REASSIGNMENT OF THE STRUCTURE FOR THE ANTITUMOR AGENT RR-150

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7-Cysteaminomitosane (RR-150) has been reported to be superior to mitomycin C against P388 leukemia and B-16 melanoma in mice and is less leukopenic. Studies reported here indicated the absence of a free thiol group in RR-150 and therefore the structure was incorrectly assigned. Reaction of mitomycin A with either 2-aminoethanethiol or cystamine gave the same disulfide, 7-*N*,7′-*N*′-dithiodiethylenedimitomycin C, which is the newly proposed structure for RR-150. Attempts to produce 7-cysteaminomitosane by reduction of the disulfide have not succeeded because of its apparent instability.

The broad spectrum antitumor activity of mitomycin C (MMC) has spawned significant interest in the development of MMC analogues that show high activity and decreased cumulative myelosuppression[1,2]. RR-150, one of the most active MMC derivatives to date, is more active *in vivo* than MMC against several murine tumor models, and causes significantly less leukopenia under chronic administration[1,3].

As originally described, RR-150 was prepared from mitomycin A (MMA, **1**) by the displacement of the 7-methoxy group with 2-aminoethanethiol[1]. The structure **3** was assigned based on spectral evidence and on related reactions of MMA. We have prepared RR-150 according to this reported method and have found the product to be at variance with the assigned structure. Because of the importance of RR-150 as an antitumor agent and as a prototype for other related MMC analogues, we wish to report the studies that have led us to reassign the structure.

RR-150 was prepared under anaerobic conditions from MMA and 2-aminoethanethiol as previously described[1] (Scheme 1). The product was characterized by ^{1}H NMR, ^{13}C NMR (Fig. 1A), IR, UV and HPLC and was consistent with the compound reported earlier[1]. The presence of a free thiol in RR-150 was called into question when it did not react with maleimide, iodoacetamide or various disulfide containing compounds under conditions where simple mercaptans such as mercaptoethanol or 2-aminoethanethiol clearly reacted. Further evidence for the absence of a free thiol in RR-150 was obtained by the isolation of a single acetate derivative in high yield when RR-150 was treated with excess acetic anhydride in pyridine. The ^{1}H NMR indicated the presence of an aziridinyl acetate (2.15 ppm) and there was no evidence for the formation of an *S*-acetate.

The synthesis of the dimer **2** from MMA and 0.5 equivalent of cystamine was undertaken in order to explore the possibility that RR-150 might be a symmetrical disulfide. The product obtained (mp 153~156°C) was identical in all respects (see ^{13}C NMR, Fig. 1B) to that produced when MMA was treated with 2-aminoethanethiol (mp 154~156°C, see ^{13}C NMR, Fig. 1A). Therefore, the two products are identical and the correct structure of RR-150 is the symmetrical dimer **2**.

Attempts to form the free thiol, **3** from the disulfide **2**, by disulfide reduction have thus far been

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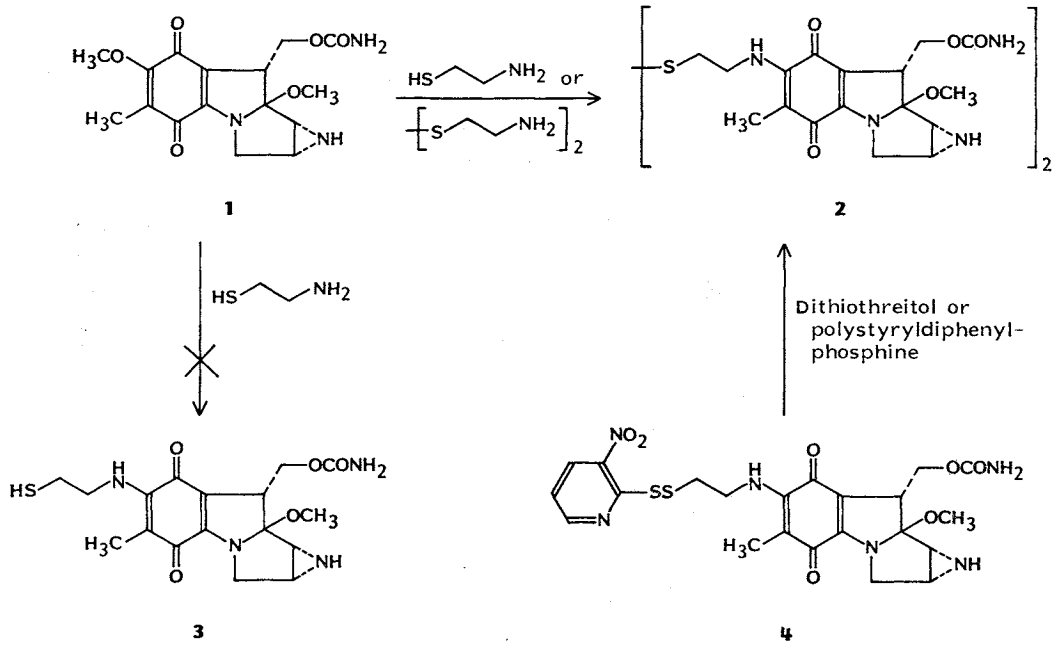
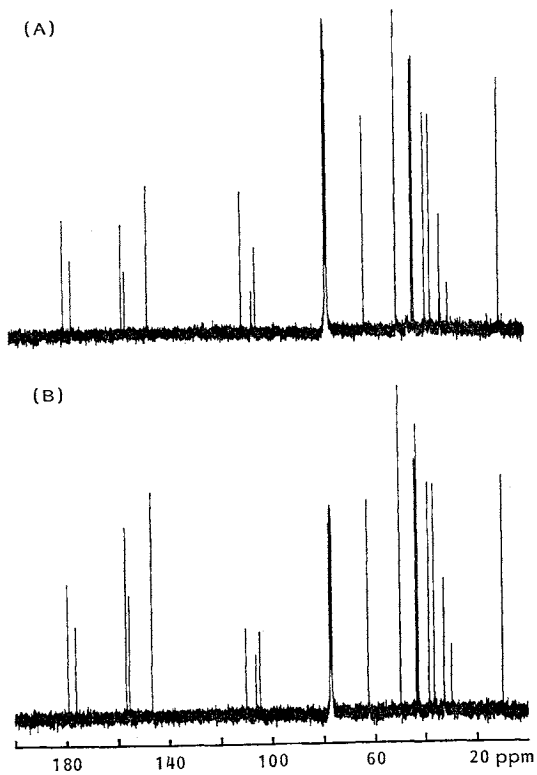
Scheme 1.



unsuccessful. Extensive decomposition of RR-150 occurred upon reaction with mild reducing agents such as dithiothreitol[4]. Reduction of the nipsyl disulfide 4[5] with polystyryldiphenylphosphine[6] or with exactly 1 equivalent of dithiothreitol led to the exclusive formation of **2**. Based on these findings, we feel that if the free thiol **3** does indeed exist, it is quite unstable.

The unusual ease with which the disulfide **2** is formed may result from the close proximity of the thiol to the quinone ring. Thiol oxidation with concomitant quinone reduction would lead to the observed product once the reduced quinone was reoxidized on exposure to air. RR-150 homologues having longer sulfur-containing amino-alkyl side chains are not nearly as active antitumor agents as RR-150 itself (W. T. BRADNER and W. A. REMERS; personal communication), indicating that the spatial relationship between the sulfur and the 7-amino group is critical for activity. Studies to further characterize the chemistry and the biological properties of the products obtained from the reaction of MMA with mercapto-containing amines are now underway.

Fig. 1. ^{13}C NMR spectrum of the product obtained by treating mitomycin A with 2-aminoethanethiol (A) or with cystamine (B) (90 MHz, $CDCl_3$).



Experimental

NMR spectra were recorded using a Bruker WM 360 NMR operating at 360 MHz for proton and 90 MHz for carbon. HPLC was performed using a Waters C-18 Radial-Pak column and the following conditions: 30~95% MeOH in H_2O over 8 minutes, 95% aq MeOH for 4 minutes; flow rate 2 ml/minute; detection at 340 nm. Retentions for RR-150 and MMA were 8.46 and 8.85 minutes respectively. All reactions and chromatographic separations were performed under an atmosphere of dry N_2 with solvents and solutions that were thoroughly degassed with N_2 prior to use.

Reaction of MMA with 2-Aminoethanethiol

MMA (50 mg, 0.14 mmol) was added to a solution of 2-aminoethanethiol hydrochloride (33 mg, 0.29 mmol) and triethylamine (0.18 ml, 1.22 mmol) in 4 ml of MeOH. Stirring was continued under an atmosphere of N_2 for 1.5 hours. Water was added to the solution, and the product was extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$), filtered and evaporated. Purification of the product by flash chromatography on silica gel using 20% MeOH in $CHCl_3$ as eluant afforded RR-150 (40 mg, 72% yield) as a fine blue solid; mp 154~156°C (literature[1] 152~154°C).

Reaction of MMA with Cystamine

MMA (50 mg, 0.14 mmol) was treated with cystamine dihydrochloride (15.8 mg, 0.07 mmol) and triethylamine (0.06 ml, 0.43 mmol) in 4 ml of MeOH as described for the reaction of MMA with 2-aminoethanethiol. The product was isolated and purified as previously described. Yield of a fine blue solid: 40 mg, 72% yield; mp 153~156°C. The product from this reaction was identical to the product obtained from MMA and 2-aminoethanethiol by ^1H NMR, ^{13}C NMR, IR, UV and HPLC. A mixture of the two solids did not result in a depressed mp. Molecular ions were not observed for either of the products using fast atom bombardment mass spectrometry.

Reaction of RR-150 with Acetic Anhydride

To a solution of RR-150 (48 mg, 0.061 mmol) in 3 ml of pyridine was added 400 mg (3.9 mmol) of acetic anhydride, and stirring was continued until all of the RR-150 was consumed. The volatile material was removed under vacuum and the residue was purified on silica gel using 4% MeOH in CH_2Cl_2. A pure blue solid was obtained (47 mg) which was characterized by ^1H NMR (pyridine-d_5); δ 2.15 (6H, s, 6-CH_3 and $NCOCH_3$), 2.9~3.1 (2H, t, SCH_2), 3.2 (3H, s, OCH_3), 3.4~3.6 (1H, m, 2-H), 3.65 (1H, m, 3-H), 3.8~4.2 (4H, m), 4.5~4.9 (4H, m), 5.5~5.7 (1H, dd, 10-H).

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