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A Re-examination of the Circular Dichroism of the Calicheamicin Enediyne/Dienone Chromophoric Interaction

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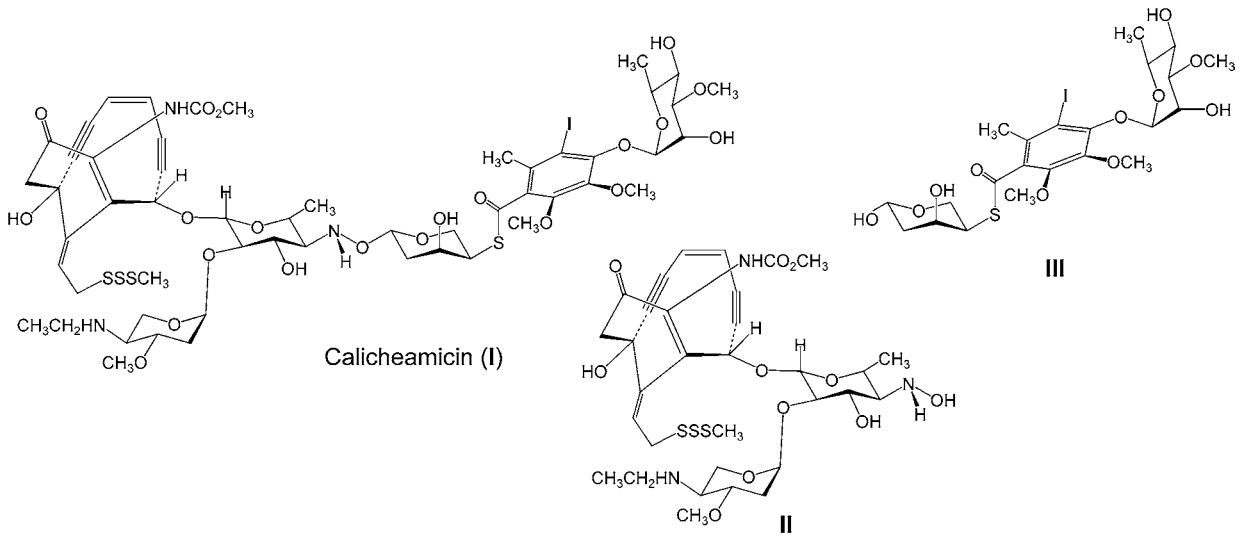
ABSTRACT



A series of calicheamicin derivatives have been made in an effort to delineate the origin of the strong circular dichroism (CD) of calicheamicin reported previously. The CD spectrum of calicheamicin (I) was compared with that of fragments II and III, which contain either the enediyne/dienone or a thiobenzoate chromophore alone. $NaBH_4$ reduction of calicheamicin produced two analogues (IV and V) that have no dienone. This allowed the assessment of possible exciton coupling between the enediyne on the warhead and the thiobenzoate on the tail. It was found that the strong negative 312/272 nm exciton split in the CD of calicheamicin is due largely to the enediyne/dienone interaction. Contributions from the thiobenzoate or its interaction with the enediyne have been ruled out.

A number of potent enediyne-containing antitumor antibiotics have been isolated and characterized from a variety of microbial and marine sources during the past 17 years.[1] All of these compounds appear to bind to DNA and, through some remarkable chemistry involving carbon-centered radicals, initiate oxygen-mediated strand cleavage. This strand cleavage appears to account for their extreme cytotoxicity that precludes their use as stand-alone chemotherapeutic agents. Due to the recent approval of Mylotarg, a calicheamicin−antibody conjugate and a first-in-class agent for the treatment of relapsed acute myeloid leukemia, there is renewed interest in the anticancer properties of enediynes.[2] The antibody portion of the conjugate specifically targets

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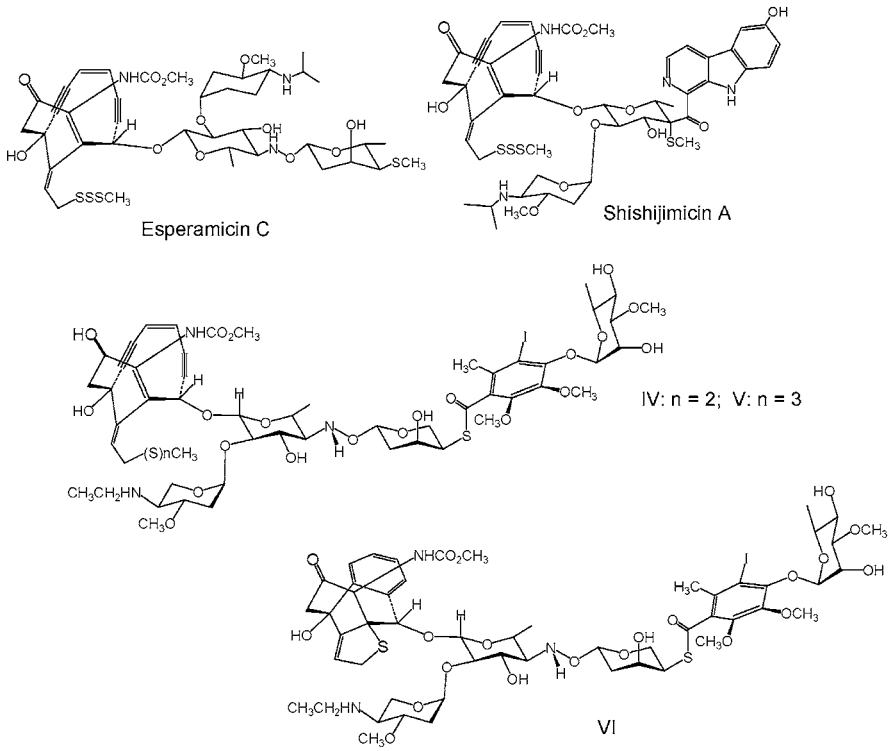

Figure 1. Some related enediyne-containing antibiotics, $NaBH_4$-reduced calicheamicins IV and V, and the aromatized analogue VI.

the cytotoxin to the tumor cell-surface antigen, thus greatly reducing nonspecific toxicity.

Surprisingly, several enediyne natural products have been obtained from tunicates. It is speculated that these marine enediynes originate from a microorganism that grows symbiotically on the surface of the tunicate.[3] A recent publication described the isolation and structural characterization of the shishijimicins (Figure 1), enediynes from the Fijian tunicate *Polysyncraton lithostrotum*.[4] The absolute stereochemical assignment of the enediyne-containing moiety of these compounds, based on circular dichroism (CD) measurements, is identical to that of calicheamicin and esperamicin.[5] The absolute stereochemistry and conformation of the aglycone portion of calicheamicin were established as depicted in I and II on the basis of X-ray and stereospecific total synthesis.[6,7] However, the authors' assignment of the Cotton effect of the dienone chromophore is at odds with that made previously with calicheamicin.[8] For shishijimicin, an extremum[4] at 325 nm ($\Delta\epsilon = -5$) was assigned to the carboline chromophore, 272 nm ($\Delta\epsilon = +7$) to the enediyne,

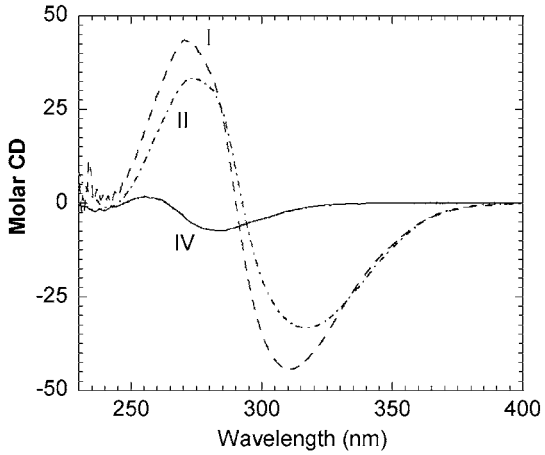

Figure 2. CD spectra of calicheamicin (I) and its truncated (II) and reduced (IV) products in ethyl alcohol.

and 239 nm ($\Delta\epsilon = -3$) to the dienone. The authors also suggested that in the CD spectrum of calicheamicin (312 nm ($\Delta\epsilon = -44$) and 272 nm ($\Delta\epsilon = +46$)) the thiobenzoate chromophore might be better assigned to the negative extremum at 312 nm. We, on the other hand, attributed the 312 nm extremum to the dienone chromophore. This was

(3) McDonald, L. A.; Capson, T.L.; Krishnamurthy, G.; Ding, W.-D.; Ellestad. G. A.; Bernan, V. A.; Maiese, W. M.; Lassota, P.; Discafani, C.; Kramer, R. A.; Ireland, C. M. *J. Am. Chem. Soc.* **1996**, *118*, 10898−10899.

(4) Oku, N.; Matsunaga, S.; Fusetani, N. *J. Am. Chem. Soc*. **2003**, *125*, 2044−2045.

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(8) Lee, M. D. In *Enediyne Antibiotics as Antitumor Agents*; Borders, D. B., Doyle, T. W., Ed.; Marcel Dekker: New York 1995; p 70.

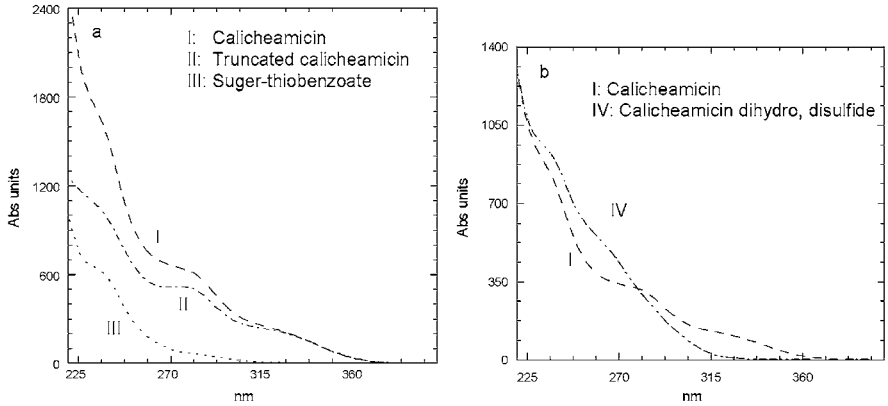

Figure 3. UV absorption profiles of calicheamicin and its derivatives based on diode array detection on HPLC.[12]

based primarily on Woodward's rules as well as the UV of certain steroidal dienones that show a band at 290 nm or above.[9]

Because of the questions raised as to the UV and CD assignments of the dienone and benzoate chromophores in calicheamicin and the unique spatial relationship of the enediyne and dienone chromophores in calicheamicin, esperamicin, namenamicin, and shishijimicin, we have made calicheamicin analogues that contain either the enediyne and dienone (II) or a thiobenzoate chromophore alone (III) and compared their CD spectra. The cleavage of calicheamicin into II and III was carried out as described previously.[10,11] Fragments II and III were characterized by LC-MS (MW 741 for II and 689 for III + formate in the negative mode electrospray detection; the formate came from the HPLC mobile phase). The CD spectrum of II (Figure 2) nearly matches that of calicheamicin (Figure 2) with two symmetrical extrema at 317 nm ($\Delta\epsilon = -36$) and 274 nm ($\Delta\epsilon = +36$). Due to the absence of the thiobenzoate in II, the two extrema are assigned to the dienone and enediyne chromophores, respectively. Therefore, the strong CD split of calicheamicin is primarily due to the enediyne/dienone chromophoric interaction.

The thiobenzoate III, on the other hand, has no CD absorption from 220 to 340 nm (data not shown). It exhibits a very weak UV absorption at ~280 nm with even weaker tailing around 310 nm (Figure 3a). Thus, the thiobenzoate itself makes no contribution to the negative exciton split at 312 and 272 nm in calicheamicin.

To obtain evidence of any possible thiobenzoate/enediyne interaction that may contribute to the negative CD extremum at 312 nm, calicheamicin was treated with $NaBH_4$. Primarily two reduction products, IV and V (Figure 1), were obtained in which the dienone carbonyl group is reduced to the alcohol.[12,13]

The UV spectrum of IV (Figure 3b) is noteworthy in that the prominent shoulder between 300 and 320 nm present in calicheamicin itself is absent in IV with a broad shoulder visible at about 270 nm assigned to the enediyne and dienecarbamate chromophores (Figure 3b). In the most prominent reaction product, disulfide IV, one of the sulfur atoms has been removed. We interpret its formation as arising from hydride cleavage of the terminal thiomethyl group followed by methylation of the remaining disulfide by methyl iodide used in the reaction. The role of methyl iodide in this reduction is necessary to methylate any thiols formed in the reaction, preventing further transformations.

Compound IV (Figure 2) shows a markedly weaker CD spectrum than that of calicheamicin with a negative extremum (283 nm) and weak positive extremum at 255 nm attributable to the dienecarbamate[14] and enediyne[15] chromophores, respectively. The origin for this very unsymmetrical exciton split is not clear but probably is due to a nonoptimal relationship of the electric transition moments. Although the direction of the electric transition dipole moments is speculative, it is clear that the relationship of the two chromophores describes a left-handed helix.[16] The absence of the 312/272 nm exciton split in IV indicates that

(9) Scott, A. I. In *Ultraviolet Spectra of Natural Products*; Pergamon Press: Oxford, UK, 1964; pp 58 and 65.

(10) Walker, S.; Landovitz, R.; Ding, W.-D.; Ellestad, G. A.; Kahne, D. *Proc. Natl. Acad. Sci*. **1992**, *89*, 4608−4612.

(11) II was quantified by comparing the 320 nm UV absorption peak area of II with that of standard calicheamicin on HPLC because they both have the same dienone chromophore.

(12) UV spectra (Figure 3a) of II and III (1:1 molar ratio) were recorded using a diode array detector on HPLC with a mobile phase of CH_3CN/H_2O/0.1% TFA. The UV spectrum of calicheamicin was normalized with II at 320 nm. UV spectra in Figure 3b were normalized at 220 nm.

(13) IV and V were purified by reverse-phase HPLC with an acetonitrile−water gradient containing 0.1% trifluoroacetic acid followed by immediate neutralization of the collected peaks with Tris buffer, pH 8. Compounds IV and V were characterized by LC-MS and UV spectroscopy (MW: 1338 M + H^+ for IV, 1370 M + H^+ for V).

(14) Correlation of the 283 nm extremum to the dienecarbamate chromophore seems reasonable on the basis of the broad UV shoulder at about 270 nm, which we believe is due mainly to an overlap of both the enediyne and dienecarbamate chromophores. An unsubstituted acyclic dienecabamate has a UV maxima of 258 nm. The addition of four alkyl groups is expected to shift the UV maxima to a longer wavelength, perhaps by as much as 20 nm; Oppolzer, W.; Frostl, W. *Helv. Chim. Acta* **1975**, *58*, 587−589.

(15) An unsubstituted cis acyclic enediyne has UV maxima in methanol at 250 nm (ϵ 14 500) and 262 (ϵ 12 500). Okamura, W. H.; Sondheimer, F. *J. Am. Chem. Soc*. **1967**, 89, 5991−5992.

there is no chromophoric interaction between the thiobenzoate and enediyne in IV.

The CD spectrum of the aromatized calicheamicin, VI (Figure 1), is of course dramatically different and weak as anticipated. In this case, a small negative extremum at 297 nm ($\Delta\epsilon = -5$) and a weak positive extremum at 260 nm ($\Delta\epsilon = +5$) are observed and assigned, respectively, to the displaced $n-\pi^*$ transition of the saturated carbonyl and the π to π^* transition of the aromatic ring.

In summary, the strong and negative exciton split between the dienone/enediyne chromophores is consistent with the absolute stereochemistry and conformation of the aglycone portion of calicheamicin. Contributions from the thiobenzoate or its interaction with the enediyne were ruled out. Esperamicin C (Figure 1), which also contains only the dienone-enediyne chromophores, exhibits essentially the same CD spectrum as calicheamicin.[17]

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