NOTES

Isolation of Albomitomycins from Solutions of 7-Amino Substituted Mitomycins; Mitomycin C and KW-2149

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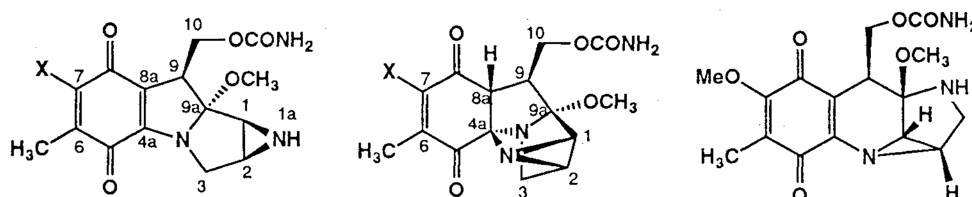
Mitomycins are represented by mitomycin C (**1**) that has been used in clinical cancer chemotherapy for a long time.[1] However **1** has also strong side effects such as myelosuppression and gastrointestinal toxicities.[2] The investigation of new potent analogs with less toxicity has been active[1] and we have found a new promising clinical candidate of KW-2149 (**2**)[3,4] in a program to develop C-7 modifications. During our approach to obtain diverse mitomycins from fermentation, we found variants of mitomycin A (**3**); albomitomycin A (**4**) and isomitomycin A (**5**), and their tripartite interconversion referred to as the mitomycin rearrangement.[5] The C-7 substituent of **3** is a methoxy group, while those of **1** and **2** are amines with amide character. To clarify the effects of C-7 substituents on the isomerism at C-4a in the mitomycin skeleton, we studied the physico-chemical properties of **1** and **2** and revealed that they are in equilibrium with albomitomycins; **6** and **7**, respectively in aqueous solutions. However isomitomycins were not found either in solutions of **1** nor **2**. Here we report on the isolation of albomitomycins from **1** and **2**.

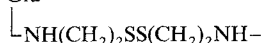
In a protic solution of **1** the conversion to an equilibrium mixture started when **1** was solubilized. Within few hours **6** reached the routinely detectable concentration and gradually increased to 6% (relative area ratio, obsd. at 254 nm, by ODS HPLC) at room temperature and the mixture attained equilibrium within few days in various conditions. Weak Lewis acids such as triisopropoxyaluminum accelerated this rearrangement reaction as previously reported.[5] However the presence of isomitomycin C was not confirmed by ordinary methods including a photo-diode array detector. Low solubility of **1** and instability of **6** in various solvents made it difficult to isolate **6**. The conditions, *e.g.*, triisopropoxyaluminum (1 equivalent)/MeOH, NaH (1 equivalent)/DMF-benzene, and DBU (1 equivalent)/DMF gave a good conversion ratio with adequate solubility. We employed the fastest conditions; MeOH reflux, since we needed multiple experiments due to low isolated yield (1 to 1.6%) of **6** caused by **6**'s reconversion to **1** in the isolation process. Synthetic conversion of **1** to **6**, that was successful in the conversion of **3** to **4**,[6] resulted in only trace amounts of **6** due to **6**'s instability.

The investigation of physico-chemical properties of **2** revealed that **2** was also in equilibrium with the albomitomycin of **2**; **7**, in aqueous solutions. However the isomitomycin of **2** was not found. Separation was made by preparative HPLC. A fraction containing **7** was trapped at −78°C, lyophilized, and stored at −20°C. However, directly after the lyophilization, it was observed by HPLC that a part of **7** converted to **2** and the purity was only 95% (relative area ratio, obsd. at 254 nm, by ODS HPLC). Even under storage at −20°C, **7** gradually converted to **2** accompanied by a change in hue to **2**'s characteristic bluish purple color in several weeks. The half life of **7** in aqueous solution (5 mg/ml, 26°C) was *ca.* 50 minutes. However highly purified **2** was obtained by the same procedure as in the case of **7**, since the



	X	No.		X	No.		
Mitomycin C	NH_2	**1**	Albomitomycin A	OMe	**4**	Isomitomycin A	**5**
KW-2149	R	**2**	Albomitomycin C	NH_2	**6**		
Mitomycin A	OMe	**3**		R	**7**		

R: L-Glu



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equilibrium trended to **2**.

It is noteworthy that both **1** and **2** could not be converted to isomitomycins as an energetically stable form, compared with **3**. Thermodynamic stability of an albomitomycin or an isomitomycin is determined by its C-7 substituent. The equilibrium ratio and rate between mitomycin and albomitomycin are governed by conditions, *e.g.*, solvent, pH, and temperature.

Since **6** and **7** were unstable in aqueous solutions and readily converted to **1** and **2**, inherent activity of these compounds might originate from mitomycin skeleton. The rates of metabolism and cellular uptake of **6** and **7** might differ from those of **1** and **2**, resulting in different biological responses. However preliminary *in vitro* and *in vivo* evaluations of **6** and **7** showed quite similar results in comparison with **1** and **2** (data not shown). In particular consideration of their fast equilibrium under physiological conditions, the potency of an albomitomycin should be comparable to that of a corresponding mitomycin. These preliminary results should not reflect the sole activity of an albomitomycin, because its conversion to a corresponding mitomycin should continue during the evaluation experiments, although quantitative analysis failed. In conclusion we could not find any particular characteristic of **6** and **7** that differed from **1** and **2** in biological activities.

Physical and Spectroscopic Data

Melting points were recorded on a Yanagimoto melting-point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer. MS spectra were recorded on a JMS-01SG-2 for EI-MS and a Hitachi M-80B spectrometer for SI-MS. IR spectra were recorded on a Shimadzu IR-27-G spectrometer. UV spectra were recorded on a Shimadzu spectrometer MPS-50L. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter.

Separation of 1 and 6

Compound **1** (200 mg) was dissolved in methanol (12 ml), and the solution was refluxed for 4 hours. The solution was applied to the column packed with Florisil (40 ml) and eluted with chloroform - methanol (93 : 7, v/v) within 10 minutes. The fractions containing **6** were concentrated *in vacuo* and applied to a flash column chromatography with CIG ID-10 packed with silica gel using chloroform - methanol (9 : 1, v/v) within 10 minutes. The colorless fractions were concentrated *in vacuo* to give a colorless paste (2.4 mg, yield 1.2%). The paste was stored at -15°C for 7 days to give colorless prisms.

6: mp 120°C (decomp.); $[\alpha]_D^{26}$ $+34.7°$ ($c=0.3$ in chloroform); EI-MS: Experimental m/z 334.1273 (M^+, molecular formula: $C_{16}H_{19}N_3O_6$; Calculated: 334.1277); UV (methanol): λ_{max} (log ε) 242 nm (3.7), 344 nm (3.8); ^{1}H NMR ($CDCl_3$) δ 1.86 (3H, s, 6-CH_3), 2.33 (1H, d, $J=3.1$ Hz, H-1), 2.79 (1H, br. d, $J=2.8$ Hz, H-2), 2.88 (1H, br. d, $J=11.3$ Hz, H-3), 2.91 (1H, d, $J=11.3$ Hz, H'-3), 3.22 (1H, br. q, $J=6.1$ Hz, H-9), 3.26 (1H, d, $J=5.6$ Hz, H-8a), 3.37 (3H, s, 9a-OCH_3), 4.28 (1H, dd, $J=11.2$, 6.1 Hz, H-10), 4.49 (1H, dd, $J=11.2$, 5.8 Hz, H'-10), 5.05 (2H, br. s, $CONH_2$), 5.16 (2H, br. s, 7-NH_2); ^{13}C NMR ($CDCl_3$) δ 9.5 (q, $J=128$ Hz, 6-CH_3), 32.3 (d, $J=191$ Hz, C-1), 32.8 (d, $J=191$ Hz, C-2), 37.7 (d, $J=137$ Hz, C-9), 49.4 (t, $J=145$ Hz, C-3), 51.1 (d, $J=142$ Hz, C-8a), 52.5 (q, $J=144$ Hz, 9a-OCH_3), 63.5 (t, $J=144$ Hz, C-10), 86.9 (s, C-4a), 107.4 (s, C-9a), 116.2 (s, C-6), 147.0 (s, C-7), 156.7 (s, $CONH_2$), 188.6 (s, C-5), 192.8 (s, C-8). Assignments of the carbon signals were done by SEL and LSPD experiments; IR (KBr) 3345, 1710, 1605, 1407, 1333 cm^{-1}.

Separation of 2 and 7

Compound **2** (45 mg, containing **7**) was dissolved in water (1.8 ml). The solution was applied to preparative HPLC using a packed column YMC D-ODS-7 (Yamamura Kagaku K.K., Japan). Elution was effected with a solvent system of water - acetonitrile (3 : 1, v/v) at a flow rate of 9.6 ml/minute. The first colorless fraction was eluted at 7.8 ~ 8.4 minutes after the injection. Directly after the elution, the fraction was cooled at -78°C and lyophilized to afford slightly bluish powders of **7** (1.5 mg). The resulting powders were subjected to HPLC using a packed column YMC AM-312 (Yamamura Kagaku K.K., Japan) and a solvent system of water - methanol (1 : 1, v/v) at a flow rate of 1 ml/minute. At a retention time of 4.2 minutes, a main peak at 254 nm was observed (area intensity: 95%). Second, in the elution of preparative HPLC blue fractions observed at 11.7 ~ 13.5 minutes after the injection were combined. The combined fractions were lyophilized to afford grayish blue powders of **2** (33 mg). The resulting powders were analyzed by the same manner as **7** (retention time: 6.0 minutes, $>99\%$ at 254 nm).

7: SI-MS (matrix: glycerol): m/z 599 $(M+1)^+$ (molecular formula $C_{24}H_{34}N_6O_8S_2$: 598.7); ^{1}H NMR (D_2O, 400 MHz) δ 1.92 (3H, s, 6-CH_3), 2.14 (2H, m, Glu βCH_2), 2.43 (2H, m, Glu γCH_2), 2.70 (1H, d, $J=3.3$ Hz, H-1), 2.84 (1H, d, $J=6.6$ Hz, H-3α, tentative), 2.85 (2H, m, 7-*N*-CγH_2), 2.95 (1H, d, $J=3.2$ Hz, H-2), 3.00 (2H, m, 7-*N*-CβH_2), 3.02 (1H, d, J was not observed by overlapping, H-3β, tentative), 3.44 (3H, s, 9a-OCH_3), 3.51 (2H, m, H-8a), 3.51 (1H, m, H-9), 3.53 (2H, t, $J=6.3$ Hz, 7-*N*-CδH_2), 3.76 (1H, t, $J=6.2$ Hz, Glu αCH), 3.82 (1H, m, 7-*N*-CαH), 3.94 (1H, m, 7-*N*-CαH), 4.31 (1H, dd, $J=11.5$, 6.5 Hz, H-10), 4.41 (1H, dd, $J=11.5$, 5.5 Hz, H'-10); IR (KBr) 3420, 3350, 3080, 2950, 1705, 1630, 1570, 1510, 1450, 1400, 1335, 1240 cm^{-1}.

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