Use of ^{13}C-^{1}H Spin-coupling Constants in the Determination of Side-chain Conformations of Amino-acids

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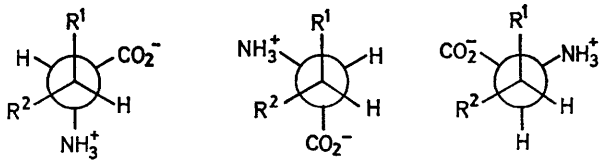
Summary It is shown that by considering the ^{13}C–C–C–H coupling constant between the α-CO_2^- and the β-protons in amino-acids it is possible to obtain side-chain conformational information concerning the rotamer populations which is not available from studies of H–H coupling constants alone.

THE use of Karplus-type relationships between three-bond ^{1}H–^{1}H spin coupling constants and the dihedral angle in H–C–C–H and H–C–N–H fragments of amino-acids and peptides to obtain conformational information has become a well established procedure.[1] In amino-acids and small peptides, measurements of side-chain conformations can be made if the β-CH_2 proton absorptions can be resolved and if there is no complicating additional coupling from protons at the γ-position. An ABX analysis on such a spectrum gives the two vicinal H_α–H_β coupling constants and approximate estimates of the fractional rotamer populations for rotamers (I), (II), and (III) can then be obtained from these values. However, it is usually impossible to assign the individual β-CH_2 signals to their respective protons and consequently one cannot assign the fractional populations of rotamers (I) and (II).

There is already considerable experimental and theoretical evidence[2] that three-bond ^{13}C–^{1}H coupling constants follow a Karplus type relationship with the dihedral angle



(I) (II) (III)

(χ) so that the *gauche*-coupling constant is expected to be much smaller than the *trans*-coupling constant. This information is sufficient to enable an assignment to be made for rotamers (I) and (II), as can be illustrated by considering the data for aspartic acid at high pH. From the proton coupling constants [J (H_α–H_β) = 9·4 and 4·2 Hz] the approximate rotamer populations can be calculated to be either:

(i) $p_I = 0{\cdot}15$, $p_{II} = 0{\cdot}62$, $p_{III} = 0{\cdot}23$ or

(ii) $p_I = 0{\cdot}62$, $p_{II} = 0{\cdot}15$, $p_{III} = 0{\cdot}23$

TABLE

Measured $^{13}CO_2^-$–βCH and αCH–βCH coupling constants (Hz) and rotamer populations in amino-acids in aqueous solution

Compound	R¹	R²	pH	$^3J(^{13}C$–$^{1}H)$	$^3J(^{1}H$–$^{1}H)$	Rotamer populations p_I	p_{II}	p_{III}
Alanine	H	H	−0·5	4·8	7·5			
			6·5	4·2	7·5			
			12·0	4·1	7·5			
Aspartic acid	CO_2H	H	11·0	½(5·1)	9·4	0·15[a]	0·62[a]	0·23[b]
					4·2			
Valine	Me	Me	5·7	3·1	4·4	0·17[a]	*ca.* 0·6	*ca.* 0·2[c]

[a] Magnitudes determined from ^{1}H–^{1}H coupling constants; assignment based on ^{13}C–^{1}H coupling constants. [b] Determined from ^{1}H–^{1}H coupling constants. [c] Determined from ^{13}C–^{1}H coupling constants.
Errors in fractional populations ±10%.

This method of determining side-chain conformations has serious limitations for molecules where $\nu_A \sim \nu_B$ or for molecules such as valine which possess only one proton on the β-carbon. For such molecules only the fractional population of one rotamer (p_I) is available from the ^{1}H–^{1}H coupling constant. We report how considerations of vicinal ^{13}C–^{1}H coupling constants can be used in conjunction with the ^{1}H–^{1}H coupling constants to give fractional populations for all three rotamers in aspartic acid and valine. The method is a general one for determining side-chain conformations of amino-acids and is thus of potential value in the conformational study of peptides.

The ^{13}C–^{1}H coupling constants between the α-$^{13}CO_2^-$ and the β-protons for three amino-acids have been measured (Table) from their natural abundance ^{13}C spectra recorded using a Varian XL100 spectrometer with Fourier transform and gated decoupling facilities.

The sum of the ^{13}C–^{1}H coupling constants [$J(^{13}CO_2^-$–$H_A)$ + $J(^{13}CO_2^-$–$H_B)$] is 5·1 Hz for aspartic acid at this pH.† (For aspartic acid at some pH values only the sum of the coupling constants is obtainable from the ^{13}C spectrum; this information is sufficient to permit a choice to be made between these two possibilities).

For possibility (i) we calculate

$$0{\cdot}38\ J_t + 1{\cdot}62\ J_g = 5{\cdot}1 \quad (1)$$

whereas for possibility (ii)

$$0{\cdot}85\ J_t + 1{\cdot}15\ J_g = 5{\cdot}1 \quad (2)$$

From the $J(^{13}CO_2^-$–H) coupling constant in alanine (4·2 Hz) it is seen that

$$J_t + 2J_g = 12{\cdot}6\text{Hz} \quad (3)$$

† At this pH the chemical shift difference between H_A and H_B in aspartic acid is sufficiently large (ν_{AB} = 0·31 p.p.m.) to prevent the α-$^{13}CO_2^-$ spectrum being deceptively simple and the observed triplet splitting indicates two approximately equal $^{13}CO_2^-$–H_β coupling constants.

From equations (1) and (3) we obtain reasonable values for J_t and J_g (11·9 and 0·4) whereas if we solve equations (2) and (3) unreasonable values with $J_g > J_t$ are obtained; clearly the correct fractional populations are shown in (i) where p_{II} is the dominant rotamer. This result is in agreement with the original assignments which relied on equivocal chemical shift arguments.[3]

It should be emphasised that using these estimated values of J_t and J_g to calculate fractional populations from the ^{13}C–C–C–H coupling constant of valine it is possible to show that rotamer (II) is most abundantly populated. This qualitative conclusion can be made with some confidence. However, owing to the uncertainties in the values of J_t and J_g it is not possible at present to deduce accurate quantitative values for the rotamer populations from ^{13}C–C–C–H spin coupling constants alone.

Although this method is a general one for the study of side-chain conformations in amino-acids, the above results were obtained on concentrated solutions (*ca.* 1M) with ^{13}C in natural abundance. To extend this method to larger molecules such as hormonal peptides it will probably be necessary to synthesise molecules enriched with ^{13}C at the peptide carbonyl groups to overcome the sensitivity problem.

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