STUDIES ON TOMAYMYCIN. I

THE STRUCTURE DETERMINATION OF TOMAYMYCIN ON THE BASIS OF NMR SPECTRA

ZENZABURO TOZUKA and TAKAO TAKAYA*

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.
Kashima, Yodogawaku, Osaka, Japan

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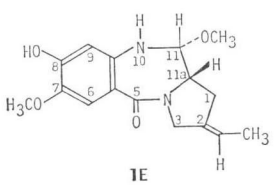
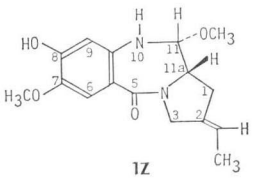
The structure of an antitumor antibiotic, tomaymycin, was determined as (11*R*, 11*aS*) (*E*)-2-ethylidene-2,3,5,10,11,11a-hexahydro-8-hydroxy-7,11-dimethoxy-5-oxo-1*H*-pyrrolo(2,1-c)-(1,4)benzodiazepine on the basis of ^{1}H and ^{13}C NMR spectra.

Tomaymycin[1] is one of the pyrrolo(1,4)benzodiazepine antibiotics, such as anthramycin[2], neothramycin[3], and sibiromycin[4]. It fascinated us as a synthetic target because of the strong antitumor activity in addition to its interesting structure. The configuration at the asymmetric C-11 and the geometrical isomerism of the 2-ethylidene group in tomaymycin were not clear when we started the total synthesis of tomaymycin[5], although the plane structural formula had been determined by the chemical degradation method[6]. In this paper, we wish to describe the structure determination of tomaymycin on the basis of NMR spectra. The 100 MHz ^{1}H NMR spectra of both *E*- (**1E**) and *Z*-tomaymycin (**1Z**) in DMSO-d_6 were the very similar as shown in Table 3. It was impossible to distinguish between the *E*- and *Z*-isomer by using the technique of NOE between the ethylidene group and the adjacent methylene groups. However, ^{13}C NMR spectrometry is potentially an extremely attractive tool for structural elucidation of the geometrical isomerism of the ethylidene moiety of tomaymycin. A high field shift arising from the steric shielding effect in the ^{13}C NMR spectra was found for an extensive series of methylbenzenes by WOOLFENDEN and GRANT[7] and also for ethylidenecyclopentane by LIPPMAA *et al.*[8] In order to clarify the configuration of the ethylidene moiety, two geometrical isomers of 4-ethylidene-L-proline diphenylmethyl ester were prepared from L-hydroxyproline as model compounds. The structure determination of tomaymycin on the basis of NMR spectra agreed with the crystal structure determined by the X-ray analysis of tomaymycin which was recently reported by ARORA.[9]



1E

E-Tomaymycin



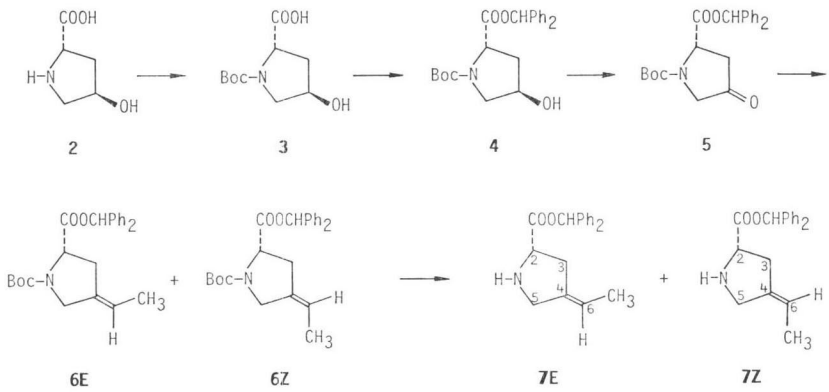
1Z

Z-Tomaymycin

Syntheses of (*E*)- and (*Z*)-4-Ethylidene-L-proline Diphenylmethyl Esters

4-Ethylidene-L-proline diphenylmethyl esters (**7E** and **7Z**) were prepared according to the modified procedure reported by BETHELL *et al.*[10] (Scheme 1). *N*-*t*-Butoxycarbonyl-L-hydroxyproline diphenylmethyl ester (**4**) was prepared by the reaction of L-hydroxyproline (**2**) with 2-phenyl-2-*t*-butoxycarbonyloxyiminoacetonitrile (Boc-ON), followed by esterification using diphenyldiazomethane, in an excellent yield. The improved MOFFATT oxidation of **4** with DMSO-trifluoroacetic acid (TFA)-pyridine-dicyclo-

Scheme 1.



hexylcarbodiimide (DCC)[11] or with dimethyl sulfoxide (DMSO)-trifluoroacetic anhydride (TFAA)-triethylamine (TEA)[12] gave the corresponding 4-oxo-L-proline derivative (**5**) in a good yield. The WITTIG reaction of **5** with the ylide obtained from the reaction of ethyltriphenylphosphonium bromide and sodium amide in liquid ammonia gave a mixture of (*E*)- and (*Z*)-ethylidene derivatives (**6E** and **6Z**) in 66% yield. They were separated by repeated column chromatography on silica gel with benzene. Removal of the *t*-butoxycarbonyl group of **6** with hydrogen chloride in methanol gave **7** in more than 90% yield.

The ^{13}C NMR Spectra of 4-Ethylidene-L-proline Diphenylmethyl Esters

Table 1 shows the ^{13}C NMR spectra of (*E*)- and (*Z*)-ethylidene-L-proline diphenylmethyl ester (**7E** and **7Z**) in which signals were assigned by analysis of both off-resonance ^{1}H-decoupled and fully ^{1}H-coupled ^{13}C NMR spectra. The chemical shift (32.6 ppm) of the methylene group at C-3 of the *E*-isomer (**7E**), which is influenced by the steric shielding effect of the methyl group in the ethylidene moiety, is 4.0 ppm higher field than that of the *Z*-isomer (**7Z**) (36.6 ppm). On the other hand, the chemical shift (47.8 ppm) of the methylene group at C-5 of the *Z*-isomer is 3.3 ppm higher field than that of the *E*-isomer (51.1 ppm) due to the same steric shielding effect. Thus the geometrical isomerism of the ethylidene moiety of 4-ethylidene-L-proline diphenylmethyl ester was determined on the basis of the steric shielding effect in the ^{13}C NMR spectra.

Table 1. ^{13}C NMR spectra of 4-ethylidene-L-proline diphenylmethyl esters.

	7E	7Z	Difference
2	60.1 (d)	60.1 (d)	0
3	32.6 (t)	36.6 (t)	4.0
4	—	—	—
5	51.1 (t)	47.8 (t)	−3.3
6	115.2 (d)	115.7 (d)	0.5
6-CH_3	14.7 (q)	14.7 (q)	0
CO_2	173.5 (s)	173.5 (s)	0

ppm (multiplicity on off-resonance)
Run in benzene with TMS as internal standard.

The ^{13}C NMR Spectra of Tomaymycin

The same steric shielding effect was observed in the ^{13}C NMR spectra of both *E*- and *Z*-tomaymycin (**1E** and **1Z**) as shown in Table 2. The chemical shift (32.3 ppm) of the methylene group at C-1 of **1E**, which is influenced by the steric shielding effect of the methyl group, is 1.4 ppm higher field than that of **1Z** (33.7 ppm). On the other hand, the chemical shift (51.2 ppm) of the methylene group at C-3 of **1Z** is 2.6 ppm higher field than that of **1E** (53.8 ppm). Thus the geometrical isomerism of the ethylidene

Table 2. ^{13}C NMR spectra of *E*- (**1E**) and *Z*-tomaymycin (**1Z**).

	1E (ppm)	1Z (ppm)		1E (ppm)	1Z (ppm)
1	32.3	33.7	9	115.0*	114.8*
2	108.9	108.4	9a	140.0*	140.7*
3	53.8	51.2	11	88.6	88.2
5	165.2	165.1	11a	53.4	53.8
5a	114.7*	114.6*	12	103.7	104.1
6	134.9*	135.0*	12-CH_3	14.1	14.1
7	139.4*	138.9*	7-OCH_3	58.1	59.0
8	150.5*	150.4*	11-OCH_3	55.9	55.9

Run in DMSO-d_6 with TMS as internal standard.
* Assignments within any vertical column may be reversed.

moiety of tomaymycin was determined on the basis of ^{13}C NMR spectra.

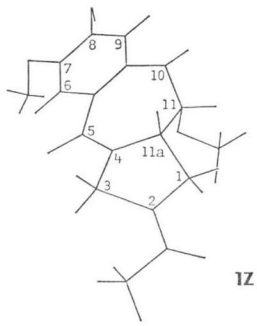
The ^1H NMR Spectra of Tomaymycin

The KARPLUS rule[13], the relation between vicinal coupling in the ^1H NMR spectrum and the dihedral angle, was applied to estimate the configuration of C-11 of tomaymycin. Table 3 shows the ^1H NMR spectra of *E*- and *Z*-tomaymycin (**1E** and **1Z**) in which H-10 (7.10 ppm, d, J=6Hz) and H-11 (4.50 ppm, d, J=6 Hz) are coupled with each other. On exchange with D_2O, the former doublet peak disappeared, and the later doublet peak was changed to a singlet peak. The coupling constant ($J_{10,11}$= 6 Hz) indicates that the dihedral angle of H_{10}–N_{10}–C_{11}–H_{11} is 0~35° or 145~180°. The H-11a (3.90 ppm, dd, J=9 Hz, 5 Hz) was not affected by exchange with D_2O and coupled only with the two protons at C-1. As there is no coupling between H-11 and H-11a, the dihedral angle of H_{11}–C_{11}–C_{11a}–H_{11a} is approximately 90°. In order to visualize the above results in the ^1H NMR spectra of **1E** and **1Z**, Dreiding models were constructed. The configuration of the asymmetric C-11 of tomaymycin was estimated to have the *R* configuration from the Dreiding models.

Table 3. ^1H NMR spectra of *E*- (**1E**) and *Z*-tomaymycin (**1Z**).

	1E	1Z
1-Ha	2.96 (1H,dd,18,9)	2.96 (1H,dd,18,9)
1-Hb	2.55 (1H,d,18)	2.56 (1H,d,18)
3-H	4.12 (2H,s)	4.12 (2H,s)
6-H	7.20 (1H,s)	7.20 (1H,s)
9-H	6.23 (1H,s)	6.23 (1H,s)
11-H	4.49 (1H,d,6)	4.50 (1H,d,6)
11a-H	3.88 (1H,dd,9,5)	3.90 (1H,dd,9,5)
12-H	5.2~5.6 (1H,m)	5.2~5.6 (1H,m)
12-CH_3	1.59 (3H,s)	1.61 (3H,s)
7-OCH_3	3.66 (3H,s)	3.68 (3H,s)
11-OCH_3	3.20 (3H,s)	3.21 (3H,s)
10-NH	7.08 (1H,d,6)	7.10 (1H,d,6)
8-OH	9.32 (1H,s)	9.35 (1H,s)

ppm (integration, multiplicity, coupling Hz)
Run in DMSO-d_6 at 100 MHz with TMS as internal standard.



1Z *z*-Tomaymycin Dreiding models

Experimental

^1H NMR and ^{13}C NMR spectra were measured at 100 MHz on a JEOL-MH 100 NMR spectrometer and at 60 MHz on a JNM-PMX 60 NMR spectrometer using TMS as an internal standard.

The ^1H NMR spectra of compounds **4**, **5**, **6E**, and **6Z** at room temperature predict the rotational isomerism due to restricted rotation of the two bulky groups (*t*-butoxycarbonyl group and diphenylmethoxycarbonyl group, of which peaks were split). IR spectra were measured on a Hitachi 260–10 spectrophotometer. Melting points were taken with an Arthur H. Thomas melting point apparatus and were uncorrected. Elemental analyses were measured on a Yanaco CHN coder MT-2.

N-t-Butoxycarbonyl-L-hydroxyproline (**3**)

A mixture of **2** (131 g), TEA (303 g), and Boc-ON (270 g) in 50% (v/v) aqueous dioxane (2 liters) was stirred at room temperature for 5 hours. The resulting solution was washed with CH_2Cl_2 (3× 300 ml) and the separated aqueous layer was adjusted to pH 3.0 with conc. HCl. The acidified solution was extracted with EtOAc (5×500 ml). The extracts were washed with brine, dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give **3** (177 g, 76.7%). An analytical sample was recrystallized from EtOAc, mp 126~127°C: IR (Nujol) 3350, 1730, 1655 cm^{-1}: NMR (DMSO-d_6) δ 1.4 (9H, s), 1.8~2.4 (2H, m), 3.07~3.7 (2H, m), 4.0~4.53 (2H, m).

Anal. Calcd. for $C_{10}H_{17}NO_5$: C 51.97, H 7.41, N 6.06.
Found: C 52.01, H 7.57, N 6.20.

N-t-Butoxycarbonyl-L-hydroxyproline Diphenylmethyl Ester (**4**)

To a solution of benzophenone hydrazone (196 g) in EtOAc (one liter) was added nickel oxide (483 g) at 0~10°C. The mixture was stirred for one hour and filtered. The filtrate was added to a solution of **3** (177 g) in EtOAc (one liter). The solution was stirred at room temperature overnight and the excess of diphenyldiazomethane was decomposed with AcOH. The solution was washed with 5% aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered, and evaporated *in vacuo*. The residue was dissolved in a little EtOAc and the solution was added to *n*-hexane with stirring. The resulting powder was filtered and air-dried to give **4** (256 g, 84.2%), mp 103~104°C: IR(Nujol) 3500, 1720, 1690 cm^{-1}: NMR (DMSO-d_6) δ 1.17, 1.38 (9H, two s), 1.77~2.33 (2H, m), 3.13~3.67 (2H, m), 5.10 (1H, d, J=4 Hz), 6.83, 6.90 (1H, two s), 7.38 (10H, s).

Anal. Calcd. for $C_{23}H_{27}NO_5$: C 69.50, H 6.85, N 3.52.
Found: C 69.55, H 6.89, N 3.53.

N-t-Butoxycarbonyl-4-oxo-L-proline Diphenylmethyl Ester (**5**)

A solution of **4** (236 g) in DMSO (500 ml) was added to a solution of TFA (24 ml) in pyridine (48 ml) at 3°C. To the solution was added DCC (372 g) at the same temperature and the mixture was stirred at room temperature overnight. After the reaction mixture was diluted with Et_2O (3 liters), a solution of oxalic acid (162 g) in MeOH (500 ml) was added to the mixture. After the evolution of gas had ceased, H_2O (500 ml) was added to the mixture and the insoluble substances were filtered off. The filtrate was washed with 5% aqueous $NaHCO_3$ and water, dried over $MgSO_4$, filtered, and evaporated to dryness *in vacuo*. The residue was triturated with 50% (v/v) *n*-hexane - Et_2O to give **5** (197 g, 83.9%), mp 97~98°C: IR (Nujol) 1760, 1740, 1700 cm^{-1}: NMR (DMSO-d_6) δ 1.27, 1.4 (9H, two s), 2.45 (1H, dd, J= 18 Hz, 3 Hz), 3.27 (1H, dd, J=18 Hz, 10 Hz), 3.65 (1H, d, J=18 Hz), 3.98 (1H, d, J=18 Hz), 4.82 (1H, dd, J=10 Hz, 3 Hz), 6.86 (1H, s), 7.35 (10 H, s).

Anal. Calcd. for $C_{23}H_{25}NO_5$: C 69.86, H 6.37, N 3.53.
Found: C 69.60, H 6.47, N 3.72.

N-t-Butoxycarbonyl-4-ethylidene-L-proline Diphenylmethyl Ester (**6**)

All operations were carried out in an atomosphere of oxygen-free nitrogen. To freshly distilled liquid ammonia (400 ml) was added sodium (3.8 g) and a trace of ferric chloride. When the blue color had disappeared, ethyltriphenylphosphonium bromide (61.4 g) was added with stirring. After stirring for 2 hours, ammonia was removed and the reaction mixture was heated at 50°C for 20 minutes. To the mixture were added dry tetrahydrofuran (THF, 400 ml) and dry ether (100 ml). To the suspension was added a solution of **5** (10 g) in THF (300 ml) at room temperature with stirring. The resulting mixture was refluxed for 24 hours, cooled with water bath, and filtered. The filtrate was evaporated *in vacuo*. The residue was chromatographed on silica gel with benzene to give the mixture of **6E** and **6Z** (6.8 g, 66%) which were separated by repeated chromatography on silica gel with benzene. The earlier fraction gave pure **6Z** as colorless oil (0.95 g, 9.2%), IR (film) 1740, 1700 cm^{-1}: NMR ($CDCl_3$) δ 1.27, 1.47 (9H,

two s), 1.58 (3H, d, J=8 Hz), 2.53 (2H d, J=18 Hz), 4.10 (2H, s), 4.33~4.73 (1H, m), 5.1~5.57 (1H, m), 6.92 (1H, s), 7.3 (10H, s).

Anal. Calcd. for $C_{25}H_{29}NO_4$: C 73.68, H 7.17, N 3.44.
Found: C 73.37, H 7.12, N 3.45.

The latter fraction gave approximately pure **6E** as pale yellow oil (1.2 g, 11.6%), IR (film) 1740, 1700 cm^{-1}: NMR (DMSO-d_6) δ 1.22, 1.42 (9H, two s), 1.55 (3H, d, J=8 Hz), 2.2~2.7 (2H, m), 4.0 (2H, m), 4.5 (1H, dd, J=10 Hz, 3Hz), 5.1~5.5 (1H, m), 6.88 (1H, s), 7.37 (10H, s).

(*Z*)-4-Ethylidene-L-proline Diphenylmethyl Ester (**7Z**)

To an ice-cooled solution of **6Z** (3.83 g) in MeOH (20 ml) was added a solution of MeOH saturated with HCl gas. The solution was stirred at room temperature for one hour and neutralized with TEA. The resulting precipitate was filtered and washed with ether. The filtrate and the washing were combined and evaporated *in vacuo*. The residue was dissolved in EtOAc, washed with water, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was chromatographed on silica gel. The eluate with EtOAc was evaporated to give **7Z** (2.65 g, 91.7%), IR (film) 3350, 1730 cm^{-1}: NMR (DMSO-d_6) δ 1.56 (3H, m), 2.20 (1H, dd, J=18 Hz, 6Hz), 3.10 (1H, dd, J=18 Hz, 12 Hz), 3.48 (2H, br. s), 3.95 (1H, dd, J=12 Hz, 6 Hz), 5.16~5.52 (1H, m), 6.84 (1H, s), 7.40 (10H, s).

(*E*)-4-Ethylidene-L-proline Diphenylmethyl Ester (**7E**)

7E was similarly prepared from **6E** (1.1 g) in 90.4% yield, IR (film) 3340, 1740 cm^{-1}. NMR (DMSO-d_6) δ 1.56 (3H, m), 2.20 (1H, dd, J=18 Hz, 6 Hz), 3.10 (1H, dd, J=18 Hz, 12 Hz), 3.48 (2H, br. s), 3.95 (1H, dd, J=12 Hz, 6 Hz), 5.16~5.5 (1H, m), 6.84 (1H, s), 7.40 (10H, s).

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References

1) ARIMA, K.; M. KOHSAKA, G. TAMURA, H. IMANAKA & H. SAKAI: Studies on tomaymycin, a new antibiotic. I. Isolation and properties of tomaymycin. J. Antibiotics 25: 437~444, 1972
2) LEIMGRUBER, W.; V. STEFANOVIC, F. SCHENKER, A. KARR & J. BERGER: Isolation and characterization of anthramycin, a new antitumor antibiotic. J. Amer. Chem. Soc. 87: 5791~5793, 1965
3) TAKEUCHI, T.; M. MIYAMOTO, M. ISHIZUKA, H. NAGANAWA, S. KONDO, M. HAMADA & H. UMEZAWA: Neothramycins A and B, new antitumor antibiotics. J. Antibiotics 29: 93~96, 1976
4) BRAZHNIKOVA, M. G.; N. V. KONSTANTINOVA & A. S. MESENTSEV: Sibiromycin: Isolation and characterization. J. Antibiotics 25: 668~673, 1972
5) TOZUKA, Z.; H. TAKASUGI & T. TAKAYA: Studies on tomaymycin. II. Total syntheses of antitumor antibiotics, *E*- and *Z*-tomaymycin. J. Antibiotics 36(3): in press.
6) KARIYONE, K.; H. YAZAWA & M. KOHSAKA: The structure of tomaymycin and oxotomaymycin. Chem. Pharm. Bull. 19: 2289~2293, 1971
7) WOOLFENDEN, W. R. & D. M. GRANT: Carbon-13 magnetic resonance. V. Conformational dependence of the chemical shifts in the methylbenzenes. J. Amer. Chem. Soc. 88: 1496~1502, 1966
8) LIPPMAA, E.; T. PEHK, J. PAASIVIRTA, N. BELIKOVA & A. PLATE: Carbon-13 chemical shifts of bicyclic compounds. Org. Magn. Resonance 2: 581~604, 1970
9) ARORA, S. K.: Structure of tomaymycin, a DNA binding antitumor antibiotic. J. Antibiotics 34: 462~464, 1981
10) BETHELL, M. & G. W. KENNER: Peptides. XV. 4-Methyleneproline and 4-hydroxymethylproline. J. Chem. Soc. 1965: 3850~3854, 1965
11) PLITZNER, K. E. & J. G. MOFFATT: Sulfoxide-carbodiimide reactions. II. Scope of the oxidation reaction. J. Amer. Chem. Soc. 87: 5670~5678, 1965
12) OMURA, K.; A. K. SHARMA & D. SWERN: Dimethyl sulfoxide-trifluoroacetic anhydride, a new reagent for oxidation of alcohols to carbonyls. J. Org. Chem. 41: 957~962, 1976
13) JACKMAN, L. M. & S. STERNHELL: "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", 2nd ed., pp. 280~304, Pergamon Press, Oxford, London, Edinburgh, New York, Toront, Sydney, Paris, Braunschweig, 1969