THE STRUCTURE OF TAUTOMYCIN, A DIALKYLMALEIC ANHYDRIDE ANTIBIOTIC

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The chemical structure of tautomycin ($C_{41}H_{66}O_{13}$) was determined by chemical degradation, spectroscopic analysis and 2D INADEQUATE of tautomycin labeled with [1,2-^{13}C]acetate. Tautomycin exists in methanol-buffer solution (1% diethylamine-formic acid, pH 7.3) as an equilibrium mixture of a 2,3-dialkylmaleic anhydride and its dicarboxylic acid in a ratio of approximately 5:4.

In the course of screening of soil microorganisms for new antibiotics for agricultural use, a strain of *Streptomyces spiroverticillatus* was found to produce a new antibiotic, tautomycin (**1**), which has high activity against *Sclerotinia sclerotiorum*. Later, the antibiotic was found to induce the morphological change (bleb formation) of human leukemia cells K562, which is correlated with protein phosphorylation. The biological activity of tautomycin has been described[1,2]. This paper presents detailed experimental results and discussion of the structure elucidation of tautomycin.

The purified tautomycin gave two peaks on the HPLC column[1]. Each peak was collected and analyzed by HPLC in the same condition. Immediately after the separation, each fraction showed virtually a single peak. However, a counter peak appeared and gradually increased. Equilibrium was reached after standing over 12 hours at room temperature. The ratio of the mixture was approximately 5:4.

HR positive FAB-MS of the pseudo molecular ion $(M+Na)^+$ from tautomycin was recorded as *m/z* 789.4380, and corresponds to a formula of $C_{41}H_{66}O_{13}Na$. FD-MS (*m/z* 767, $(M+H)^+$) and the total number of carbons detected by ^{13}C NMR spectrum confirmed the molecular formula. IR absorption bands at 1825 and 1755 cm^{-1}, and UV maximum at 250 nm in CH_3CN suggested the presence of a 2,3-dialkylmaleic anhydride structure[3]. IR absorption bands at 1730 and 1700 cm^{-1} suggested the presence of ester and ketone. ^{1}H NMR spectrum of tautomycin in DMSO-d_6 showed three deuterium exchangeable hydroxy protons (5.84, 4.66 and 4.46 ppm). After a week in a NMR tube in DMSO-d_6, two carboxyl protons at 12.5 ppm appeared. This suggests that the acid anhydride had been hydrolyzed by a trace amount of water in DMSO-d_6. The quaternary carbon at 95.4 ppm in ^{13}C NMR spectrum suggests the presence of a six membered spiroketal moiety[4]. Homonuclear proton spin decoupling experiment in connection with ^{1}H-^{13}C COSY, ^{1}H-^{1}H COSY and heteronuclear multiple bond correlation (HMBC) spectra of **1** revealed the partial structures (A), (B), (C), (D) and (E) as shown in Fig. 1. Treatment of **1** at pH 9 (20% Cs_2CO_3, MeOH) resulted in the hydrolysis of an ester bond, dehydration between C-21 and C-22 and gave the anhydrodeacyltautomycin **2** and an acid **3** as shown in Fig. 2. The fully-decoupled (COM) and INEPT ^{13}C NMR spectrum and ^{1}H-^{13}C COSY spectrum of **2** indicated the presence of $8\times CH_3$, $8\times CH_2$, $6\times CH$, $5\times CHO$, $2\times CH{=}$, $2\times C{=}O$, $1\times OCO$ and $1\times OCH_3$. SI-MS (*m/z* 567 $(M+H)^+$) and the NMR data of **2** indicated the molecular formula of $C_{33}H_{58}O_7$ accounting for three double bonds and two rings. The coupling constant ($J=16$ Hz) between 21-H at 6.36 ppm and 22-H at

Fig. 1. Partial structure of tautomycin.

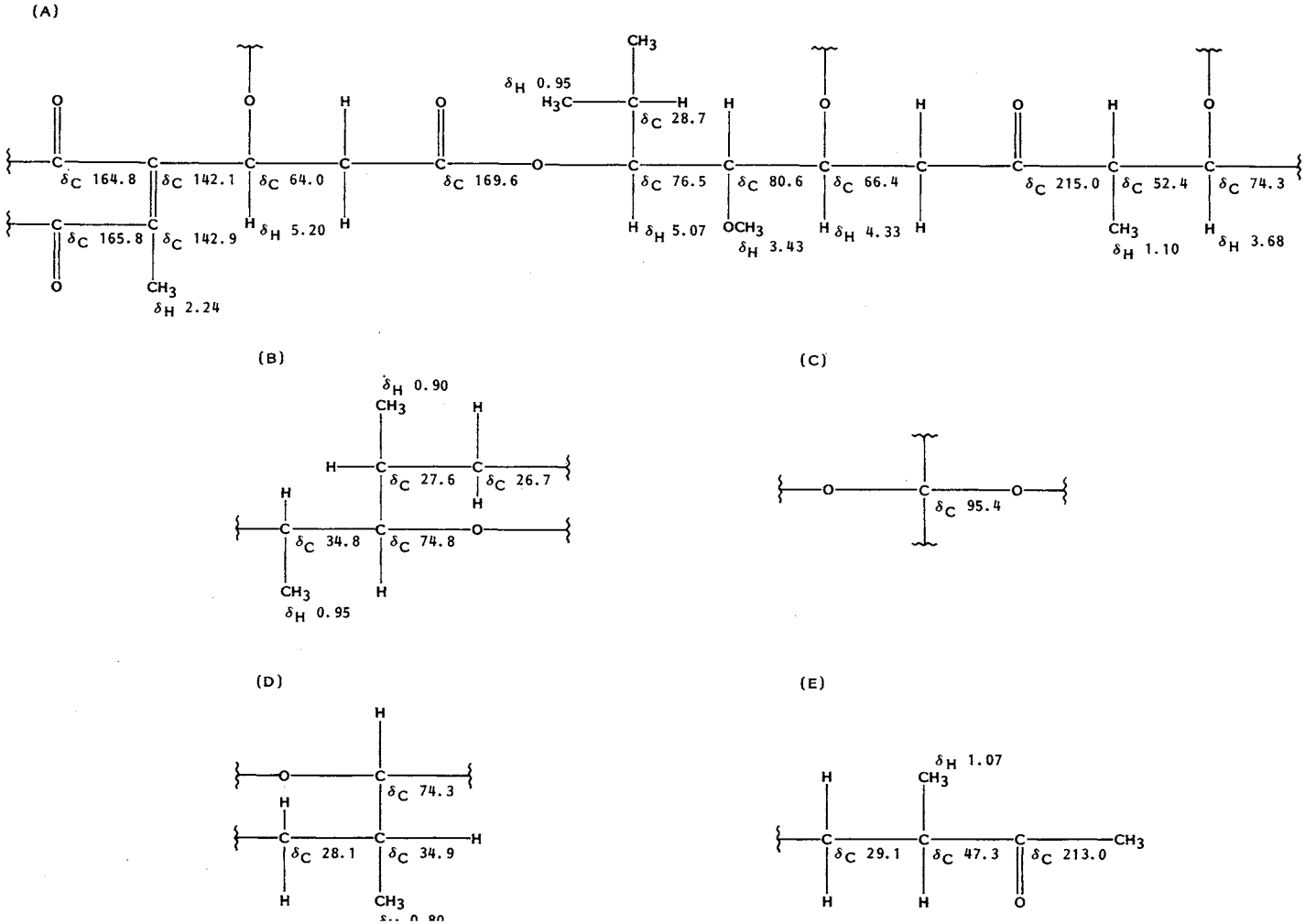
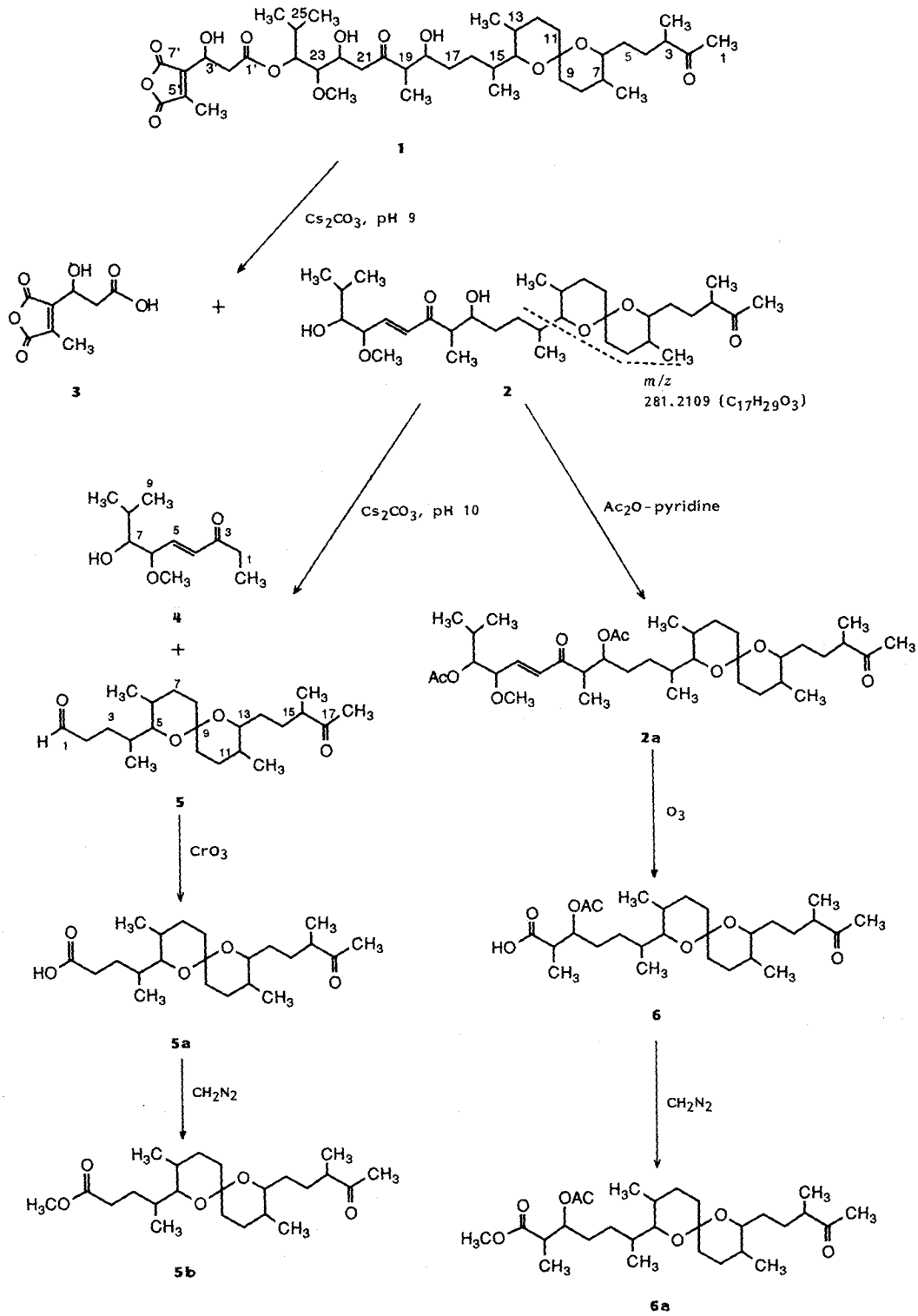


Fig. 2. Degradation of tautomycin.



6.83 ppm suggested the *E*-configuration of the double bond. HREI-MS gave an intense fragment ion (*m/z* 281.2109, $C_{17}H_{29}O_3$, Δ 0.5 mmu) as shown in Fig. 2.

Acetylation of **2** (Ac_2O-pyridine) gave a diacetate **2a** (*m/z* 651 $(M+H)^+$). SI-MS and the number of carbons led to the molecular formula of $C_{37}H_{62}O_9$ which is consistent with the molecular formula of **2**. Table 1 presents the NMR data of **2** and **2a**.

When the methanol solution of **2** was adjusted to pH 10 (20% Cs_2CO_3), retro-aldol cleavage[5] occurred and compounds **4** and **5** were obtained (Fig. 2). Table 2 presents NMR data for **4**. The data of SI-MS (*m/z* 201 $(M+H)^+$) and the number of carbons indicated the molecular formula of $C_{11}H_{20}O_3$ for **4**. Homonuclear proton spin decoupling experiment and ^{13}C NMR data established the structure of **4**. The coupling constant ($J=17$ Hz) between 4-H (6.25 ppm, d) and 5-H (6.75 ppm, dd) indicated *E*-configuration of the double bond. Table 3 presents the NMR data of **5**. The molecular formula ($C_{22}H_{38}O_4$) of **5**

Table 1. ^{13}C and ^1H NMR chemical shifts of **2** and **2a** in $CDCl_3$.

	2		**2a**	
	^{13}C (100 MHz) ppm	^1H (400 MHz) ppm (*J*, Hz)	^{13}C (100 MHz) ppm	^1H (400 MHz) ppm (*J*, Hz)
C-1	28.1 q	2.15 s	28.0 q	2.15 s
C-2	212.5 s	—	213.0 s	—
C-3	47.3 d	2.55 m	47.2 d	2.55 m
3-CH_3	16.2 q	1.10 d, $J=6.5$	16.2 q	0.98 d, $J=6.5$
C-4	29.9 t	1.65 m	29.0 t	1.62 m
C-5	30.2 t	1.25, 1.55 m	30.2 t	1.25, 1.55 m
C-6	74.3 d	3.17 dt, $J=9.9$, 2.4	75.0 d	3.15 dt, $J=9.9$, 2.4
C-7	34.9 d	1.26 m	34.5 d	1.25 m
7-CH_3	18.0 q	0.80 d, $J=6.5$	17.9 q	0.80 d, $J=6.5$
C-8	28.1 t	1.45 m	27.9 t	1.45, 1.58 m
C-9	36.1 t	1.45, 1.62 m	36.0 t	1.45, 1.62 m
C-10	95.7 s	—	95.5 s	—
C-11	30.7 t	1.37, 1.55 m	30.6 t	1.37, 1.55 m
C-12	26.8 t	1.38, 2.00 m	26.7 t	1.37, 2.00 m
C-13	27.6 d	1.85 m	27.5 d	2.00 m
13-CH_3	11.0 q	0.90 d, $J=6.5$	10.8 q	0.90 d, $J=6.5$
C-14	74.9 d	3.27 dd, $J=9.9$, 2.4	74.6 d	3.22 dd, $J=9.9$, 2.4
C-15	34.9 d	1.55 m	34.5 d	1.55 m
15-CH_3	15.8 q	1.00 d, $J=6.5$	16.6 q	0.90 d, $J=6.5$
C-16	27.9 t	1.52, 1.83 m	27.6 t	1.52, 1.86 m
C-17	31.8 t	1.34, 1.62 m	30.6 t	1.25, 1.62 m
C-18	74.3 d	3.70 m	74.5 d	5.08 dt, $J=2.0$, 7.1
$COCH_3$	—	—	170.5 s	—
	—	—	20.9 q	2.00 s
C-19	48.7 d	2.96 m	46.5 d	3.15 m
19-CH_3	14.8 q	1.18 d, $J=6.5$	12.0 q	0.90 d, $J=6.5$
C-20	204.0 s	—	200.0 s	—
C-21	132.1 d	6.36 d, $J=16$	131.0 d	6.35 dd, $J=16$
C-22	142.7 d	6.83 dd, $J=16$, 6.7	143.0 d	6.78 dd, $J=16$, 6.7
C-23	82.1 d	3.83 dd, $J=8.7$, 4.4	81.0 d	3.85 dd, $J=8.7$, 4.4
23-OCH_3	57.2 q	3.35 s	57.4 q	3.35 s
C-24	77.9 d	3.50 dd, $J=10.2$, 2.0	77.5 d	4.90 dd, $J=10.2$, 2.0
$COCH_3$	—	—	170.5 s	—
	—	—	20.9 q	2.00 s
C-25	29.1 d	1.67 m	28.5 d	1.95 m
25-CH_3	18.9 q	0.90 d, $J=6.5$	19.2 q	1.08 d, $J=6.5$
C-26	18.2 q	1.00 d, $J=6.5$	17.4 q	1.08 d, $J=6.5$

Table 2. ^{13}C and ^{1}H NMR chemical shifts of **4** in $CDCl_3$.

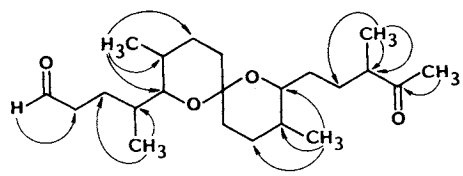
	^{13}C (100 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)		^{13}C (100 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)
C-1	8.0 q	1.10 t, J=7.7	C-7	77.5 d	3.47 dd, J=3.8, 7.7
C-2	30.0 t	2.65 q, J=7.7	C-8	33.5 d	1.67 m
C-3	201.0 s	—	C-9	19.0 q	1.00 d, J=7.7
C-4	133.0 d	6.25 d, J=17	C-10	18.5 q	0.90 d, J=7.7
C-5	141.0 d	6.75 dd, J=17	C-11	57.0 s	3.33 s
C-6	82.5 d	3.80 dd, J=3.8, 7.7			

Table 3. ^{13}C and ^{1}H NMR chemical shifts of **5**.

	^{13}C (100 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)		^{1}H (100 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)
C-1	203.0 d	9.8 t, J=2.5	C-10	36.0 t	1.45, 1.62 m
C-2	40.5 t	2.45, 2.50 m	C-11	24.0 t	1.45, 1.58 m
C-3	27.5 t	1.60 m	C-12	34.9 d	1.25 m
C-4	34.0 d	1.68 m	12-CH_3	18.0 q	0.80 d, J=7.6
4-CH_3	16.4 q	1.00 d, J=7.6	C-13	74.2 d	3.15 dt, J=3.0, 9.6
C-5	74.8 d	3.28 dd, J=7.6, 6.8	C-14	30.5 t	1.25, 1.54 m
C-6	27.5 d	1.85 m	C-15	29.0 t	1.62 m
6-CH_3	10.9 q	0.90 d, J=7.6	C-16	47.4 d	2.55 m
C-7	27.0 t	1.38, 2.00 m	16-CH_3	16.4 q	1.10 d, J=7.6
C-8	30.0 t	1.38, 1.55 m	C-17	213 s	—
C-9	95.4 s	—	C-18	28.1 q	2.15 s

was established by HR positive FAB-MS (m/z 367.2857, (M+H), Δ 0.9 mmu), negative FAB-MS (m/z 365 (M−H)). The signal at 9.8 ppm in ^{1}H NMR spectrum shows the presence of aldehyde. Heteronuclear multiple bond correlation (HMBC) and correlation spectroscopy *via* long-range coupling (COLOC) spectra of **5** showed ^{1}H-^{13}C long range coupling pattern as shown in Fig. 3. The aldehyde **5** could be converted to carboxylic acid by Jones oxidation. Esterification of **5a** with diazomethane gave compound **5b** ($C_{23}H_{40}O_5$, EI-MS m/z 396 M^+) (Fig. 2).

Fig. 3. ^{1}H-^{13}C Long range coupling pattern of **5**.



Ozonolysis of **2a** (O_3, −78°C, H_2O_2) gave carboxylic acid **6** (Fig. 2). EI-MS (m/z 482 M^+) and the ^{13}C NMR data gave the molecular formula of $C_{27}H_{46}O_7$ HREI-MS of **6** gave a fragment ion (m/z 281, $C_{17}H_{29}O_3$), which is the same fragment ion in EI-MS of **2a**. Esterification of **6** with diazomethane gave the methyl ester **6a** (EI-MS, m/z 496 M^+).

Treatment of acid **3** with methanolic H_2SO_4 gave monomethyl ester **3a** (EI-MS, m/z 215 $(M+H)^+$) and trimethyl ester **3b** (EI-MS, m/z 261 $(M+H)^+$) (Fig. 4). Table 4 presents NMR data for **3a** and **3b**. The IR (1830 and 1760 cm^{-1}) and UV (250 nm in CH_3CN) spectra suggested the dialkylmaleic anhydride structure of **3a**. No carboxyl proton was detected in ^{1}H NMR spectrum of a fresh solution of **3a** in DMSO-d_6. However, after a week in a NMR tube at room temperature, two carboxyl protons at 12.6 ppm appeared and reached an equilibrium. NOE between CH_3 at 2.11 ppm and 3-H at 4.97 ppm (3.5% enhancement, DMSO-d_6) confirmed the 2,3-dialkylmaleic anhydride structure and excluded the possibility of presence of lactone structure. The IR and UV spectra of **3b** also revealed the absence of anhydride moiety

Fig. 4. Structure of **3a** and **3b**.

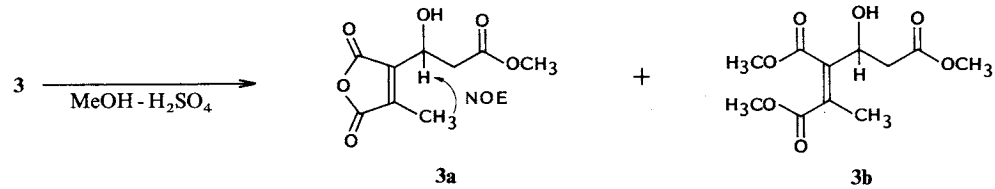


Table 4. ^{13}C and ^{1}H NMR chemical shifts of **3a** and **3b** in $CDCl_3$.

	3a		**3b**	
	^{13}C (100 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)	^{13}C (100 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)
C-1	171.6 s	—	171.5	—
OCH_3	52.0 q	3.78 s	52.0 q	3.72 s
C-2	39.0 t	2.88 m	39.5 t	2.58 dd, J=4.0, 17.0, 2.95 dd, J=10.0, 17.0
C-3	64.0 d	5.13 dd, J=4.0, 8.5	66.0 d	5.10 dd, J=4.0, 10.0
C-4	141.7 s	—	131.0 s	—
C-5	143.1 s	—	140.0 s	—
C-6	166.0 s	—	167.0 s	—
OCH_3	—	—	51.5 q	3.72 s
C-7	164.6 s	—	167.5 s	—
OCH_3	—	—	52.0 q	3.80 s
C-8	10.0 q	2.28 s	14.0 q	2.00 s

(disappearance of 1830 and 1760 cm^{-1} in IR and 250 nm in UV spectrum). The above data in connection with HMBC spectrum and homonuclear proton spin decoupling experiments established the structure of **3a** and **3b**.

Both **1** and **3** exist in methanol - buffer (1% diethylamine - formic acid, pH 7.3) solution as an equilibrium mixture (approximately, 5:4). No carboxyl proton was detected in ^{1}H NMR spectrum of a fresh solution of **1** in DMSO-d_6. After a week two carboxyl protons at 12.5 ppm, new signals of 3'-H at 4.90 ppm and 3'-OH at 5.45 ppm appeared. The ratio between the original and new signals was approximately 6:4, which remained unchanged even after addition of water.

Ring opening and closure of **1** were shown by oxygen exchange experiment. Treatment of **1** with $H_2{}^{18}O$ for 4 hours in CH_3CN gave peaks 2, 4 and 6 mass units higher as detected by FD-MS $(M+Na)^+$ m/z 789 (100%), 791 (80%), 793 (45%), 795 (5%). The ratio of incorporation of ^{18}O into **1** increased with time. Extraction with ethyl acetate at pH 4 resulted in the ring closure. Above data gave the clear evidence of 2,3-dialkylmaleic anhydride structure of tautomycin. 2,3-Dimethylmaleic anhydride as a model compound also showed the similar behavior in ^{1}H and ^{13}C NMR spectra. The equilibrium of 2,3-dialkylmaleic anhydride was reported in the earlier paper[6].

To confirm the skeleton of **1**, a biosynthetic study[7] was carried out. Feeding experiments using ^{13}C-labeled acetate and propionate showed clearly the labeling pattern as shown in Fig. 5, and supported the spiroketal ring structure. Feeding of [1,2-^{13}C]acetate resulted in considerable randomization in incorporation into **1**. Thus the carbons that should be derived from C-1, C-2 and C-3 of propionate were also enriched. A similar randomization was also observed in cationomycin[8]. 2D INADEQUATE spec-

Fig. 5. ^{13}C Labeling pattern of partial structure of tautomycin.

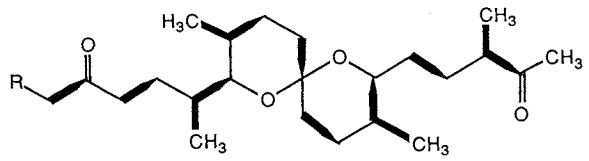


Fig. 6. 2D INADEQUATE spectrum of tautomycin labeled with [1,2-^{13}C]acetate.

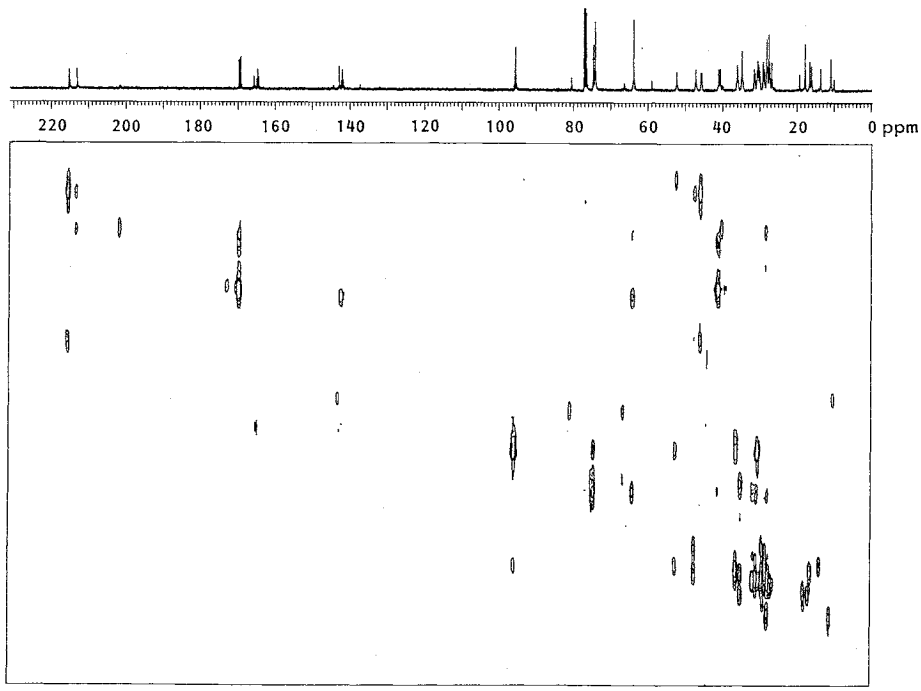
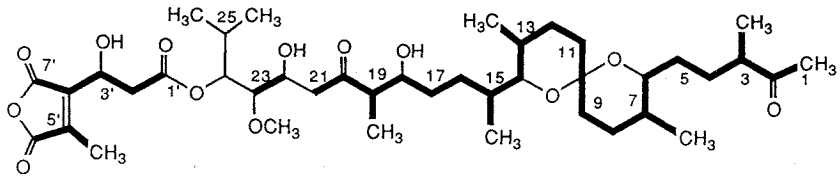


Fig. 7. Carbon-carbon connectivity pattern of tautomycin labeled with [1,2-^{13}C]acetate.



trum (Fig. 6) of [1,2-^{13}C]acetate labeled tautomycin gave the carbon-carbon connectivity pattern of **1** as shown in bold lines (Fig. 7)[9]. Table 5 presents the total ^{13}C and ^{1}H NMR assignment of tautomycin.

Tautomycin has a unique 2,3-dialkylmaleic anhydride moiety. Some secondary metabolites, such as byssochlamic acid[10], glauconic acid[11], gluacanic acid[11], heveadride[12], sipitatonic acid[13] and 2-carboxymethyl-3-hexylmaleic anhydride[3] also contain dialkylmaleic anhydride. However, their structures are totally different from tautomycin. The biological activities are also different. Later, we found another new antibiotic, tautomycetin[14], from another species of *Streptomyces*, whose structure is similar to tautomycin. They have the common 2-(3'-hydroxycarboxyethyl)-3-methylmaleic anhydride moiety.

Table 5. ^{13}C and ^{1}H NMR chemical shifts of tautomycin in $CDCl_3$.

	^{13}C (100 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)		^{13}C (100 MHz) ppm	^{1}H (400 MHz) ppm (J, Hz)
C-1	28.1 q	2.12 s	19-CH_3	13.7 q	1.08 d, J=7.4
C-2	213.0 s	—	C-20	215.0 s	—
C-3	47.4 d	2.52 m	C-21	45.8 t	2.63, 2.97 dd, J=8.9, 13.1 overlaping
3-CH_3	16.2 q	1.07 d, J=7.4			
C-4	29.1 t	1.62 m			
C-5	30.7 t	1.25, 1.54 m	C-22	66.4 d	4.33 ddd, J=2.2, 4.7, 8.9
C-6	74.3 d	3.13 dt, J=9.0, 1.8			
C-7	34.9 d	1.24 m	C-23	80.6 d	3.25 dd overlaping
7-CH_3	17.9 q	0.80 d, J=7.4	OCH_3	59.1 q	3.42 s
C-8	28.1 t	1.45, 1.58 m	C-24	76.5 d	5.07 dd, J=6.3, 6.3
C-9	36.1 t	1.45, 1.62 m	C-25	28.7 d	2.08 m
C-10	95.4 s	—	25-CH_3	19.4 q	0.95 d, J=7.4
C-11	30.2 t	1.38, 1.55 m	C-26	17.8 q	0.95 d, J=7.4
C-12	26.7 t	1.38, 2.00 m	C-1′	169.6 s	—
C-13	27.6 d	1.82 m	C-2′	40.9 t	2.75, 2.90 dd, dd, J=9.4, 16.0; 3.0, 16.0
13-CH_3	10.9 q	0.90 d, J=7.4			
C-14	74.8 d	3.23 dd overlaping			
C-15	34.8 d	1.54 m	C-3′	64.0 d	5.18 dd, J=9.4, 3.0
15-CH_3	16.7 q	0.95 d, J=7.4	C-4′	142.1 s	—
C-16	27.4 t	1.52, 1.83 m	C-5′	142.9 s	—
C-17	31.8 t	1.27, 1.61 m	5′-CH_3	10.2 q	2.24 s
C-18	74.3 d	3.68 m	C-6′	165.8 s	—
C-19	52.0 d	2.62 m	C-7′	164.8 s	—

Tautomycin and tautomycetin are a new class of antibiotics having unique structures and biological activities.

Experimental

General Methods

The ^{1}H NMR spectra and 2D NMR spectra were recorded on a JMN GX-400 FT-NMR spectrometer, and ^{13}C NMR spectra were recorded on a Jeol FX 100 FT-NMR spectrometer. UV spectra were recorded on a Hitachi 220 A spectrophotometer, IR spectra were recorded on a Shimadzu IR spectrophotometer IR-27 G. Optical rotations were measured on Perkin-Elmer 241 MC polarimeter. EI-MS, SI-MS and HREI-MS were recorded on Hitachi M-80 spectrometer. Positive and negative FAB-MS were performed on JMS DSX-300 and SX-102 mass spectrometers.

All reactions were monitored by TLC carried out on 0.25 mm E. Merck Silica gel plates ($60F_{254}$). Preparative TLC was performed on 0.5 mm 20×20 cm E. Merck Silica gel plates ($60F_{254}$). E. Merck Silica gel (60 particle size 63~200 μm) was used for column chromatography.

Preparation and Purification of Tautomycin

Fermentation was carried out at 28°C for 72 hours in a jar fermenter containing 18 liters of the medium which is composed of glucose 1%, soluble starch 0.5%, meat extract 0.05%, dry yeast 0.2%, soybean flour 1.25%, NaCl 0.1% and K_2HPO_4 0.0025%. The filtered broth (36 liters) was extracted with EtOAc at pH 4. The mycelial cake was extracted with acetone (15 liters). The extract was evaporated *in vacuo* to give an aqueous solution which was extracted with EtOAc twice (5 liters). The combined organic layer was washed with H_2O, dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. A portion of an oily residue, 5.27 g (total amount 7.93 g) was applied onto a silica gel column (size 7.5×30 cm) and developed successively with the solvents, $CHCl_3$-MeOH, 10:1 (2 liters), 4:1 (2 liters), 2:1 (1 liter) and 1:1 (1 liter) into 1 liter portions. Active portions were collected and concentrated *in vacuo* to give a yellowish oily material (1.20 g). Further purification of the oily material carried out by HPLC (Senshu-Pak-ODS-H,

250 × 20 mm, i.d., MeOH - H_2O - buffer (1% diethylamine - formic acid, pH 7.3) 80 : 10 : 10) gave a pure sample of **1** (472 mg). **1**: Hygroscopic amorphous white powder, $[\alpha]_D^{25}$ +3.4° (*c* 1, $CHCl_3$); UV $\lambda_{max}^{CH_3CN}$ nm (ε) 217 (46.5), 250 (sh); IR ν_{max} (KBr) cm^{-1} 3400, 1825, 1755, 1730, 1700; HRFAB-MS (*m/z* 789.4376 $(M+Na)^+$).

Anal Calcd for $C_{41}H_{66}O_{13}$: C 63.48, H 8.25.
Found: C 63.36, H 8.69.

Hydrolysis of Tautomycin

To a stirred solution of tautomycin **1** (83 mg 0.11 mmol) in MeOH (2 ml) was added dropwise 20% Cs_2CO_3 solution until the mixture solution reached pH 9. The reaction mixture was stirred at room temperature for 3 hours. The solution was adjusted to pH 4 with 0.1 N HCl, and the MeOH was evaporated off. The resulting mixture was extracted with EtOAc. The organic solution was washed with H_2O, and dried over Na_2SO_4. After filtration, the EtOAc was evaporated off. The residue was subjected to preparative TLC (silica gel, MeOH - $CHCl_3$, 1 : 50, Rf 0.30) to give compound **2** (40 mg, 64% yield). Further purification could be achieved by HPLC (Senshu-Pak-ODS-H, MeOH - H_2O, 86 : 14). **2**: Colorless oil, Rf 0.30 (silica gel TLC; MeOH - $CHCl_3$, 1 : 50); $[\alpha]_D^{25}$ −21.9° (*c* 2.5, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 223 (213); IR ν_{max} (film) cm^{-1} 3450, 1720, 1690, 1650; SI-MS (*m/z* 567 $(M+H)^+$).

Anal Calcd for $C_{33}H_{58}O_7$: C 69.96, H 10.25.
Found: C 69.75, H 10.45.

Acetylation of **2**

Compound **2** (20 mg, 0.035 mmol) was dissolved in dry pyridine (0.5 ml) and acetic anhydride (0.5 ml). The reaction mixture was stirred at room temperature for 12 hours. After quenching with ice, the mixture was extracted with EtOAc (30 ml). The EtOAc extract was washed successively with 1 N HCl (10 ml), saturated aqueous $NaHCO_3$ (10 ml) and H_2O (30 ml), and then dried over Na_2SO_4. After concentration *in vacuo* to dryness, the residue was subjected to preparative TLC (silica gel, EtOAc - benzene, 25 : 75) to give compound **2a** (12.7 mg, 56% yield). Further purification could be achieved by HPLC (Senshu-Pak-ODS-H, MeOH - H_2O - buffer (1% diethylamine - formic acid, pH 7.3) 85 : 5 : 10) **2a**: Colorless oil; Rf 0.40 (silica gel TLC; EtOAc - benzene, 25 : 75); $[\alpha]_D^{25}$ +23.3° (*c* 0.6, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 223 (276); IR ν_{max} (film) cm^{-1} 1730, 1700, 1600; SI-MS (*m/z* 651 $(M+H)^+$).

Anal Calcd for $C_{37}H_{62}O_9$: C 68.31 H 9.54.
Found: C 68.33 H 9.67.

Retro-aldol Cleavage of **2**

To a stirred solution of **2** (100 mg, 0.18 mmol) in MeOH (10 ml) was added dropwise 20% Cs_2CO_3 solution until the solution reached pH 10. The mixture was stirred for 3 hours at room temperature, and evaporated to remove MeOH. The aqueous solution was extracted with EtOAc. The organic solution was dried over Na_2SO_4. After filtration, the solution was evaporated to dryness. The residue was subjected to preparative TLC (silica gel, MeOH - $CHCl_3$, 1 : 50) to give compound **4** (21 mg, 58% yield) and compound **5** (16 mg, 24% yield). Further purification of **4** could be achieved by HPLC (Senshu-Pak-ODS-H, MeOH - H_2O, 86 : 14). **4**: Rf 0.87 (silica gel TLC; MeOH - $CHCl_3$, 1 : 50); $[\alpha]_D^{25}$ −27.0° (*c* 1, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 220 (134); IR ν_{max} (film) cm^{-1} 3400, 1700, 1650; SI-MS (*m/z* 201, $(M+H)^+$).

Anal Calcd for $C_{11}H_{20}O_3$: C 66.00, H 10.00.
Found: C 65.73, H 9.93.

Compound **5**: Rf 0.68 (silica gel TLC; MeOH - $CHCl_3$, 1 : 50); $[\alpha]_D^{25}$ −45.8° (*c* 1.35, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 220 (17); IR ν_{max} (film) cm^{-1} 2820, 1725, 1700; FAB positive (*m/z* 367 $(M+H)$), negative (*m/z* 365 $(M-H)$).

Anal Calcd for $C_{22}H_{38}O_4$: C 72.13, H 10.38.
Found: C 71.90, H 10.47.

Preparation of **5a** and **5b**

To a stirred solution of compound **5** (45 mg, 0.12 mmol) in acetone (10 ml) was added Jones reagent (CrO_3, H_2SO_4 in acetone, 10 drops) at 0°C. The mixture solution was stirred for 15 minutes at 0°C. After concentration to remove acetone, the aqueous solution was extracted with EtOAc. The organic layer was

washed with water and dried over Na_2SO_4. After filtration, the solution was evaporated to dryness. The residue was subjected to preparative TLC (silica gel, MeOH - $CHCl_3$, 1 : 50) to give compound **5a** (40 mg, 83% yield). To a solution of **5a** (40 mg, 0.10 mmol) in ether (20 ml) was added excess diazomethane in ether (20 ml) at 0°C. The reaction mixture was stirred at 0°C for 3 hours. After concentration to dryness *in vacuo*, the residue was subjected to preparative TLC (silica gel, benzene - EtOAc, 8 : 1) to give compound **5b** (36 mg, 90% yield). **5b**: Rf 0.50 (silica gel TLC; benzene - EtOAc, 8 : 1); $[\alpha]_D^{25}$ −37.3° (*c* 1.7, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 220 (7); IR ν_{max} (film) cm^{-1} 1730, 700; EI-MS (*m/z* 396 M^+).

Anal Calcd for $C_{23}H_{40}O_5$: C 69.70, H 10.00.
Found: C 69.72, H 10.14.

Ozonolysis of **2a**

Ozone was passed through the solution of **2a** (86 mg, 0.13 mmol) in CH_2Cl_2 (2 ml) at −78°C for 40 minutes. Excess ozone was removed by concentration *in vacuo*, and the residue was dissolved in a mixture of CH_2Cl_2 (2 ml), methanol (1 ml) and water (1 ml). 30% H_2O_2 (52 μl) was added at −10°C with stirring. The reaction mixture was adjusted to pH 4 with 0.1 N HCl and kept at 10°C for 2 hours. The solvent was evaporated *in vacuo*. The aqueous solution was extracted with EtOAc. The EtOAc solution was evaporated to dryness *in vacuo*. The residue was subjected to preparative TLC (silica gel, EtOAc - benzene, 1 : 4) to give compound **6** (34.6 mg, 55% yield). Further purification could be achieved by HPLC (Senshu-Pak-ODS-H, MeOH - H_2O - buffer (1% diethylamine - formic acid, pH 7.3) 86 : 4 : 10). **6**: Rf 0.1 (silica gel TLC; EtOAc - benzene, 1 : 4); $[\alpha]_D^{25}$ −27.6° (*c* 2, $CHCl_3$); UV λ_{max}^{MeOH} nm (ε) 205 (15); IR ν_{max} (film) cm^{-1} 1740, 1700; EI-MS (*m/z* 482 M^+).

Anal Calcd for $C_{27}H_{46}O_7$: C 67.22, H 9.54.
Found: C 66.40, H 9.65.

Methylation of **6**

To a stirred solution of **6** (24 mg, 0.05 mmol) in ether (20 ml) excess diazomethane in ether solution was added at 0°C. The reaction mixture was stirred at 0°C for 3 hours. After concentration to dryness *in vacuo* the residue was subjected to preparative TLC (silica gel, benzene - EtOAc, 8 : 1) to give compound **6a** (18 mg, 73 yield). **6a**: Rf 0.3 (silica gel TLC; benzene - EtOAc, 8 : 1); $[\alpha]_D^{25}$ −16.35° (*c* 1.1, $CHCl_3$): UV λ_{max}^{MeOH} nm (ε) 205 (8); IR ν_{max} (film) cm^{-1} 1730, 1700; EI-MS (*m/z* 496 M^+).

Anal Calcd for $C_{28}H_{48}O_7$: C 67.47, H 9.69.
Found: C 67.87, H 9.81.

Preparation of Monomethyl Ester of **3**

A water layer of the hydrolysate of **1** (tautomycin 1.1 g, 30% purity, 0.44 mmol) was acidified to pH 4 and lyophilized. The dry powder was suspended in methanol (10 ml). To the stirred suspension was added conc H_2SO_4 (1 ml) dropwise at 0°C. After 2 hours at room temperature water was added, and the mixture solution was extracted with EtOAc. The organic solution was washed with water and dried over Na_2SO_4. After filtration, the solution was evaporated to dryness *in vacuo*. The residue was subjected to preparative TLC (silica gel, MeOH - $CHCl_3$, 1 : 1) to give compound **3a** (12.6 mg, 13% yield). **3a**: Rf 0.42 (silica gel TLC, 50% MeOH in $CHCl_3$); UV $\lambda_{max}^{CH_3CN}$ nm (ε) 218 (22), 250 (24); IR (film) cm^{-1} 1830, 1760; EI-MS (*m/z* 215 $(M+H)^+$).

Anal Calcd for $C_9H_{10}O_6$: C 50.47, H 4.67.
Found: C 50.02, H 5.57.

Preparation of Trimethyl Ester of **3**

A water layer of the hydrolysate of **1** (tautomycin 259 mg, 0.33 mmol) was acidified to pH 4 and lyophilized. The dry powder was suspended in methanol (5 ml). To the stirred suspension was added dropwise conc H_2SO_4 (0.5 ml) at 0°C. After 48 hours at room temperature, the MeOH was removed by evaporation. The aqueous mixture solution was extracted with EtOAc. The organic solution was washed with H_2O and dried over Na_2SO_4. After filtration, the EtOAc solution was evaporated to dryness. The residue was subjected to preparative TLC (silica gel; MeOH - $CHCl_3$, 1 : 25) to give compound **3b** (48.5 mg,

56% yield); **3b**: $[\alpha]_D^{25}$ $-16.3°$ (*c* 0.3, $CHCl_3$); UV $\lambda_{max}^{CH_3CN}$ nm (ε) 225 (33); IR ν_{max} (film) cm^{-1} 1720; EI-MS (*m/z* 261 $(M+H)^+$).

Anal Calcd for $C_{11}H_{16}O_7$: C 50.77, H 6.15.
Found: C 50.62, H 6.31.

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