Natural Abundance Nitrogen-15 Nuclear Magnetic Resonance Spectroscopy —Amino-acid Derivatives

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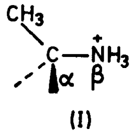
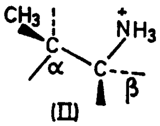
Summary. High-resolution ^{15}N n.m.r. spectra of a series of amino-acid methyl esters as their hydrochlorides have been measured in natural abundance and reveal the presence of a γ-alkyl substituent effect, similar to that observed in ^{13}C n.m.r.

THE development of nitrogen nuclear magnetic resonance has been slow relative to other nuclei (*e.g.* ^{1}H, ^{19}F, ^{31}P) for reasons that are now well known.[1] The abundant nitrogen-14 isotope possesses an electric quadrupole moment which results in considerable line broadening, while the ^{15}N isotope (natural abundance 0.36%) has provided an almost insurmountable sensitivity problem on all but the isotopically enriched molecules. Recent advances in both technique and instrumentation, specifically pulsed Fourier techniques, have overcome the latter problem[2] with the result that n.m.r. studies on ^{15}N in natural abundance are now feasible.



(I)



(II)

In order to investigate the practicability of applying natural abundance nitrogen n.m.r. to biological systems, we have measured the nitrogen-15 chemical shifts of some amino-acid methyl esters as their hydrochlorides. The spectrum of arginine methyl ester di-hydrochloride is shown in the figure and provides a typical example of the signal-to-noise attainable after approximately seven hours of pulsing. The two low-field resonances arise from the nitrogens in the guanidine moiety, while the high-field resonance is produced by the NH_3 group nitrogen. The appearance of only three nitrogen resonances is consistent with the expectation that the two NH_2 groups will be equivalent.

The nitrogen positions of all compounds studied are shown in the Table. It can be seen that the observed range of ^{15}N chemical shifts is small relative to the known total range (> 900 p.p.m.) and if one excludes the glycine derivative (entry 1), the chemical shift variation becomes only 5 p.p.m. Clearly, ^{14}N n.m.r. is unsuitable for measuring such subtle differences.[3] Examination of the data (see Table) revealed the nitrogen absorption for the glycine derivative to be furthest up-field. Substitution of a methyl group on the glycine methylene produces a large down-field shift (13·2 p.p.m.) and the lowest-field nitrogen resonance in this series. An effect of similar magnitude and direction has been observed in going from the methylammonium to the ethylammonium ion (15 p.p.m.).[4] Further aliphatic substitution (entries 3—10) produces an up-field shift relative to the NH_3^+ nitrogen in alanine.

Nitrogen-15 chemical shifts[a] *in some amino-acid methyl ester hydrochlorides* $RCH(\overset{+}{N}H_3)CO_2CH_3$

	R	$\delta^{15}N$	$\Delta\delta$
1	H	10·0	0
2	CH_3	23·2	13·2
3	$(CH_3)_2CH$	18·3	8·3
4	$(CH_3)_2CHCH_2$	22·0	12·0
5	$CH_3CH_2(CH_3CH)$	18·9	8·9
6	$PhCH_2$	20·4	10·4
7	$4\text{-}OH\text{-}C_6H_4CH_2$	19·4	9·4
8	$[\overline{NH:CH\cdot \underset{+}{N}H\cdot CH:C}]\text{–}CH_2\text{–}$ ..	21·7	11·7
9	$\overset{+}{N}H_3(CH_2)_4{}^{b,d}$	21·5	11·5
10	$NH_2C(=\overset{+}{N}H_2)NH(CH_2)_3{}^{b,e}$ ..	21·0	11·0

[a] Chemical shifts are reported down-field relative to the external reference.[6] Spectra were measured on 5–9 molar aqueous solutions with pH values in the range 0·5–2·0. Values are estimated to be correct to ± 0·3 p.p.m. [b] Measured as the dihydrochlorides. [c] Imidazole nitrogen-15 resonances at 144·2 and 146·3 p.p.m. [d] Additional $^{15}NH_3^+$ resonance at 5·8 p.p.m. [e] Guanidine nitrogen-15 resonances at 43·3 (NH_2) and 54·8 p.p.m.(NH).

If, by analogy with ^{13}C n.m.r., substitution of a methyl group on the carbon attached to nitrogen is described as a β-effect (see I) and substitution at the immediately adjacent carbon considered a γ-effect, then the magnitude and direction of the observed changes in these compounds can be more readily understood. The ^{13}C β-methyl substituent effect in open-chain aliphatic systems has been demonstrated[5] to be down-field in sense and to have a magnitude of *ca.* 9 p.p.m.

The corresponding stereochemically-dependent γ-effect is known[5] to be opposite in sense and of the order of 2–3 p.p.m. Thus the low-field position of the nitrogen resonance in the alanine derivative relative to compounds (3—10) results from the absence of a γ-alkyl effect while the nitrogen resonances in the valine and isoleucine derivatives (entries 3 and 5) appear at higher fields due to the presence of two γ-alkyl effects.

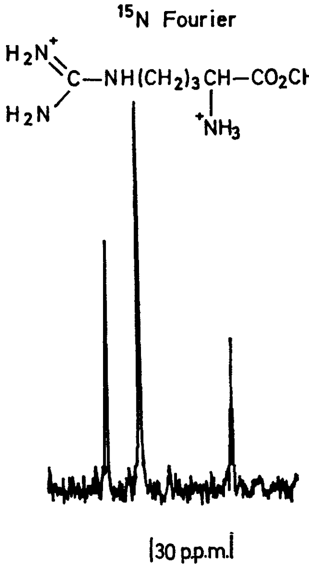


FIGURE. *Natural abundance, proton-decoupled* ^{15}N *"Fourier" power spectrum of arginine methyl ester di-hydrochloride at* 21·14 kG. *The spectrum is the result of* 66,680 *pulses each of* 6 μs *duration and repeated every* 0·4 *s.*

To date there are insufficient data to attempt a more quantitative analysis of the individual substituent effects although a comparison of entries 3 and 5 suggests that the δ-effect is small.

The introduction of a remote nitrogen grouping (entries 8—10) in which the nitrogen lone pair is either protonated or involved in π-bonding appears to produce only a small change in the position of the nitrogen found α to the methoxycarbonyl group.

The observed 10 p.p.m. down-field shift of the glycine nitrogen resonance from the reference[6] is reasonable if one assumes that the methoxycarbonyl group will induce a nitrogen shift comparable in magnitude and direction to that observed in the analogous ^{13}C resonance.[7]

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[1] J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy", Vol. 2, p. 1031, Pergamon London, 1966.

[2] J. M. Briggs, L. F. Farnell, and E. W. Randall, *Chem. Comm.*, 1971, 680.

[3] The half-height width of the ^{14}N resonance of the methylammonium ion exceeds 7 p.p.m. (see ref. 4).

[4] M. Witanowski and H. Januszewski, *Canad. J. Chem.*, 1969, **47**, 1321.

[5] D. M. Grant and E. G. Paul, *J. Amer. Chem. Soc.*, 1964, **86**, 2984.

[6] 5M-$^{15}NH_4NO_3$ in 2M-nitric acid. The selection of this particular gegenion is based on studies which have revealed significant concentration and gegenion effects on the position of the ^{15}N resonance of the ammonium ion; J. M. Briggs and E. W. Randall, unpublished results.

[7] See E. Lippmaa and T. Pehk, *Eesti NSV Tead. Akad. Toim., Keem., Geol.*, 1968, **17**, 210: J. B. Grutzner, M. Jautelat, J. B. Dence, R. A. Smith, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1970, **92**, 7107.