A NEW ANTITUMOR SUBSTANCE, BE-18591, PRODUCED BY A STREPTOMYCETE

II. STRUCTURE DETERMINATION

SHIGERU NAKAJIMA, KATSUHISA KOJIRI and HIROYUKI SUDA

New Drug Discovery Research Laboratories,
Tsukuba Research Institute,
Banyu Pharmaceutical Co., Ltd.,
Tsukuba Techno-Park Oho, Okubo 3,
Tsukuba 300-33, Japan

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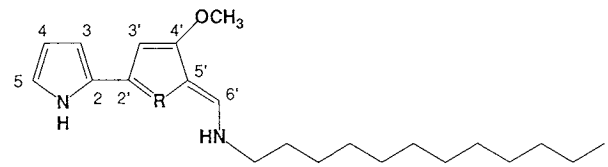
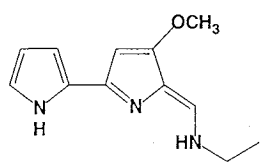
In the course of screening for new antitumor substances, BE-18591 (**1a**) was isolated from cultures of *Streptomyces* sp. BA18591[1]. The isolation and physico-chemical properties together with biological activities are reported in the preceding paper. In this paper, the structure elucidation is described.

The physico-chemical properties of **1a** were summarized in a previous paper[1]. The molecular formula of **1a** was determined as $C_{22}H_{35}N_3O$ from HRFAB-MS (calcd: *m/z* 358.2903, found: *m/z* 358.2881 $(M+H)^+$) and ^{13}C NMR spectral data. BE-18591 was found to be basic in nature, its hydrochloride salt (**1b**) giving different ^1H NMR spectrum from that of the free base **1a**. Since a D_2O-exchangeable proton at δ_H 13.7 was newly observed and other D_2O-exchangeable protons at δ_H 9.48 and δ_H 10.6 were clearly observed in the ^1H NMR spectrum of **1b**, the hydrochloride salt **1b** was used for further NMR studies. The ^1H and ^{13}C NMR data for **1a** and **1b** are shown in Table 1. In decoupling experiments of **1b**, irradiation of the D_2O-exchangeable proton at δ_H 10.6 caused the signals at δ_H 6.27, 6.73 and 7.05 to collapse to sharp signals. Those signals were also found to be mutually coupled one another (1.4~3.6 Hz). From these data and the coupling constant (3.6 Hz) between δ_H 6.27 and 6.73, the presence of a 2-substituted pyrrole ring was suggested[2]. A second partial structure (**A** in Fig. 2) was revealed by analysis of the ^1H-^{13}C COSY spectrum, and the results from the decoupling experiments of **1b**. The nature of the remaining unit C_6H_6NO depicted in partial structure **B**, was suggested from LSPD experiments and the HMBC spectrum of **1b** (Fig. 2). The D_2O-exchangeable proton at δ_H 13.7 was coupled to the carbons at δ_C 90.9, 110.6, 142.0 and 163.4. The methoxyl proton at δ_H 3.92 was coupled to the carbon at δ_C 163.4. The D_2O-exchangeable proton in the partial structure **A** at δ_H 9.48 was coupled to the carbon at δ_C 110.6. The olefinic proton at δ_H 7.32 was coupled to the carbon at δ_C 163.4. The ^{13}C-^1H long range coupling data and ^1H-^1H COSY data of **1b** show the connectivity of the partial structures **A** and **B**. The chemical shift of C-3′ (δ_C 90.9) implies that the methine carbon was adjacent to the carbon bearing a methoxyl moiety. The

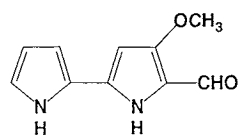
Fig. 1. Structures of BE-18591, tambjamine E and tambjamine aldehyde.



BE-18591 (**1a**) R = N
BE-18591 HCl (**1b**) R = $\overset{+}{N}H \cdot Cl$



Tambjamine E



Tambjamine aldehyde (**2**)

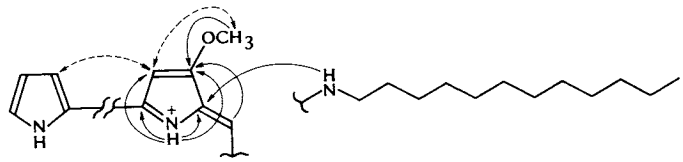
Table 1. ^{1}H and ^{13}C NMR data for BE-18591 (**1a**), BE-18591 HCl (**1b**) and tambjamine E in $CDCl_3$.

		BE-18591[a]		BE-18591 HCl[a]		Tambjamine E[b]	
		^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
	NH	10.8 (1H, br s)[c]	—	10.6 (1H, br s)	—	9.92 (1H, br s)	—
Pyrrole-1	2	—	122.3 (s)	—	122.5 (s)	—	123.7
	3	6.74 (1H, m)	113.2 (d)	6.73 (1H, ddd, 1.4, 2.6, 3.6)	112.9 (d)	7.07 (1H, br m)	111.2
	4	6.28 (1H, m)	110.6 (d)	6.27 (1H, ddd, 2.0, 2.6, 3.6)	110.5 (d)	6.28 (1H, br m)	113.0
	5	7.06 (1H, m)	124.1 (d)	7.05 (1H, dt, 1.4, 2.6)	123.9 (d)	6.76 (1H, br m)	123.8
	NH′	—	—	13.7 (1H, br s)	—	—	—
	2′	—	142.4 (s)	—	142.0 (s)	—	142.2
Pyrrole-2	3′	5.96 (1H, s)	91.9 (d)	5.94 (1H, d, 1.6)	90.9 (d)	5.97 (1H, s)	92.3
	4′	—	163.6 (s)	—	163.4 (s)	—	164.6
	5′	—	110.6 (s)	—	110.6 (s)	—	111.4
	OCH_3	3.93 (3H, s)	58.4 (q)	3.92 (3H, s)	58.3 (q)	3.93 (3H, s)	59.1
	CH=	7.33 (1H, br d, 9.0)	140.0 (d)	7.32 (1H, d, 14.8)	140.0 (d)	7.36 (1H, br m)	142.8
	NH	9.50 (1H, br s)	—	9.48 (1H, br s)	—	9.50 (1H, br s)	—
		0.88 (3H, t, 7.0)	14.1 (q)	0.88 (3H, t, 7.0)	14.1 (q)		
		1.20～1.45 (18H, m)	22.7 (t)	1.19～1.45 (18H, m)	22.6 (t)		
Dodecyl moiety			26.5 (t)		26.5 (t)		
			29.1 (t)		29.1 (t)		
			29.3 (t)		29.3 (t)		
			29.4 (t)		29.4 (t)		
			29.6 (t) × 3		29.5 (t)		
			31.9 (t)		29.6 (t) × 2		
		1.75 (2H, m)	30.2 (t)		31.8 (t)		
		3.47 (2H, br t, 7.0)	51.0 (t)	1.75 (2H, m),	30.2 (t)		
				3.47 (2H, dt, 6.3, 6.7)	50.9 (t)		

[a] ^{1}H NMR at 300 MHz and ^{13}C NMR at 75 MHz.
[b] Data in ref 5.
[c] Multiplicity, J in Hz.

Fig. 2. Partial structures **A**, **B** and pyrrole for **1b**.

The solid-line arrows indicate ^{13}C-^{1}H long range couplings and the dotted-line arrows indicate NOEs.



pyrrole **B** **A**

remaining connectivity of the 2-substituted pyrrole ring and partial structure **B** was confirmed from the difference NOE experiments of **1b**. When the 3'-H signal (δ_H 5.94) was irradiated, NOEs were observed for the 3-H (δ_H 6.73) and methoxyl (δ_H 3.92) protons (Fig. 2). The double bond geometry of C-5' was determined to have the Z configuration because of the small ^{13}C-^{1}H coupling constant ($^{3}J_{C\text{-}4'\text{-}6'\text{-}H} < 2.8$ Hz). Based on the above results, the structure of **1b** was determined as shown in Fig. 1.

The structure of the free base **1a** should be represented by an enamine structure shown in Fig. 1 from the results of the homonuclear decoupling experiments of **1a**. The signals at δ_H 3.47 and δ_H 7.33 were collapsed by irradiation of the D_2O-exchangeable proton at δ_H 9.50.

After the structure determination of BE-18591 had been completed[3], we noticed that BE-18591 was a member of the Tambjamines group isolated from ascidians and nudibranchs as defensive metabolites against their predators (Fig. 1)[4,5]. UV and ^{1}H NMR data for the pyrrole rings of BE-18591 were essentially identical with those of the Tambjamines. However the ^{1}H and ^{13}C NMR assignments for the pyrrole rings of Tambjamines required correction[5]. The assignments were obtained unambiguously from difference NOE data described above, the coupling constants of protons on the pyrrole rings and the ^{1}H-^{13}C COSY spectrum of **1b** (Table 1).

BE-18591 can be considered to be derived biogenetically from a precursor Tambjamine aldehyde (**2**)[6]. In fact, the aldehyde **2** was isolated under neutral conditions from the mycelium of the strain *Streptomyces* sp. BA18591. The physico-chemical data for **2** was as follows: FAB-MS; *m/z* 191 $(M+H)^+$, UV λ_{max}; 251 and 362, ^{1}H NMR; δ_H 3.84 (3H, s, OCH_3), 6.12 (1H, m, 4-H), 6.27 (1H, s, 3'-H), 6.75 (1H, m, 3-H), 6.90 (1H, m, 5-H), 9.32 (1H, s, CHO), 11.2 (1H, br s, NH) and 11.4 (1H, br s, NH'), ^{13}C NMR; δ_C 57.8 (OCH_3), 90.9 (C-3'), 108.3 (C-3), 109.3 (C-4), 117.4 (C-5'), 120.4 (C-5), 123.4 (C-2), 133.2 (C-2'), 158.7 (C-4') and 171.7 (CHO).

References

1) KOJIRI, K.; S. NAKAJIMA, H. SUZUKI, A. OKURA & H. SUDA: A new antitumor substance, BE-18591, produced by a streptomycete. I. Fermentation, isolation, physico-chemical and biological properties. J. Antibiotics 46: 1799~1803, 1993

2) ABRAHAM, J. R. & H. J. BERNSTEIN: The proton resonance spectra of furan and pyrrole. Can. J. Chem. 37: 1056~1065, 1959

3) KOJIRI, K.; H. SUZUKI, S. NAKAJIMA, A. OKURA, H. SUDA & M. OKANISHI (Banyu): Antitumor substance BE-18591. Jpn. Kokai 210676 ('92), July 31, 1992

4) CARTE, B. & D. J. FAULKNER: Defensive metabolites from three nembrothid nudibranchs. J. Org. Chem. 48: 2314~2318, 1983

5) LINDQUIST, N. & W. FENICAL: New tambjamine class alkaloids from the marine ascidian *Atapozoa* sp. and its nudibranch predators. Origin of the tambjamines in *Atapozoa*. Experientia 47: 504~506, 1991

6) MODY, S. R.; V. HEIDARYNEJAD, A. M. PATEL & P. J. DAVE: Isolation and characterization of Serratia marcescens mutants. Defective in prodigiosin biosynthesis. Curr. Microbiol. 20: 95~103, 1990