

THE STRUCTURES OF BUNDLIN A (LANKACIDIN) AND BUNDLIN B

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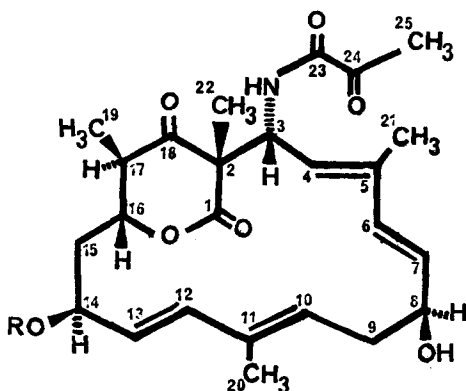
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In the course of our screening program of new antibiotics effective against "Shirahagare" disease caused by the pathogenic bacteria, Xanthomonas oryzae in green house test, we found that a strain of Streptomyces sp. 6642-GC₁ produced two active substances which were identified with bundlin A and bundlin B isolated by Sakamoto et al. (1) from the cultured broth of Streptomyces griseofuscus. Bundlin A is identical with lankacidin produced by Streptomyces violaceoniger together with lankamycin (2), (3).

This paper concerns with the structural elucidation of bundlin A and B as shown in formulae I-A and I-B. (4).

Bundlin A (B.A.) is colorless needles and has the molecular formula of C₂₅H₃₃NO₇ (M⁺, m/e 459) m.p. 205-207°C, [α]_D²⁵ -221.0° (C=1, methanol). The UV



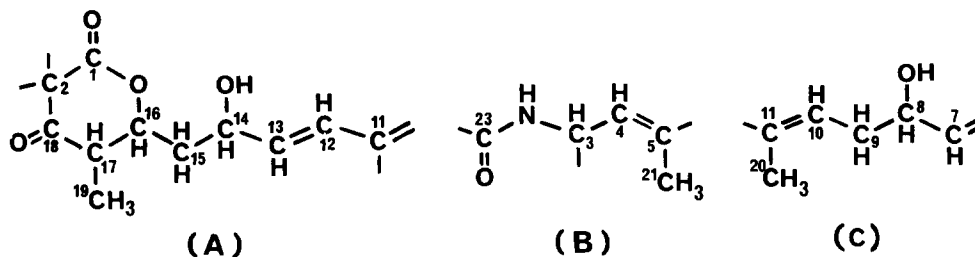
I-A: R=H

I-B: R=CO-CH₃

spectrum in methanol has λ_{\max} 227 m μ (ϵ 80,000). The IR spectrum shows absorption bands at $\nu_{\max}^{\text{CHCl}_3}$ 3600, 3440, 1755, 1735(shoulder), 1715, 1690, 1360 and 965 cm^{-1} .

Bundlin B (B.B.) is colorless needles and has the molecular formula of $\text{C}_{27}\text{H}_{35}\text{NO}_8$, m.p. 213-215°C, $[\alpha]_D^{25}$ -212.1° (C=1, methanol). The UV and IR spectra of B.B. are quite similar to those of B.A. The NMR spectrum of B.B. in d_1 -chloroform shows the presence of one more methyl singlet (2.08 ppm) assignable as acetyl methyl protons than that of B.A. Treatment of B.A. with acetic anhydride in pyridine afforded corresponding diacetate; $\text{C}_{29}\text{H}_{37}\text{NO}_9$, m.w. 543 (M^+ , m/e 543) m.p. 142-144°C. Similarly, B.B. gave the monoacetate which was indistinguishable with B.A. diacetate on comparison of their IR and NMR spectra, thus, the relationship between B.A. and B.B. was established.

B.A. was treated with active manganese dioxide in methylene dichloride to give oxobundlin A (O.B.A.); $\text{C}_{25}\text{H}_{31}\text{NO}_7$, $\lambda_{\max}^{\text{MeOH}}$ 233 m μ (ϵ 40,600), 292 m μ (ϵ 19,000). The NMR spectra of B.A. and O.B.A. (Table 1) together with their spin decoupling experiments demonstrated the presence of partial structures A, B and C.



Two multiplets at δ 4.00 and 4.30 in NMR spectrum of B.A. are assigned to the protons linked to C_8 and C_{14} bearing hydroxyl groups, however, the signal at δ 4.30 shifts to δ 5.35 in the spectrum of B.B. and disappears in that of O.B.A. In consequence, the position of an acetyl substituent of B.B. must link to C_{14} hydroxyl group of B.A.

The presence of a β -keto- δ -lactone system was demonstrated by the following experiments. When B.A. was treated with an equimolar of sodium hydroxide in

Table 1 NMR spectra of O.B.A. (in CDCl₃) and B.A. (in CD₃COCD₃)

	spin decoupling experiments of O.B.A.				spin decoupling experiments of B.A.			
	δ-value (ppm)		J (c/s)		δ-value (ppm)		J (c/s)	
	irr.	obs.			irr.	obs.		
A	H-19, 1.17	H-17, 2.18	7	dq→d	H-19, 1.21	H-17, 1.56	7	dq→d
	H-17, 2.18	H-16, 4.56	12	dq→m	H-17, 2.56	H-16, 4.64	12	dq→m
	H-16, 4.56	H-15a, 2.70	3	dd→d	H-13, 5.62	H-12, 6.10	15	bd→bs
		H-15b, 3.25	4	dd→d				
	H-15a, 2.70	H-15b, 3.25	14	dd→d				
	H-12, 6.52	H-13, 7.14	15	d→s				
B	H-3, 5.43	H-N, 8.00	10	d→s	H-3, 5.3	H-N, 8.00	10	d→s
	H-3, 5.43	H-4, 4.66	10	d→bs	H-3, 5.3	H-4, 4.75	10	d→bs
	H-21, 1.94	H-4, 4.66		bd→shd	H-21, 1.82	H-4, 4.75		bd→shd
C	H-20, 1.59	H-10, 5.86		bt→sht	uncoupled and overlapped signals			
	H-9, ca2.5	H-10, 5.86	9	bt→bs				
	H-9, ca2.5	H-8, 4.08		m→m	H-22, 1.34	3H,s		
	H-7, ca5.55	H-8, 4.08		m→m	H-20, 1.51	3H,s		
	uncoupled and overlapped signals				H-25, 2.36	3H,s		
	H-22, 1.42	3H,s			H-15, ca2.25			
	H-25, 2.45	3H,s			H-9, ca2.25			
	H-6, ca5.55				H-8, 4.63	1H,m		
	H-0, ca2.5	1H,			H-14, 4.28	1H,m		
					H-6, ca5.5			
				H-7, ca5.5				
				H-10, ca5.3				
				H-0, 2.87	2H,s			

s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, b: broad, sh: sharp

aqueous methanol, the IR absorption band at 1755 cm⁻¹ was replaced by those of 1610-1630 and 1400 cm⁻¹ corresponding to the carboxylate ion, hence this band was assigned to ν_{C=O} of the lactonic function. The band at high frequency of 1755 cm⁻¹ seemed unusual for δ-lactone, however, it shifted to 1730 cm⁻¹ in the reduction product of B.A. with sodium borohydride. This frequency shift is consistent with assignment of a β-keto-δ-lactone system as partial structure A. (5).

Spin decoupling experiments of the doublet at characteristically low field of δ 8.00 (assigned as an amide proton because it disappeared with the addition of D_2O and CF_3COOH) and its environmental protons establish the partial structure B.

The whole molecular structure and the absolute configuration was determined by three-dimensional X-ray structure analysis because of the small quantity of material available.

The p-bromophenylsulfonylhydrazone of bundlin A was prepared by the treatment of bundlin A with p-bromophenylsulfonylhydrazine in methanol at room temperature. The crystals are colorless plates; $C_{31}H_{38}N_3O_8SBr$, m.w. 692, m.p. 194.5-197°C. They are monoclinic, space group $P2_1$, with two molecules in the unit cell of dimensions; $a=11.24 \text{ \AA}$, $b=26.51 \text{ \AA}$, $c=6.17 \text{ \AA}$ and $\beta=111.37^\circ$. Intensities of the 2419 independent reflections were measured visually from the equi-inclination Weissenberg photographs around b and c axes taken with $CuK\alpha$ radiation ($\lambda=1.5418 \text{ \AA}$). A three-dimensional Patterson function was then calculated. The locations of the bromine and sulfur atoms could be easily deduced. The first electron density map which was synthesized using phases based on the contribution of the bromine and sulfur atoms revealed the positions of six lighter atoms. From successive calculations of three-dimensional Fourier and difference synthesis all the atomic positions were fixed. Parameters thus obtained were refined by the least-squares method. The reliability factor is 14.9 % at the present stage.

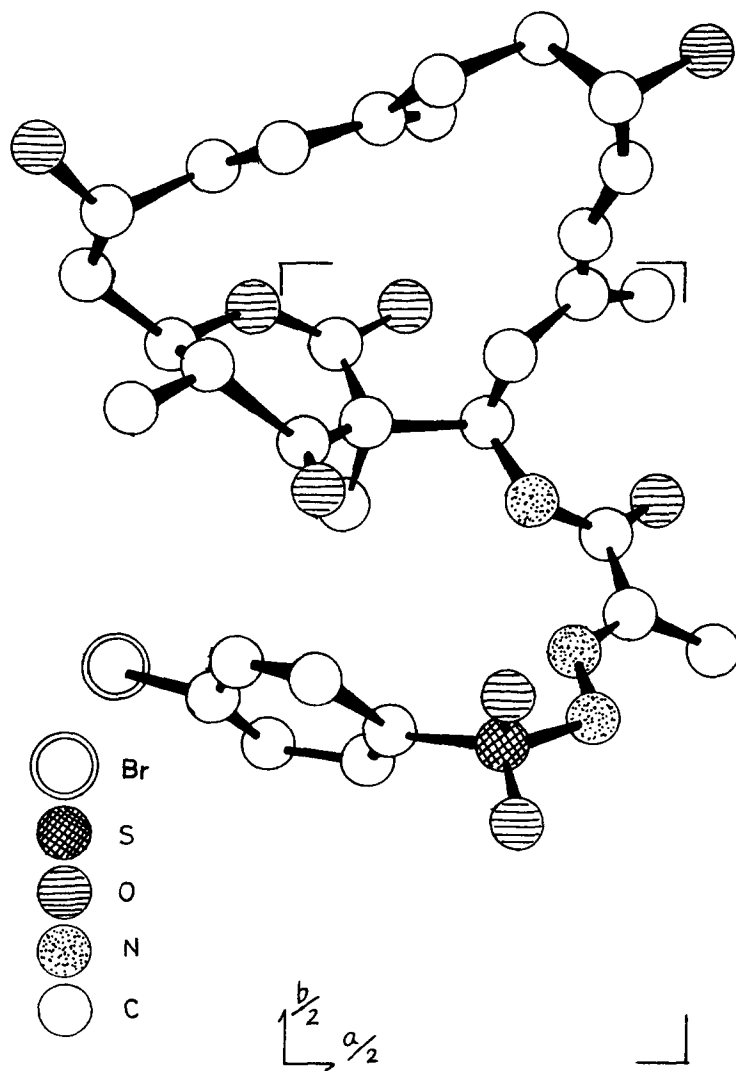
The absolute configuration was determined by use of the anomalous dispersion effect of the bromine atom for $CrK\alpha$ radiation ($\lambda=2.2909 \text{ \AA}$, $\Delta f'=-0.6$, $\Delta f''=2.7$). In Table 2 some of the calculated intensities and the observed relations between (hkl) and $(h\bar{k}l)$ are compared.

Table 2 Determination of the Absolute Configuration

Indexes	Fc(hkl) ²	obs.	Fc(h \bar{k} l) ²
0 2 1	1835	<	2246
0 14 1	2880	<<	3133
0 15 1	3234	>	2988
2 11 1	445	<	710
4 5 1	3355	>	3109
4 6 1	3432	<<	3815
4 7 1	1567	>	1214

The resulting molecular structure of bundlin A p-bromophenylsulfonylhydrazone viewed along the c axis is illustrated in Figure 1. Thus, bundlin A has a stereochemical structure I-A. The absolute configurations of six asymmetric carbon atoms are 2(S), 3(R), 8(S), 14(S), 16(R) and 17(R), respectively.

Fig. 1



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