Application of the Homonuclear and Heteronuclear Two-Dimensional Chemical-Shift Correlation NMR Spectroscopy to the Complete Assignment of ^1H and ^{13}C NMR Spectra of Ionophorous Antibiotic X.14547 A

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^1H and ^{13}C NMR spectra of ionophorous antibiotic X.14547 A have been completely assigned by parallel use of homo- and heteronuclear two-dimensional NMR techniques. This result was obtained with 0.1 mmol of material.

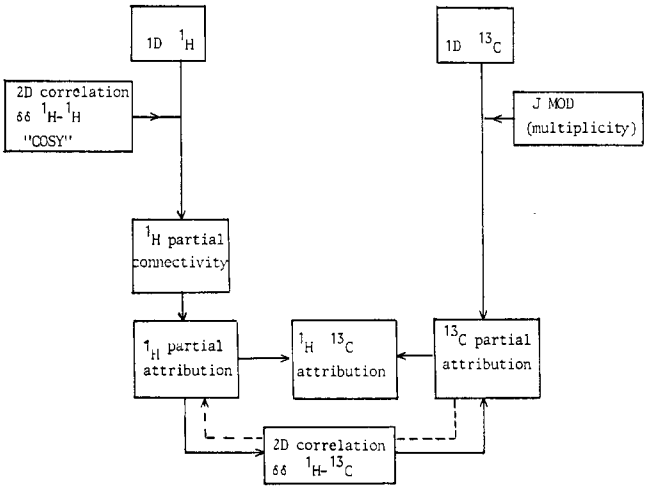
The study of the behavior of a molecule in solution falls mainly in the application field of NMR. When the structure of the product is known, the first and essential step of any NMR study is to achieve the complete signal assignments of NMR spectra. Even with high-field spectrometers, some regions of ^1H NMR spectra of medium- to large-size molecules remain unresolved; see, for example, region A of the ^1H NMR spectrum of antibiotic X.14547 A (Figure 1). Assignments in these regions can sometimes be made with classical one-dimensional (1-D) experiments (double resonance, etc.). In the case of ^{13}C NMR spectra, in spite of some advanced 1-D techniques (selective decoupling, spin population inversion, etc.), assignment is generally made by comparison with parent compounds, a method that may give rise to many errors. When 1-D NMR is used, the only secure method for total assignment of ^{13}C NMR spectra is the time-consuming chemical and biological synthesis of ^{13}C-labeled compounds.

Two-dimensional (2-D) NMR methods have been shown to be of great help in solving the problems described above. Both J–δ[1] and δ–δ (COSY, SECSY)[1,3] ^1H correlations allow the elucidation of most parts of ^1H NMR spectra but do not, necessarily, lead to total assignments. On the other hand, δ–δ ^{13}C correlation (INADEQUATE)[1,2] leads to complete assignment of ^{13}C NMR spectra, but this method is not very sensitive, requiring at least 1 to 2 mmol and is, therefore, often unsuitable. In fact, it should be possible to deduce, in most cases, total ^1H and ^{13}C assignments by an intercorrelation of ^1H and ^{13}C NMR spectral information. The 2-D heteronuclear chemical-shift correlation gives this relation.

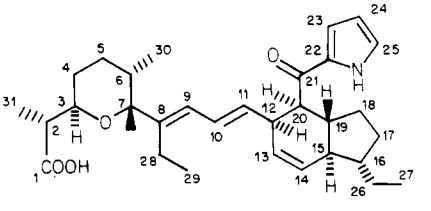
We describe here the complete assignment of ^1H and ^{13}C NMR spectra of an ionophorous antibiotic, X.14547 A, with less than 0.1 mmol of material, by parallel use of homo- and heteronuclear chemical-shift correlation ^1H–^1H[1,3] and ^1H–^{13}C[1,4] (Scheme I).

The "COSY" ^1H–^1H chemical-shift correlation (Figure 3) provides a ^1H scalar coupling relationship. Groups of coupled protons, separated by quaternary carbons of heteroatoms, can be detected. A partial assignment is deduced. The modulated spin–echo ^{13}C NMR spectrum[5]

Scheme I



(Figure 2) gives ^{13}C signal multiplicity leading to a preliminary ^{13}C assignment. Then the ^1H–^{13}C chemical-shift correlation, which is the backbone of the method, allows us to deduce ^{13}C assignments from ^1H ones. Since carbon connectivity is obtained through ^1H relations, the method fails when a quaternary carbon is encountered. This difficulty is overcome by use of "long-range" ^1H–^{13}C chemical-shift correlation,[1] which relates ^1H and ^{13}C through ^2J or ^3J scalar couplings. This experiment allows us to "jump" over quaternary carbon but also identifies them through their relation with a distant proton. At this stage, it is necessay to refer back to the ^1H NMR spectrum. Assignment of carbons of CH_2 groups bearing nonequivalent protons, one proton being previously assigned, leads to the assignment of the second proton. After a small number of steps back and forth between ^1H and ^{13}C assignments, the ^1H and ^{13}C spectrum may finally be fully assigned. We applied this strategy to the ionophorous antibiotic X.14547 A (1).



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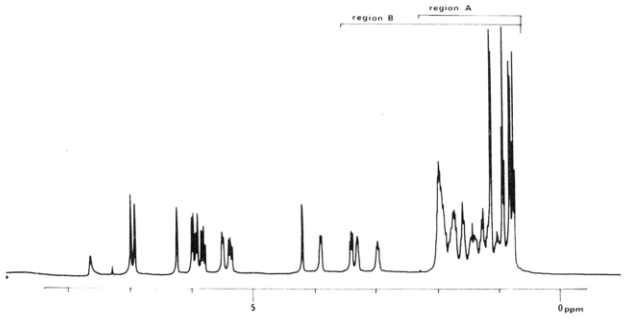

Figure 1. ^1H NMR (400 MHz) spectrum of X.14547 A (1) in $CDCl_3$.

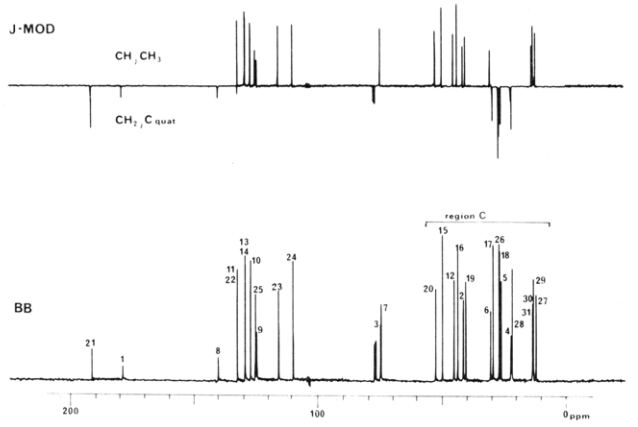

Figure 2. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of X.14547 A (1). Lower trace: broad-band spectrum. Upper trace: *J*-modulation spectrum.

X.14547 A is a member of the polyether family,[6] recently isolated by Westley and co-workers from a strain of *Streptomyces antibioticus* NRRL 8167. Its biological properties have been fully investigated.[7] Structurally, it presents a novel feature with a *trans*-butadienyl moiety connecting a pyrrolcarbonyl and a tetrahydroindan "right-part" group trans-fused to a tetrahydropyryl-propionic acid "left-part" group. This unique structure has attracted much interest in the total synthesis field.[8]

This substance was characterized as a monovalent/divalent cation ionophore in apolar solvents.[7c] In methanol, the formation of 2:1 neutral complexes was observed either with mono or divalent cations.[9] In the mitochondrial membrane, X.14547 A is primarily a K^+ carrier. NMR conformational studies of this ionophore and its complexes with various cations have never been undertaken. As a first step, it was necessary to obtain total assignments of ^1H and ^{13}C NMR spectra.

Since classical one-dimensional NMR methods failed to provide total ^1H[10] and ^{13}C[11] assignments, we resorted to 2-D NMR. To our knowledge, the only other 2-D NMR study carried out on such compounds led to the complete

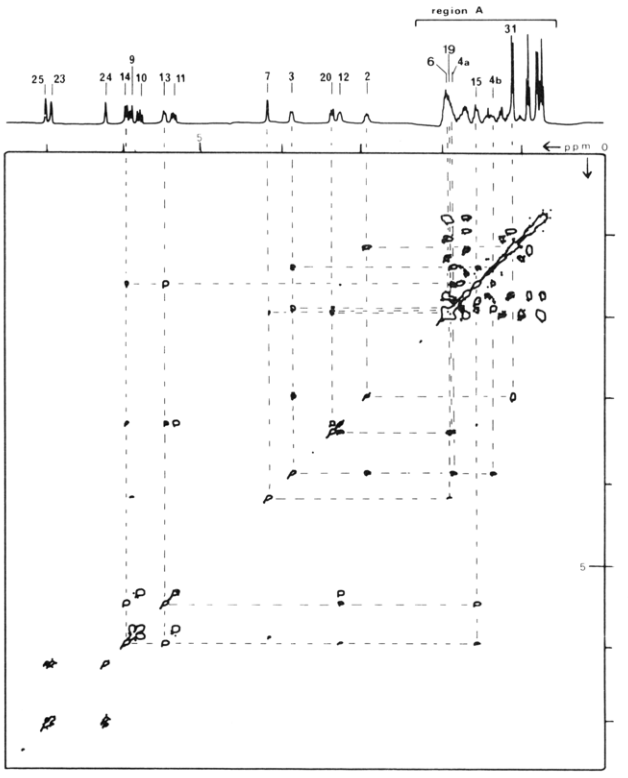

Figure 3. COSY (400 MHz, $CDCl_3$) spectrum of X.14547 A (1) presented as a contour plot (δ ^1H in two dimensions).

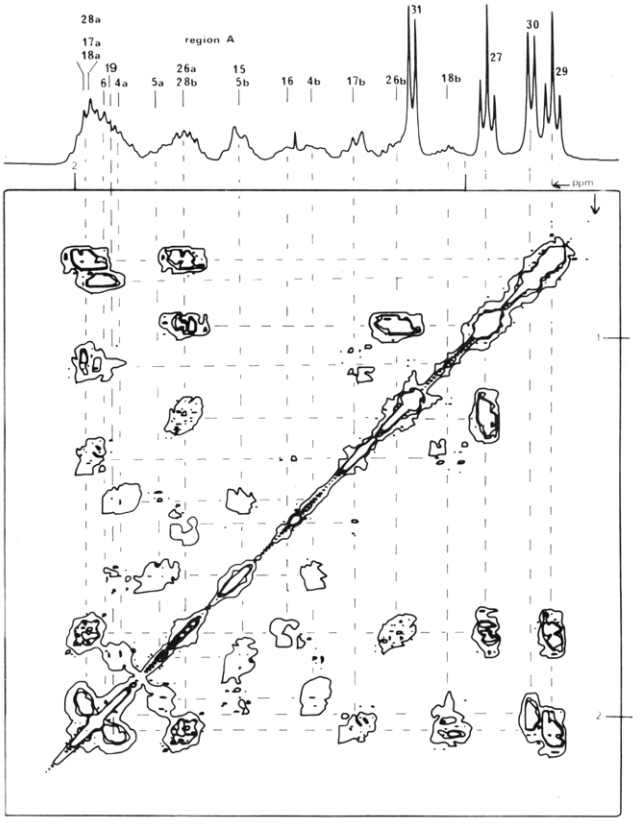

Figure 4. COSY (400 MHz, $CDCl_3$) spectrum of X.14547 A (1) (region A; see Figure 1) presented as a contour plot (δ ^1H in two dimensions).

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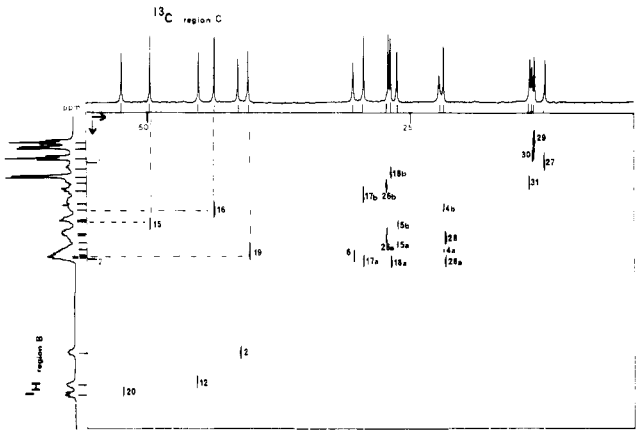

Figure 5. ^1H–^{13}C 2-D chemical-shift correlation NMR (100 MHz, $CDCl_3$) spectrum of X.14547 A (1) (region B for ^1H; region C for ^{13}C; see Figures 1 and 2) presented as a contour plot.

assignment of the ^{13}C NMR spectrum of monensin by the INADEQUATE method.[12]

Results and Discussion

The strategy described above was implemented. The ionophorous antibiotic X.14547 A (1) bears two ethyl groups. Its 400-MHz ^1H NMR spectrum (Figure 1) shows two methyl triplets at 0.79 and 0.96 ppm. The δ–δ ^1H correlation (COSY) (Figures 3 and 4) shows that the methyl at 0.96 ppm is coupled with two intercoupled protons, one of which is also coupled with another proton. This arrangement can only correspond to the Me_{27}–H_{26a}, H_{26b}–H_{16} linkages.[13] The resonances of methyl-29 and methylene-28, which form an isolated set, may then be deduced.

Out of the remaining two methyl doublets, one is coupled to a strongly deshielded proton (δ 2.94). H_{31} and H_2 are thus identified and the "COSY" spectrum gives Me_{31}–H_2–H_3–H_{4a}, H_{4b}–H_{5a}, H_{5b} filiation. The other doublet corresponds to the Me_{30} resonance, and the Me_{30}–H_6–H_7 sequence is, therefore, characterized.

In addition, the "COSY" procedure gives unequivocally the H_9–H_{10}–H_{11}–H_{12}–H_{20}–H_{19} and H_{12}–H_{13}–H_{14}–H_{15} connectivities and also pyrrole protons H_{23}–H_{24}–H_{25}; the positions of these protons on the pyrrole ring are obtained by comparison with assignments of Deber[14] and David[15] for calcimycin, which also bears an acylpyrrole moiety. The signal due to the pyrrole NH is identified by homonuclear decoupling.

The H_{18} protons and one of the methylene-17 protons remain to be identified (the other H_{17} is correlated with H_{16}).

The *J*-modulated spin–echo spectrum[5] gives multiplicity of ^{13}C signals. The assignment of the ^{13}C NMR spectrum (Figure 2) is deduced from the preceding ^1H NMR spectrum by δ–δ ^1H–^{13}C correlation[1,4] (Figure 5). In this way, 26 out of the 31 carbons are easily identified (Table I, assignment methods D, G). At the same time, this procedure verifies previous proton assignments. Five carbons remain to be identified, namely, the four quaternary ones, C_1, C_{21}, C_8, and C_{22}, and the methylene C_{18}. The signals at 179.4 and 191.6 ppm are respectively assigned to C_1 and C_{21} of the acid[11] and ketone[15] functions.

The signals at 140.0 and 132.6 ppm are identified as the sp_2 quaternary carbons C_8 and C_{22} by long-range heteronuclear δ–δ ^1H–^{13}C correlation.[1] Experimental conditions were set up to enhance the signal of carbons weakly coupled to protons (J = 12 Hz). Figure 6 shows C_8–H_7, H_{29} (b) and C_{22}–H_{23}, H_{24}, H_{25} (a) correlations obtained by this method.

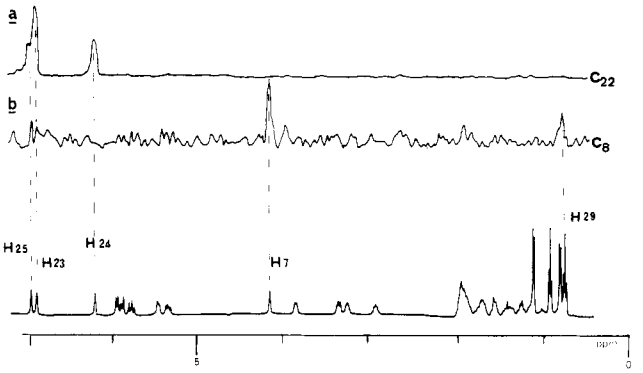

Figure 6. ^1H–^{13}C 2-D long-range chemical-shift correlation lower trace ^1H NMR (400 MHz, $CDCl_3$) spectrum of X.14547 A (1) presented as cross-section plots: (a) carbon C-22; (b) carbon C-8.

Table I. ^1H (400.13 MHz) and ^{13}C (100.627 MHz) NMR Spectral Data[a] ($CDCl_3$) of X.14547 A (1) and Assignment Methods[b]

C no.	^{13}C NMR shift,[a] δ	^{13}C NMR assignment method[b]	^1H NMR[c] shift, δ	^1H NMR[c] assignment method
1	179.4	A		
2	41.3	D, G	2.94	B, C
3	75.1	D, G	3.90	C
4	22.0	D, G	1.90-1.45	C-C
5	26.4	D, G	1.82-1.61	C-C
6	30.4	D, G	1.95	C
7	74.4	D, G	4.20	C
8	140.0	E, G		
9	124.5	D, G	5.93	C
10	127.2	D, G	5.79	C
11	132.4	D, G	5.42	C
12	45.7	D, G	3.32	C
13	129.4	D, G	5.50	C
14	129.5	D, G	5.98	C
15	49.9	D, G	1.60	C
16	43.8	D, G	1.48	C
17	29.7	D, G	1.99-1.30	D, F-C
18	27.2	D	2.00-1.06	D, F-D, F
19	40.8	D, G	1.93	C
20	52.7	D, G	3.41	C
21	191.6	A		
22	132.6	E, G		
23	116.1	D, G	6.92	C
24	110.2	D, G	6.27	C
25	125.4	D, G	7.00	C
26	27.4	D, G	1.74-1.18	C-C
27	12.6	D, G	0.96	B, C
28	21.8	D, G	2.00-1.75	C-C
29	13.4	D, G	0.79	B, C
30	13.5	D, G	0.84	B, C
31	14.3	D, G	1.15	B, C

[a] The ^{13}C NMR data are for the carbons indicated and ^1H NMR data for the corresponding hydrogens on these carbons. [b] Assignment methods: A = ^{13}C NMR spectrum (BB and *J* modulated); B = ^1H NMR spectrum; C = 2-D shift-correlated ^1H NMR COSY spectrum; D = 2-D shift-correlated ^1H and ^{13}C NMR spectrum; E = long-range shift-correlated ^1H and ^{13}C NMR spectrum; F = assignment ^{13}C → assignment ^1H; G = assignment ^1H → assignment ^{13}C. [c] NH, δ 7.65; COOH, δ 2.3-2.8.

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Correlation of one carbon with the H_{17a} gives C_{17}, and thus assignment of the remaining H_{17b} is deduced. This proton identification is a good example of the effectiveness of going back and forth between ^1H and ^{13}C identifications through ^1H–^{13}C correlation. Only one methylene carbon remains unassigned in the ^{13}C NMR spectrum and must therefore be C_{18}. The chemical shifts of H_{18a} and H_{18b} are then deduced, and a confirmation of this result is found in the "COSY" spectrum, which shows that H_{18a} and H_{18b} are coupled together and with H_{17b}.

We have demonstrated that, following the procedure described above, it is possible to completely assign ^1H and ^{13}C NMR spectra of a natural product with only 0.1 mmol of material. This procedure is quite reliable, ^1H assignments were often verified a second time through ^1H–^{13}C correlation and, except for the positioning of H_{23}–H_{24}–H_{25} of the pyrrole, no comparison with parent compounds was made. Total 2-D NMR experimentation time (acquisition, computation, and plot of spectra) was 68 h, 14 h for "COSY" correlation (0.1 mmol), and 27 h for each δ–δ ^1H–^{13}C correlation (0.1 mmol).

Experimental Section

Production of X.14547 A. The antibiotic X.14547 A was produced and isolated from a strain of *Streptomyces antibioticus* NRRL 8167 according to ref 7b and 9.

NMR Spectra. All the spectra were recorded on a Brucker WM 400.

COSY. The two-dimensional correlated ^1H NMR experiment was also performed on the Brucker WM 400. The applied pulse sequence was $(\pi/2)$-(t1)-$(\pi/4)$-(FID, t2). The spectral width in F_1 and F_2 was 4000 Hz; the number of data points in F_2 was 2048, and 512 increments were recorded. Before Fourier transformation, the data were multiplied with unshifted sine bell. Zero filling was applied in each dimension. Total acquisition time was 4 h. The $\pi/2$ pulse was 8 μs.

^1H–^{13}C Shift Correlation. The experiment was also performed on the Brucker WM 400. The applied pulse sequence was $(\pi/2, {}^1H)$-$(t_{1/2})$-$(\pi, {}^{13}C)$-$(t_{1/2})$-(τ_1)-$(\pi/2, {}^1H; \pi/2, {}^{13}C)$-$(\tau_2)$-(BB, ^1H; FID, t_2) with $\tau_1 = 0.0033$ s and $\tau_2 = 0.00167$ s. The spectral width in F_1 was 2700 Hz and in F_2, 14 200 Hz; the number of data points in F_2 was 4096, and 256 increments were recorded. Before Fourier transformation, the data wre multiplied with sine-bell shifted $\pi/10$ in F_2, and Lorentz–Gauss in F_1. Zero filling was applied in each dimension. Total acquisition time was 17 h. The $\pi/2$ pulse was 11 μs for ^{13}C, and the decoupler $\pi/2$ pulse for ^1H was 47 μs.

^1H–^{13}C "Long Range" Shift Correlation. Id to ^1H–^{13}C shift correlation, except: $\tau_1 = 0.0417$ s, $\tau_2 = 0.0417$ s; the data were multiplied with sine-bell squared shifted $\pi/6$ in F_1 and $\pi/4$ in F_2 before Fourier transformation.

Registry No. 1, 66513-28-8.

Mechanistic Aspects of the Annelation Reactions of Benzocyclobutenedione Monoketals with Vinyllithium Reagents

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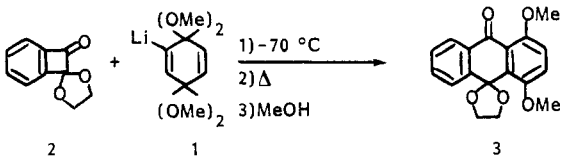
The mechanism of the annelation reaction of vinyllithium reagents with benzocyclobutenedione monoketals has been investigated. The results of these studies strongly support a mechanism involving addition of the organolithium reagent to form the lithium salt of a benzocyclobutenol followed by ring opening and cyclization to produce the tricyclic product. This reaction was examined with benzocyclobutenone and with the ethylene glycol and ethanedithiol monoketals of benzocyclobutenedione as the carbonyl components. The lithio derivatives of the bisketal of 2-bromobenzoquinone, the ethylene glycol ketal of α-bromocyclohexenone, and the ethanedithiol ketal of β-bromocyclohexenone were explored as the organometallic components. These studies have established the major mechanistic aspects and the scope of this annelation reaction.

In connection with past synthetic studies, a mild, regiospecific method for the construction of linear polycyclic systems was required. The reaction of a lithiated quinone bisketal, **1**,[1] with a benzocyclobutanedione monoketal, **2**,[2] to form **3**[3] reported in 1979 met this need; this type of



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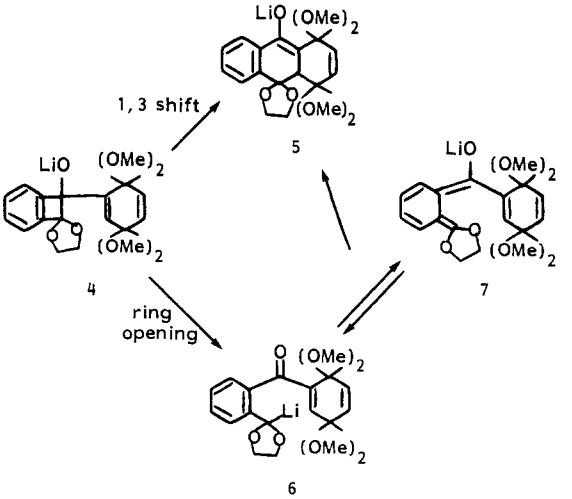
annelation was later employed in the synthesis of 4-demethoxydaunomycinone,[3a] daunomycinone,[3b] and α-citromycinone.[4] Applications to the synthesis of other polycyclic natural products would be facilitated by an understanding of the mechanism of the reaction. The studies reported herein provide this information for the

Scheme I. Mechanistic Possibilities for Annelation



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