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REACTIONS OF THE TRISULFIDE MOIETY IN CALICHEAMICIN

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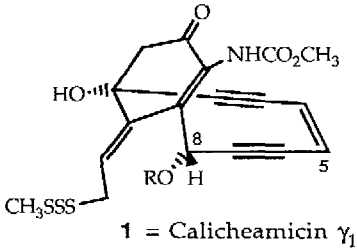
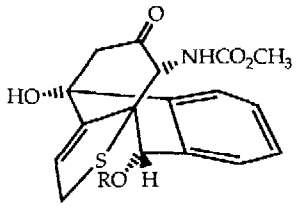
Summary: Calicheamicin γ_1^I (1) reacts with Ph_3P to form a dimeric trisulfide (3), MeSSMe, Ph_3PS, and Ph_3PO, as well as an aromatic degradation product (2). The oxygen of the Ph_3PO is derived from O_2. Calicheamicin also reacts with thiols to produce disulfides (eg. 4) with high selectivity. Dimeric trisulfide 3 is generated during this reaction.

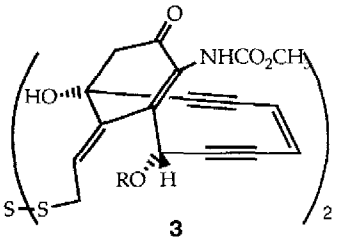
The calicheamicins[1] and esperamicins[2] are two new classes of potent antitumor antibiotics which appear to target DNA *in vivo* and to cause single- and double-strand cleavage. Understanding the chemistry of the trisulfide moiety in these molecules is crucial, since it is cleavage of the trisulfide which triggers reaction of the aglycone and leads to diradical formation.[1,2,3] In this publication we present the unusual chemical properties we have observed for the trisulfide of calicheamicin γ_1^I (1).



1 = Calicheamicin γ_1^I
(γ_1^I-SSSMe)

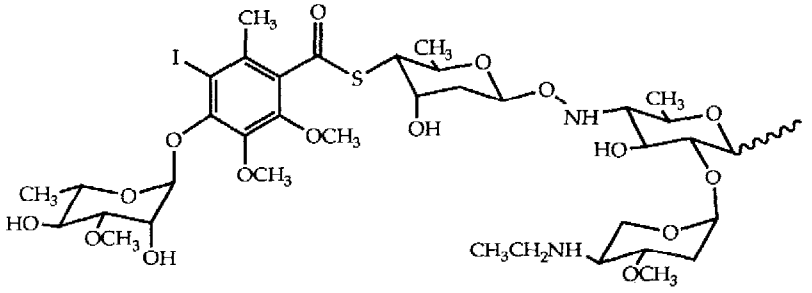


2



3

R =



The reaction of 1 with triphenyl phosphine (Ph_3P) to give the aromatic derivative 2 (5-15% yield) was invaluable in the structure elucidation of this compound.[4] An unprecedented product which has been more recently isolated and identified from this reaction is the dimeric trisulfide 3. This product precipitates from the reaction mixture in about 30% yield, thereby preventing further reaction with Ph_3P. Compound 3 has been characterized by ^{1}H-NMR, ^{13}C-NMR, FAB-MS, and elemental analysis,[5] and has *in vivo* antitumor activity virtually identical to 1.[1]

Although the multitude of unidentified minor products makes detailed mechanistic studies impractical, several by-products of this reaction do have mechanistic implication. The by-products which were identified are Ph_3PO and Ph_3PS, isolated in roughly equal amounts, and MeSSMe and MeSH, identified by GC-MS. Most of the products of this reaction can be explained by the following scheme.[6]

$$\gamma_1^{I}\text{-SSSMe (1)} + Ph_3P \rightleftharpoons \gamma_1^{I}\text{-S}^- + Ph_3PSSMe^+$$

$$\gamma_1^{I}\text{-S}^- \xrightarrow{\text{references 1 and 2}} \mathbf{2}$$

$$\gamma_1^{I}\text{-SSSMe} + Ph_3P \rightleftharpoons \gamma_1^{I}\text{-SS}^- + Ph_3PSMe^+$$

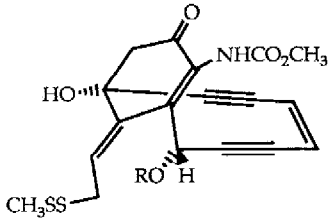
$$\gamma_1^{I}\text{-SSSMe} + Ph_3P \rightleftharpoons \gamma_1^{I}\text{-SSP}Ph_3^+ + MeS^-$$

$$\gamma_1^{I}\text{-SS}^- + \gamma_1^{I}\text{-SSP}Ph_3^+ \longrightarrow \gamma_1^{I}\text{-SSS-}\gamma_1^{I}\ (\mathbf{3}) + Ph_3PS$$

The only identified product unaccounted for in this scheme is Ph_3PO. The oxygen in this compound originates from molecular oxygen, since no Ph_3PO is formed when oxygen is excluded from the reaction, and the use of an $^{18}O_2$ atmosphere yields $Ph_3P^{18}O$. At least two mechanisms are possible. The p-benzyne diradical intermediate involved in the formation of 2 (or another carbon-centered radical derived from it) could react with molecular oxygen to form a peroxide which is subsequently reduced by Ph_3P. Although numerous unidentified minor products are formed in this reaction, no analogues of 2 with an oxidized aryl ring have been isolated. Alternatively, superoxide could be generated under the reaction conditions,[7] and this could react with Ph_3P.

Attempts to form di- or monosulfide analogs of 1 using Ph_3P were unsuccessful, as were attempts with the more reactive $P(NMe_2)_3$.[8] Subsequently, disulfide 4 was isolated from the fermentation broths. This compound has been characterized by both ^{1}H-NMR and FAB-MS.[9] While its *in vivo* and *in vitro* properties are almost identical to 1, it is considerably less reactive chemically toward Ph_3P and dithiothreitol, as expected. More recently we have found that treatment of 1 with a large excess of MeSH in acetonitrile produces disulfide 4 in good yield. Treatment in methylene chloride is unproductive, while in methanol the only product observed is the aromatic derivative 2. Further work showed that this disulfide formation is general for

non-reducing thiols (primary, secondary, tertiary, and aryl), that it is catalyzed by the amine present in the starting material,[10] and that only a slight excess of thiol is needed. Evans and Saville[11] have shown that the reaction of two symmetrical trisulfides with a primary or a secondary thiol gives disulfides. This work with calicheamicin is the first example to our knowledge of the application of this reaction to an unsymmetrical trisulfide. The highly regioselective attack of the thiolate on this allyl-methyl trisulfide is most surprising. This reaction allows the formation of altered, more stable compounds for further study which still have the aglycone intact.



4, Ras in structure **1**

Close monitoring of this reaction revealed that the dimeric trisulfide **3** is formed in significant amounts early in the reaction. Its concentration, as monitored by RP-HPLC, increases rapidly until roughly equivalent to that of **1**, at which point the concentrations of the two species fall off in parallel until the disulfide is the only significant product (generally 85-95% by RP-HPLC). These results can be explained by the following scheme.

$$\gamma_1^I\text{-SSSMe} + \text{RSH} \longrightarrow \gamma_1^I\text{-SSR (eg } \mathbf{4}) + \text{MeSS}^-$$

$$\gamma_1^I\text{-SSSMe} + \text{MeSS}^- \rightleftharpoons \gamma_1^I\text{-SS}^- + \text{MeSSSMe}$$

$$\gamma_1^I\text{-SSSMe} + \gamma_1^I\text{-SS}^- \rightleftharpoons \gamma_1^I\text{-SSS-}\gamma_1^I\ (\mathbf{3}) + \text{MeSS}^-$$

Initial generation of $MeSS^-$ (or possibly other catalytically active species) leads to a rapid equilibrium between γ_1^I-SSSMe, γ_1^I-SSS-γ_1^I, and MeSSSMe. This is most likely due to the greater reactivity of trisulfides versus disulfides, as shown by control experiments, and by the greater reactivity of $MeSS^-$ versus MeS^- due to the alpha effect of the lone pair of electrons on the neighboring sulfur.[12] Although other pathways may be occurring to a minor extent, the high yield of **4** and similar disulfides indicates that attack of the thiols on **1** must be selective for the allylic sulfur.

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(3) For some recent modeling of this reaction see: (a) Magnus, Philip; Carter, Paul A. J. Am. Chem. Soc. 1988, 110, 1626. (b) Nicolaou, K.C.; Zuccarello, G.; Ogawa, Y.; Schweiger, E.J.; Kumazawa, T. J. Am. Chem. Soc. 1988, 110, 4866. (c) Danishefsky, S.J.; Mantlo, N.B.; Yamashita, D.S. J. Am. Chem. Soc. 1988, 110, 6890. (d) Magnus, P.; Lewis, R.T.; Huffman, J.C. J. Am. Chem. Soc. 1988, 110, 6921. (e) Kende, A.S.; Smith, C.A. Tet. Lett. 1988, 29, 4217.

(4) (a) Lee, M.D.; Dunne, T.S.; Chang, C.C.; Ellestad, G.A.; Siegel, M.M.; Morton, G.O.; McGahren, W.J.; Borders, D.B. J. Am. Chem. Soc. 1987, 109, 3466. (b) We have revised the stereochemistry at C_8 in calicheamicin so the the $J_{5,8}$ NMR coupling constant seen in calicheamicin (and esperamicin) is consistent with a comparable coupling seen by Kende and Smith in a model system.[3e]

(5) The ^{1}H-NMR and ^{13}C-NMR of **3** are virtually identical to those of **1**, except that the signals for the methyl group of the trisulfide (δ_H-2.52 and δ_C-22.8) are missing. FAB-MS shows an M+1 ion at 2609.

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(7) (a) Misra, H.P.; J. Biol. Chem. 1974, 249, 2151. (b) Also cf.: Chin, D-H.; Goldberg, I.H.; Biochemistry, 1986, 25, 1009.

(8) Harpp, D.N.; Gleason, J.G. J. Am. Chem. Soc. 1971, 93, 2437.

(9) ^{1}H-NMR: -SSMe at δ 2.36 (versus -SSSMe at δ 2.52). See Block, E. "Reactions of Organosulfur Compounds", Academic Press, N.Y. 1978, p 287. FAB-MS: M+1 at 1336.

(10) Calicheamicin γ_1^1 is usually handled as the acetate salt. The disulfide formation is successful with this material without any added catalyst. However, the addition of small amounts of triethyl amine increases the rate greatly. Experiments show that no reaction occurs without the addition of triethyl amine or triethyl ammonium acetate when this reaction is conducted with members of the series where the aminosugar is missing.

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