The Structure of Tautomycin, a Regulator of Eukaryotic Cell Growth

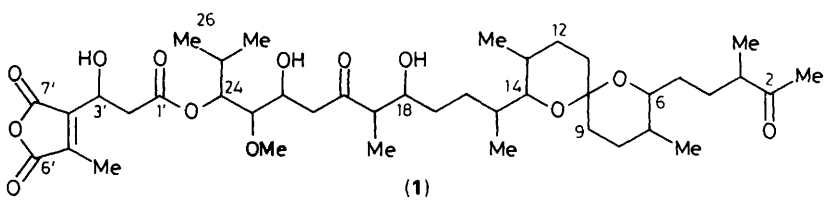
Makoto Ubukata, Xing-Chun Cheng, and Kiyoshi Isono*

Antibiotics Laboratory, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama 351-01, Japan

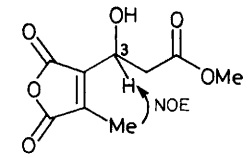
Structure (**1**) is proposed for tautomycin produced by *Streptomyces spiroverticillatus*, on the basis of chemical degradation and spectroscopic evidences including 2D INADEQUATE of tautomycin labelled with [1,2-^{13}C]acetate.

In our laboratory, an antifungal antibiotic, tautomycin, was isolated from a culture of *Streptomyces spiroverticillatus*.[1] Besides antifungal activity, tautomycin induced a morphological change (bleb formation) of human leukaemia cells K562, which is correlated with protein phosphorylation.[2] On the basis of chemical degradation and spectroscopic evidence, we propose structure (**1**) for tautomycin.

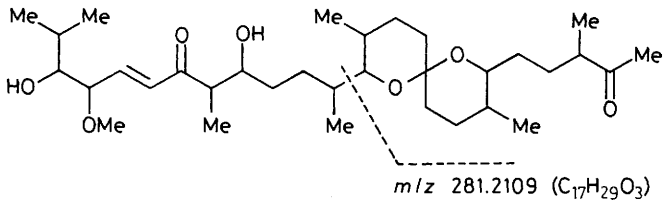
The field desorption (FD) mass spectrum (*m/z* 767, MH^+), high resolution positive FAB mass spectrum [*m/z* 789.4380 (M + Na)$^+$, Δ 2.1], and the total number of carbons detected by



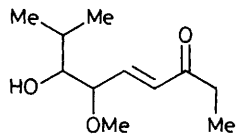
(**1**)



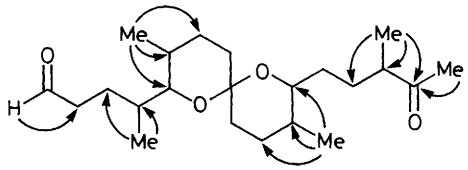
(2a)



(3)



(4)



(5)

^{13}C NMR spectra indicated the molecular formula of $C_{41}H_{66}O_{13}$.† $UV_{max.}$ (MeCN) at 250 nm and IR bands at 1755 and 1825 cm^{-1} suggested the presence of a 2,3-dialkylmaleic anhydride structure. ^1H NMR of (**1**) in [$^2H_6$]DMSO (DMSO = dimethylsulphoxide) showed three hydroxy protons [δ 5.84 (3′-OH), 4.64 (18-OH), 4.46 (22-OH)]. After a week in an NMR tube in [$^2H_6$]DMSO, two carboxyl protons at δ 12.5, and new signals of 3′-H at δ 4.9 and 3′-OH at δ 5.45 appeared. The ratio between the original signals and new signals was approximately 6:4, which remained unchanged even after addition of water.‡ This data and ^1H-^1H COSY, ^1H-^{13}C COSY, and heteronuclear multiple bond correction (HMBC)[3] spectra revealed the partial structures (C-18–C-24–C-1′–C-7′), (C-1–C-4), (C-6–C-8), and (C-12–C-15). The quaternary carbon at δ 95.4 suggested the presence of a six membered spiroketal moiety.[4]

Alkaline hydrolysis of (**1**) (20% Cs_2CO_3, pH 9, room temp., 2 h) gave an acid (**2**) and anhydrodeacyltautomycin (**3**). Treatment of (**2**) with methanolic H_2SO_4 gave monomethyl ester (**2a**) and trimethyl ester (**2b**). Monomethyl ester (**2a**) [EI mass spectrum (*m/z* 215, *M*H$^+$), IR (1760, 1830 cm^{-1}), $UV_{max.}$ (250 nm in MeCN)] showed an equilibrium mixture on HPLC like (**1**). Difference NOE (3.5% between Me at δ 2.11 and H-3 at δ 4.97, [$^2H_6$]DMSO) and HMBC spectra established the structure of (**2a**). The IR and UV spectra of (**2b**) revealed the absence of the 2,3-dialkylmaleic anhydride moiety (disappearance of 1760 and 1830 cm^{-1} in IR and 250 nm $UV_{max.}$ in MeCN). No carboxyl proton was detected in the ^1H NMR spectrum of a fresh solution of (**2a**) in [$^2H_6$]DMSO. After a week in an NMR tube, two carboxyl protons at δ 12.6 appeared and reached an equilibrium.

Both (**1**) and (**2a**) exist in methanol–buffer (1% diethylamine–formic acid, pH 7.3) solution as equilibrium mixtures (*ca.* 5:4). Ring opening and closure of (**1**) were shown by an oxygen exchange experiment. Treatment of (**1**) with $H_2{}^{18}O$ in MeCN, followed by evaporation gave peaks 2, 4, and 6 mass units higher as detected by FD mass spectrometry. The ratio of incorporation of ^{18}O into (**1**) increased with time.§ Similar treatment of (**3**) with $H_2{}^{18}O$ gave no higher peaks. Extraction with ethyl acetate at pH 4 resulted in the ring closure.‡

The secondary ion (SI) mass spectrum (*m/z* 567, *M*H$^+$) and the total number of carbons detected by ^{13}C NMR spectra of (**3**) suggested the molecular formula of $C_{33}H_{58}O_7$. High resolution EI mass spectrometry¶ gave a dehydrated fragment ion [$C_{33}H_{54}O_5$, $(M - 2H_2O)^+$, Δ 1.7 mmu] and an intense fragment ion [*m/z* 281.2109, $(C_{17}H_{29}O_3)^+$, Δ 0.5 mmu] as shown. Acetylation of (**3**) gave diacetate (**3a**) (*m/z* 651, *M*H$^+$).

Retro-aldol cleavage (20% Cs_2CO_3, pH 10, room temp., 3 h) of (**3**) gave (**4**) and (**5**). The data from SI MS (*m/z* 201, *M*H$^+$), a homonuclear proton spin decoupling experiment, and ^{13}C NMR established the structure of (**4**). The coupling constant (17 Hz) between H-4 (δ 6.25, d) and H-5 (δ 6.75, dd) indicated (*E*) configuration of the double bond. The molecular formula ($C_{22}H_{38}O_4$) of (**5**) was established by high resolution positive FAB MS (*m/z* 367.2857, *M*H$^+$, Δ 0.9 mmu), negative FAB MS (*m/z* 365, *M*H$^-$)‖ and the total number of carbons (22C) detected by ^{13}C NMR. HMBC spectra of (**5**) showed a ^1H-^{13}C long range coupling pattern as shown.

† FD MS, Hitachi M80 instrument. FAB MS, JMS-SX102. We revised the molecular formula $C_{42}H_{70}O_{12}$ in ref. 1.

‡ 2,3-Dimethylmaleic anhydride as a model compound also showed similar behaviour. See also ref. 5.

§ FD MS; (**1**) treated with $H_2{}^{18}O$ for 4 h: $(M + Na)^+$, *m/z* 789 (100%); 791 (80%); 793 (45%); 795 (5%).

¶ SI MS and high resolution EI MS, Hitachi M80 instrument.

‖ FAB MS, JMS DX 300 and SX 102 instruments.

†† 2D INADEQUATE spectra revealed the correlation of C-1 with C-2, C-2 with C-3, C-3 with 3-Me, C-3 with C-4, C-4 with C-5, C-5 with C-6, C-6 with C-7, C-7 with 7-Me, C-7 with C-8, C-8 with C-9, C-9 with C-10, C-10 with C-11, C-11 with C-12, C-12 with C-13, C-13 with 13-Me, C-13 with C-14, C-14 with C-15, C-15 with 15-Me, C-15 with C-16, C-16 with C-17, C-17 with C-18, C-18 with C-19, C-19 with 19-Me, C-19 with C-20, C-20 with C-21, C-22 with C-23, C-1′ with C-2′, C-2′ with C-3′, C-3′ with C-4′, C-4′ with C-7′, C-6′ with C-5′, and C-5′ with 5′-Me. 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. 2D INADEQUATE spectra of [1,2-^{13}C]acetate labelled tautomycin gave the carbon–carbon connectivity pattern of (**1**) as described here. A similar randomization was also observed in cationomycin.[6]

2D INADEQUATE spectroscopy†† of tautomycin labelled with [1,2-^{13}C]acetate permitted the complete assignments of ^{13}C and ^{1}H NMR signals‡‡ and established the total structure. The stereochemistry remains to be solved.

‡‡ NMR spectra were measured using JEOL GSX 400 and GSX 500 instruments. All NMR were measured in $CDCl_3$ except the solvent shown in the text; (**1**), ^{1}H NMR $CDCl_3$ [trimethylsilane (TMS)]: 2.12 (s, 3H, H-1), 2.52 (m, 1H, H-3), 1.62 (m, 2H, H-4), 1.25, 1.54 (m, each 2H, H-5), 3.13 (dt, 1H, H-6), 1.24 (m, 1H, H-7), 1.45, 1.58 (m, each 1H, H-8), 1.45, 1.62 (m, each 1H, H-9), 1.38, 1.55 (m, each 1H, H-11), 1.38, 2.00 (m, each 1H, H-12), 1.82 (m, 1H, H-13), 3.25 (dd, 1H, H-14), 1.54 (m, 1H, H-15), 1.52, 1.83 (m, each 1H, H-16), 1.27, 1.61 (m, each 1H, H-17), 3.68 (m, 1H, H-18), 2.62 (m, 1H, H-19), 2.63, 2.97 (m, each 1H, H-21), 4.33 (ddd, 1H, H-23), 3.25 (dd, 1H, H-23), 5.07 (dd, 1H, H-24), 2.08 (m, 1H, H-25), 0.95 (d, 3H, H-26), 1.07 (d, 3H, 3-Me), 0.8 (d, 3H, 7-Me), 0.9 (d, 3H, 13-Me), 0.95 (d, 3H, 15-Me), 1.08 (d, 3H, 19-Me), 0.95 (d, 3H, 25-Me), 3.42 (s, 3H, 23-OMe), 2.75, 2.90 (dd, each 1H, H-2′), 5.18 (dd, 1H, H-3′), 2.24 (s, 3H, 5′-Me).

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