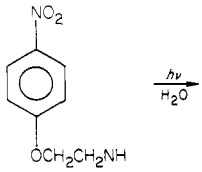
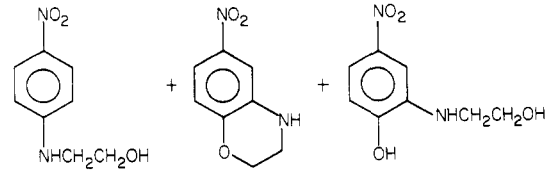


III

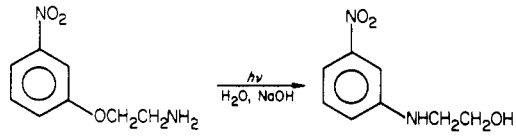


(thermal product) VII VIII

VI

with nitrogen or by vacuum degassing had no influence on the rate of the photoreaction.

The meta isomer II showed no change in its UV spectrum after heating at 99 °C for 15 min in aqueous sodium hydroxide (0.01 M). On photolysis of II (1.1×10^{-4} M) in a cuvette in aqueous sodium hydroxide (0.01 M) at 313 nm or with Pyrex-filtered light from a mercury lamp, λ_{max} at 332 nm ($A = 0.10$) and 274 nm ($A = 0.29$) shifted to 383 nm ($A = 0.11$) and 241 nm ($A = 0.81$), respectively; isosbestic points were observed at 358, 306, 292, 272, and 218 nm. The final UV spectrum was superimposable on that of V at 1.1×10^{-4} M. The preparative reaction was carried out by using 273 mg of the hydrochloride of II in 250 mL of 0.01 M NaOH and irradiating with a 200-W Hanovia immersion lamp for 4 h. Workup was performed by extracting with ether and concentrating the extract to an oily orange solid which was identified as nearly pure V.



II V

The quantum yield for the photo-Smiles rearrangment of II (1.3×10^{-3} M) in aqueous sodium hydroxide (0.01 M) was determined at 313 nm on a merry-go-round apparatus by using degassed valerophenone (0.1 M) in benzene as the actinometer.[11] At less than 10% conversions, the quantum yield is 0.23.

Remarkable contrast to these results is shown by the intermolecular photosubstitutions by amines on 3- and 4-nitroanisoles. Though quantitative efficiency data are not available, Cornelisse and Havinga note[1] that the photodisplacements of methoxide from 4-nitroanisole in water by amines such as methyl-, dimethyl-, or ethylamine to give 4-nitroanilines are clean and efficient reactions.[12] The opportunity for the analogous reaction, if the mechanism involves nucleophilic attack on C-1 of the excited aromatic molecule, is clearly presented by III since it undergoes Smiles rearrangement in the ground state. Such reaction is, however, not seen in the excited state; intramolecular attack at C-2 of III prevails instead. Also, whereas the *intermolecular* substitution on 3-nitroanisole by amines is relatively inefficient among nitroanisole photosubstitution,[1] we observed the *intramolecular* meta photosubstitution of II to be highly efficient.

An attractive explanation for these differences is that, in contrast to the intermolecular reactions, the intramolecular reactions of II and III allow no direct interaction of the nucleophile with the nitro group in the excited state. The strong meta-activating influence of the nitro group in the π,π^* state[13,14] prevails and directs the incoming nucleophile in II and III to a ring carbon atom meta to nitro. Thus, the intermolecular photosubstitutions on 4-nitroanisole may be initiated not by nucleophilic ring attack but by an electron-transfer interaction in an n,π^* state[15] involving the nitro group and the amine. This interaction cannot occur in III. The electron-transfer interaction may lead to quenching as well as to substitution for 4-nitroanisole[13] but largely to quenching for 3-nitroanisole because the odd electron distribution in the anion radical is unfavorable for radical coupling at the ring position bearing the nucleofugic methoxy group.[16] This quenching interaction would be unfavorable in II, the absence of which may enable its efficient photosubstitution.

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Natural Abundance ^{13}C–^{13}C Coupling Observed via Double-Quantum Coherence

Sir:

We propose a new technique for observing ^{13}C–^{13}C spin–spin coupling in the NMR spectra of compounds with ^{13}C in natural abundance. The basic problem is that of identifying the appropriate weak satellite signals in the flanks of strong ^{13}C lines, and the limit is set not so much by the sensitivity or dynamic range of the NMR spectrometer but rather by the presence of a jumble of other weak lines from spinning sidebands, from incomplete proton decoupling, or simply from small amounts of impurities. Investigations of carbon–carbon coupling[1-5] have consequently relied heavily on specific isotopic enrichment, particularly for weak long-range couplings, which are important in conformational studies. The new method filters out the desired ^{13}C satellite signals, suppressing the much stronger signals from molecules with an isolated ^{13}C nucleus, together with any modulation sidebands of the latter.

This discrimination is achieved on the basis of the homonuclear spin–spin coupling J_{C-C}. The idea is to create double-quantum coherence[6-18] in the manner of recent two-dimensional Fourier

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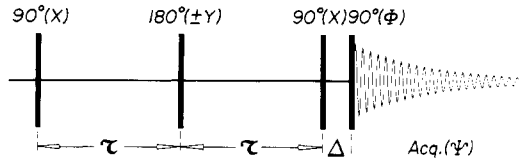

Figure 1. Pulse sequence used for the creation of double-quantum coherence followed by regeneration of single-quantum transverse magnetization. The phases Φ and Ψ are cycled according to Table I.

Table I. Phase of the Read Pulse (Φ) and the Receiver Reference Phase (Ψ) Compared with the Phase of the Three Principal Signal Components, S_0, S_1, and S_2

Φ	S_0	S_1	S_2	Ψ
$+x$	$-y$	$+x$	$+x$	$+x$
$+y$	$+x$	$+y$	$-y$	$-y$
$-x$	$+y$	$+x$	$-x$	$-x$
$-y$	$-x$	$+y$	$+y$	$+y$

transform experiments,[9,11] converting this into detectable transverse nuclear magnetization by means of a radiofrequency pulse. The unwanted conventional signals are filtered out by exploiting the unique phase properties of the double-quantum signals.[11,12] Because of the low natural abundance of ^{13}C, the spectra are always of the AB or AX type, and the theory is correspondingly simple; all the spectra are proton noise decoupled throughout the experiment.

A pulse sequence 90° (x)–τ–180° (y)–τ–90° (x) creates two principal signal components, S_0 from molecules with only isolated carbon-13 nuclei and S_2 from molecules with two coupled carbon-13 spins. S_0 is a strong signal and must be suppressed; it is represented by a magnetization vector aligned along the $-z$ axis if pulse imperfections are neglected. The much weaker component, S_2, is the one of interest. It is not a true magnetization but represents a coherence between the energy levels $\alpha\alpha$ and $\beta\beta$, that is to say a double-quantum coherence. We require the maximum transfer of nuclear magnetization into this double-quantum coherence, and for weakly coupled spins this condition[15] is

$$\tau = (2n + 1)/(4J_{C\text{-}C}) \quad (1)$$

Note that molecules containing only a single carbon-13 nucleus cannot generate any multiple-quantum coherence. The key to discrimination between S_0 and S_2 lies in the different sensitivities to a change in the phase of a 90° "read" pulse used to convert S_0 and S_2 into observable transverse nuclear magnetizations. The complete pulse sequence is set out in Figure 1, where Δ is a very short delay (in practice 10 μs) needed for resetting the radiofrequency phase.

The phase Φ of the read pulse is cycled through four settings, $+x$, $+y$, $-x$, and $-y$. The transverse signal generated from S_0 cycles in step with Φ and in the same sense (Table I). In contrast, the signal generated from double-quantum coherence shifts by 270° for each 90° step in Φ and thus appears to rotate in 90° steps in the opposite sense to the sense of rotation of Φ. The receiver reference phase (Ψ) is made to follow this latter signal, causing cancellation of the signal originating from S_0 but revealing the weak signals from molecules with two coupled carbon-13 spins.

Since S_0 is two orders of magnitude stronger than S_2, careful consideration has to be given to pulse imperfections, which have the effect of generating spurious transverse magnetization (S_1) at the time of the Δ delay. For example, if the radiofrequency field strength is weaker than the nominal value, S_1 components will be generated as indicated in Table I. This is the reason for employing a four-step sequence. Further attenuation of spurious signals is achieved by alternating the phase of the 180° pulse as indicated in Figure 1, giving a cycle of eight steps.

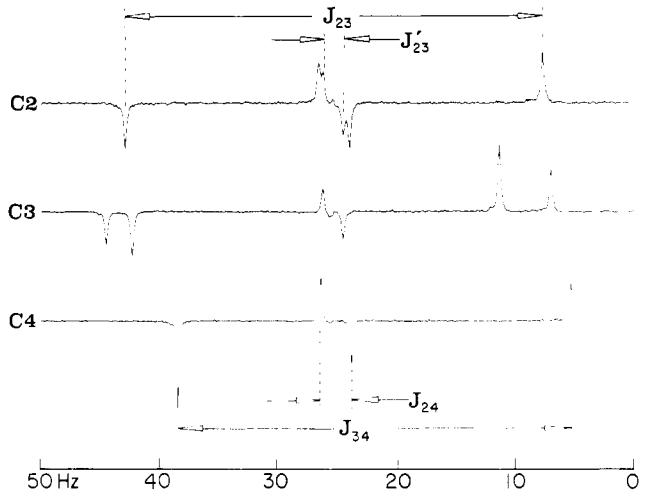

Figure 2. Sections from the carbon-13 spectrum of piperidine, showing ^{13}C–^{13}C direct and long-range couplings. Note the effective suppression of the signals from the much more abundant molecules with isolated ^{13}C nuclei.

The final degree of suppression of these unwanted signals is obtained by repeating the entire eight-step sequence four times with all pulse phases and the receiver reference phase incremented together in 90° steps. This corrects for small errors in the 90° phase shifts in the transmitter and receiver channels and for any inequality of the audiofrequency filters in the quadrature detection system.

Experiments were carried out at 50 MHz on a Varian XL-200 spectrometer with appropriate programming for pulse sequencing and data acquisition. The natural abundance carbon-13 spectrum of piperidine was chosen as an illustrative example. There are four observable carbon–carbon couplings, $^1J_{2,3} = 35.2$, $^3J_{2,3'} = 1.7$, $^2J_{2,4} = 2.6$, and $^1J_{3,4} = 33.0$ Hz. The first step is to set the condition for optimum transfer into double-quantum coherence for the two direct couplings according to eq 1. This allows $^1J_{2,3}$ and $^1J_{3,4}$ to be evaluated, and with the assumption that the long-range couplings would be of the order of 5 Hz, both the direct and long-range couplings may be observed in the same spectrum by setting $\tau = 51$ ms and letting $n = 3$ and $n = 0$, respectively. The conditions for satisfying eq 1 are not critical when $n = 0$, and the signals corresponding to long-range coupling are not far below maximum intensity.

Figure 2 shows 50-Hz sections from the spectrum of piperidine centered on the three carbon-13 chemical shifts. Both $^1J_{2,3}$ and $^1J_{3,4}$ are obtained from AB-type spectra with significant displacements of the centers of the doublets from the chemical shift frequencies and noticeable asymmetries in the intensities. The two components of each J doublet always appear in antiphase. Whether they appear in the "up–down" or "down–up" configuration depends upon whether n is even or odd. In the piperidine spectra, the doublets due to direct couplings ($n = 3$) are all "down–up" while those from long-range couplings ($n = 0$) are all "up–down". (Where necessary to avoid interference between adjacent doublets, a simple extension of the pulse sequence restores the more familiar "up–up" configuration.)

When there has been optimum transfer into double-quantum coherence, the spectra from coupled ^{13}C–^{13}C molecules appear with the same sensitivity as the conventional spectrum. Signals from impurities and modulation artifacts are effectively suppressed, leaving a clean spectrum of the desired isotopomers. For complicated spectra, two-dimensional Fourier transformation methods can be used to avoid overlap between different multiplets.[19]

This technique is not limited to the example of carbon–carbon coupling but could be extended to heteronuclear systems or applied

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to the simplification of proton spectra in a manner similar to that of spin-echo difference spectroscopy.[20]

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Electrochemistry of Vitamin B_{12}. 6. Diaquocobinamide

Sir:

The chemistry of cobinamides has been widely investigated since it may provide a source of information for a better understanding of the properties of cobalamins.[1,2] The comparison between the two classes of compounds may indeed help in the evaluation of the role of the nucleotide side chain Bzm ligand. There have been, in this connection, few studies on electrochemistry of cobinamides.[3-6] The polarographic investigation of an extended series of cobinamides[3] has led to a rather puzzling observation concerning diaquocobinamide(III): its reduction wave is located at −0.74 V vs. SCE, i.e., considerably negative (700 mV) to the standard potential of the Co(III)/Co(II) couple in aquocobalamin.[7,8] This observation has been later taken as evidence that diaquocobinamide(III) has a standard potential more negative than that of aquocobalamin(III) in a discussion of the electrochemical reduction mechanism of the later compound.[9] One would have, on the contrary, anticipated that due to the electron-donating ability of Bzm being clearly higher than that for water the reduction of Co(III) to Co(II) would be much more difficult for aquocobalamin than for diaquocobinamide. It is noted that the pH (6.2) where the polarographic determination was carried out is slightly higher than the pK_a (6) of the ionization of a first axial water molecule[10] and that the half-wave potential may be affected by electron-transfer kinetics and/or adsorption phenomena. It seems unlikely, however, that these effects could reverse the expected behavior to such a large extent. On the other hand, one could invoke stereochemical differences between cobinamides and cobalamins such as bent-up conformation of the corrin ring in the latter case as compared to the former,[11] leading to different electron-donating properties of the ring which might counterbalance the influence of the axial ligands. Again, however, the large magnitude of the inversion in reducibility appears difficult to rationalize within this context.

Previous determinations of the Co(III)/Co(II) thermodynamics of cobalamins have suggested, through extrapolation of the data obtained in strongly acidic media, that the standard potential would be +0.270 V vs. SCE for the base-off forms.[7] It is expected that the behavior of diaquocobinamide is similar to that of the base-off aquocobalamin, leading to an $E°$ value in a more reasonable agreement with the anticipated effect of the axial ligands. It was the purpose of the work reported hereafter to determine directly the main redox characteristics of the Co(III)/Co(II) and Co(II)/Co(I) couples in diaquocobinamide and to see whether or not the expectations derived from the extrapolation of the aquocobalamin behavior are legitimate.

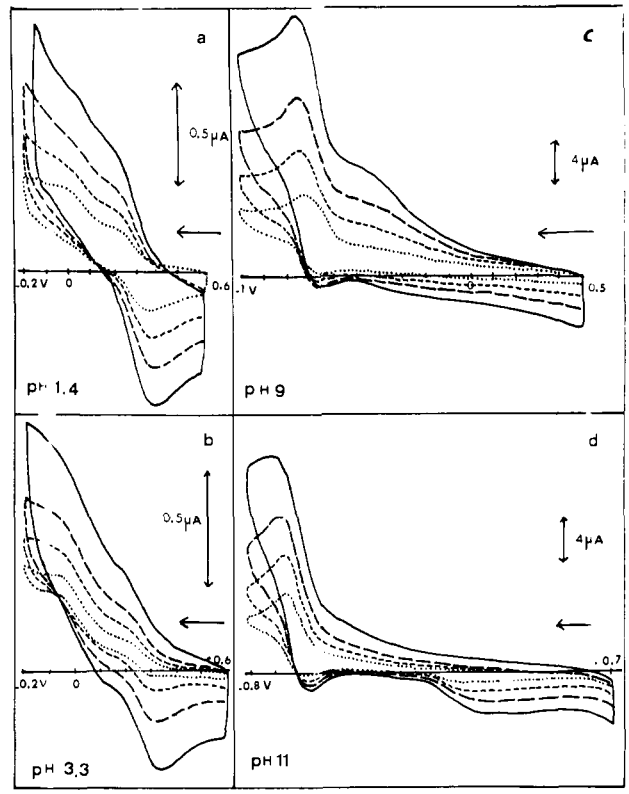

Figure 1. Cyclic voltammetry of diaquocobinamide as a function of pH. Electrodes: (a and b) glassy carbon, (c and d) platinum. Buffer: (a) $HClO_4$; (b-d) Britton-Robinson. Concentration: (a and b) 1, (c) 0.9, (d) 0.8 mM. Sweep rate (from bottom upward): 0.02, 0.05, 0.1, and 0.2 V s^{-1}.

Instrumentation and procedures for cyclic voltammetry were the same as already described.[7,8,12] Diaquocobinamide was prepared from aquocobalamin and purified according to previously described procedures.[13]

The general features of the cyclic voltammetry of the Co(III)/Co(II) couples as a function of pH (Figure 1) are similar to what was observed with aquocobalamin:[7,8] while in acidic media, the Co(III)/Co(II) wave is clearly separated from the Co(II)/Co(I) wave, there is a negative shift of the former upon increasing the pH over the pK_a (6) of the aquo-hydroxo interconversion, finally leading to an almost complete merging of the two waves. For pH < 6, it is noted, however, that the Co(III)/Co(II) cathodic pattern is split into two waves, which was not the case for aquocobalamin. Determination of $E°$ on the first of these waves and on the anodic Co(II)/Co(III) wave leads to an approximate value of 0.265 V, i.e., close to the figure found for the base-off aquocobalamin in strongly acidic medium.

A spectroelectrochemical investigation of the oxidoreduction of diaquocobinamide was carried out at pHs 1.6 and 3.8 on a platinum-grid electrode in order to reach a more reliable determination of the Co(III)/Co(II) $E°$. The results obtained upon reduction at pH 3.8 are summarized in Figure 2. The logarithms of the analyses (Figure 2c) have a slope close to 60 mV, as expected for a reversible process. The reversibility has actually been reached and confirmed by a reoxidation experiment in which the

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