over-states method,[16] σ_p is calculated using the unfilled CNDO/2 molecular orbitals to approximate the excited states of the molecule. Unlike the average energy method it does not require special corrections to prevent remote changes in excitation energy from unduly influencing the results.

In the α-amino and α-carboxyl titrations, both methods give close agreement with experiment for the ring carbons of phenylalanine and reasonable agreement for the ring carbons of histidine. In the average energy method calculations for these carbons, ΔE was fixed in both of these titrations. It would appear then that for carbons remote from the ionization site the ^{13}C chemical shift is proportional to electron density, as in various aromatic derivatives.[12]

For the other carbons the agreement between experimental and calculated ^{13}C nmr shifts is quite poor. To a large extent the lack of agreement is due to the approximations involved in the calculation of σ_p, some of which are inherent in the use of the CNDO/2 method. Recent finite perturbation calculations[17] have indicated a need for new atomic parameters for CNDO calculations. The use of spherical orbitals in calculating electron repulsion integrals involving charged lone pairs on oxygen and nitrogen atoms can be expected to overemphasize charge delocalization.[18] The results are influenced strongly by the choice of bond lengths and angles, and to a lesser extent by the choice of conformation where rotation about a single bond is possible. Finally, the calculated charge densities are appropriate to the gas phase and neglect the probably significant influence of changes in solvation in the course of the experimental titrations.

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The relationship between calculated charge densities and chemical shifts for the α, β, and carbonyl carbons is of some interest. Horsley and Sternlicht[2] noted that the ^{13}C nmr titration shifts of C_α and C_β for several amino acids were in the direction of decreased shielding on deprotonation, while the ^1H nmr titration shifts of their directly bonded protons were in the direction of increased shielding. Other authors[3-6] have tended to follow them in interpreting these results as evidence that the carbon atoms become slightly more positive in each deprotonation step, due to an electric field induced polarization of the C–H bonds. Our CNDO/2 carbon charge densities (Table III) indicate that although C_β does become more positive in both the amino and carboxyl titrations, C_0 becomes more negative, and C_α becomes more negative overall. The same CNDO/2 calculations indicate that the α and β hydrogens become more negative in each deprotonation step,[19] in agreement with proton nmr results (2). Thus our results suggest that although some bond polarization occurs, the observed deshielding on deprotonation is often the result of a decrease in excitation energy that more than compensates for an increase in electron density. Most of our calculated chemical shifts agree in sign with the observed shifts, lending some support to this model.

It would appear that some caution is needed in using either ^{13}C chemical shifts or the ^1H chemical shifts of directly bonded hydrogens as an indication of changes in carbon charge density close to a site of ionization.

Conclusions

This study has shown clearly the usefulness of computer fitting of individual ^{13}C nmr titration curves in order to obtain accurate ionization constants (pK values) and chemical shift changes from the data. The technique should have great potential in peptide and protein systems, particularly when a carbon atom is influenced by more than one ionization.

The amino acids provide a challenging test for theoretical investigations of the electronic features important in determining ^{13}C chemical shifts. On the basis of CNDO/2 calculations we have rationalized the overall trends in the amino acid titration shifts. For carbons near the titration sites the overall deshielding results from competition between the deshielding effect of a decrease in excitation energy and the shielding effect of an increase in electron density on deprotonation. For more remote carbons the titration shifts follow electron density. The extension of this qualitative model to provide a detailed explanation of all of the observed titration shifts requires an improved molecular orbital method, possibly based on finite perturbation techniques.

(19) Typical changes are −0.10 charge unit for α and −0.07 charge unit for β hydrogens on going from cation to anion.

Experimental Section and Details of Calculation

Materials. The amino acids and histidine derivatives (Schwarz/Mann and Sigma) were dissolved in H_2O at concentrations near 0.1 g/ml; at pH values where solubility was limited saturated solutions were used. Titrations with HCl and NaOH were performed directly in the nmr sample tubes using a micro combination electrode and digital pH meter.

Nmr. ^{13}C spectra at 25.16 MHz and 31 ± 2° were recorded on a Varian XL-100-15 spectrometer in pulsed Fourier transform/proton noise decoupled mode. Peak positions were measured relative to 5% dioxane dissolved in the external D_2O used for locking;[6] bulk susceptibility corrections and the effect of changes in salt and solute concentrations during the titrations were determined to be less than the experimental error of ±0.1 ppm. Chemical shifts in the figures are reported relative to TMS using the relation $\delta_{TMS} = \delta_{dioxane} - 67.3$ ppm.

pK Calculations. The amino acid titration curves were computer fitted as a sum of simple proton association equilibria using a model equation of the form

$$\delta_{obsd} = \delta_{min} + \sum_{i=1,n} [\Delta_i \, 10^{(pK_i - pH)}]/[1 + 10^{(pK_i - pH)}]$$

where δ_{obsd} is the observed ^{13}C chemical shift, δ_{min} is the minimum chemical shift in the protonated form of the amino acid, and Δ_i and pK_i are the chemical shift and ionization constants, respectively, for the ith protonation transition. Details of the least-squares program used are given in ref 6.

Molecular Orbital Calculations. A modified version[12] of the standard CNDO/2 computer program[11] was used to calculate ^{13}C chemical shifts. The diamagnetic contribution (σ_d) was evaluated from local charge densities.[20] The paramagnetic contribution (σ_p) was calculated by the average energy approximation of Pugmire and Grant[14] and by the sum-over-states method[16] as described above. All C, N, and O atoms were included in the σ_p calculation. Bond angles, bond distances, and conformation averaging for alanine, phenylalanine, and histidine were as previously described.[12] For glutamic acid a single conformation was used in which all backbone carbons were trans and all other groups were staggered.

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Communications to the Editor

An Anomalous Brønsted Relationship for β-Diketones

Sir:

Considerable interest has recently been expressed in the various interpretations[1-3] given, to what after all has been known for some time, namely that the acidities of the nitroalkanes, CH_3NO_2, $CH_3CH_2NO_2$, and $(CH_3)_2CHNO_2$, increase in this order whereas the rates of hydroxide-catalyzed deprotonation decrease in the same sequence. Subsequent studies[4] illustrate the greater sensitivity of substituent effects on rates than on

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