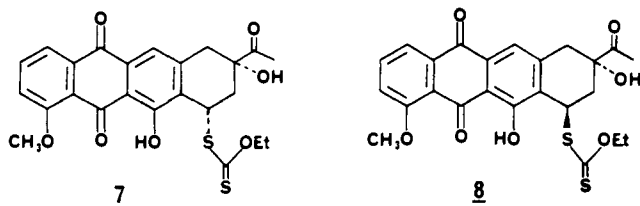


ature, the reaction is worked up, yielding two products in a combined yield of 87%. The spectroscopic properties identify these as C-7 ethyl xanthyl diastereomers, present in an 85/15 ratio. The major isomer **7** is assigned a 7*S* configuration on the basis of a



pseudoaxial C-9 hydroxyl (determined by  $J_{8e,10e} = 2.1$  Hz)<sup>8</sup> and the coupling constants of the 8a, 8e, and 7e (ABX) hydrogens ( $J_{AB} = 14.6$ ,  $J_{AX} = 5.8$ ,  $J_{BX} = 2.4$  Hz) and by analogy to the aclacinomycin adducts (below). The minor isomer has an identical A ring conformation ( $J_{8e,10e} = 2.2$  Hz) and the appropriate couplings for a pseudoaxial hydrogen at C-7 ( $J_{AB} = 15.0$ ,  $J_{AX} = 5.0$ ,  $J_{BX} = 7.7$  Hz). In a likewise manner, the aclacinomycin A quinone methide reacts with ethyl xanthate to give an 80/20 diastereomer ratio, in a combined yield of 90%. A 7*S* assignment to the major isomer ( $J_{AB} = 15.3$ ,  $J_{AX} = 6.0$ ,  $J_{BX} = 1.2$  Hz) is corroborated by Krueger and Prairie, from the circular dichroism spectrum.<sup>9</sup> The generality of this reaction has been extended to thiol nucleophiles and to alternative methods of quinone methide generation. Reaction of aclacinomycin in the presence of 15 mM *N*-acetylcysteine gives a single product (90% yield), to which is assigned a 7(*S*)-(N-acetylcysteinyl)-7-deoxyaklavinone structure. Marcellomycin also gives a single N-acetylcysteinyl adduct (77%) using NADH as a reductant and spinach ferredoxin/NADP<sup>+</sup> oxidoreductase<sup>10</sup> as a catalyst.

Several experiments establish the quinone methide as the pivotal intermediate. No reaction occurs in the absence of NADH or enzyme. The isolated adducts are converted anaerobically in the absence of nucleophiles to the 7-deoxyanthracyclones, while in the presence of substoichiometric NADH and a second nucleophile, the C-7 substituent is interchanged. The circumstances under which stable adduct formation occurs explain our previous failure to observe xanthate trapping of the 7-deoxyaklavinone quinone methide.<sup>6</sup> As an excess of reductant was used, the adduct (although certainly formed) was converted to 7-deoxyaklavinone and 7,7'-bis(7-deoxyaklavinone).<sup>11</sup> Last, under these conditions nucleophile adducts are not obtained from an 11-hydroxy-lanthracycline, daunomycin. Either its quinone methide is unreactive or more readily protonated, or the adduct is more poorly trapped by disproportionation.

The present study carries two implications. First, it outlines a strategy for the direct conversion of 11-deoxyanthracyclines (and presumably 11-deoxyanthracyclones) to new derivatives. Second, it indicates that these quinone methides have electrophilic character and provides precedent for such behavior in vivo.

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(11) The present observations and those of Kleyer and Koch<sup>7</sup> suggest an alternative mechanism for dimer formation (rather than that of neutral radical coupling<sup>3,6</sup>), that of nucleophilic addition of one quinone methide to a second quinone methide, behaving as an electrophile.

to us their circular dichroism studies on these compounds. The support of the National Institute of General Medical Sciences is acknowledged.

**Supplementary Material Available:** Experimental protocols, spectroscopic data, and <sup>1</sup>H NMR and CIMS spectra (12 pages). Ordering information is given on any current masthead page.

### Sensitivity-Enhanced Correlation of <sup>15</sup>N and <sup>1</sup>H Chemical Shifts in Natural-Abundance Samples via Multiple Quantum Coherence

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We demonstrate the feasibility of a new and sensitive technique for determining <sup>15</sup>N-<sup>1</sup>H chemical-shift correlation in natural-abundance samples. The poor sensitivity of <sup>15</sup>N NMR detection is caused by its low natural abundance (0.37%) and its low magnetogyric ratio. Additional problems often arise because of long <sup>15</sup>N relaxation times and a negative magnetogyric ratio. Investigations of <sup>15</sup>N chemical shifts and <sup>15</sup>N-<sup>1</sup>H shift correlation have consequently heavily relied on specific isotopic enrichment and on the use of large sample volumes with high sample concentrations. Another approach, proposed by Bodenhausen and Ruben,<sup>1</sup> initially transfers proton magnetization to the (natural abundance) <sup>15</sup>N nuclei. Then the time evolution of the <sup>15</sup>N signals, and thus the <sup>15</sup>N chemical shifts, can be monitored indirectly in a two-dimensional experiment.<sup>2,3</sup> This latter technique has the advantage of detecting the <sup>15</sup>N chemical shifts and the <sup>1</sup>H-<sup>15</sup>N shift correlation via the protons, which, due to their high magnetogyric ratio, give sensitivity that is several orders of magnitude better than what is obtained in a direct detection of <sup>15</sup>N. In practice this very large gain in sensitivity is not easily achieved. The large number of proton pulses in the experiment complicates suppression of large signals or selective excitation of the signals of interest. We propose a much simpler experiment, based on an idea of Jeener,<sup>4</sup> which offers similar advantages as the experiment proposed by Bodenhausen and Ruben<sup>1</sup> but avoids most of the practical problems. Experiments very similar to the one described in this paper have been developed independently by Bendall et al.<sup>5</sup> and have been applied to <sup>13</sup>C studies of small organic compounds.

The simplest possible pulse scheme employs the sequence  $\alpha^{\circ}_x(^1\text{H})-1/(2J_{\text{NH}})-90^{\circ}_y(^{15}\text{N})-t_1-90^{\circ}_x(^{15}\text{N})$ -acquire(<sup>1</sup>H), where the flip angle  $\alpha$  equals 90° or smaller. The basic idea is that a 90°(<sup>1</sup>H)-1/(2J<sub>NH</sub>)-90°(<sup>15</sup>N) sequence converts all longitudinal magnetization of protons directly coupled to <sup>15</sup>N into equal amounts<sup>6</sup> of heteronuclear zero- and double-quantum coherence.<sup>7-11</sup> The frequencies of the zero- and double-quantum

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Table I. Radio-Frequency Phase of the First  $^{15}\text{N}$  Pulse,  $\phi$ , and the Relative Phases of the Transverse Proton Magnetizations Originating from Heteronuclear Zero- ( $M_{ZQ}$ ) and Double-Quantum ( $M_{DQ}$ ) Coherence

$\phi$	$M_{ZQ}$	$M_{DQ}$
$x$	$x$	$x$
$y$	$y$	$-y$
$-x$	$-x$	$-x$
$-y$	$-y$	$y$

coherences equal  $\delta_{\text{H}} + \delta_{\text{N}}$  and  $\delta_{\text{H}} - \delta_{\text{N}}$ , respectively,<sup>12</sup> where  $\delta_{\text{H}}$  and  $\delta_{\text{N}}$  are the  $^1\text{H}$  and  $^{15}\text{N}$  chemical shift frequencies of the NH pair considered. The multiple-quantum coherence is converted into transverse proton magnetization by the second  $^{15}\text{N}$  90° pulse. The evolution of the heteronuclear multiple-quantum coherence can be mapped out in a two-dimensional experiment. The phase  $\phi$  of the first  $^{15}\text{N}$  pulse is cycled through four settings,  $+x$ ,  $+y$ ,  $-x$ , and  $-y$ , in the four steps of the experiment (Table I). This gives good suppression of proton signals that are not coupled to a  $^{15}\text{N}$  nucleus<sup>13</sup> and allows discrimination between the zero- and double-quantum signals,<sup>8,10,11</sup> i.e., determination of the sign of the  $^{15}\text{N}$  offset frequency from the  $^{15}\text{N}$  transmitter. The coordinates of the two components of a heteronuclear doublet in, for example, the zero-quantum 2D spectrum are  $(F_1, F_2) = (\delta_{\text{N}} + \delta_{\text{H}}, \delta_{\text{H}} \pm J_{\text{NH}}/2)$ . The effect of  $\delta_{\text{H}}$  in the  $F_1$  dimension can be removed,<sup>14-16</sup> leaving a pure chemical-shift correlation map with coordinates of the center of the heteronuclear doublet at  $(\delta_{\text{N}}, \delta_{\text{H}})$ .

Experiments were carried out on a Nicolet-360 spectrometer, equipped with a 1180 computer and a 293A' pulse programmer. An ADNIC phase programmer<sup>17</sup> was used for controlling the phases of  $^{15}\text{N}$  pulses. A triply tuned probehead ( $^1\text{H}, ^2\text{D}, ^{15}\text{N}$ ) was used.<sup>16</sup> Gramacidin S, 12 mg, dissolved in  $\text{Me}_2\text{SO}-d_6$ , in a 5-mm sample tube, giving a 18 mM concentration, was chosen as an illustrative example. Note that due to the symmetry of gramacidin S, the effective concentration of the sites of interest corresponds to 36 mM. First, the proton pulse length and power are set to give a proton flip angle of approximately 60°<sup>18</sup> near resonance, with minimal excitation of signals outside the proton region of interest. In our case, a 1 msec proton pulse served this purpose. In another experiment,<sup>19</sup> a 2-1-4 Redfield pulse<sup>20</sup> allowed measurement of imino proton signals from samples of  $^{15}\text{N}$ -labeled tRNA in a 92%  $\text{H}_2\text{O}/8\%$   $\text{D}_2\text{O}$  mixture. The delays  $\Delta_1$  and  $\Delta_2$  were set to 4.5 ms, and the  $^{15}\text{N}$  90° pulse was carefully measured<sup>6</sup> by using a  $^{15}\text{N}$ -labeled compound and determined to be 550  $\mu\text{s}$ . The evolution period was incremented in 32 equal steps of 1 ms, giving a spectral width in the  $F_1$  dimension of  $\pm 500$  Hz. For each value of  $t_1$  1200 transients were recorded, and the total measuring time was approximately 3 h.

Figure 1a shows the amide region of the conventional proton spectrum of gramacidin S. Unfortunately, the valine amide proton resonance is not observed in this spectrum, possibly due to fast exchange with a small amount of  $\text{H}_2\text{O}$  present in the sample. Figure 1b is a projection of the 2D heteronuclear-shift correlation spectrum (Figure 1c) derived from the double-quantum signals and represents the  $^{15}\text{N}$ -coupled proton spectrum, with signals from protons not directly bonded to  $^{15}\text{N}$  effectively suppressed. A small signal in this projection at 7.3 ppm is due to not completely suppressed signal from the aromatic protons and shows that

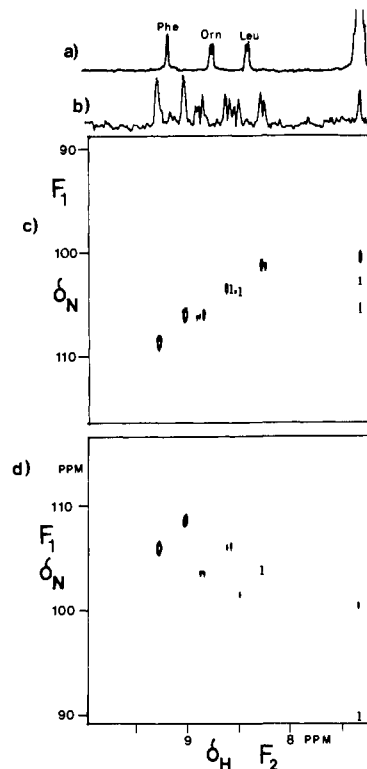


Figure 1. (a) Imino region of the conventional  $^1\text{H}$  spectrum of gramacidin S. (b) Projection of the two-dimensional shift correlation spectrum (c) derived from the double-quantum signals onto the  $F_2$  axis, representing the  $^{15}\text{N}$ -coupled proton spectrum, with resonances from protons not bonded to  $^{15}\text{N}$  effectively suppressed. (d) Heteronuclear shift correlation spectrum derived from the zero-quantum signals. Both 2D spectra result from the same experiment and have uncorrelated noise. A 18 mM solution of gramacidin S (12 mg) in  $\text{Me}_2\text{SO}-d_6$  in a 5-mm sample tube was used, and the total measuring time was 3 h.

suppression of unwanted signals is of the order of 2000. By storing the results of steps 1 and 3 separate from those of steps 2 and 4 (Table I), both the zero- and the double-quantum correlation signals were retrieved from the same sets of acquired data. The noise is uncorrelated in the two spectra, and availability of two independent spectra can be used as a check on experimental results in the case of complicated spectra: the center of the heteronuclear doublets in the 2D spectra have to be at symmetrical positions in the  $F_1$  dimension with respect to the  $^{15}\text{N}$  transmitter frequency. Because the double-quantum frequency equals  $\delta_{\text{H}} - \delta_{\text{N}}$ , in the 2D shift-correlation spectrum derived from the double-quantum signals the chemical-shift axis is reversed (Figure 1c). The spectra in Figure 1, parts c and d, give  $^{15}\text{N}$  shifts for the Phe, Orn, and Leu of 106.5, 103.5, and 102 ppm, respectively, confirming the assignment made by Hawkes et al.<sup>21</sup> It has been demonstrated elsewhere<sup>16</sup> that sensitivity of the experiment can be improved by almost a factor of 2 by employing broad-band  $^{15}\text{N}$  decoupling during data acquisition, but suppression of the signals from protons not directly coupled to  $^{15}\text{N}$  is harder in this case, due to the influence of the  $^{15}\text{N}$  irradiation on the deuterium lock signal.

We have demonstrated that this technique can be used to correlate  $^1\text{H}$  and  $^{15}\text{N}$  chemical shifts in natural-abundance samples. The sequence can be used in aqueous solutions.<sup>19</sup> While no actual comparison of the sensitivity of our method with the directly detected, INEPT-enhanced<sup>22</sup> signals has been made (no 5-mm  $^{15}\text{N}$  probe is available in our laboratory), theory shows that an increase in sensitivity by at least an order of magnitude can be expected if probe design and experimental setup are optimized.<sup>23</sup>

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