bration under basic conditions. Conversion into the thiopyranone **122** followed by treatment with sodium iodide generated the sulfonium ylide **123**, which rearranged to form the sulfur-bridged cytochalasan **124** (65%) together with minor isomeric products.[38] Further modification gave rise to zygosporin E (**125**)[39] (Scheme XIV).

Another approach to the 11-membered-ring system of the cytochalasans based on fragmentation of keto toluene-*p*-sulfonates has been described.[40]

Conclusion

Intramolecular Diels–Alder reactions of 3-acylpyrrol-2(5*H*)-ones would appear to provide useful access to macrocyclic compounds including [11]cytochalasans, although improved methods are required for stereoselective 13-membered-ring formation. Conformational factors affecting the endo–exo selectivities of these reactions have not been elucidated, although it is likely that they are under kinetic control. Further work on other approaches to cytochalasan synthesis is expected, thus making these complex, biologically active molecules, and their analogues, more readily available for chemical and biological study. Further insights into the processes involved in their biosynthesis are of interest. Is an enzymically mediated Diels–Alder reaction involved?[41,42]

I thank all my co-workers who have been involved in cytochalasan synthesis including S. J. Bailey, A. P. Craven, H. Dyke, S. A. Harkin, E. Merifield, R. Sauter, O. Singh, P. G. Steel, D. J. Tapolczay, S. M. Vather, J. P. Watts, and J. W. F. Whitehead. I also thank the SERC, ICI Pharmaceuticals, and the Wellcome Research Laboratories for support of parts of this program.

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Calicheamicins: Discovery, Structure, Chemistry, and Interaction with DNA

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Introduction

The enediyne class of antitumor antibiotics represented in Chart I by calicheamicin γ_1^I (**1**),[1] esperamicin A_1 (**2**),[2] dynemicin A (**3**),[3] and neocarzinostatin (**4**)[4] are some of the most potent antitumor agents ever discovered. Calicheamicin γ_1^I is over 1000 times more potent than adriamycin, a clinically useful antitumor antibiotic, when tested in murine tumor models. The remarkable biological properties of this class of compounds appear to be a consequence of their ability to interact with cellular DNA and initiate double-stranded cleavage by carbon-centered diradical hydrogen abstraction processes. The elucidation of the chemical structures of **1** and **2** has stimulated intense synthetic efforts in a number of academic laboratories.

Similarities between the calicheamicins and the esperamicins were recognized quite early in our studies of the calicheamicins due to the extreme potency of both families of compounds, the presence of a thio sugar in both, and characteristic proton signals of their aglycons. However, the close structural relationships between these two families of compounds were not recognized until the structures of calicheamicin γ_1^I and the esperimicins were published simultaneously.[1,2] Dynemicin A shares with the calicheamicins in having a 10-membered enediyne ring system, although the remainder of its structure is very different. The chemical structure of the neocarzinostatin chromophore is only remotely related to that of the calicheamicins. The conjugated diacetylenic ring systems of these potent antitumor antibiotics can undergo cycloaromatization

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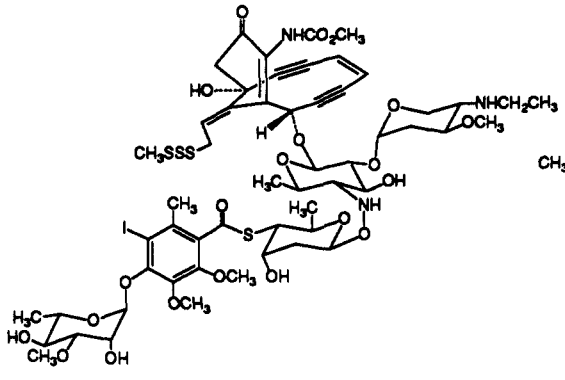
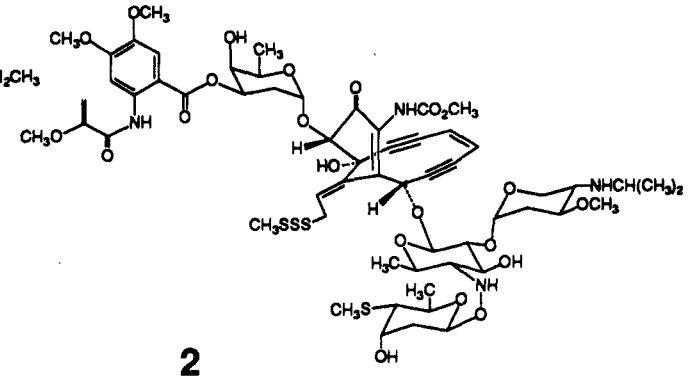
Chart I



1



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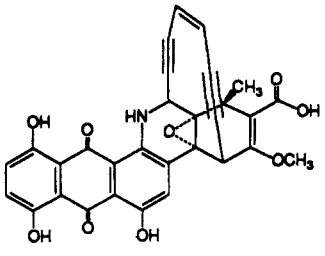

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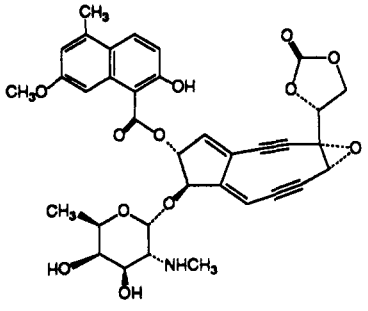

4

via carbon-centered diradicals and cause DNA cleavage.[5,6] The discovery and the structural elucidation of the calicheamicins and the DNA cleavage chemistry of calicheamicin γ_1^I will be presented in the following account.

Discovery of the Calicheamicins

The calicheamicins are produced by the fermentation of *Micromonospora echinospora* ssp *calichensis*, a bacterium isolated from a chalky, or caliche, soil sample collected in Texas. They were discovered in the mid-1980s in a fermentation products screening program through the use of the biochemical induction assay (BIA), which utilized a genetically engineered strain of *Escherichia coli* to detect DNA damaging agents.[7] Crude preparations of the BIA positive fermentation broths were evaluated in murine tumor models P388 and B16. Only those demonstrating good efficacy were pursued further. A crude preparation of calicheamicin, estimated to be <1% pure, showed activity and potency comparable to pure adriamycin. A series of 7–10 BIA positive components were detected in the crude antibiotic complex, and they were named according to their relative chromatographic mobilities and structural features. Calicheamicins β_1^{Br} (5) and γ_1^{Br} (6) were the first members of the family to be isolated.[8] The fermentation broths contained ~0.1 mg/L calicheamicin β_1^{Br}, the major component of the complex. The first 18 mg of calicheamicin β_1^{Br} was isolated from the processing of 1500 L of the fermentation broth. In order to obtain enough calicheamicin β_1^{Br} for structural elucidation and thorough biologically evaluation, a strain and fermentation improvement program was undertaken.[9]

Strains of *M. echinospora* ssp *calichensis* producing larger amounts of calicheamicins were obtained by screening survivors after exposure to ultraviolet light, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and increasing concentrations of calicheamicin β_1^{Br}. In an attempt to further increase the fermentation yields of these new strains, various bromides were added to the fermentation medium, unfortunately with no success. However, when the fermentation medium was supplemented with sodium iodide, a dramatic increase in the fermentation yield was observed based on the BIA. Scale-up of the fermentation resulted in the isolation of the iodine-containing calicheamicins, with calicheamicin γ_1^I produced as the major component of this new complex. The iodinated calicheamicins cochromatographed with their brominated counterparts in TLC and were named accordingly. During the course of our study, approximately 100000 L of fermentation broth containing 2–10 mg/L calicheamicin γ_1^I was processed in 1500- or 4500-L batches, with the initial pilot-plant-scale extraction and concentration steps being the most difficult

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Scheme I

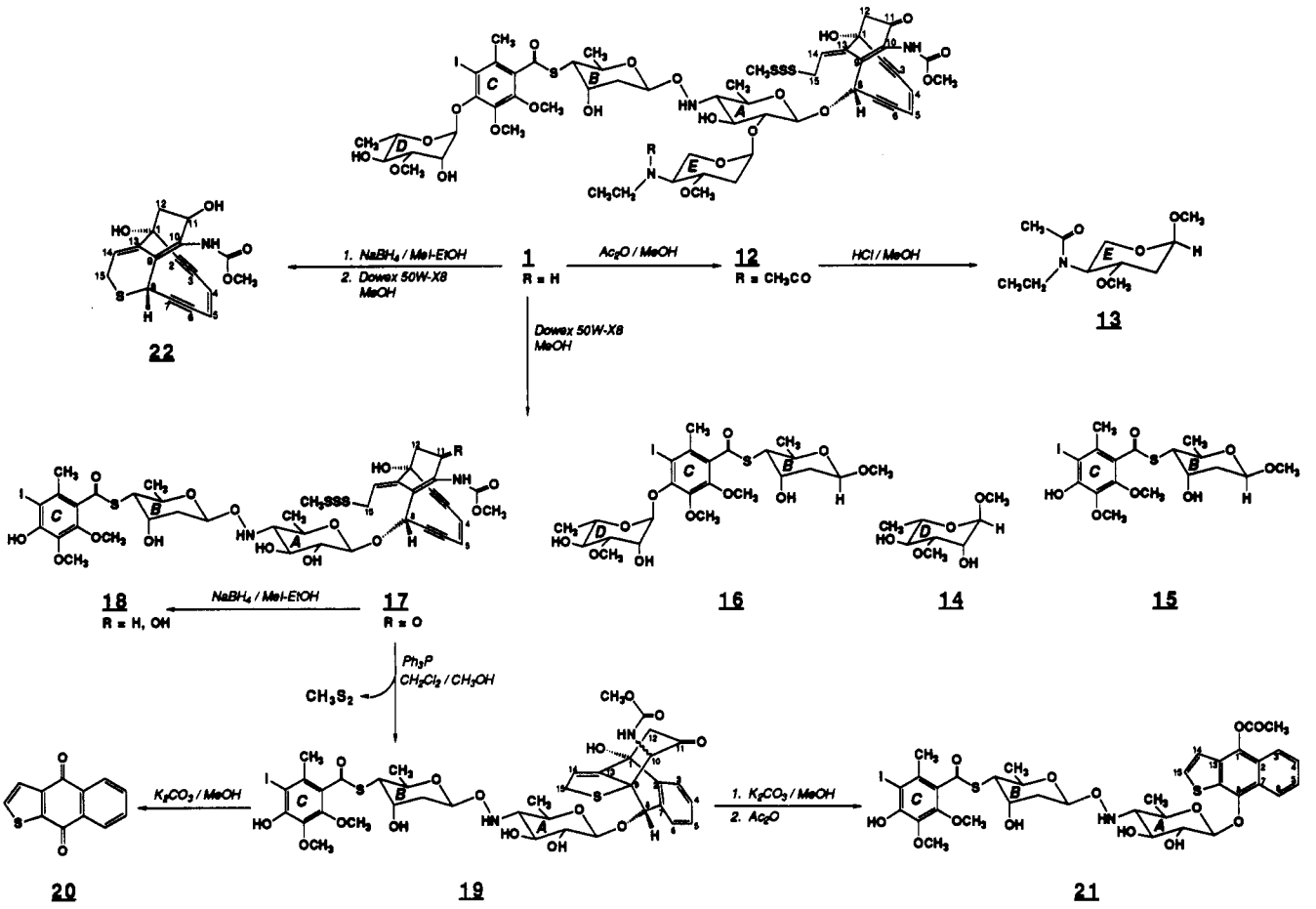
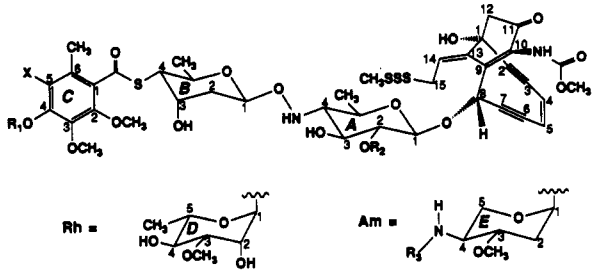


Table I.
Chemical Structures of Calicheamicins β_1^{Br}, γ_1^{Br}, α_2^I, α_3^I, β_1^I, γ_1^I, and δ_1^I



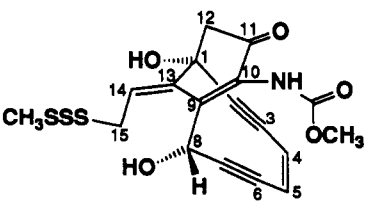
calicheamicin	X	R_1	R_2	R_3
β_1^{Br} (5)	Br	Rh	Am	$(CH_3)_2CH$
γ_1^{Br} (6)	Br	Rh	Am	CH_3CH_2
α_2^I (7)	I	H	Am	CH_3CH_2
α_3^I (8)	I	Rh	H	
β_1^I (9)	I	Rh	Am	$(CH_3)_2CH$
γ_1^I (1)	I	Rh	Am	CH_3CH_2
δ_1^I (10)	I	Rh	Am	CH_3

and the least reproducible. Once the crude calicheamicin complex was obtained as a precipitated solid, it could be stored at −20 °C with no appreciable decomposition. The crude calicheamicin complex was further purified by repetative chromatography on silica gel and C_{18}-bonded silica.[8]

The chemical structures of the fully characterized calicheamicins are shown in Table I. One series is brominated on the aromatic ring, while the other contains iodine. The β_1, γ_1, and δ components differ by having an isopropyl, ethyl, or methyl substitution on the aminopentose (ring E). The 3-methoxyrhamnose unit (ring D) is missing in the α_2 component, while the aminopentose unit is missing in α_3.

Structure and Chemistry of the Calicheamicins[1]

Spectroscopic data (FABMS, ESCA, ^1H NMR, and ^{13}C NMR) and elemental analysis on calicheamicin γ_1^I suggested a molecular formula of $C_{54-55}H_{73-77}N_3S_4I$, as well as the presence of four glycosides and basic nitrogens. Two approaches toward degradation and derivatization studies were pursued: first, selective *N*-acetylation in order to provide a more suitable derivative for molecular formula determination by high-resolution FABMS; second, carefully controlled methanolysis in order to isolate and identify the methyl glycosides of the individual glycosidic units, as well as to isolate partially degraded larger fragments containing the aglycon of calicheamicin γ_1^I—calicheamicinone (11).



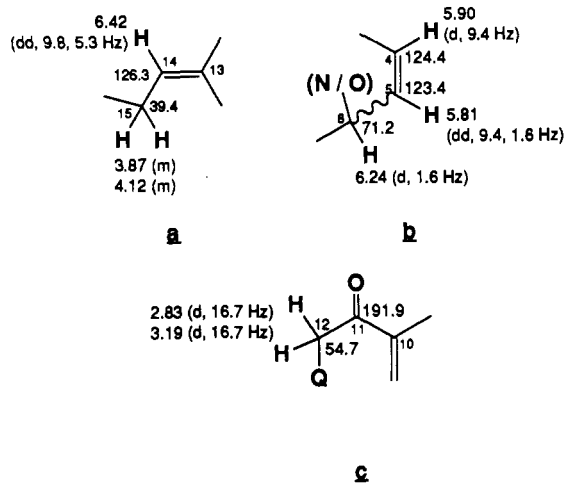
11 calicheamicinone

We anticipated that the NMR spectra of these fragments would be relatively easy to interpret and assign with the use of 2D NMR techniques. These assign-

ments could then be correlated to the NMR data of calicheamicin γ_1^I itself and allow us to elucidate its chemical structure by 2D NMR techniques.

N-Acetylcalicheamicin γ_1^I (**12**, Scheme I) gave a strong M + H ion at m/z 1410 and permitted the determination of its molecular formula ($C_{57}H_{76}IN_3O_{22}S_4$) by high-resolution FABMS. Methanolysis of **12** using methanolic HCl and of **1** using Dowex 50W-X8 (H^+) in methanol resulted in the isolation of key degradation products, **13**–**17** (Scheme I). The exact configuration of **13** was confirmed by total synthesis,[10] while that of **14** was determined by its specific rotation. The attachment of the ethylamino sugar (E ring) to the A ring was first suggested by the mass spectral fragmentation pattern. The exact location of the glycosidic linkage was determined by ^1H NMR analysis of peracetylated derivatives of **12**, **17**, **15**, and **16**.

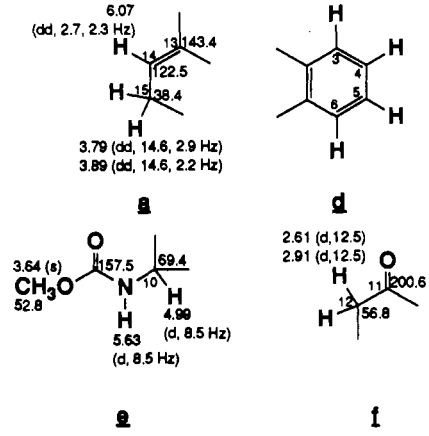
The chemical structure of **11**, calculated to have a molecular formula of $C_{18}H_{17}NO_5S_3$ containing 11 double bond equivalents, could not be assigned based on NMR data alone. The only definitive structural information gleaned from extensive NMR studies carried out on calicheamicin pseudoaglycon (**17**) was the presence of an isolated methylene (C-12), an α,β-unsaturated ketone (C-11), and partial structures a and b. **17** was converted to dihydrocalicheamicin pseudoaglycon (**18**), and the coupling pattern of the new proton at C-11 and the methylene protons on C-12 allowed us to combine the isolated methylene and the enone to partial structure c. Partial structures a–c and two methyl groups attached to heteroatoms accounted for all nonexchangeable protons in **11**.



a b c

In an attempt to elucidate the nature of the three sulfur atoms in calicheamicinone, **17** was treated with triphenylphosphine, and compound **19** containing four contiguous aromatic protons was isolated. One puzzling piece of data concerning the transformation from **17** to **19** stood out immediately: ^1H and ^{13}C NMR data showed that a methyl group (δ_H 2.52, s) was absent in **19**, while the molecular formula of **19** differed from that of **17** by the elements CS_2. In fact, prominent ions due to the loss of CS_2 from M + Na were observed during the FABMS analysis of **17** and **12**. During the conversion to **19** via the reaction with triphenylphosphine, **17** must have lost CH_3S_2 and acquired three new protons along the way. The obvious source of these protons was the solvent used for the reaction, CH_2Cl_2/CH_3OH, specifically, the exchangeable proton of methanol. In order to locate these newly acquired protons in **19**, the reaction of **17** with triphenylphosphine was carried out in CH_2Cl_2/CD_3OD (2:1). Surprisingly, no deuterium incorporation in **19** was observed. The reaction was then carried out in CD_2Cl_2/CD_3OD (2:1), and two deuterium atoms were incorporated into the para positions (C-3, C-6) of the new aromatic ring in **19**, suggesting a free-radical mechanism for the formation of this aromatic ring. The deuterium labeling pattern and the source of the deuterium atoms suggested the existence of a benzene-1,4-diyl as an intermediate for the conversion of **17** to **19**. The significance of this observation became apparent when the chemical structure of **11** was determined.

Extensive NMR studies on **19**, including long-range ^1H–^{13}C correlation experiments, established the presence of partial structures a, d, e, and f in **19**. The NMR data, however, did not permit us to arrive at a satisfactory structure for the aglycon of **19**. As a result, degradation studies on **19** were undertaken. Treatment of **19** with methanolic K_2CO_3 (30 min) afforded a number of new compounds; however, only **20** could be isolated. Sequential treatment of **19** with methanolic K_2CO_3 (5 min) and excess acetic anhydride trapped the intermediate of the above conversion as crystalline **21**. The chemical structure of **21** including its absolute configuration was determined by X-ray crystallography. This established that (1) both the hydroxyamino sugar (ring A) and the thio sugar (ring B) were in the D configuration, (2) there is an unusual N–O glycosidic linkage between the two sugars, (3) there is a basic carbon skeleton of the aglycon of **19**, and (4) the hydroxyamino sugar was linked by a glycosidic bond to the aglycon of **19** at C-8. During the mild base treatment of **19**, a retro-aldol reaction and subsequent aromatization to the naphthothiophene ring system in **21** must have occurred, which resulted in the elimination of structural units e and f from **19**. Consequently, the chemical structure of **19** was assigned.



a d e f

Methanolysis of dihydrocalicheamicin γ_1^I using Dowex 50W-X8 (H^+) as the acid catalyst afforded **22** instead of the expected **18**. The chemical structure of **22** was determined by comparing its NMR data with that of the aglycon portion of **18** and by consideration of the chemical structure of **19**. The unexpected carbon chemical shift, 30.7 ppm, of C-8 of **22** prompted us to propose a displacement reaction at C-8, instead of the normal methanolysis reaction, during the formation of

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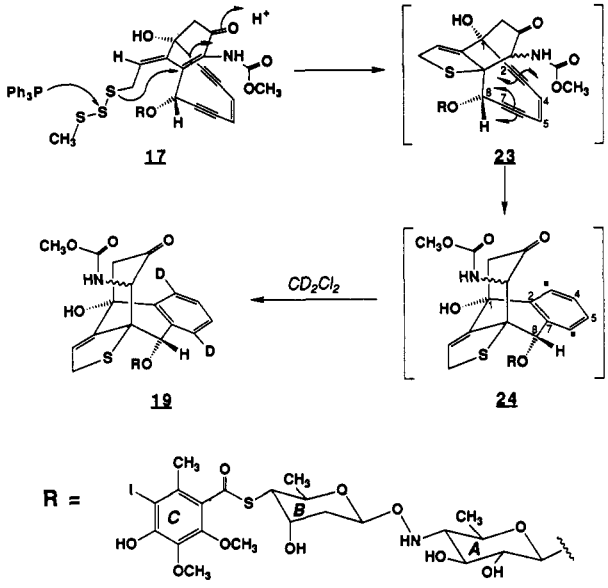
Scheme II



22 from dihydrocalicheamicin γ_1^I. The symmetrical conjugated enediyne system was supported by the presence of four quaternary carbon signals (86.1, 87.6, 99.7, and 102.0 ppm) in its ^{13}C NMR spectrum, a weak IR absorption at 2190 cm^{-1}, and the observation that the olefinic protons in b appeared as a two-proton singlet in 17 and 8. The structure of 22 required that the S–C-9 bond in 19 be absent in calicheamicinone and be formed during the transformation from 17 to 19. As a result, the chemical structure of the calicheamicin pseudoaglycon was assigned as 17. The stereochemistry at C-8 was revised since our original publication of the structure of 1, by biosynthetic analogy to the structure of esperamicin X, which was determined by X-ray crystallography.[2b]

As described earlier, the aromatization of 17 to 19 occurred via a benzene-1,4-diyl intermediate. In order to account for this experimental observation, the following sequence of events was proposed (Scheme II): (1) triphenylphosphine attack at the allylic trisulfide, (2) Michael reaction of the resulting thiolate or thiol with the α,β-unsaturated ketone, (3) tautomerization of the resulting enol to the corresponding keto (23), (4) cyclization of 23 to generate the 1,4-diyl 24, and (5) deuterium atom abstraction from solvent CD_2Cl_2 to give the isolated reaction product 19.[1b] The cyclization of the enediyne system via a 1,4-diyl is supported by Bergman's work on 1,4-dehydrobenzene.[11] A number of recent publications have cleverly confirmed the validity of this sequence of events (Scheme II). Indeed, it has recently been shown that the tendency for ring closure is governed by the difference in strain between the enediyne and biradicaloid intermediate.[12] The

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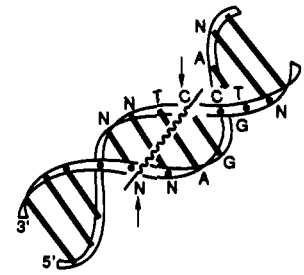

Figure 1. Depiction of minor groove DNA cleavage by calicheamicin. Arrows denote the positions of hydrogen abstraction by the diradical intermediate.

calicheamicins, the calicheamicin pseudoaglycon (17), the dihydro calicheamicin pseudoaglycon (18), and dihydrocalicheamicin γ_1^I are potent DNA damaging agents as demonstrated by their activity in the BIA. Compound 19 and calicheamicin ϵ (the 19 equivalent derived from calicheamicin γ_1^I), however, are completely inactive in the BIA. These observations led us to propose that the aromatization process shown in Scheme II is responsible for the potent DNA damaging effects of the calicheamicins and that the 1,4-diyl 24 is the active species in the DNA cleavage process.[1b]

Interaction of Calicheamicin γ_1^I with DNA

Although the exact biochemical basis responsible for the cytotoxicity of calicheamicin remains speculative, it seems that DNA strand scission is at least partially involved because of the potent in vivo cleavage properties of this drug.[13] Recent studies on the mechanism of calicheamicin cytotoxicity in HL-60 leukemia cells suggested that cell death could be due to the dramatic lowering of NAD^+ levels as a result of irreversible activation of poly(ADP-ribose) polymerase involved in DNA repair.[13a] Cellular DNA damage by calicheamicin has also been documented in an experiment with human lung diploid fibroblast cells where extensive chromosome abberations were observed at drug concentrations as low as a few picograms/milliliter.[14] Furthermore, studies with HeLa cells showed that, at approximately 50 pg/mL drug concentration, 50% of the DNA synthesis was inhibited as determined by the incorporation of tritiated thymidine.[15] But it is certainly possible that other cellular components are also damaged.

Cleavage of Plasmid DNA. Cleavage studies with supercoiled plasmid DNA (bacteriophage *φX174*) showed that, in the presence of reducing thiols, calicheamicin caused single- and double-stranded scission at concentrations as low as 7 nM as demonstrated by the appearance of essentially equal amounts of open-circular and linear DNA forms. The formation of linear DNA at these low drug concentrations strongly suggests a concerted cleavage process consistent with the biradical nature of the cleaving species.[14] No cutting of single-strand *φX174* (+strand) or single-strand ^{32}P end-labeled DNA from restriction fragments has been observed at these low drug concentrations, indicating

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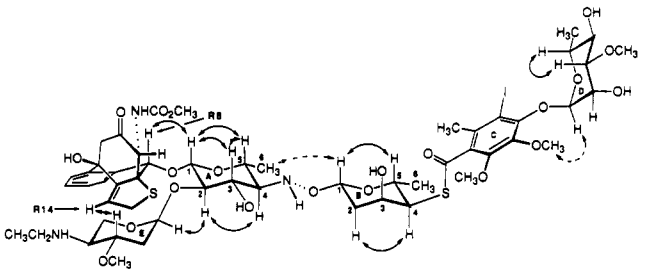


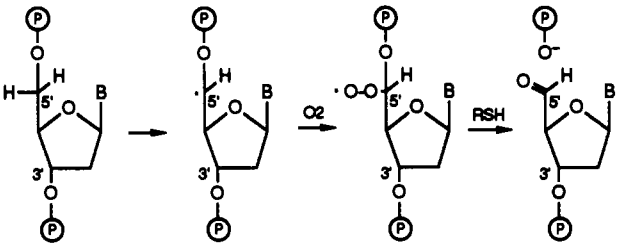
Figure 2. Selected through-space connectivities of calicheamicin ϵ from ROESY experiments as indicated by bold (strong NOEs) or dashed (weak NOEs) arrows.[18]

the preference of calicheamicin for binding and cleaving double-stranded DNA.

Sequence Specificity. Analysis of calicheamicin-produced DNA oligomers, derived from 5′-^{32}P end-labeled restriction fragments, on high-resolution denaturing poly(acrylamide) gels showed a remarkable degree of sequence discrimination, especially for a 1367-dalton molecule. By using Maxam–Gilbert chemically produced markers as controls, tetramer tracts such as TCCT and CTCT were shown to be principal cleavage sites, although other regions were also cleaved but to a lesser extent. These experiments showed that the 5′-cytidine in the TCCT or 5′-thymidine in the CTCT tract was attacked to give fragments ending in 3′-phosphates since the electrophoretic mobilities of the calicheamicin-produced oligonucleotides matched exactly those of the controls. Cleavage on the complementary strand, always less than observed on the TCCT strand, was offset by two nucleotides toward the 3′ end from the AGGA tetramer as shown in Figure 1. This 3′ offset of the duplex strand scission indicates that the minor groove of the DNA is the binding region for calicheamicin, similar to other known microbial minor groove binders such as netropsin, distamycin, and CC-1065. Indeed, competition experiments with netropsin altered the calicheamicin cleavage pattern, suggesting possible overlap in binding regions of the two drugs.

The fact that calicheamicin γ_1^I seems to recognize several oligopyrimidine/oligopurine tracts, depending on the flanking sequences,[14] suggests that it senses a three-dimensional minor groove structure rather than specific base pair contacts. The cleavage specificity must arise from a reasonably strong association between the complementary binding surfaces of the drug and those of the preferred sequences of the DNA minor groove. The importance of the thiobenzoate tail in binding and specificity has recently been shown by the sequence-independent cleavage properties of calicheamicinone at concentrations 1000 times that required for calicheamicin γ_1^I.[16] The specificity also suggests that there is probably some rigidity in the molecule, a characteristic that is known to enhance binding affinity.[17] The conformation of the aglycon-hydroxylamino sugar linkage appears to be an especially important factor for aligning the diradical with the 3′-offset DNA target sites so that optimal directionality

Scheme III
Probable Mechanism of DNA Cleavage at the 5′-Carbon of the Preferred Deoxyribose



for the hydrogen abstraction step is obtained.

A solution conformation study by Walker and Kahne using ^{1}H NOE NMR led them to suggest that calicheamicin does exist in an extended and substantially preorganized conformation.[18] These NOE experiments were carried out on calicheamicin ϵ, Figure 2, and assumed that the solution conformation of this inactive but more stable derivative was similar to that of the parent drug. This is reasonable since calicheamicin ϵ contains the identical oligosaccharide tail portion as γ_1^I and exhibits a DNA binding affinity similar to that of γ_1^I.[19] Only the aglycon portion is altered between ϵ and the parent drug. However, it is well-known that NOE NMR measurements provide only an average conformation on the NMR time scale. But because torsional oscillations occur over a narrow range in the majority of glycosidic linkages, the NOE method is considered useful for developing a preferred conformation in oligosaccharides.[20] Furthermore, a recent conformational analysis of the N–O bond by the same group indicates that this linkage plays a key role in enforcing a curvature to the molecule.[21] In this regard, a crystal structure of a calicheamicin degradation product[1b] shows that the C–N–O–C grouping adopts an eclipsed conformation consistent with the solution conformational analysis of the N–O bond.

Other factors that are likely to be important in the calicheamicin/DNA association include hydrophobic interactions[22] (calicheamicin is extremely water insoluble), electrostatic interactions involving the basic ethylamino sugar and hydroxylamino moieties, and hydrogen bond associations originating from the deoxysugar hydroxyl groups. In any event, more definitive insight as to the nature of the binding parameters will have to await an X-ray analysis of the DNA/calicheamicin complex.

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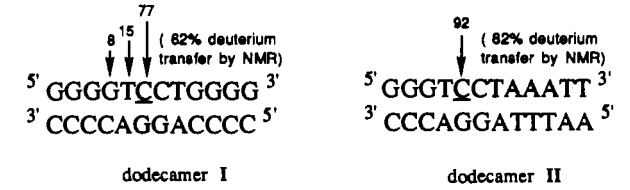

Figure 3. Comparison of sequencing gel analyses with ^1H NMR results from cleavage of dodecamers I and II.

Cleavage Site Chemistry. The identity of the hydrogen atom abstracted from the deoxyribose was inferred to be one of the geminal hydrogens at the 5′ carbon (probably the pro-*S* based on models). Cleavage experiments using a 3′ ^{32}P end-labeled fragment from pBr322 containing a number of TCCT sites resulted in oligonucleotide fragments that migrated as if they were two nucleotides longer than the chemically produced markers. This provides strong evidence that strand scission is initiated by hydrogen abstraction of one of the 5′ hydrogens on the target deoxyribose to give 3′-labeled fragments terminating in 5′-aldehydes (Scheme III). On treatment with base, β-elimination occurs, whereby this terminal subunit is removed, yielding an oligomer shortened by one nucleotide.[14] This was identical with the observations of Goldberg and colleagues on similar experiments with neocarzinostatin.[23]

Conclusive evidence that calicheamicin does indeed abstract nonexchangeable hydrogen atoms from the DNA backbone came from experiments in which sonicated and deuterium-exchanged calf-thymus DNA was cleaved with calicheamicin in the presence of deuterated methyl thioglycolate, perdeuterated ethanol (for solubilization of the drug), and deuterium-exchanged Tris buffer.[24] High-resolution NMR analysis of the isolated aromatic end product (calicheamicin ε) showed no incorporation of deuterium into the aromatic ring, suggesting that the hydrogen atoms at C-3 and C-6 originated solely from the DNA.

The above results suggested that short synthetic DNA oligomers,[25] specifically labeled at C-5′ of the 5′-cytidine in the TCCT site, could be used to carry out atom-transfer experiments. Integration of the aromatic NMR signals of calicheamicin ε would then provide direct chemical evidence for the extent of atom transfer from DNA. The site (C-3 or C-6) of deuterium incorporation in the aromatized end product would also provide evidence for the binding orientation of the drug/DNA complex given the absolute configuration of calicheamicin. This would then resolve the difference in orientations proposed by us (tail portion toward the 5′ side of the TCCT tract)[24] based on the dodecamer cleavage experiments and that proposed by the Schreiber group (tail portion of the drug toward the 3′ side)[26] based on molecular modeling studies.

In collaboration with the groups of Schreiber and Townsend at Harvard and Johns Hopkins, specifically labeled 5′ dideuterated cytidine was synthesized and incorporated into the dodecamers shown in Figure 3.[27] The cleavage of these dodecamers at a drug/DNA molar ratio of 0.5 resulted in a remarkably specific transfer of deuterium from the labeled cytidine to carbon-6 of the aromatic end product as determined by high-resolution NMR. Only carbon-6 incorporation was detected, indicating that the drug does indeed bind in the minor groove with the aglycone portion oriented toward the 5′ side of the TCCT and the noncovalent binding oligosaccharide tail portion of the molecule pointed toward the 3′ end as suggested by Schreiber's modeling studies. However, as shown in Figure 3, the ^1H NMR spectra also indicated that the deuterium incorporations from the isotope-transfer experiments were not complete, 62 ± 5% from dodecamer I and 82 ± 5% from dodecamer II, taking into account of the actual heavy isotope contents (95 ± 1%) of the labeled substrates.

Control experiments with deuterium-labeled dodecamers in a deuterated medium resulted in no additional capture of deuterium from the reaction components. Clearly, hydrogen incorporation must have originated either by abstraction from a nearby unlabeled deoxyribose or from another position on the labeled deoxycytidine itself. Analysis of the reaction mixture from 5′ ^{32}P end-labeled dodecamers on sequencing gels did indicate other cleavage sites with dodecamer I as indicated by the arrows in Figure 3. The same analysis on dodecamer II showed a much tighter cutting pattern. Surprisingly, no isotope-induced branching was observed in these reactions as shown by the gel experiments using labeled and unlabeled dodecamer.

In an attempt to provide insight concerning the rate-determining step in the DNA recognition/cleavage event, the cycloaromatization of calicheamicin by variable-temperature NMR was studied by Townsend's group.[28] By monitoring the reaction of calicheamicin with n-Bu_3P in methanol-d_4, they identified the dihydrothiophene intermediate **23** as having a significantly long solution half-life of 4.5 ± 1.5 s at 37 °C. Even though the hybridization at C-9 is now sp^3, the enediyne does not instantaneously aromatize at 37 °C. Based on the first-order rate constant of $(5 \pm 2) \times 10^{-4}$ s^{-1} determined over three half-lives for the conversion of **23** to **19**, a $\Delta G^{\ddagger}$ of 19.3 ± 0.2 kcal/mol was estimated, which was similar to the analogous rearrangement step of neocarzinostatin.[6a] It has been observed in a recent study assessing the kinetics of trisulfide cleavage in calicheamicin that the bimolecular thiol chemistry leading to intermediate **23** is at least 50 times slower than the rate of the Bergman cyclization. Thus, the formation of intermediate **23**, with its relatively long half-life, may be the kinetically significant event in the DNA recognition/cleavage reaction at the preferred sites.[29] Whether the cleavage properties of calicheamicin are due solely to the thermodynamic binding properties of **23** to the preferred sequences and/or whether the DNA serves as a template and actually accelerates the cyclization of **23** to **19** remains to be

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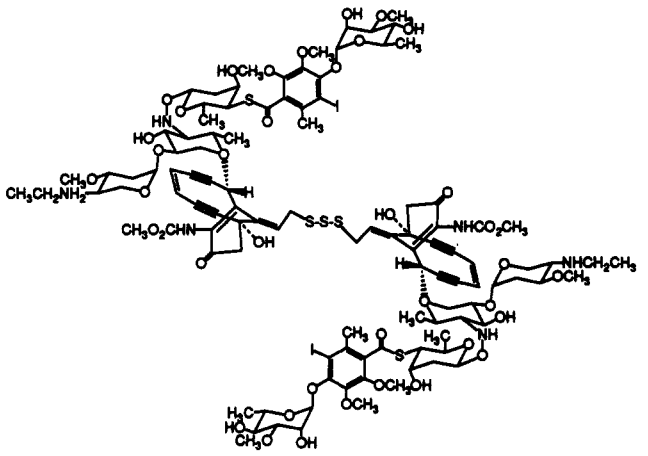
determined. Although NMR signals attributable to **23** were observed in the reaction of calicheamicin with reducing thiols, the very complex nature of the sulfur chemistry made it difficult to obtain a clear picture of the reaction pathway.

Sulfur-Exchange Chemistry

During the structural studies on calicheamicin γ_1^I, it was observed that reaction of the drug with Ph_3P in methylene chloride gave the symmetrical trisulfide dimer **25** as an insoluble precipitate.[30] Careful HPLC



25 Calicheamicin γ_1^I dimer

monitoring of the reaction of calicheamicin γ_1^I with nonreducing thiols in acetonitrile showed that this dimer was also an intermediate on the pathway to very stable disulfide derivatives of calicheamicin. These disulfide derivatives showed a DNA cleavage pattern identical with that of the parent trisulfide. Significantly, so did the symmetrical trisulfide **25**.[31] This result provides support for a common intermediate, perhaps **23**, in the binding/cleavage event.

A surprising and important feature of the trisulfide cleavage with nonreducing thiols is the highly regioselective attack on the allylic sulfur of the methyl trisulfide moiety.[30] For example, treatment of calicheamicin with a large excess of MeSH in acetonitrile produced the corresponding methyl disulfide analogue of calicheamicin, which was also isolated as a natural product from fermentation broths. Reaction in methylene chloride was not productive, whereas in methanol only the aromatic end product was obtained. The reaction has proved general for primary, secondary, tertiary, and aryl nonreducing thiols and appears to be catalyzed by the basic nitrogen of the ethylamino sugar. **12** and calicheamicin α_3 (**8**) do not react with thiols in the absence of a base such as triethylamine or triethylammonium acetate. The disulfide analogues are less susceptible to cleavage with Ph_3P or reducing thiols compared to the parent trisulfide. Attempts to form di- or monosulfide analogues of calicheamicin γ_1^I with Ph_3P were unsuccessful, as were attempts with the more reactive $P(NMe_2)_3$.

Future Development

The unique structural features and remarkable biological properties of the calicheamicins and esperamicins have stimulated intense synthetic efforts, and a total synthesis of calicheamicin γ_1^I may soon be accomplished. Most of the initial synthetic efforts have been directed at **11**, which is particularly interesting because of its facile bond reorganization involving a *p*-benzyne intermediate (Scheme II). Since calicheamicinone is highly functionalized, preliminary synthetic efforts have focused on simplified models that defined the requirements for the bond reorganization process. A number of these prototype molecules were synthesized in the laboratories of Danishefsky,[12g,h,32] Nicolaou,[12c,33] Kende,[34] Magnus,[12e,35] and Tomioka.[36] Synthesis of **11** itself was recently reported by Danishefsky.[37] The various subunits of the oligosaccharide fragment of calicheamicin γ_1^I have been stereoselectively synthesized.[38] Recently the total synthesis of the completely assembled and enantiomerically pure oligosaccharide was reported by Nicolaou.[39]

Although the calicheamicins are extremely potent antitumor agents that are very effective in murine tumor models, long-term toxicity observed in mice and rats has restricted the development of the parent antibiotics as clinically useful antitumor agents.[40] The extreme potencies of these compounds, however, have made them ideal candidates for monoclonal antibody (MoAb) conjugation to obtain targeted chemotherapeutic agents. Initial studies have utilized the facile sulfide exchange chemistry discussed above to provide a mild and efficient method for the attachment of linkers such as 3-mercaptopropionyl hydrazide without triggering the cyclization. The resulting calicheamicin–mercaptopropionyl hydrazide was then conjugated to periodate-oxidized monoclonal antibodies.[41]

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N-Acetylcalicheamicin γ_1^I has been linked to an internalizable MoAb (7F11C7) specific for the human milk fat globule membrane antigen present on a variety of carcinomas. This MoAb conjugate caused tumor regression of the MX-1 and Lung 78 nonsmall lung carcinoma xenographs and resulted in long-term tumor free survivors in both murine models.[41] These very promising results suggest that it may be possible to prepare MoAb–calicheamicin conjugates that will be suitable for clinical treatment of human carcinomas.

Registry No. 1, 108212-75-5; 2, 99674-26-7; 3, 124412-57-3.

Reactions of Hydroperoxides with Metallotetraphenylporphyrins in Aqueous Solutions

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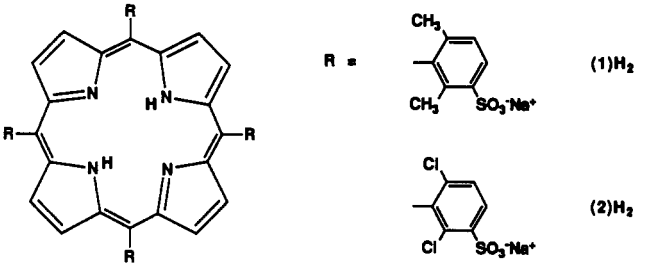
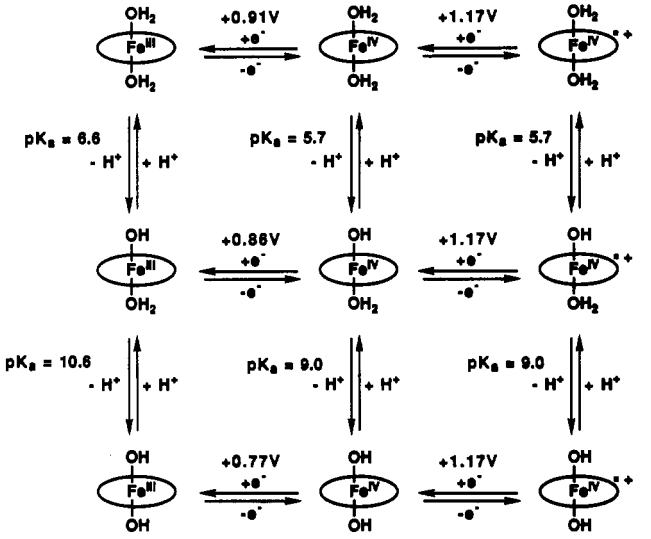
A marked renewal of interest in the mechanisms of reaction of metal ions with hydroperoxides[1] has come about due to interest in the biochemical reactions of peroxidases[2] with alkyl hydroperoxides and catalases[3] with hydrogen peroxide. Interest also stems from the finding that organic molecules in the presence of hydroperoxides and cytochrome P-450s[4] are oxidized to products that are comparable to those obtained in biochemical reactions that generate unidentified oxidants by stepwise reaction of $1e^- + O_2 + 1e^-$ with the Fe^{III} moiety of cytochrome P-450. This Account describes our studies of the reaction of hydroperoxides with Fe(III) and Mn(III) tetraphenylporphyrins in aqueous solution. It is in water that conditions (ionic strength, acidity, ligand species concentration) are best controlled and data (kinetic, electrochemical, etc.) are interpretable. A synopsis of our studies in organic solvents has been provided in a recent review.[5]

We have prepared and used the water-soluble Fe(III) and Mn(III) complexes of *meso*-tetrakis(2,6-dimethyl-3-sulfonatophenyl)porphyrin, $(1)H_2$, and *meso*-tetrakis(2,6-dichloro-3-sulfonatophenyl)porphyrin, $(2)H_2$ (Chart I). The Fe(III) and Mn(III) complexes of $(1)H_2$ and $(2)H_2$ are abbreviated as $(1)Fe^{III}(X)_2$, $(1)Mn^{III}(X)_2$, $(2)Fe^{III}(X)_2$, and $(2)Mn^{III}(X)_2$, respectively, where X = H_2O and HO^- axial ligands.[6-8] These metal complexes do not form μ-oxo dimers nor do they aggregate due to steric hindrance by the eight ortho substituents (CH_3 or Cl). Due to the four *m*-sulfonate substituents (Chart I), $(1)Fe^{III}(X)_2$, $(1)Mn^{III}(X)_2$, $(2)Fe^{III}(X)_2$, and $(2)Mn^{III}(X)_2$ exist as mixtures of four atropisomers. The electronic environments of the metal centers of a set of atropisomers have been shown to be comparable.

Chart I



Scheme I



Dynamics, Electrochemical, and Product Studies with $(1)Fe^{III}(X)_2$ and $(2)Fe^{III}(X)_2$

Pertinent Information Obtained from Electrochemical Studies with $(1)Fe^{III}(X)_2$ in Water.[9]

The pK_a and $E^{\circ\prime}$ values of Scheme I were calculated from appropriate Nernst–Clark plots of potentials (E_m) for

After dropping out of high school to serve in the military, Thomas C. Bruice attended the University of Southern California (B.S., 1950; Ph.D., 1954) and received his postdoctoral training at UCLA. Prior to moving to the University of California, Santa Barbara, in 1964, Professor Bruice held faculty positions at Yale, Johns Hopkins, and Cornell Universities. His research has involved the study of the mechanisms of reactions of biochemical interest; it has been recognized by a number of awards.

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