Application of Long-Range Spin–Spin Couplings in Biosynthetic Studies

Summary: The long-range ^{13}C–^{1}H couplings of anthramycin and pyrrolnitrin are utilized to locate the ^{13}C-enriched carbon atoms of the biosynthetically labeled antibiotics which were isolated from feeding experiments with L-[Me-^{13}C]methionine and DL-[alanine-3-^{13}C]tryptophan.

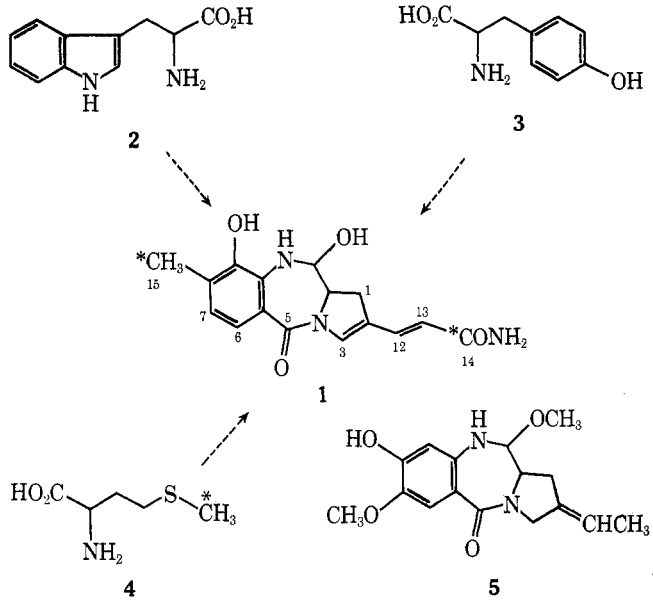
Sir: Stable isotope labeling techniques, such as NMR methods in conjunction with ^{13}C labeling, have attracted much attention as a tool in biosynthetic studies in recent years.[1] The advent of sensitive NMR spectrometers, particularly ones operating in the pulse Fourier transform mode, has provided two elegant methods, the measurement of ^{13}C–^{1}H satellite signals by proton NMR (^{1}H NMR) and ^{13}C-enriched resonance signals by carbon-13 NMR (^{13}C NMR), to detect the labeled carbon atoms. These are especially useful in biosynthetic studies of microbial metabolites because of the ease with which high enrichments can be obtained.

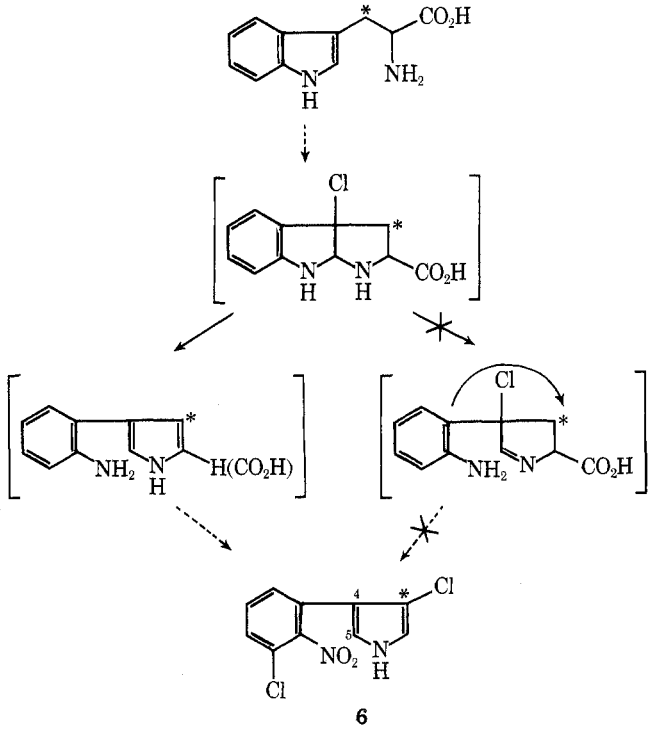
The ^{13}C NMR method generally gives more straightforward information, but access to a highly stable CMR spectrometer is still limited for many researchers and the sensitivity is relatively poor. Therefore, the ^{1}H NMR method is still valuable. On the other hand, it has been noted critically[1] that only carbon atoms with directly attached hydrogens can be analyzed for ^{13}C enrichment by ^{1}H NMR. In this communication, however, we want to illustrate that the presence of a directly attached hydrogen is not a necessary requirement for this type of analysis.

So far the major effort in the analysis of ^{13}C–proton couplings has been directed toward the determination of ^{13}C–^{1}H coupling constants through one bond,[2] which provide the basis for the classical procedure of establishing biosynthetic ^{13}C-labeling patterns by ^{1}H NMR. The ^{13}C–^{1}H long-range interactions have received little attention,[2,3] presumably because of the limited resolution of older NMR spectrometers. By virtue of the gated decoupling technique, we recently have very successfully applied specific ^{13}C–^{1}H long-range coupling information in the spectral analysis of drugs,[4] antibiotics,[5] and other organic molecules.[6] This success has led us to further explore the usefulness of long-range ^{13}C–^{1}H coupling such as in biosynthetic applications.

In a recent publication,[7] we have shown that the antitumor antibiotic anthramycin (**1**) is biosynthetically derived from tryptophan (**2**) and tyrosine (**3**) (Scheme I). Methionine (**4**) was demonstrated to contribute two C-1 units, the aromatic methyl group (C-15) and one of the two amide groups (C-5 or C-14). The ^{1}H NMR spectrum of anthramycin biosynthetically enriched from L-[Me-^{13}C]methionine (Figure 1) clearly indicates the ^{13}C satellite ($J_{C\text{-}15\text{–}H\text{-}15}$ = 126.7 Hz) in agreement with the direct ^{13}C NMR measurement. Using the direct^{13} C NMR method, there is a possible ambiguity regarding the assignment of the two amide carbonyls which appear at 163.4 and 167.7 ppm. Our original assignment was based upon a comparison with tomaymycin (**5**). However, by means of the indirect ^{1}H NMR method we can now resolve this problem and unequivocally assign the position of the label. Comparing the ^{1}H NMR spectrum of the ^{13}C-enriched sample with that of the unenriched one, it is noticed that the H-12 signal intensity is significantly reduced relative to the H-6 signal intensity. This reduction in intensity can be ascribed to the line-broadening effect which results from the ^{13}C–^{1}H long-range coupling (^{3}J(C-14–H-12)).[8] A similar intensity reduction or line-broadening effect can also be detected in the H-13 and H-7 signals, due to the two-bond coupling between C-14 and H-13, and three-bond coupling between C-15 and H-7, respectively.[6,8] None of these long-range couplings can be measured accurately because of the limited resolution. These spectral data firmly verify our earlier ^{13}C chemical shift assignments[7] and validate the conclusion that the enriched carbon atom is located at the terminal amide group (C-14). Another illustration of this new ^{1}H NMR method is the analysis of biosynthetically ^{13}C-labeled pyrrolnitrin (**6**), an antifungal antibiotic. Previous investigations have shown that pyrrolnitrin is derived from tryptophan,[9] and that no 1,2-aryl rearrangement is involved in its biosynthesis[10] (Scheme II).

Scheme I





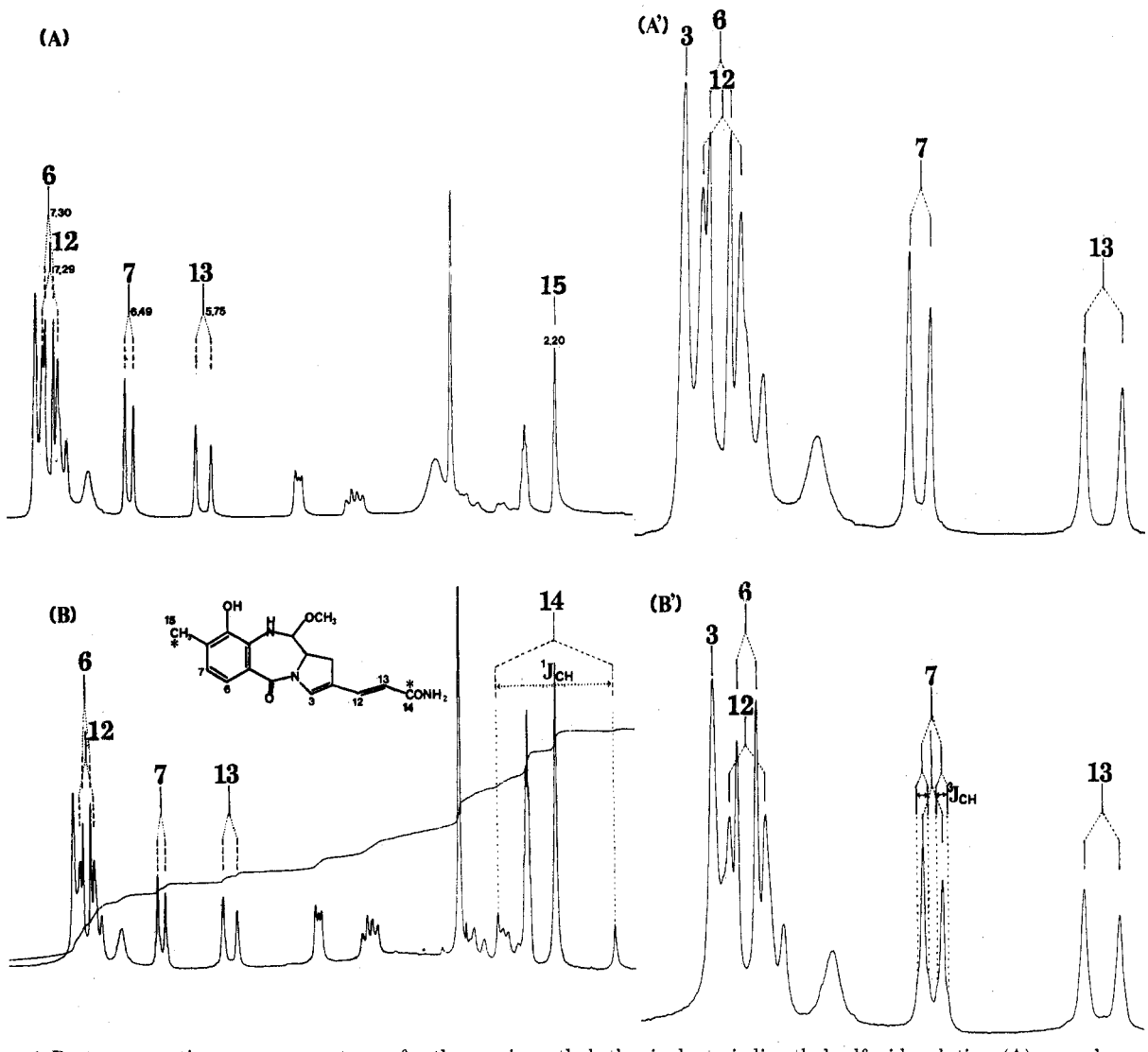


Figure 1. Proton magnetic resonance spectrum of anthramycin methyl ether in deuteriodimethyl sulfoxide solution: (A) normal sample; (A′) expanded downfield portion of (A); (B) biosynthetically ^{13}C-enriched sample; (B′) expanded downfield portion of (B).

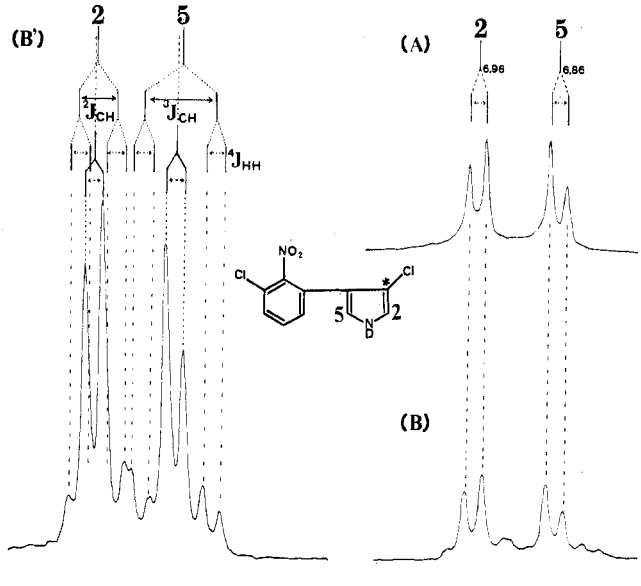


Figure 2. Proton magnetic resonance spectrum of pyrrolnitrin in deuterioacetone solution. Only the H_2 and H_5 signals are shown: (A) normal sample; (B) biosynthetically ^{13}C-enriched sample, 28.5 atom % excess ^{13}C; (B′) expanded portion of (B).

In the ^1H NMR spectrum of the pyrrolnitrin isolated from a feeding experiment with DL-[alanine-3-^{13}C]tryptophan, the H-2 and H-5 signals of the pyrrole ring display extra side bands (Figure 2) which arise from the two-bond [^2J(C-3–H-2) = 5.3 ± 0.3 Hz] and three-bond [^3J(C-3–H-5) = 9.0 ± 0.3 Hz] couplings. The assignments of the H-2 and H-5 chemical shifts are based on specific deuteration experiments.[11] Obviously, the location of the ^{13}C-enriched quaternary carbon can therefore be determined.

These results indicate that the analysis of ^{13}C–^1H long-range coupling can be very useful in biosynthetic studies. It extends the applicability of ^1H NMR spectroscopy for the determination of ^{13}C labeling patterns from only protonated carbon atoms to most nonprotonated ones. In view of the much higher sensitivity of detection of the hydrogen nucleus, this can be particularly important when only very minute amounts of enriched compound are available. We believe that other long-range couplings, for example, those of ^{15}N–^1H and ^{15}N–^{13}C, can also be profitably applied in biosynthetic studies.

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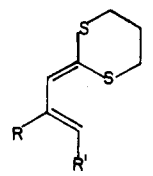
Conjugate and Diels–Alder Reactions of an Activated Allylidenedithiane

Summary: Peterson olefination of 2-methoxyacrolein and 2-lithio-2-trimethylsilyl-1,3-dithiane gives 2-(2-methoxy)-allylidene-1,3-dithiane; this compound reacts in a Michael sense with some electron-deficient unsaturated systems and in a Diels–Alder process with others.

Sir: We have been studying the preparation and utilization of extensively functionalized dienes which might impart to their cycloaddition products relatively complex oxygenation and unsaturation patterns. This facet of the Diels–Alder reaction appears not to have received the degree of study commensurate with its potentialities in organic synthesis.[1-3]

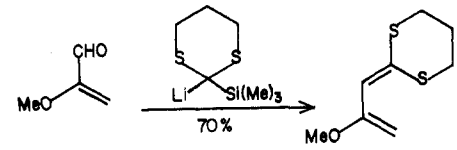
Carey and Court[4] reported the preparation of alkylated allylidenedithianes and their study as potential enophiles. For instance, compound **1a** reacts with tetracyanoethylene and



1a R = R' = Me
1b R = R' = H

with maleic anhydride to give Diels–Alder adducts. However, it does not appear to react with weaker dienophiles.

We reasoned that greater reactivity might be achieved if the terminal carbon of the allylidene group were unsubstituted, and if an additional electron-donating group were introduced at the 3 position of the diene. The synergism of such a group with the electron-donating capabilities of the sulfur atoms might be particularly helpful in promoting cycloadditions with electron-deficient dienophiles. Our precise objective thus became compound **2.**



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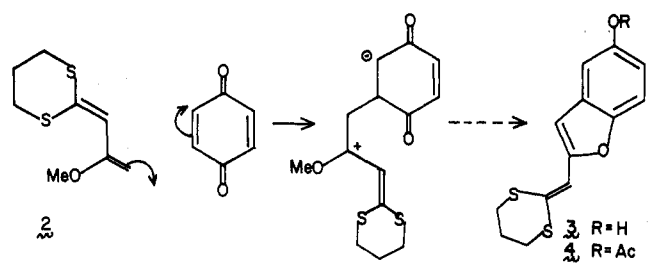
In practice, Peterson olefination[5] of 2-methoxyacrolein[6] with 2-lithio-2-trimethylsilyl-1,3-dithiane[7] gave a 70% yield (distilled) of virtually[8] pure **2**: $\delta_{ppm}^{CDCl_3}$ 1.8–2.4 (m, 2), 2.8–3.1 (m, 4), 3.58 (s, 3), 4.13 (d, J = 2 Hz, 1), 4.18 (d, J = 2 Hz, 1), 6.18 (s, 1).[9] In the light of the reports of Seebach[10] that the parent compound **1b** was unstable, it was useful to discover that compound **2** can be prepared on a reasonably large scale and can be distilled [bp 92–94 °C (0.07 mm)] without serious decomposition.

Compound **2** reacts with 1,4-benzoquinone at room temperature. After 3 hr, a 62% yield of a crystalline product, mp 92–95 °C, was isolated. Its formula, $C_{13}H_{12}O_2S_2$, shows it to be a 1:1 adduct minus CH_3OH. Its infrared spectrum ($\lambda_{max}^{CHCl_3}$ 2.85 μm) suggests the presence of a hydroxyl group but lacks absorptions which are characteristic of a carbonyl group. Acetylation with pyridine and acetic anhydride gives a monoacetate, mp 95–96 °C, $\lambda_{max}^{CHCl_3}$ 5.70 μm. The NMR spectrum of this compound measured at 250 MHz [$\lambda_{ppm}^{CDCl_3}$ 2.05–2.20 (m, 2), 2.23 (s, 3), 2.85–3.05 (m, 4), 6.69 (s, 1), 6.83 (s, 1), 6.91 (d, d, J_1 = 8.0 Hz, J_2 = 2.5 Hz, 1), 7.20 (d, J = 2.5 Hz, 1), 7.35 (d, J = 8 Hz, 1)] defines its structure to be **4.**[9]



2 **3** R = H **4** R = Ac

The formation of benzofuran **3** corresponds to Michael addition of diene **2** to the quinone followed by cyclization (with elimination of methanol) and tautomerization in unspecified order. Such a sequence finds analogy in the reaction of ketene acetals with quinones.[11]

The tendency of compound **2** to participate in Michael-type processes was also exhibited in its reactions with dimethyl acetylenedicarboxylate (**5**), diethyl azodicarboxylate (**6**), and 4-phenyl-2,4-triazoline-3,5-dione (**7**)[12] to give **8**,[9] **9**,[9] and **10**,[9] in the yields shown in Scheme I. In the latter two cases, homogeneous products were obtained as the ketones after hydrolysis of the intermediate enol ethers with dilute acid. Though full accounting of the reaction course was not achieved owing to the formation of complex products,[13] we found no evidence for the formation of any [4 + 2] cycloaddition adducts from these reactions.

These results may be understood in terms of the strongly nucleophilic character of diene **2** which is attacked by highly reactive unsaturated electrophiles (cf. **11**) in orientational arrangements which are not suitable for concerted cycloaddition. Dipolar structures of the type **12** which are produced find other pathways for charge dissipation which are of lower energy than cyclization.

On the basis of the arguments cited above, it seemed possible that *less reactive electrophiles might be more prone to give Diels–Alder products.* It was hoped that cycloaddition, which presumably requires more exacting orientational ar-