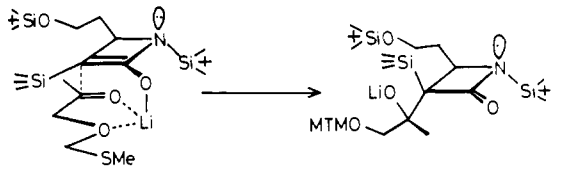
Scheme II



A B

+47.82° (c 1.18, $CHCl_3$). The final phase of the synthesis or the construction of the bicyclic system was performed in a straightforward manner according to the known procedures. The β-keto ester **12** was obtained in 63% yield from **11** (4 steps) and conversion of **12** to the enol phosphate followed by the direct treatment with NaI (11.2 equiv) and powdered silver (*E*)-2-acetamido-1-ethenethiolate (1.1 equiv) in CH_3CN[14] afforded the desired *E* isomer **13** in 82% yield along with *Z* isomer[4a] in 17% yield.[15] Catalytic hydrogenolysis of **13** (H_2, 40 psi, 10% Pd–C, phosphate buffer solution–dioxane, pH 7.5, 49% yield) completed the total synthesis of **3**[16] identical in all respects[16] with natural asparenomycin C including the antibacterial activity. It should be mentioned here that the optically active half-ester in (*S*) form prepared by an enzyme-mediated hydrolysis of the prochiral dimethyl β-aminoglutarate can be now converted to any type of naturally occurring carbapenem antibiotics[3,5] in principle.

Acknowledgment. This work was financially supported in part by a Grant-in-Aid Special Project Research 57118002 of the Ministry of Education, Science, and Culture of Japan. We express our cordial thanks to Drs. W. Nagata and N. Tsuji of Shionogi Research Laboratories, Shionogi & Co., Ltd., for kind donation of natural asparenomycins.

Supplementary Material Available: Listings of physical properties of new compounds (9 pages). Ordering information is given on any current masthead page.

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(15) The ratio of silver thiolate and **12** was critical in this reaction. The use of excess silver thiolate decreased the ratio of **13** and *Z* isomer, and *Z* isomer was easily lactonized after removal of the protected group.
(16) All materials described here gave satisfactory MS, IR, NMR spectra consistent with their structure (supplementary material).

Nucleophilic Trapping of 7,11-Dideoxyanthracyclinone Quinone Methides

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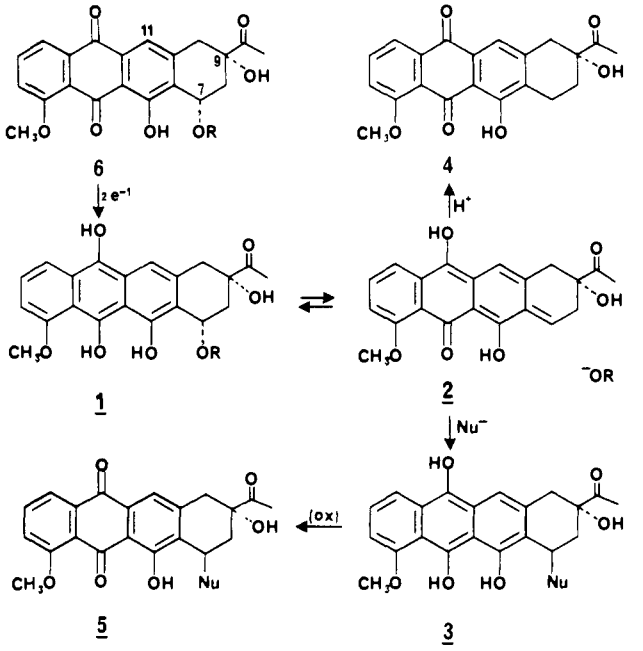
The anthracycline glycosides comprise an important class of antitumor antibiotics. A chemical basis for the expression of some of their biological activities has been sought in the ability of their anthraquinone moiety to undergo enzyme-catalyzed reduction to semiquinone and hydroquinone states. In the presence of O_2, both reduced states are oxidized;[1] in the absence of O_2, the hydroquinone eliminates the C-7 glycoside to provide a quinone methide.[2-4] This quinone methide has been suggested as a plausible intermediate in the covalent labeling of cellular macromolecules.[2,4] Until recently, the only known reaction of the quinone methide was irreversible solvent protonation at C-7.[5,6]

Scheme I



Kleyer and Koch[7] have now observed that the 7-deoxydaunomycinone quinone methide is efficiently trapped by a second electrophile, benzaldehyde. We report here that the quinone methides of 11-deoxyanthracyclinones possess reactivity as electrophiles, reacting with thiol and thiolate nucleophiles by addition at C-7.

Since the circumstances required for the isolation of the resultant adducts are unusual, a brief discussion of the quinone methide is necessary. All evidence indicates that the equilibrium between the hydroquinone **1** and the quinone methide **2** and free glycoside strongly favors quinone methide formation. Thus, nucleophile addition will provide an *unstable* adduct, **3**, as quinone methide formation remains favored. In order to prevent the eventual (and irrevocable) loss of the quinone methide to solvent protonation (to give **4**), it is necessary to provide to the nucleophile an oxidant, to trap **3** and convert it to the stable quinone adduct **5** (Scheme I). In searching for the requisite conditions, we have observed that the anthracycline glycoside itself is a most suitable oxidant and that the nucleophile adducts may be isolated under the following circumstances. After initial quinone methide formation and nucleophile addition, the hydroquinone adduct is trapped by disproportionation. The anthracycline glycoside hydroquinone from the disproportionation eliminates to a second quinone methide; this is also sequentially trapped by nucleophile addition and disproportionation. Hence, in the presence of a suitable nucleophile, quinone methide formation is autocatalytic. If the rate of nucleophile addition and of the disproportionation exceeds that of solvent protonation at C-7, an excellent yield for conversion of the anthracycline to the adduct may be expected. This has proven to be the case for the 11-deoxyanthracycline glycosides 11-deoxydaunomycin (**6**, R = daunosamine), aclacinomycin A, and marcellomycin, with thiol and thiolate nucleophiles.

The method that is used to initiate (and sustain) quinone methide formation is enzyme-catalyzed reduction. A typical reaction is described: To a 10.0-mL anaerobic solution of potassium ethyl xanthate (10 mM), 11-deoxydaunomycin (0.91 mM), and NADH (0.18 mM) in 35 mM potassium phosphate pH 7.0 buffer is added *V. harveyi* oxidoreductase (0.26 μmol min^{-1} NADH oxidized by riboflavin).[6] After 6 h at ambient temper-

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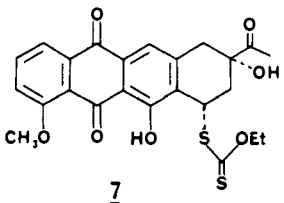
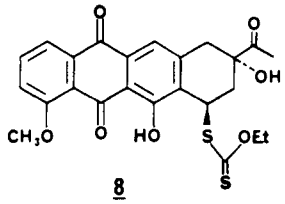
ature, the reaction is worked up, yielding two products in a combined yield of 87%. The spectroscopic properties identify these as C-7 ethyl xanthyl diastereomers, present in an 85/15 ratio. The major isomer **7** is assigned a 7*S* configuration on the basis of a



7



8

pseudoaxial C-9 hydroxyl (determined by $J_{8e,10e}$ = 2.1 Hz)[8] 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.[9] 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$^+$ oxidoreductase[10] 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-deoxyanthracyclinones, 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.[6] As an excess of reductant was used, the adduct (although certainly formed) was converted to 7-deoxyaklavinone and 7,7′-bis(7-deoxyaklavinone).[11] Last, under these conditions nucleophile adducts are not obtained from an 11-hydroxylanthracycline, 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-deoxyanthracyclinones) to new derivatives. Second, it indicates that these quinone methides have electrophilic character and provides precedent for such behavior in vivo.

Acknowledgment. We thank Dr. Federico Arcamone, Farmitalia Carlo Erba, Dr. Akihiro Yoshimoto, Sanraku-Ocean, and Dr. William Bradner, Bristol-Myers, for their gifts of the anthracyclines. We are indebted to Dr. James Becvar, University of Texas, El Paso, for the *V. harveyi* oxidoreductase and to M. Prairie and W. C. Krueger of the Upjohn Co. for communicating 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 ^{1}H NMR and CIMS spectra (12 pages). Ordering information is given on any current masthead page.

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

Sensitivity-Enhanced Correlation of ^{15}N and ^{1}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 ^{15}N-^{1}H chemical-shift correlation in natural-abundance samples. The poor sensitivity of ^{15}N NMR detection is caused by its low natural abundance (0.37%) and its low magnetogyric ratio. Additional problems often arise because of long ^{15}N relaxation times and a negative magnetogyric ratio. Investigations of ^{15}N chemical shifts and ^{15}N-^{1}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,[1] initially transfers proton magnetization to the (natural abundance) ^{15}N nuclei. Then the time evolution of the ^{15}N signals, and thus the ^{15}N chemical shifts, can be monitored indirectly in a two-dimensional experiment.[2,3] This latter technique has the advantage of detecting the ^{15}N chemical shifts and the ^{1}H-^{15}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 ^{15}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,[4] which offers similar advantages as the experiment proposed by Bodenhausen and Ruben[1] 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.[5] and have been applied to ^{13}C studies of small organic compounds.

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

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‡ Regional NMR Facility.

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