Combined Use of Multiple and Selective Proton-decoupled ^{13}C and ^{1}H N.M.R. Spectra for Complete Proton and Carbon-13 Resonance Assignments of Polypeptides

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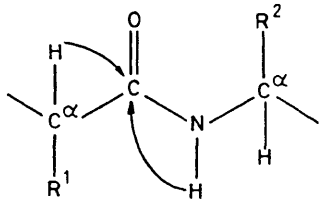
Summary Selective proton irradiations during proton and carbon-13 n.m.r. observation demonstrate unequivocal assignments of ^{1}H and ^{13}C resonances of the model peptide valinomycin and the usefulness of this technique in peptide sequencing is noted.

ASSIGNMENT of the resonances is the essential first step in the n.m.r. determination of the molecular conformations of peptides and proteins. Assignments of the peptide N*H* proton and the *C*=O carbon nuclei are of particular importance since they can provide valuable information on the secondary structure of a peptide.[1] Expensive and time-consuming synthetic isotope enrichments have been used for unequivocal n.m.r. signal assignment. However, off-resonance[2] and selective decouplings[2-4] have been used to assign the ^{13}C resonances provided their respective ^{1}H resonances have been previously assigned. We report here the use of selective irradiation of ^{1}H for the simultaneous and co mplete assignment of both the ^{1}H and ^{13}C resonances of peptides.

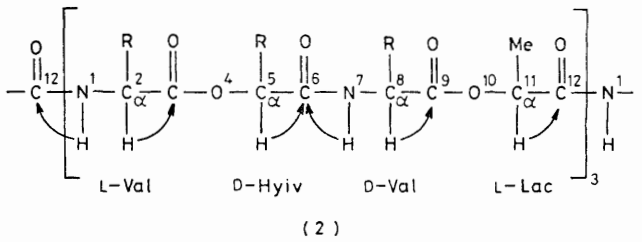


(1)

A peptide fragment can be written as in structure (**1**) where R^1 and R^2 are the amino side chains. The peptide *C*=O carbon is ^{2}J coupled to the α-proton of the same residue and to the N*H* proton of the subsequent residue

(shown by the arrows). Selective irradiation of these protons can be made to determine which two protons are coupled to a particular *C*=*O* carbon. Once a single residue is identified either from the side-chain spin pattern or by perturbing the end groups in the case of a linear peptide, the rest of the residues in the molecule can be assigned by stepwise selective irradiation of ^1H resonances.

This technique is illustrated below by assigning the peptide ^1H and ^{13}C resonances of the valinomycin model system. Valinomycin is a cyclic dodecadepsipeptide with a tetramer sequence repeated three times as shown in structure (**2**; R = Pr^i).



(2)

The ^1H (α- and N*H* proton region) and the ^{13}C (*C*=O carbon region) spectra are depicted in Figure 1. Irradiation of the upfield N*H* proton at δ 7·88 decouples the upfield α-proton at δ 4·23 as shown in Figure 1(B). Selective irradiation of this N*H* proton during ^{13}C observation sharpens the most upfield *C*=O carbon resonance at 168·7 p.p.m. [see Figure 1(E)] which, in the difference spectrum presented in Figure 1(F), appears as the only resonance. In accordance with the peptide fragment (**1**) this *C*=O carbon

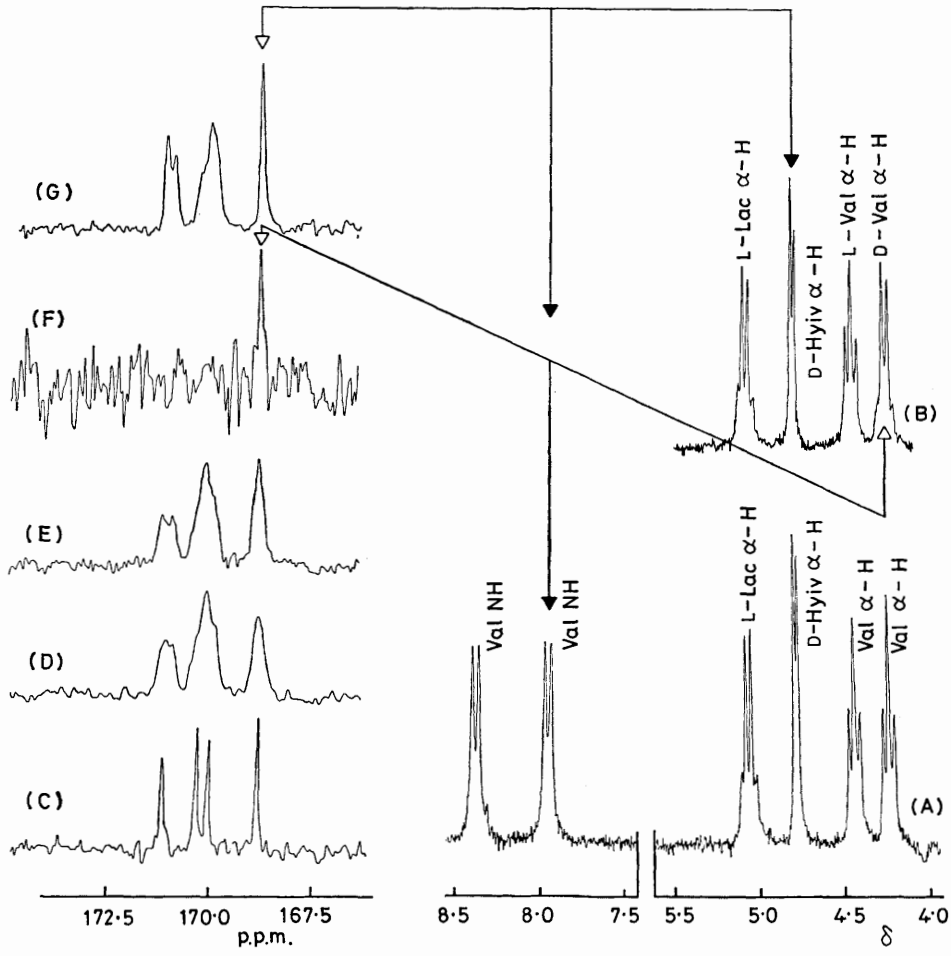


FIGURE 1. (A) and (B) are the ^1H spectra obtained with a Varian HR-220 spectrometer operating at 20 °C. (A) N*H* and α-^1H regions only. The assignments of protons are made by inspection of fine structure. The L- and D-Val residues, of course, can not be delineated without synthetic isotopic enrichment or the irradiation approach outlined herein. (B) With the N*H* proton at δ 7·88 saturated (▲) showing the observed decoupling effect (Δ). The *C*=O spectra, (C)—(G), were obtained on a JEOL FX-100 spectrometer, equipped with a multinuclear probe, using a pulse width of 10 μs for a 45° magnetization vector and with 2·5 s repetition time. Each spectrum is the result of an average of 1800 scans for a 25 mM sample of valinomycin in [$^2H_6$]dimethyl sulphoxide. Me_4Si was used as an internal standard. (C) Completely ^1H broad-band decoupled. (D) No ^1H irradiation (undecoupled). (E) Peptide N*H* proton at δ 7·88 is selectively irradiated. (F) Difference spectrum obtained by subtracting (D) from (E). (G) With both the N*H* proton at δ 7·88 and the D-Hyiv α-^1H at δ 4·77 simultaneously and selectively irradiated; the D-Hyiv *C*=O is clearly seen as the narrow line.

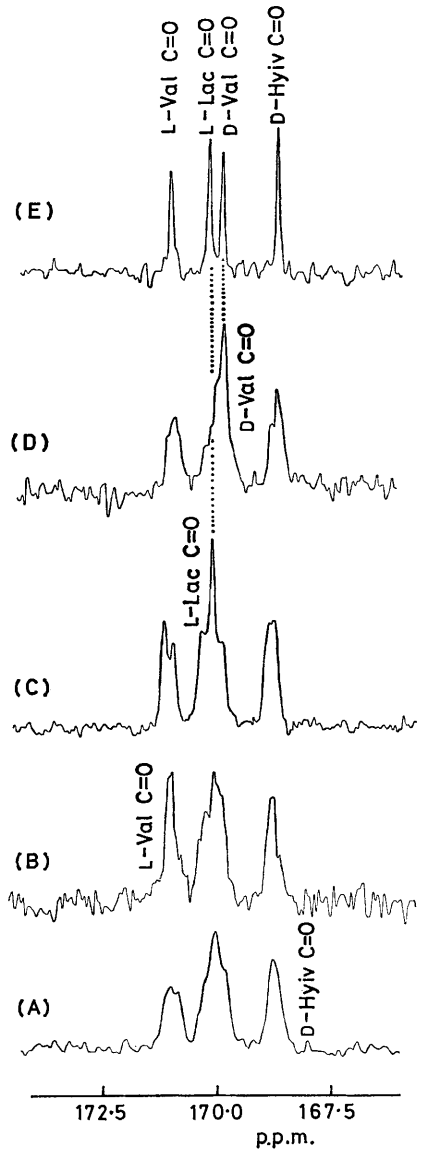


FIGURE 2. 25 MHz ^{13}C spectra obtained on a JEOL FX-100 spectrometer equipped with a multinuclear probe. Conditions are the same as in Figure 1 (C—G). (A) Undecoupled. (B) The L-Val α-^1H at δ 4·41 is selectively irradiated. (C) The L-Lac α-^1H at δ 5·05 and the L-Val-N*H* at δ 8·31 are simultaneously irradiated to give the narrow L-Lac *C*=O peak. (D) The D-Val α-^1H at δ 4·23 is selectively irradiated. (E) Completely ^1H broad-band decoupled.

should also be coupled to an α-proton. Selective double irradiation of the D-Hyiv α-^1H at δ 4·77 (as recognized from its coupling pattern with the side-chain β-^1H) and of the δ 7·88 N*H* proton almost completely decouples the expected *C*=O carbon [see Figure 1(G)]. Therefore, this *C*=O carbon at 168·7 p.p.m. is assigned to the D-Hyiv residue. The valinomycin sequence (**2**) shows that the D-Hyiv *C*=O carbon is ^2J coupled to the D-Val N*H* proton (shown by the arrow). Accordingly the irradiated upfield N*H* proton at δ 7·88 and the corresponding α-^1H at δ 4·23 are assigned to the D-Val residue. By elimination, the N*H* proton at δ 8·31 and the α-^1H at δ 4·41 p.p.m. are assigned to the L-Val residue. The appropriate couplings here are verified below.

Selective irradiation of the L-Val α-^1H reduces the linewidth of the *C*=O carbon signal at 171·1 p.p.m. as shown in Figure 2(B) which, therefore, belongs to this residue. Selective double irradiation of the L-Lac α-^1H at δ 5·05 and of the L-Val N*H* proton at δ 8·31 shows the line narrowing at 170·2 p.p.m. [see Figure 2(C)] and so it is assigned to the L-Lac residue. The remaining *C*=O carbon resonance at 169·9 p.p.m. is obviously assigned to the D-Valine. This is verified by irradiating the D-Val α-^1H at δ 4·23 and the decoupled resonance is shown in Figure 2(D). The complete assignment of the *C*=O carbons and ^1H resonances of valinomycin, demonstrated in this study by stepwise selective irradiation of ^1H resonances, is in total agreement with the previously reported assignments[4] obtained by synthesizing the [^{15}N]-enriched D-Val residue in valinomycin.

Since the peptide fragment (**1**) shows that a single *C*=O carbon nucleus is ^2J coupled to two protons belonging to two residues across the peptide bond, stepwise selective irradiation of ^1H resonances could, of course, be employed for sequencing a peptide.

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