SYNTHESIS AND ANTITUMOR ACTIVITY OF A NOVEL WATER SOLUBLE MITOMYCIN ANALOG; 7-*N*-[2-[[2-(γ-L-GLUTAMYLAMINO)ETHYL]DITHIO]ETHYL]MITOMYCIN C

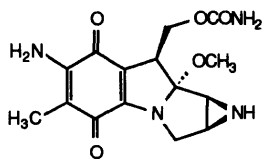
Motomichi KONO,*[a,1)] Yutaka SAITOH,[a] Masaji KASAI,[a,1)] Akira SATO,[a] Kunikatsu SHIRAHATA,[a] Makoto MORIMOTO,[b] and Tadashi ASHIZAWA[b]

Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.,[a] 3-6-6, Asahimachi, Machida, Tokyo 194, Japan and Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co., Ltd.,[b] 1188 Shimotogari, Nagaizumi, Sunto, Shizuoka 411, Japan

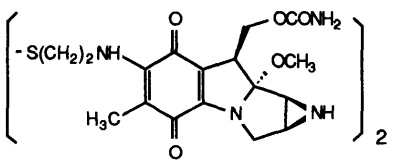
Introducing the mercaptoethyl group at the 7-N position of mitomycin C **1** has led to the isolation of 7-*N*,7'-*N*'-dithiodiethylenedimitomycin C **2**. The compound **2** showed excellent antitumor activity against sarcoma 180 (sc-ip) and leukemia P388 (ip-ip) in mice. As an extension of this study, we synthesized mitomycin dimers with symmetrical disulfide and mitomycin derivatives with unsymmetrical disulfide at the 7-N side chain. Among these compounds, the water soluble conjugate **3** with ethyl γ-L-glutamyl-L-cysteinylglycinate was far more effective against sarcoma 180 and leukemia P388 than **1**. During the subsequent stage of inquiry for the potent congeners of **3**, the compound **4** (water solubility : >500 mg/ml), designated as KW2149, with the γ-L-glutamylcystamino group at the 7th position was finally selected for further evaluation.

KEYWORDS mitomycin; symmetrical disulfide; unsymmetrical disulfide; glutathione relative; water solubility; sarcoma 180; leukemia P388

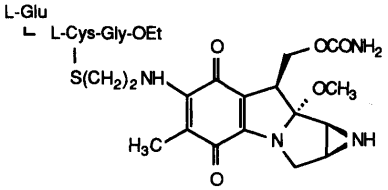
Mitomycin C **1** is a valuable antitumor antibiotic because of its broad spectrum and strong activity, and is widely used in clinical chemotherapy.[2)] The investigation of new analogs of the mitomycin family has been active[3)] since **1** has strong side effects, e.g., myelosuppression. Concerning the mode of action, recent direct evidence showed the cross-linking of the DNA double strand[4)] by the activated mitomycin caused by the reduction of the quinone as a trigger reaction. The observation that the quinone of mitomycin was easily reduced by a thiol with subsequent decomposition[5)] envisaged us the possibility of the intramolecular reductive activation of mitomycin by an auxiliary at the 7-N position. Introducing the mercaptoethyl group at the 7-N position of **1** led to the isolation of the novel mitomycin dimer **2**[6)] with symmetrical disulfide. As an extension of this study, we synthesized various mitomycin derivatives with symmetrical and unsymmetrical disulfides,[9)] and found that the conjugate **3** with a glutathione relative was far more effective than **1** against several rodent tumors. During the subsequent stage of inquiry for the potent congeners of **3**, 7-*N*-[2-[[2-(γ-L-glutamylamino)ethyl]dithio]ethyl]-mitomycin C **4** was finally selected for further evaluation. Here we describe the development of mitomycin derivatives with disulfide at the 7-N side chain, and the synthesis and antitumor effects of **2**, **3**, and **4**.



1 (mitomycin C)



2



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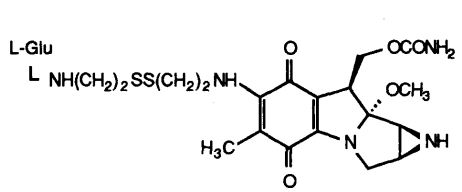

4 (KW2149)

Fig 1

The reaction of mitomycin A **5** with cysteamine **6** resulted in 7-*N*, 7'-*N*'-dithiodiethylenedimitomycin C **2** (35%) and 7-*N*-[2-[(2-aminoethyl)dithio]ethyl]mitomycin C **7** (26%). Although compound **7** was unstable, directly after the purification, **7** afforded **2** in a high yield when treated with 1 equivalent of **5**. The amination of **5** by a half equivalent of cystamine **8** also gave a high yield of **2**. Thus the structures of **2** and **7** were unambiguously assigned respectively to 1:2 and 1:1 condensation products of **8** with **5**.[12] According to time observations for this reaction[13] the mechanism of the formation of **2** and **7** was based on the premise that the oxidation of **6** by the quinone of **5** caused the formation of **8** which was trapped by one or a couple of **5**.

The compound **2** showed a wide range of effective dosage against sarcoma 180 and the myelosuppression appeared to be reduced compared to **1**. In addition, the increase in the life span of **2** against leukemia P388 was superior to that of **1** (Table I).[14] These results prompted us to derive similar derivatives with several mitomycin skeletons and analogous dimers with propylene $(CH_2)_3$ to dodecamethylene $(CH_2)_{12}$ instead of ethylene $(CH_2)_2$ as a spacer.[15] Among these compounds, **2** showed the most effective activity. However the activity of **2** against human mammary cancer xenograft MX-1 (sc-iv) was inferior to that of **1** (data not shown), and the low water solubility (0.1 mg/ml) would be troublesome in practical usage.[16]

At this point, attention was turned to the synthesis of mitomycin derivatives with unsymmetrical disulfide as a means of circumventing these problems. In consideration of the solubility and the improvement of pharmacodynamics, we synthesized various derivatives with unsymmetrical disulfide.[15]

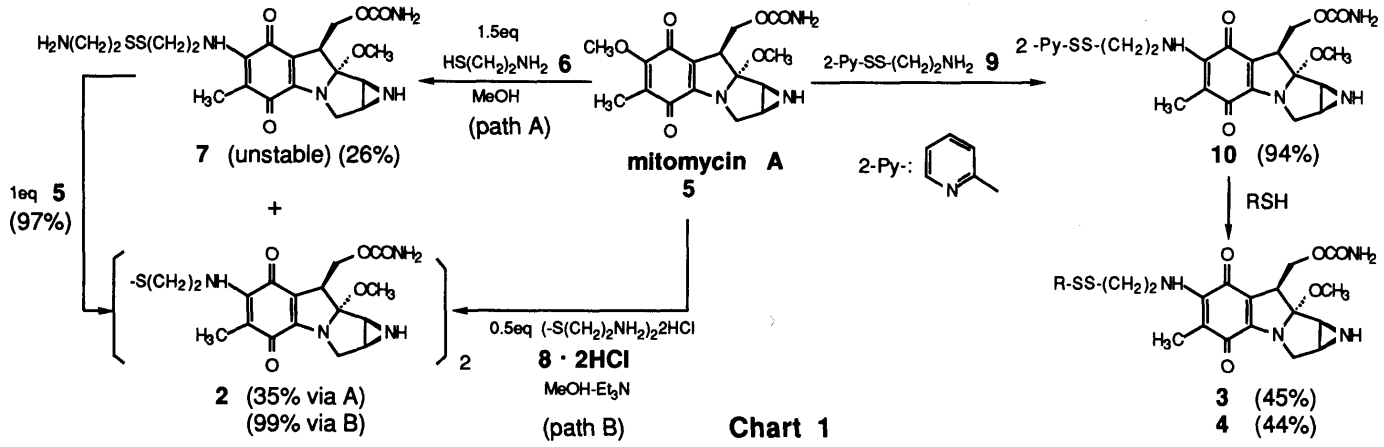

Chart 1

Most of mitomycin derivatives with unsymmetrical disulfide at the 7-N side chain were derived by the use of exchange reaction of disulfide under neutral condition from an intermediate **10**,[17] which was readily obtained from **5** and **9**.[18] This exchange reaction proceeded more rapidly than reductive side reactions, such as decomposition of mitomycin skeleton. The disproportionations of unsymmetrical disulfides developed rapidly for such molecules that had a basic group at the 7-N side chain like **7** or the metal salt of carboxylic acid. Thus no stable disulfide appeared with such groups. On the other hand, if the acidity of the side chain was high, the acid-labile skeleton of mitomycin decomposed immediately. Considering these characteristics of mitomycin derivatives with unsymmetrical disulfide, various compounds were synthesized. In these trials, acidic peptide glutathione was changed to neutral ethyl γ-L-glutamyl-L-cysteinylglycinate,[19] which formed a stable disulfide. The conjugate **3**[20] had enough water solubility (>160 mg/ml) for formulation owing to the contribution of a zwitter ion structure. The compound **3** was more effective than **1** against sarcoma 180 and leukemia P388 (Table I.). In our next efforts to find more potent compounds than **3**, the ethyl L-cysteinylglycinate moiety of **3** was substituted by cysteamine to afford water soluble **4** (>500 mg/ml).[21]

Of those mitomycins evaluated in the past, the compound **4** was one of the derivatives with the most effective activity against sarcoma 180 and leukemia P388. During the next stage, those excellent compounds were tested for various tumors and toxicity in detail.[22] Finally, **4** was selected for further evaluation for reasons that myelosuppression was decreased compared to **1** and the activity against various tumor lines was superior to **1** and other candidates. The compound **4** was designated as KW2149 and is under preclinical study.

Table I. Antitumor Activity of 2, 3, and 4

No	LD_{50} ip mg/kg	S180 sc-ip ED_{50} ip mg/kg	S180 sc-ip CI[a]	S180 sc-ip (WBC_{4000}/ED_{50})[a]	P388 ip-ip ILSmax[a]
1	8.4	2.6-4.4	1	1	1
2	18.8	6.3	1.56	2.96	2.50
3	45.0	6.8	2.21	7.94	> 1.95
4	22.5	2.7	2.58	4.12	> 2.30

a): The value of tested compound/the value of mitomycin C.
CI: LD_{50}/ED_{50}.
The LD_{50} values were determined in ddY mice (5 mice/group) after 14 days of observation and were calculated by probit analysis. Sarcoma 180 cells ($5x10^6$/mouse) were implanted s.c. into ddY mice and P388 cells ($1x10^6$/mouse) were implanted i.p. into CD_2F_1 mice (6 mice/group respectively), and the drug was administered i.p. respectively on day 1. ED_{50} values were doses which gave 50% inhibition of tumor growth on day 7. WBC_{4000} values were doses to give a WBC number of 4000/mm^3 on day 4. ILS % (Increased Life Span) values were determined after 33 days observation.

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6) Iyengar reported that the reaction of **5** with hydrochloride salt of **6** gave 7-*N*-(2-mercaptoethyl)mitomycin C refered to as RR150.[7] Recently the structure of RR150 was reassigned to **2** by Senter.[8] These studies were not related to our research.
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9) During the course of our work, analogous mitomycin derivatives with unsymmetrical disulfide were reported by Vyas.[10] Prior to that publication, we had started investigation of our unsymmetrical disulfides.[11]
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12) Spectral data consistently supported the structure of **2**. **2**: mp 142-145°C; SI-MS m/z 789 $(M+3)^+$; ^1H NMR (100MHz, Py-d_5) δ 2.14(3H, s), 2.75 (1H, dd), 3.00 (2H, t), 3.13 (1H, d), 3.22(3H, s), 3.59(1H, dd), 3.95(2H, q), 3.99(1H, dd), 4.52(1H, d), 5.02(1H, t), 5.36(1H, dd), 7.27(1H, t), 7.58(2H, br); ^{13}C NMR (25MHz, Py-d_5) δ 10.0(6-Me), 32.7(2), 36.6(1), 38.6(7-N-Cβ), 43.8(7-N-Cα), 44.3(9), 49.7(9a-OMe), 50.5(3), 62.4(10), 104.5(6), 106.8(9a), 110.8(8a), 147.1(7), 155.7(4a), 158.0(OCON), 176.7(8), 179.1(5); IR (KBr) 3290, 2920, 1714, 1632, 1554, 1507, 1448, 1325, 1217, 1062, 752cm^{-1}.
13) The time courses for the reaction of **5** and 2 equivalents of **6**·HCl in the presence of triethylamine under nitrogen atmosphere were observed by HPLC. After 2 hours the mole number of the consumed mitomycin skeletons were nearly equal to the sum of those of **2** and **7**.
14) Antitumor effects against sarcoma 180 (sc-ip) and leukemia P388 (ip-ip) were evaluated according to the method described in the literature: K. Fujimoto, T. Oka, M. Morimoto, *Cancer Res.*, **47**, 1516(1987).
15) The structure-activity relationship among these compounds will be reported elsewhere.
16) The importance of the solubility of the agents tested for antitumor activity was indicated in the report of NCI: J. P. Davignon, J. C. Cradock, *Pharm. Weekble.*, **119**, 1144(1984).
17) **10**: ^1H NMR (100MHz, Py-d_5) δ 2.06(3H, s), 2.76(1H, br), 3.08(2H, t), 3.14(1H, br), 3.23(3H, S), 3.60(1H, dd), 3.90(1H, q), 4.00(1H, dd), 4.53(1H, d), 5.06(1H, t), 5.40(1H, dd), 7.06(2H, m), 7.61(2H, m), 8.66(1H, m); IR (KBr) 3290, 2930, 1713, 1632, 1553, 1505, 1444, 1414, 1330, 1061, 755cm^{-1}; Anal. Calcd for $C_{22}H_{25}N_5O_5S_2$: C, 52.47; H, 5.00; N, 13.91. Found: C, 52.58; H, 5.06; N, 13.63.
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20) **3**: SI-MS m/z 730 $(M+3)^+$, 729 $(M+2)^+$, 728 $(M+1)^+$; ^1H NMR (400MHz, D_2O) δ 1.25(3H, t, J=7.2Hz), 1.98(3H, s), 2.15(2H, m), 2.52(2H, m), 2.97(1H, dd, J=14.3, 9.1Hz), 3.01(4H, m), 3.19(1H, dd, J=14.3, 4.8Hz), 3.28(3H, s), 3.63(1H, dd, J=10.7, 4.6Hz), 3.64(1H, br d, J=13.3Hz), 3.76(1H, t, J=6.4Hz), 3.97 and 4.01(2H, ABq, J=17.7Hz), 4.01(2H, t, J=6.2Hz), 4.18(1H, d, J=13.3Hz), 4.19(2H, q, J=7.2Hz), 4.23(1H, dd, J=10.8, 10.7Hz), 4.58(1H, dd, J=10.8, 4.6Hz), 4.72(1H, dd, J=9.1, 4.8Hz); IR (KBr) 3300, 3070, 2990, 2950, 1715, 1635, 1553, 1513, 1450, 1330, 1212, 1065cm^{-1}; Anal. Calcd for $C_{29}H_{41}N_7O_{11}S_2$: C, 47.9; H, 5.7; N, 13.5. Found: C, 47.6; H, 5.8; N, 13.2.
21) The γ-L-glutamylcysteamine required was prepared according to the conventional method of peptide synthesis. **4**: SI-MS m/z 601 $(M+3)^+$, 600 $(M+2)^+$, 599 $(M+1)^+$; ^1H NMR (400MHz, D_2O) δ 2.00(6-CH_3, s), 2.15(Glu CβH_2, m), 2.44(Glu CγH_2, m), 2.86(7-N-CεH_2, t, J=6.3Hz), 3.00(7-N-CβH_2, t, J=6.3Hz), 3.02(1-H*, br), 3.05(2-H*, br), 3.30(9a-OCH_3, s), 3.53(7-N-CζH_2, t, J=6.3Hz), 3.65(3a-H, br d), 3.65(9-H, dd, J=10.7, 4.5Hz), 3.78(Glu CαH, t, J=6.2Hz), 4.00(7-N-CαH_2, t, 6.3Hz), 4.19(3b-H, br d, J=13.7Hz), 4.26(10-Ha, t, J=10.7Hz), 4.60(10-Hb, dd, J=10.7, 4.5Hz); ^{13}C NMR (100MHz, D_2O) δ 10.24(6-Me), 27.35(Glu Cβ), 32.51(Glu Cγ), 33.56(2), 36.47(1), 37.58(7-N-Cε), 38.50(7-N-Cβ), 39.04(7-N-Cζ), 43.89(9), 44.33(7-N-Cα), 50.52(3), 50.72(9a-OMe), 55.09(Glu Cα), 62.93(10), 105.19(6), 107.18(9a), 109.76(8a), 150.39(7), 157.81(OCON), 159.73(4a), 174.70(Glu CγCO*), 175.46(Glu CαCO*), 176.51(8), 178.86(5) (*: tentative); IR (KBr) 3450, 3300, 3080, 2950, 1707, 1631, 1560, 1510, 1460, 1328, 1062cm^{-1}; Anal. Calcd for $C_{24}H_{34}N_6O_8S_2 \cdot H_2O$: C, 46.74; H, 5.88; N, 13.63. Found: C, 46.51; H, 5.97; N, 13.60.
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