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STRUCTURE OF LACTIVICIN, AN ANTIBIOTIC HAVING A NEW NUCLEUS AND SIMILAR BIOLOGICAL ACTIVITIES TO β-LACTAM ANTIBIOTICS

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Summary: The structure of a new antibiotic, lactivicin, was determined to be [4S]-2-(4-acetylamino-3-oxo-2-isoxazolidinyl)-5-oxo-tetrahydrofuran-2-carboxylic acid.

In a screening program for new inhibitors of cell wall synthesis from bacterial strains, we isolated a novel antibiotic, lactivicin (LTV, 1), from culture filtrates of Empedobacter lactamgenus YK-258 and Lysobacter albus YK-422. The structure of 1 has been determined to be a dicyclic dipeptide (Fig. 1). LTV was active against Gram-positive and negative bacteria and showed biological activities similar to those of β-lactam antibiotics in affinity for penicillin-binding proteins and susceptibility of β-lactamases.[1] This communication describes the structure determination of 1.

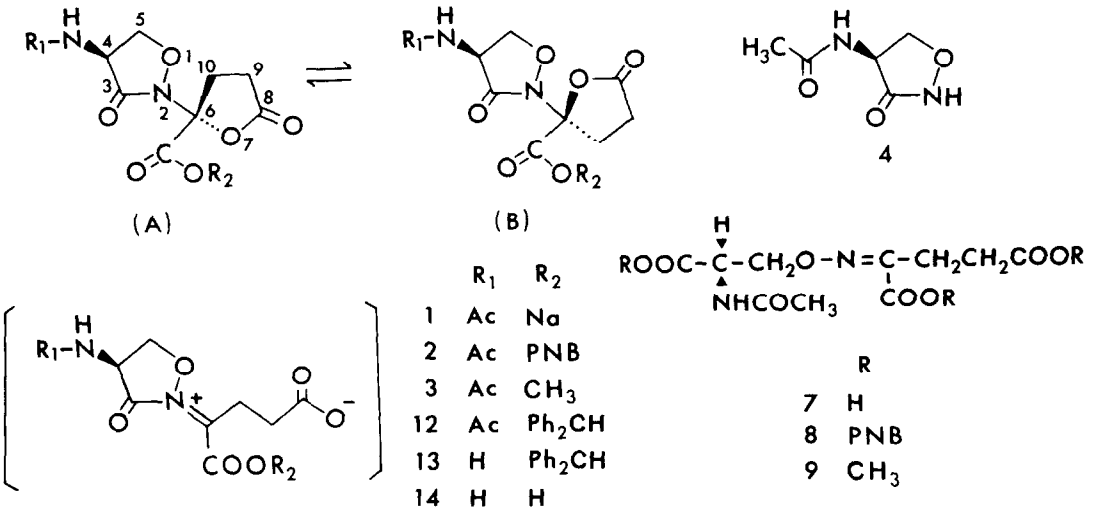

Fig. 1

Table 1 ^{13}C NMR spectral data [100 MHz, DMSO-d_6, δ_{ppm}]

Carbon	1	3A[a]	3B	4	7[b]
CO	175.84 s	174.27 s	174.39 s		179.62 s
"	172.82, 171.07 s	171.80 s	170.22 s	170.45 s	177.10 s
"	169.68, 169.64 s	169.56 s	169.59 s	169.55 s	175.55 s
"	167.21, 167.02 s	165.54 s	165.41 s		168.42 s
C-6	96.44, 96.28 s	91.48 s	91.38 s		156.19 s
C-5	71.07, 68.40 t	69.52 t	71.75 t	72.48 t	76.77 t
C-4	52.19, 51.93 d	51.60 d	51.02 d	51.07 d	55.73 d
C-9	29.06, 28.82 t[c]	28.31 t[c]	28.28 t[c]		32.84 t
C-10	28.15, 28.12 t[c]	27.17 t[c]	27.11 t[c]		23.56 t
$CO\underline{C}H_3$	22.25 q	22.12 q	22.14 q	22.23 q	24.60 q
$COO\underline{C}H_3$		53.54 q	53.53 q		

a: 67.8 MHz, b: in D_2O, c: the signals may be reversed.

This acidic, water-soluble antibiotic was isolated as a sodium salt, $[\alpha]_D$ -24.1° (H_2O), SI-MS: m/z 317 $(M+Na)^+$, UV (H_2O): 216 nm (ε 4050, sh), by column chromatographies using anion-exchange resins, QAE-Sephadex, an adsorptive resin and activated carbon, ion-paired extraction and preparative reverse-phase HPLC; methods used to isolate naturally occurring β-lactam antibiotics.[2] Two close peaks having almost the same height were observed on the HPLC of purified LTV. Peaks A and B showed almost the same antimicrobial activities in appearance and were present in a ratio of 53:47. Both peaks separated by HPLC reached the equilibrium states at 23°C after about 1 hour in the pH 3 to 7. Upon treatment with p-nitrobenzyl (PNB) bromide in DMF, 1 gave two pure epimers of PNB ester A (**2A**) and B (**2B**).[3] When **2B** was hydrogenated, 1 could only be obtained as the equilibrium mixture. LTV also afforded a pair of methyl esters (**3A**), mp 163-166°C (dec), $[\alpha]_D$ +76.7° ($CHCl_3$), CD (MeOH): [θ] +54,300 at 214 nm and -35,500 at 246 nm, and (**3B**), mp 180-181°C (dec), $[\alpha]_D$ -112° ($CHCl_3$), CD (MeOH): [θ] -30,300 at 228 nm and +4,900 at 257 nm, with a similar procedure using CH_3I. These epimers did not isomerize in 50% MeOH/0.01M phosphate buffer for 2 days at 23°C; only degradation patterns were found.

On acid hydrolysis of 1 in 6N HCl, serine was detected in the hydrolysates and assigned to be the L-form by the modified HPLC method.[4] The ^{13}C NMR spectral studies of 1 and 3 suggested the presence of the following functions in 1; a seryl, an acetyl, an ethylene, a quaternary carbon and two carbonyls (Table 1). In the ^1H NMR spectrum of **2A** (400 MHz, $CDCl_3$), the signals at δ 4.12 (dd, J=8.4, 11.0), 4.81 (dd, J=8.4, 8.5), 4.68 (ddd, J=4.9, 8.5, 11.0), 5.97 (d, J=4.9, CO$\underline{N}$H) were assigned to the serine moiety and those at 2.62 (ddd, J=4.7, 10.2, 17.9), 2.86 (ddd, J=8.1, 10.0, 17.9), 2.47 (ddd, J=8.1, 10.2, 14.1), 3.18 (ddd, J=4.7, 10.0, 14.1) to the ethylene moiety. From these all findings, the molecular formula of 1 was determined to be $C_{10}H_{11}N_2O_7Na$.

Alkaline and acidic hydrolyses gave clear results for the structure determination. When **1** was hydrolyzed in 0.1N NaOH, N-acetyl-L-cycloserine (**4**)[5], mp 174-175°C (dec), $[\alpha]_D$ -70.1° (H_2O) and α-ketoglutaric acid (**6**) were obtained. Upon treatment with Dowex 50W x 2, **1** gave a tricarboxylic acid compound (**7**), $[\alpha]_D$ +17.3° (H_2O), which was converted into a tri-PNB ester (**8**) or a trimethyl ester (**9**). In the ^{13}C NMR spectrum of **7**, the quaternary carbon signals at δ 96.44 s and 96.28 s in **1** were absent but a new signal at 156.19 s was observed. The collapse of these chemical shifts strongly suggested the formation of an oxyimino group [-C=N-O-] in **7**, from a quaternary carbon moiety [-O-C(-CO-)-N-] in **1** which was assumed from the chemical shifts at ca. 98 in the ^{13}C NMR spectra of cephabacin M_{1-6}.[6] Hydrogenolysis of **7** was carried out by 10% Pd-C to clarify this assumption, which gave N-acetyl-L-serine (**10**) and D,L-glutamic acid (**11**). These degradation patterns showed that **1** consists of 3-isoxazolidone and glutaric acid moieties and is bound between the N-oxyamide group in **4** and the ketone group in **6** to form a N-C bond. Thus, the quaternary carbon should be the α-carbon of **11** and the structure of **1** was finally deduced (Fig. 1).

As described above, **3A** and **3B** did not isomerize in aqueous solutions, but, in $Et_3N/CHCl_3$ at 23°C, they reached about 1:1 equilibrium after 22 hours. Upon treatment of **1** with $H_2{}^{18}O$ for 1.5 hours, no incorporation of ^{18}O was observed by the SI-MS. These facts indicate that this type of isomerization does not occur by attack of the hydroxyl anion at the C-6 position. Thus, the mechanism can be explained by the formation of an immonium cation as the intermediate. The carboxylate compound may attain the equilibrium before decomposition proceeds in aqueous solutions, because the carboxyl anion stabilizes the immonium cation.[7] A similar mechanism has been reported for oxapenem.[8]

LTV gave a useful starting material for chemical modification. LTV benzhydryl ester (**12**) could be deacetylated by the iminoether method[9] to afford an amino derivative (**13**). Deprotection of **13** by H_2/Pd-C gave 4-amino-lactivinic acid (4-ALA, **14**). On preferential crystallization from H_2O, **14** gave a single epimer (**14A**), mp 177-181°C (dec), $[\alpha]_D$ +124° (H_2O). The absolute configuration at the C-6 position of **14A** was determined as R by X-ray crystallographic analysis.[10] Another partially purified epimer (**14B**) was obtained from **12B** in the manner described above. Compound **13** was acylated and deprotected to afford various types of N-acyl derivatives.

CD spectral studies gave important information for determining the absolute configurations at the C-6 position (Fig. 2). LTV and related compounds occurred in about 1:1

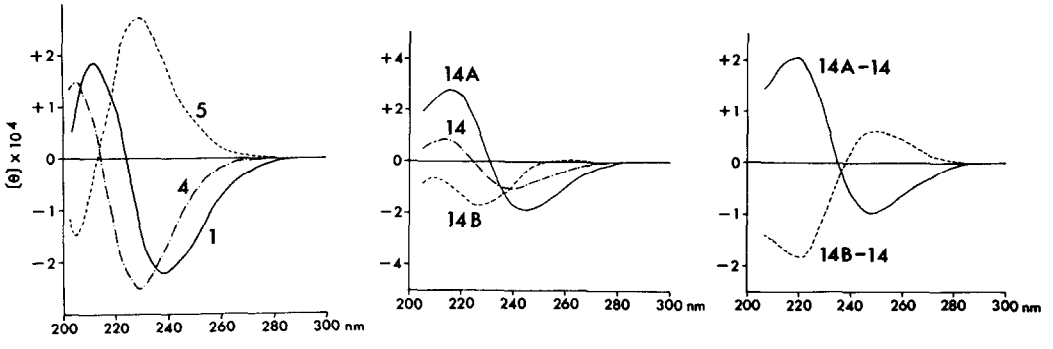

Fig. 2

ratios of their epimers seem to cause the Cotton effects of the γ-lactone carbonyl moiety to offset each other. Thus, their CD spectra only reflect the 3-isoxazolidone carbonyl moiety. The residual CD spectra obtained by subtracting the spectra of the mixture from those of the isolated epimers may only reflect γ-lactone carbonyl moiety; a positive Cotton effect at 217-219 nm and a negative one at 248-250 nm for A-typed epimers and typically opposite patterns for B-typed epimers. CD spectral data in combination with the result of X-ray crystallographic analysis showed the absolute configurations at the C-6 position to be R for A-type epimers and S for B-type ones.

The partial structure in **1** from the 4-amide group to the 6-carboxyl group through the 3-amide group resembles the active site of β-lactam antibiotics such as penicillins, cephalosporins or nocardicins. This may account for the biological activities of **1** similar to those of β-lactams in spite of the absence of the β-lactam ring in the molecule. Enzymatic hydrolysis of **1** by cephalosporinase gave the cleaved compound at the N-oxyamide group (**7**) as for the usual cephalosporins. We believe that this new skeleton could serve as the nucleus for synthesizing a new type of antibiotics.

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