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Communications to the Editor

Caution in Using ^{15}N–^{13}C Spin–Spin Coupling for Determining (Bio)synthetic Pathways

Sir:

A recent communication by Suzuki et al.[1] purported a successful application of ^{15}N,^{13}C double label technique for determining the synthetic pathway to adenine from hydrogen cyanide and formamide.[1] The idea was the same as the well-established ^{13}C double label technique which depends upon the fairly large coupling constants between directly bonded nuclei.[2] It was assumed that the doubly labeled precursors could be detected unambiguously by the coupling constants $J(^{13}C–^{15}N)$, whereas the recombined C–N bonds in the products would lack these couplings. This is a reasonable approach, but unfortunately the magnitude of directly bonded ^{15}N–^{13}C coupling constants is not fully understood.[3] In particular, in the course of the structural investigation using ^{15}N-enriched nucleic acid derivatives, we have found numerous unusually small ^{15}N–^{13}C coupling constants for directly bonded pairs,[4] and therefore the application of ^{15}N,^{13}C double label technique used to determine synthetic pathways may be unreliable.

About 100 mg of the fully ^{15}N-labeled adenosine[5] (95% ^{15}N enrichment based on a mass spectrometric analysis) dissolved in 1.5 mL of deuteriodimethyl sulfoxide showed a complex ^{13}C NMR spectrum under proton wide-band irradiation (Figure 1A). The fine structure of the spectrum, which does not exist in the case of normal adenosine (Figure 1B), should be due to ^{15}N coupling constants. The tentative assignment of these coupling constants, which is shown in Figure 1A, was made by comparing the data for the other nucleic acid derivatives[4] and also by the spectral data for [^{15}N]adenine derived from [^{15}N,^{13}C]hydrogen cyanide.[1] The directly bonded coupling constants obtained are shown below with the tentative assignment: $J(C_6–N_6)$, 20.5 (20.5); $J(C_4–N_3)$, 4.4 (9.5); $J(C_4–N_9)$, 19.3; $J(C_8–N_7)$, 10.4; $J(C_5–N_7)$, 8.5[6] (7.3); and $J(C_1–N_9)$, 11.1 Hz.[6] The values in the parentheses represent the coupling constants in the [^{15}N]adenine,[1] and the uncertainty of the values in the [^{15}N]adenosine is 0.5 Hz (600-Hz spectral width and 4K real data points). Once again note that the assignments may not all be correct without doing selective ^{15}N decoupling while observing ^{13}C NMR or comparing these with spectra of selectively ^{15}N-enriched adenosines.

In any case, C-8 of the fully ^{15}N-enriched adenosine showed only one measurable spin–spin coupling constant, either with N-7 or N-9, and C-2 showed a rather broad singlet with a line width of ~7 Hz but did not show any resolved coupling to ^{15}N, even though the latter carbon also has two adjacent ^{15}N nuclei. This fact itself is an interesting subject, which we are currently investigating with respect to the sensitivity of ^{15}N–^{13}C coupling constants to the electronic structure of intervening bonds, but at the same time it introduces more complicated situations in using ^{15}N,^{13}C double label technique to ascertain the intact ^{15}N–^{13}C fragments in the products. For instance, we now know that "the presence of enhanced peaks instead of ^{13}C–^{15}N coupled peaks can *not necessarily* be explained by the thermal fission and re-formation of the C–N bond in formamide during prolonged heating procedure".[1]

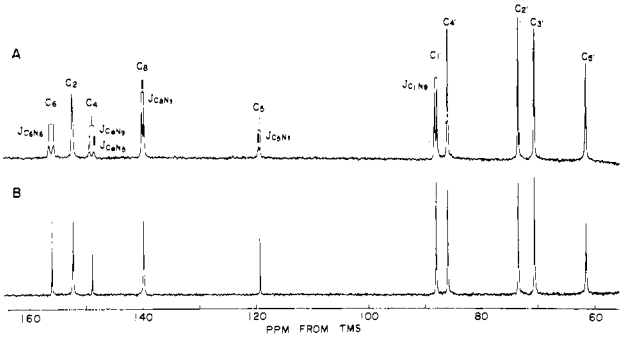

Figure 1.

In conclusion, this method can be used unambiguously only for systems in which the relevant coupling constants are large enough.[8]

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(4) Presented in part at the 17th NMR Symposium (Japan), Tokyo, Nov 1978.
(5) ^{15}N-Enriched adenosine was produced by the microbial fermentation using [^{15}N]ammonium sulfate as the sole nitrogen source, and the details of the procedure will be given in a separate paper.
(6) These values were obtained from the spectrum shown in Figure 1A and may

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therefore contain larger error.

(7) K. T. Suzuki, H. Yamada, and M. Hirobe, *J. Chem. Soc., Chem. Commun.*, 485 (1978).

(8) NOTE ADDED IN PROOF. After the manuscript was accepted, we measured the ^{13}C NMR spectrum of [$^{15}N_7$]-9-ethyladenine in Me_2SO and found that the C_8 signal appeared as a sharp singlet. This fact establishes that the unassigned coupling constant, 10.4 Hz, in the C_8 signal in [^{15}N]adenosine is $J(C_8N_9)$ (Kainosho, Watanabe, and Kyogoku, unpublished results). Therefore, disappearance of splitting in the C_8 signal of adenine derived from doubly enriched formamide is indeed due to the bond fission and re-formation of C–N bond during the thermal reaction, as Suzuki et al. have suggested.[1]

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Hydroxylation and Epoxidation Catalyzed by Iron–Porphine Complexes. Oxygen Transfer from Iodosylbenzene

Sir:

The catalytic cycle of cytochrome P-450 is believed to involve reductive activation of dioxygen at the heme center and subsequent peroxy bond cleavage to give a ferryl ion species as the active oxygen transfer agent.[1,2] Support for an iron–oxo species such as **1** is derived from the fact that a number of single oxygen donors, hydroperoxides, peroxy acids, and iodosylbenzene, effect oxygen transfer in a manner similar in many respects to the fully reconstituted enzyme system.[3-5] As part of our program to evaluate simple iron catalysts as oxygen transfer agents,[6,7] we have found that chloro-$\alpha,\beta,\gamma,\delta$-tetraphenylporphinatoiron(III) (**2**) and chlorodimethylferriprotoporphyrin IX (**3**) catalyze the hydroxylation and epoxidation of hydrocarbons with iodosylbenzene as an oxygen source.

$$\text{P-450} \cdot Fe^{3+} \xrightarrow[2e^-,\ 2H^+]{O_2} \text{P-450} \cdot Fe^{3+} \cdot H_2O_2 \rightarrow \text{P-450} \cdot FeO^{3+} + H_2O$$

1

In a typical experiment solid iodosylbenzene was added slowly to a solution of hydrocarbon and catalyst in methylene chloride under nitrogen at room temperature. Results for the oxidation of a representative family of hydrocarbons are given in Table I. Thus, cyclohexene (1 mL, 9.8 mmol) and **2** (0.035 g, 0.049 mmol) were dissolved in 6 mL of methylene chloride. Iodosylbenzene (0.066 g, 0.3 mmol) was added to this mixture over a period of 30 min. The reaction mixture was diluted with ether, washed with sodium sulfite, and analyzed by GLC. The yield of cyclohexene oxide was 55% based on iodosylbenzene. Cyclohexenol (15%) and a trace of cyclohexenone were the only other organic products. Iodobenzene was recovered in quantitative yield. Similarly, cyclohexadiene gave a 74% yield of the corresponding monoepoxide.

The reaction of *cis*- and *trans*-stilbene with iodosylbenzene using **3** as a catalyst gave the corresponding *cis*- and *trans*-stilbene oxides. The complete retention of configuration in this case contrasts with the epoxidation of *cis*- and *trans*-stilbene by tris(acetylacetonato)iron(III) hydrogen peroxide which has been reported to yield the trans epoxide from both starting materials.[8] Surprisingly, **2** catalyzed the conversion of *cis*-stilbene to *cis*-stilbene oxide while the trans isomer was inert. Indeed, a mixture of the two olefins led to efficient isolation of *cis*-stilbene oxide (82%) and recovery of *trans*-stilbene!

Such a dramatic change in selectivity with changes in the substitution pattern on the porphyrin suggests that the catalyst is intimately involved in the oxygen transfer step.[9] The nature of this selectivity is not clear, however. Space-filling models indicate that the approach of the double bond of *cis*-stilbene to the iron center of **2** is relatively unencumbered by phenyl–phenyl interactions between the catalyst and the substrate. By contrast, significant phenyl–phenyl nonbonded interactions develop between **2** and *trans*-stilbene for any geometry except parallel approach to the porphyrin plane directly from above. This apparent requirement for a restricted mode of approach could reasonably be explained by (a) the need to avoid the creation of a molecular void as the two molecules approach, (b) the specific presence of iodobenzene as oxygen transfer takes place, or (c) the stereoelectronic requirements for such an oxygen transfer. The generality of this specificity was further indicated by the observation that *cis*-2-butene was six times more reactive than the trans isomer in a competitive oxidation with **2** and iodosylbenzene.[10]

Unactivated aliphatic centers were found to be oxidized by **2** and iodosylbenzene to give alcohols. Thus, cyclohexane afforded cyclohexanol in 8% yield. Although this transformation was relatively inefficient, the lack of significant further oxidation to cyclohexanone is exceptional.[11] Adamantane gave a 13% yield of adamantanols with a strong preference (48:1, statistically corrected) for hydroxylation of the tertiary center. Hydroxylation of *cis*-decalin gave *cis*- and *trans*-9-decalol (5:1) indicating predominant retention of configuration at the oxidized center.

Reaction of chlorodioctylferriprotoporphyrin IX (**4**) with iodosylbenzene led to significant oxidation of the aliphatic side chains. Subsequent cleavage of the ester linkages with lithium aluminum hydride and GLC analysis of the product octanediols as the bistrifluoroacetates revealed a pronounced regioselectivity for C_4 and C_5 of the octyl chain (Table II). Similar oxidation of excess octyl acetate with **3** and iodosylbenzene gave a mixture of product diols with a modest selectivity for hydroxylation toward the end of the chain after identical workup and analysis.[12]

Table I. Hydrocarbon Oxidation with **2** and Iodosylbenzene

substrate	products	yield, %[a]
		55
		15
		74
		82
	*trans*-stilbene oxide	trace
		8
adamantane	1-adamantanol	12
	2-adamantanol	1

[a] Yields based on iodosylbenzene consumed. Preliminary results indicate that the lower yields with the less reactive hydrocarbons was due to competing destruction of the catalyst.

Table II. Intermolecular vs. Intramolecular Oxidation of Octyl Esters

	octanediol isomers					
	1,2	1,3	1,4	1,5	1,6	1,7
octyl acetate	<2	17	15	21	22	24
4	<2	15	28	30	13	14

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