A Method of Assigning the ^{13}C Resonances of Non-equivalent Carbon Atoms in Molecules Containing a Chiral Centre: Application to Valine

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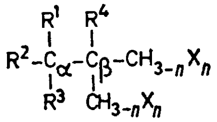
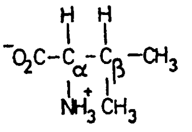
Summary A method is described for assigning the ^{13}C signals of magnetically non-equivalent γ-carbons in molecules containing a chiral centre, and is applied to valine.

It is well known that the γ-carbons in molecules of formula (**1**) are magnetically non-equivalent because of the presence of the chiral centre at C_α. This non-equivalence results in different ^{13}C chemical shifts for the γ-carbons and there are many examples in the literature.[1] Thus in valine (**2**) the



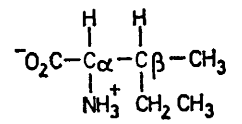
$R^2\text{-}\underset{R^3}{\overset{R^1}{C_\alpha}}\text{-}\underset{CH_{3-n}X_n}{\overset{R^4}{C_\beta}}\text{-}CH_{3-n}X_n$

(1)



$^{-}O_2C\text{-}\underset{NH_3^+}{\overset{H}{C_\alpha}}\text{-}\underset{CH_3}{\overset{H}{C_\beta}}\text{-}CH_3$

(2)



$^{-}O_2C\text{-}\underset{NH_3^+}{\overset{H}{C_\alpha}}\text{-}\underset{CH_2CH_3}{\overset{H}{C_\beta}}\text{-}CH_3$

(3)

γ-methyls have a chemical shift difference of 2·4 p.p.m. at pH 12. A substantial contribution to the ^{13}C chemical shift difference arises from differences between the populations of the rotamers about the C_α–C_β bond. This shielding non-equivalence potentially contains conformational information. However, before the usefulness of this approach to conformational analysis can be evaluated it is necessary to assign the magnetically non-equivalent γ-carbons in molecules containing a chiral centre. We have devised a method of assignment based on comparing the γ-carbon shieldings of the unassigned compound with those in carefully chosen model compounds which retain similar conformations while having unequivocally assigned γ-carbon resonances.

Table 1. γ-Carbon shift non-equivalences, $\Delta\delta$[a]

Compound	Assignment	Cation	$\Delta\delta$/p.p.m. Zwitterion	Anion
Valine	γ_A upfield	0·46	1·32	2·37
	γ_B upfield	−0·46	−1·32	−2·37
Isoleucine, CH_3	—	0·70	1·38	2·20
Isoleucine, CH_2	—	0·20	1·06	2·16

[a] Experimental details are described in ref. 2. Values are correct to ±0·04 p.p.m. Positive values indicate $C\gamma_A$ resonance upfield of $C\gamma_B$.

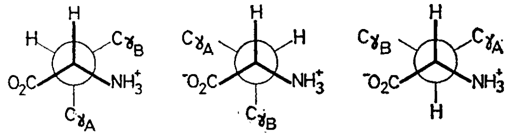


Figure. Staggered rotamers of L-valine, L-isoleucine and allo-L-isoleucine zwitterions. (a) in valine $C\gamma_A$ and $C\gamma_B$ are both methyls, (b) in L-isoleucine $C\gamma_A$ is a methylene and $C\gamma_B$ a methyl, (c) in allo-L-isoleucine $C\gamma_A$ is a methyl and $C\gamma_B$ a methylene.

In the case of valine the assignments can be made by considering the ^{13}C chemical shifts of the γ-carbons in L- and allo-L-isoleucine (**3**) where the γ-methylenes resonate *ca.* 10 p.p.m. downfield of the γ-methyls. A study of the three bond ^{1}H–^{1}H and ^{1}H–^{13}C coupling constants in valine, L-isoleucine and allo-L-isoleucine indicates that the conformational situation is similar for the C_α–C_β bond in the three compounds.† Therefore the valine γ-carbon resonances may be assigned with confidence by comparison with those of L- and allo-L-isoleucine.

TABLE 2. γ-Carbon protonation shifts.[a]

		CO_2^- Protonation		NH_2 Protonation	
Compound	Assignment	$C\gamma_A$ /p.p.m.	$C\gamma_B$ /p.p.m.	$C\gamma_A$ /p.p.m.	$C\gamma_B$ p.p.m.
Valine	γ_A upfield	−0·28	0·60	0·06	1·12
	γ_B upfield	0·60	−0·28	1·12	0·06
L-isoleucine	—	−0·34	0·52	−0·38	0·86
Allo-L-isoleucine	—	−0·16	0·52	0·04	0·74

[a] Experimental details described in ref. 2. Positive values indicate upfield shift upon protonation.

Table 1 shows the γ-carbon shift difference, $\Delta\delta = \delta(C\gamma_A) - \delta(C\gamma_B)$, for valine and the pairs of methyl carbons and methylene carbons of the isoleucine isomers (Figure). If the upfield resonance of valine is assigned to $C\gamma_A$ then the γ-carbon shift differences are seen to be positive and in very good agreement, in each ionisation state, with those of the isoleucine isomers. This assignment is confirmed by comparing the shifts of the individual γ-carbon resonances which accompany protonation of either the amino or carboxy groups (Table 2). It is clear that very similar protonation shifts are observed for valine and the isoleucine isomers only if the upfield resonance of valine is assigned to $C\gamma_A$.

Using these ^{13}C assignments for the valine γ-carbons we can assign the non-equivalent methyl proton signals by heteronuclear $\{^{1}H\}$–^{13}C double resonance experiments. The high field methyl proton doublet is connected to the high field γ-^{13}C signal ($C\gamma_A$).

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† For the anions of valine, L-isoleucine and allo-L-isoleucine $^{3}J_{CO-\beta CH}$ is 2·3, 2·7 and 1·8 Hz respectively, and $^{3}J_{\alpha CH-\beta CH}$ is 5·3, 5·2 and 4·0 Hz, respectively.

[1] J. A. Kroschwitz, M. Winokur, H. J. Reich, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1969, **91**, 5927; J. B. Stothers, 'Carbon-13 N.M.R. Spectroscopy,' Academic Press, New York, 1972, pp. 431–432.

[2] J. G. Batchelor, J. Feeney, and G. G. K. Roberts, *J. Magnetic Resonance*, in the press.