Application in Biosynthetic Studies of ^{13}C Isotope Shifts in Infrared Spectroscopy

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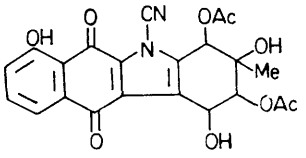
Summary The cyanide carbon atom in kinamycin D (**1**) was proved to originate from the acetate carboxy-carbon atom by ^{13}C isotope shifts in the i.r. spectra of kinamycin D prepared from 95% enriched sodium [1-^{13}C]acetate.

IN the course of biosynthetic studies of kinamycin D (**1**), a metabolite of *Streptomyces murayamaensis*,[1] by ^{13}C n.m.r. spectroscopy, the ^{13}C n.m.r. signal of the *N*-cyanide group was not observable owing either to its unusually long relaxation time or to an incidental overlap with the other

signals around 120 p.p.m.‡ The origin of the cyanide carbon atom thus could not be determined by this method, although the rest of the carbon skeleton of (**1**) was shown to be synthesized *via* a polyketide intermediate from acetate. We report here a convenient method using ^{13}C isotope shifts in i.r. spectroscopy for determination of the biosynthetic origin of the cyanide carbon atom in (**1**).



(**1**)

Labelled samples of kinamycin D were prepared by feeding 0·1% of 95% enriched sodium [2-^{13}C]acetate or 0·1% of 95% enriched sodium [1-^{13}C]acetate, after 6 h incubation, to the producing strain grown on 0·5% glucose, 1% soybean meal, and 0·3% sodium chloride at pH 8·0. The Figure shows the 2000—3000 cm^{-1} region of the i.r. spectra of labelled and unlabelled samples of kinamycin D. The C≡N stretching vibration of (**1c**) (from [1-^{13}C]acetate) shifts 16 cm^{-1} to lower frequency in comparison with its position for (**1a**) (unlabelled) and (**1b**) (from [2-^{13}C]acetate). This shift can be explained in terms of ^{13}C isotope shifts, and shows that the cyanide carbon atom in (**1c**) is labelled with the ^{13}C from [1-^{13}C] acetate.

The observed isotope shifts may be estimated to be about 36% of the total $^{13}C{\equiv}N$ isotope shifts of 44 cm^{-1}, because the isotope effects in the N–$^{13}C{\equiv}N$ system are shared by both the N–C bond and the C≡N bond. This method may be useful for compounds of low solubility in n.m.r. solvents or compounds which give undetectable or unassignable signals in their ^{13}C n.m.r. spectra.

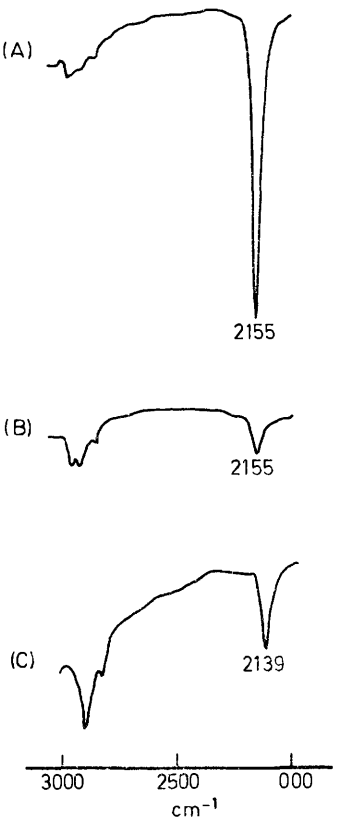


FIGURE. Partial i.r. spectra of kinamycin D: (A), unlabelled, (**1a**); (B) from 95% enriched [2-^{13}C]acetate, (**1b**); (C) from 95% enriched [1-^{13}C]acetate, (**1c**). The measurement error is ±1 cm^{-1} for each spectrum.

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‡ Chemical shifts for N–$^{13}C{\equiv}N$ in *NN*-dimethylaminocyanamide and ethyl *N*-cyano-*N*-methylaminoacetate were 119·4 and 117·8 p.p.m. respectively.

[1] T. Hata, S. Ômura, Y. Iwai, A. Nakagawa, M. Otani, S. Ito, and T. Matsuya, *J. Antibiotics*, 1971, **24**, 353; S. Ômura, A. Nakagawa, H. Yamada, T. Hata, A. Furusaki, and T. Watanabe, *Chem. Pharm. Bull.* (*Japan*), 1971, **19**, 2428; 1973, **21**, 931.