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Studies on Lankacidin-Group (T-2636) Antibiotics. V.[1] Chemical Structures of Lankacidin-Group Antibiotics. (1)

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The structures of antibiotics, lankacidin A (T-2636 A), lankacidin C (T-2636 C), lankacidinol A (T-2636 D) and lankacidinol (T-2636 F) were elucidated by chemical degradations and spectral analyses. They all have the characteristic cyclo heptadeca carbon skeltone with a β-keto-δ-lactone ring. Of them lankacidin A and C have a pyruvamide group and the others have a lactamide group in their side chain.

On the point of view from chemical and biological properties, these antibiotics might be in intermediate position between the macrolide and the polyene macrolide antibiotics.

The antibiotics, lankacidin A (I), lankacidin C (II), lankacidinol A (III) and lankacidinol (IV) which have been tentatively designated in the preceding papers as T-2636 A, C, D and F, respectively, had been isolated from the culture broth of *Streptomyces rochei* var. *volubilis*.[3—5] These antibiotics are active against Gram-positive bacteria, mycoplasma, *Neisseria gonorrhoeae*, *Vibrio cholerae*, *Xanthomonas oryzae* and clinically isolated staphylococci.[1,3] They have low toxicity and strong effect on Staphylococcal infection in mice by oral administration.[1,4] This paper deals with the chemical structural elucidation of I, II, III and IV in detail[6,7] (Chart 3).

II has been isolated from the broth filtrate as colorless needles; mp 201—203° (decomp.), $[\alpha]_D$ −235° (in ethanol) and UV[8] λ_{max}^{EtOH} 227 nm (ε=46700). The molecular formula of II is assumed to be $C_{25}H_{33}O_7N$ from various data and one nitrogen atom contained in a molecule has not basic character. And also, II is clearly different from the usual macrolide antibiotics on its UV, IR[8] and NMR[8] spectra.[4]

Acetylation of II afforded I, lankacidin C 8-acetate (V, $C_{27}H_{35}O_8N$ Mass Spectrum[8] *m/e*; 501 (M^+), 441 (M^+-60 (AcOH))) and C 8,14-diacetate[4] (VI, $C_{29}H_{37}O_9N$ Mass Spectrum *m/e*; 543 (M^+), 483 (M^+-60). Lankacidinol 2′,8,14-triacetate[5] (VII, $C_{31}H_{41}O_{10}N$ Mass Spectrum *m/e*; 587 (M^+), 528, 527 (M^+-60)) was found to be completely identical with lankacidinol A 2′,8-diacetate.[5] The chromium trioxide oxidation of III at 2′-hydroxyl group gave dehydro derivative which was identical with I and the sodium borohydride reduction of II at 2′-carbonyl group gave dihydro derivative which was identical with IV. I was deacetylated with dilute sodium bicarbonate to give II and also, III was hydrolized to IV by treatment with dilute potassium carbonate. The correlations among these compounds are shown in Chart 1.

The spectral studies of these compounds and other reduction-oxidation products shown in Chart 2 suggest the presence of the following functional groups in II;

1) Part IV: K. Tsuchiya, T. Yamazaki, Y. Takeuchi and T. Oishi, *J. Antibiotics*, **24**, 29 (1971).
2) Location: *Juso, Higashiyodogawa-ku, Osaka.*
3) Part I: E. Higashide, T. Fugono, K. Hatano and M. Shibata, *J. Antibiotics*, **24**, 1 (1971).
4) Part II: S. Harada, T. Kishi and K. Mizuno, *J. Antibiotics*, **24**, 13 (1971).
5) Part III: T. Fugono, S. Harada, E. Higashide and T. Kishi, *J. Antibiotics*, **24**, 23 (1971).
6) S. Harada, E. Higashide, T. Fugono and T. Kishi, *Tetrahedron Letters*, **1969**, 2239.
7) K. Kamiya, S. Harada, Y. Wada, M. Nishikawa and T. Kishi, *Tetrahedron Letters*, **1969**, 2245.
8) The abbreviations used are as follows; MS: mass spectrun (*m/e*), NMR: nuclear magnetic resonance (δ_{ppm}), IR: infrared absorption (ν_{max}), UV: ultraviolet absorption (λ_{max}).

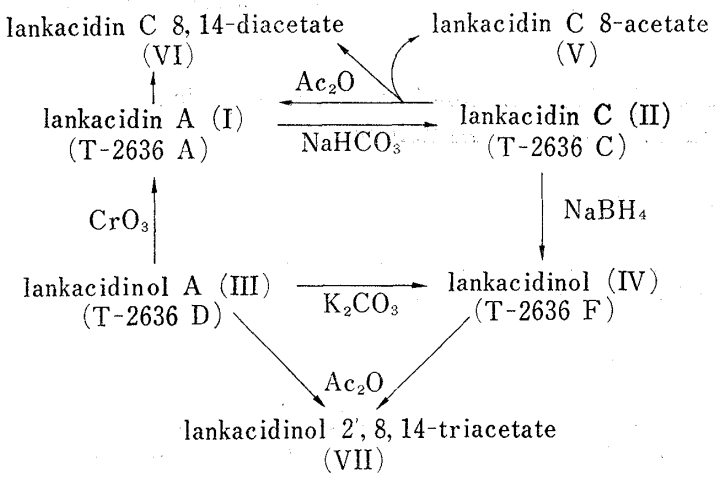
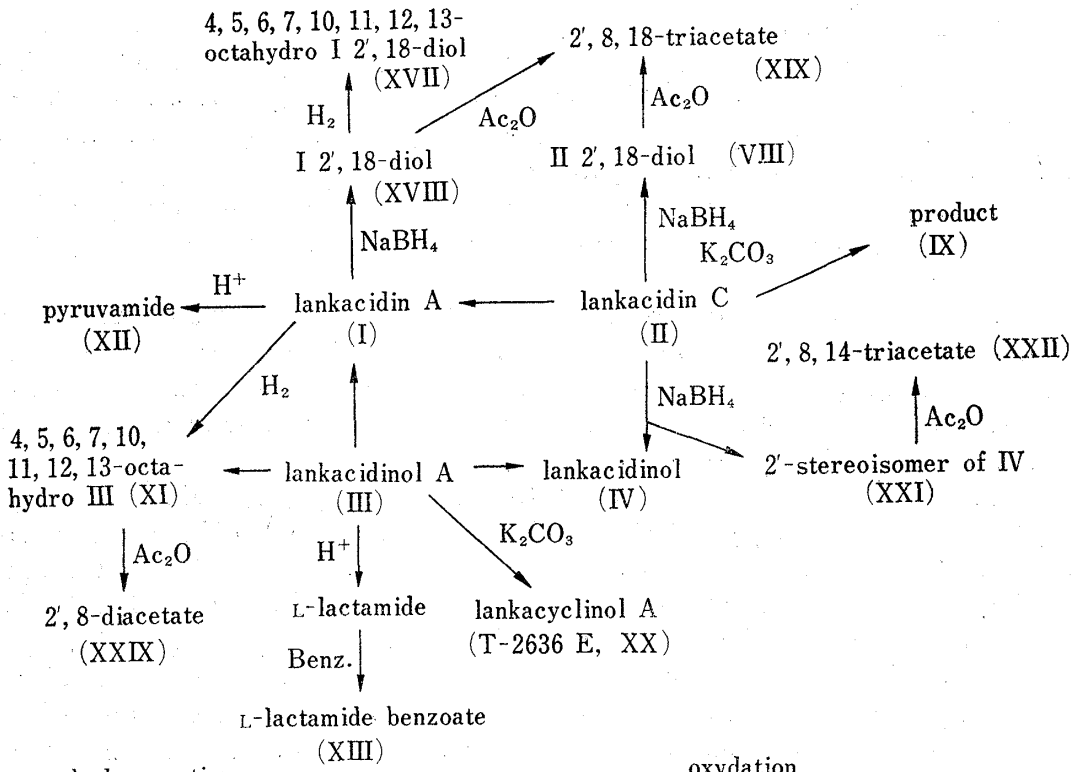


Chart 1

1. Two secondary hydroxyls, from the NMR and IR spectra of I, II,[4] V and VI ($\delta_{ppm}^{CDCl_3}$ 4.06 (1H, multiplet, H_8), 4.26 (1H, multiplet, H_{14}) in II);
2. An acid amide group, from the IR of VI (ν_{max}^{KBr} cm^{-1}: 3400 and 1680) and the NMR of II ($\delta_{ppm}^{CDCl_3}$ 8.0 (1H, doublet, J=9 Hz, which disappeared slowly with addition of D_2O));
3. A methyl ketone group, from a signal of $\delta_{ppm}^{d_6\text{-DMSO}}$ 2.32 (3H, singlet) and an absorption of ν_{max}^{KBr} cm^{-1} 1725 in II, which shifted to $\delta_{ppm}^{d_6\text{-DMSO}}$ 1.2 (3H, doublet, J=6 Hz) and disappeared from carbonyl region in IV;[5]



hydrogenation

II $\xrightarrow[EtOH]{Pd/BaSO_4}$ 6, 7-dihydro II (XIV)

II $\xrightarrow[EtOH]{Pd\text{-}C}$ 6, 7, 12, 13-tetrahydro II (X)

II $\xrightarrow[EtOH]{PtO_2}$ 4, 5, 6, 7, 10, 11, 12, 13-octahydro II 2'-ol (XXVI)

II $\xrightarrow[AcOH]{PtO_2}$ 8-deoxy-4, 5, 6, 7, 10, 11, 12, 13-octahydro II 2'-ol (XXVII)

oxydation

III $\xrightarrow{CrO_3}$ 8-oxo III (XXX), 8-oxo I (XV)

I $\xrightarrow{CrO_3}$ (XV), (XXV)

II $\xrightarrow{CrO_3}$ 14-oxo II (XXXI)

VII $\xrightarrow{MnO_2}$ 14-oxo II 8-acetate (XVI)

Chart 2

4. An isolated carbonyl group, from ν_{max}^{KBr} cm^{-1} 1710 in II and the fact that UV spectrum of I[4] was the same value as that of lankacidin C 2′,18-diol (VIII, $C_{25}H_{37}O_7N$ λ_{max}^{EtOH} nm (ε): 229 (48100)), which was obtained by sodium borohydride reduction of II;
5. A lactone group, from ν_{max}^{KBr} cm^{-1} 1750 in II and the fact that the IR spectrum of the product obtained from treatment of II with potassium carbonate[9] (IX) showed an absorption at ν_{max}^{KBr} cm^{-1} 1650 (COO^-);
6. Two trans disubstituted double bonds and two allylic methyl groups, from the results of the NMR comparison studies in I, 6,7,12,13-tetrahydro lankacidin C (X, $C_{25}H_{37}O_7N$) and 4,5,6,7,10,11,12,13-octahydro lankacidinol A (XI, $C_{27}H_{45}O_8N$) obtained by catalytic hydrogenation of II and III, respectively, as shown below;

Compound	Chemical shift ($\delta_{ppm}^{CDCl_3}$)			
	H_4	H_{10}	$5CH_3$	$11CH_3$
I	4.68	5.3—5.6	1.90	1.54
X	4.72	5.36	1.84	1.66
XI	1.2—1.8		0.9	

7. One each of tertiary methyl and secondary methyl, from $\delta_{ppm}^{CDCl_3}$ 1.38 (3H, singlet) and 1.24 (3H, doublet, J=7 Hz) in II and
8. Two methylene groups, from $\delta_{ppm}^{CDCl_3}$ 2.2—2.5 in II.

From these data, characteristics of all carbons except for carbon at 3-position are clarified, therefore bicyclic structure are suggested from the unsaturation number in II (ten).

Mild acid hydrolysis of I and III yielded a pyruvamide[10] (XII) and a lactamide as aqueous hydrolysates, which were derived to a pyruvamide 2,4-dinitro-phenyl hydrazone and an L-lactamide benzoate[11] (XIII), respectively. Therefore, the acid amide group should be combined with methyl ketone group in I and II, or methyl carbinol group in III and IV, and that, from the results of NMR spin-decoupling in I (Fig. 1) and X (Fig. 2), it is suggested that two partial structures shown below, (A′) and (C′), exist in II.

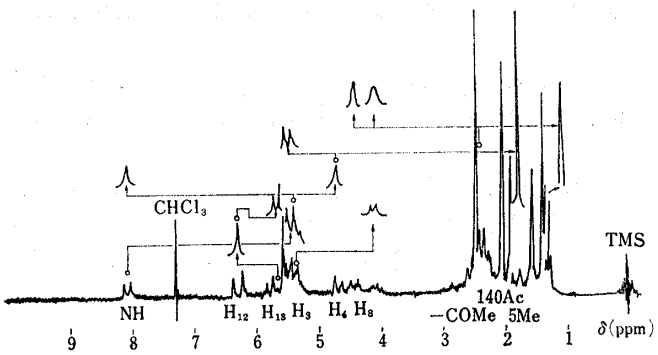


Fig. 1. NMR Spectrum of Lankacidin A (II)
100 MHz, $CDCl_3$

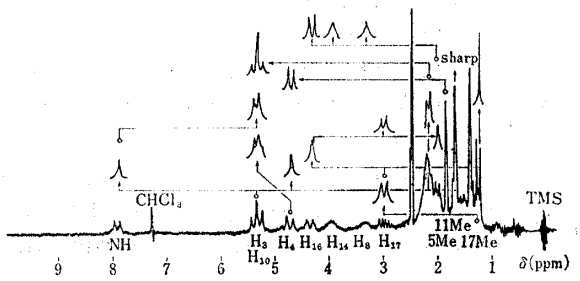
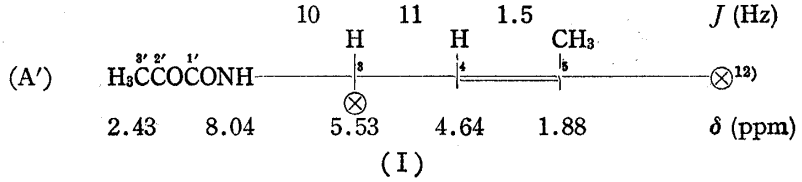


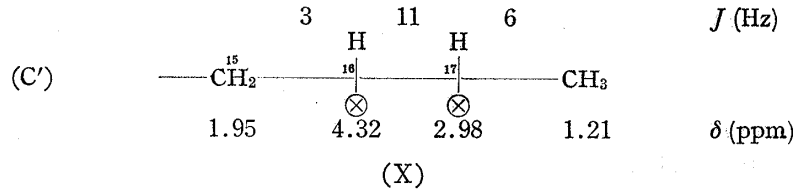
Fig. 2. NMR Spectrum of 6,7,12,13-Tetrahydro Lankacidin C (X)
100 MHz, $CDCl_3$

Part structure



(I)

9) Part VI: S. Harada, *Chem. Pharm. Bull.* (Tokyo), in preparation.
10) L. Claisen und J. Shadwell, *Chem. Ber.*, **11**, 1563 (1878).
11) C.M. Bean, J. Kenyon and H. Phillips, *J. Chem. Soc.*, **1943**, 303.
12) No coupling was observed.



(X)

On partial catalytic hydrogenation, II gave 6,7-dihydro lankacidin C (XIV, $C_{25}H_{35}O_7N$) and X, and their UV and IR spectra are shown below;

Compound	UV λ_{max}^{EtOH} nm (ε)	IR $\nu_{max}^{CHCl_3}$ cm^{-1}
II	226 (48600)	1750, 1725, 1710
XIV	234 (21600)	1740, 1725, 1700
X	245 (3140)	1745, 1725, 1710

Subtraction of the UV absorption of XIV from that of II leaves a distinct absorption maximum at 224 nm (ε=35600).

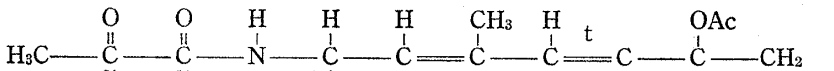
These absorptions at 224 and 234 nm may be ascribable to an α,β-unsaturated lactone[13] and a diene,[14] or two dienes. In the IR spectra of II, XIV and X, no shifts of carbonyl absorptions were observed, thus the presence of an α,β-unsaturated lactone group was ruled out.

Two single proton singnals at 4.06 and 4.26 ppm in II, which are ascribable to secondary hydroxylic methine protons, showed up-field shifts in the NMR spectra of X (Fig. 2) at 3.34 ppm (1H, multiplet, H_8) and 3.98 (1H, multiplet, H_{14}). Therefore, the two secondary hydroxyls should be present at positions α to double bonds. Furthermore, mild oxidation of I and V with chromium trioxide or manganese dioxide afforded 8-oxo lankacidin A (XV, $C_{27}H_{33}O_8N$ λ_{max}^{EtOH} nm (ε): 237 (34900), 275 (17800), $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680, 1590 ($\alpha,\beta,\gamma,\delta$-unsaturated carbonyl)) and 14-oxo lankacidin C 8-acetate (XVI, $C_{27}H_{33}O_8N$ λ_{max}^{EtOH} nm (ε): 233 (29600), 288 (16000), $\nu_{max}^{CHCl_3}$ cm^{-1}: 1680, 1590 ($\alpha,\beta,\gamma,\delta$-unsaturated carbonyl)), respectively. These spectral data and the fact that methylene and vinyl protons adjacent to newly occurring carbonyl groups in the NMR spectra of XV and XVI showed down-field shifts as compared with those in II.[15]

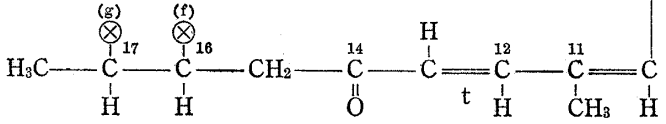
The presence of the part structures shown in Table I, (A), (B), (B') and (C) was collaborated by the above-described data and the NMR spin-decoupling of XV and XVI, which are shown in Fig. 3 and 4.

The NMR spectrum of X suggests the presence of two allylic methyl groups and its UV spectrum rules out the presence of a diene system in X. Therefore, the only possible combination of the aliphatic part structure has to be (b)—(c) and (d)—(e) as shown below in XVI;

(A)



(B)



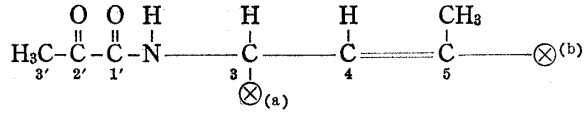
(C)

13) P.W.K. Woo, H.W. Dion and Q.R. Bartz, *J. Am. Chem. Soc.*, **86**, 2724 (1964).
14) S. Omura, H. Ogura and T. Hata, *Tetrahedron Letters*, **1967**, 609.
15) F. McCapra, A.I. Scott, P. Delmotte, J. Delmotte-Plaquée and N.S. Bacca, *Tetrahedron Letters*, **1964**, 869.

TABLE I

Part structure

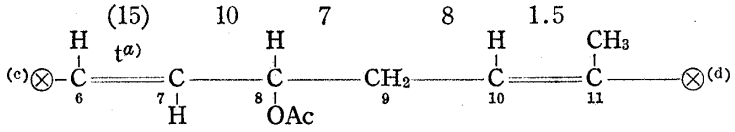
(A)



2.45 7.98 5.46 4.68 1.95 δ (ppm)

XVI

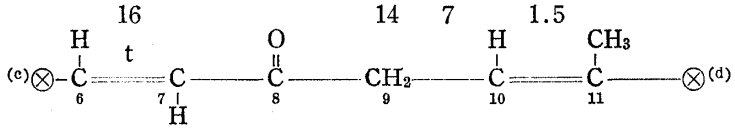
(B)



(5.6—5.5) 5.02 2.52 5.83 1.58 δ (ppm)

XVI

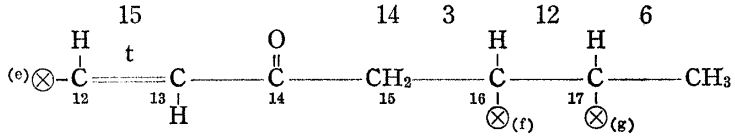
(B′)



6.70 6.0 3.85 5.70 1.63 δ (ppm)

XV

(C)



7.15 6.50 2.96 4.50 2.25 1.10 δ (ppm)

XVI

a) *trans*

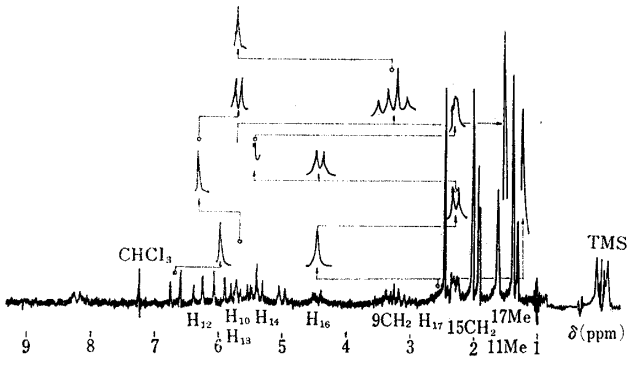


Fig. 3. NMR Spectrum of 8-Oxo Lankacidin A (XV)
100 MHz, $CDCl_3$

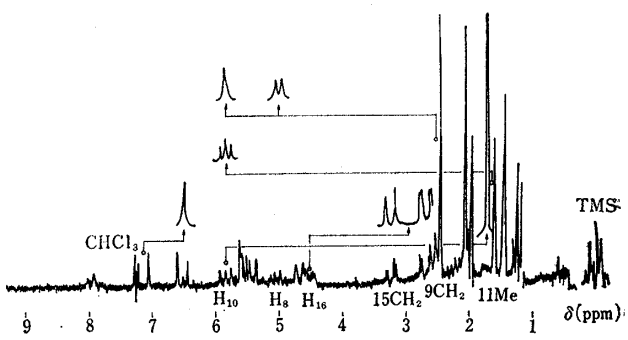


Fig. 4. NMR Spectrum of 14-Oxo-lankacidin C, 8-Acetate (XVI)
100MHz, $CDCl_3$

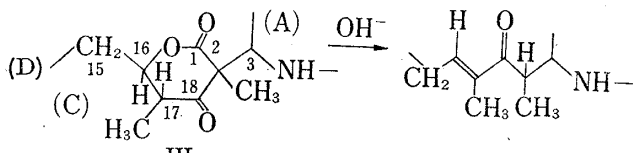
The methine proton at 2.98 ppm in X showed up-field shift to 2.0 ppm in 4,5,6,7,10,11,-12,13-octahydro 2′,18-diol (XVII, $C_{27}H_{47}O_8N$, Mass Spectrum *m/e*; 513 (M^+)) which was obtained from lankacidin A 2′,18-diol (XVIII, $C_{27}H_{39}O_8N$) by catalytic hydrogenation. The methine proton at 4.32 ppm in X was no shift and observed at 4.1 ppm in 2′,8,18-triacetate of XVIII (XIX, $C_{33}H_{45}O_{11}N$, Mass Spectrum *m/e*; 631 (M^+)) which was also obtained by acetylation of VIII.

Thus, the isolated carbonyl and lactone groups should be adjacent to the methine protons at the position (g) and (f), respectively.

The remaining part of the total molecule of XVI can now be accounted for by a single tertiary methyl group of which position is assumed to be constructed in the part structure

TABLE II

Part structure

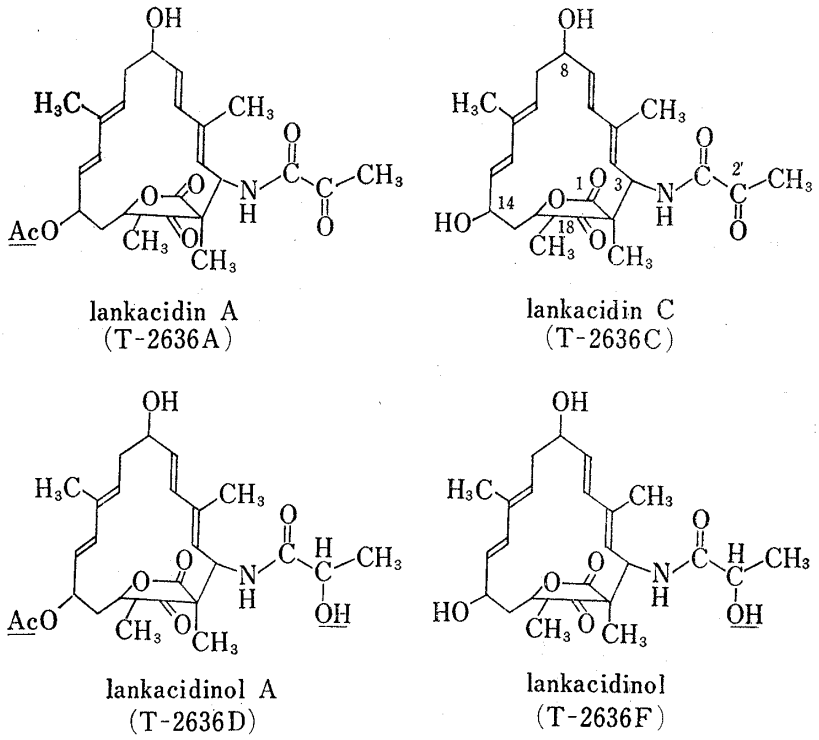


III XX

(D) enlarged with the combination of the residuous position (a), isolated carbonyl and lactone functions as shown in Table II.

And also, the six-membered ring moiety was finally deduced from the structural elucidation[9] of lankacyclinol A (T-2636E, XX, $C_{26}H_{37}O_6N$, Mass Spectrum *m/e*; 459 (M^+))[4] obtained by basic decomposition of III, in which the part structure was elucidated by NMR spin-decoupling studies of XX.

In this β-keto-δ-lactone system, the absorption of the lactone in II (ν_{max}^{KBr} cm^{-1}: 1755) shifts to ν_{max}^{KBr} cm^{-1} 1705 in that of VIII. This abnormal absorption in II to high frequency is accounted for the strain by the β-keto radical.



lankacidin A (T-2636A)

lankacidin C (T-2636C)

lankacidinol A (T-2636D)

lankacidinol (T-2636F)

Chart 3

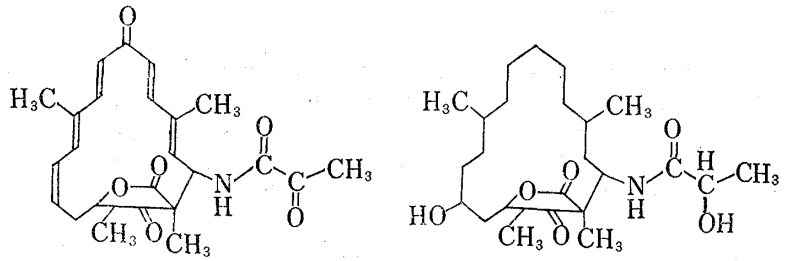
On the basis of these findings, the structures I, II, III and IV have been proposed for lankacidin-group (T-2636) antibiotics as shown in Chart 3.

In the reduction-oxidation reaction of lankacidin-group antibiotics, some interesting findings were observed. At first, 2′-stereoisomer of IV (XXI, $C_{25}H_{35}O_7N$) was synthesized together with IV by 0.25 molar sodium borohydride reduction of II and separated with preferential crystallization from methanol. The triacetate of XXI (XXII, $C_{31}H_{41}O_{10}N$) are similar to VII in the MS spectrum (*m/e* 587 (M^+)), elemental analysis and IR spectrum ($CHCl_3$), however, the chemical shifts of the NMR spectrum shown below and finger prints of the IR (KBr) spectrum are apparently different with those of VII.

Compound	Chemical shift ($\delta_{ppm}^{CDCl_3}$)			
	2-CH_3	2′-OAc	$H_{2'}$	-NHCO-
VII	1.40	2.16	5.11	7.30
XXII	1.32	2.22	5.19	7.19

On the other hand, mild acid hydrolysis of III gave L-lactamide, therefore, the chemical formula of XXI may be 2′-stereoisomer of IV (2′-D type). The same treatment of VI gave lankacidinol 8,14-diacetate (XXIII, $C_{29}H_{39}O_9N$) and its 2′-stereoisomer (XXIV) which were acetylated to afford VI and XXII, respectively.

On chromium trioxide oxidation, I gave pale yellow crystals (XXV, $C_{25}H_{29}O_6N$, Mass Spectrum *m/e*; 439 (M^+)) and XV. The NMR and IR spectra of XXV indicates that there are no acetyl, hydroxyl and one methylene group in comparison with those of I, and the UV spectrum of XXV showed three maxima in 233 nm (ε=23600), 260 (20000) and 340 (12000). These data and the results of the NMR spin-decoupling of XXV suggest the structure XXV as shown in Chart 4.



XXV XXVII

Chart 4

When II was hydrogenated over platinum dioxide in acetic acid, 4,5,6,7,10,11,12,13-octahydro lankacidin C 2′-ol (D,L-mixture, XXVI, $C_{25}H_{43}O_7N$ Mass Spectrum *m/e*; 469 (M^+)) and its 8-deoxy compound (XXVII, $C_{25}H_{43}O_6N$ Mass Spectrum *m/e*; 453 (M^+)) were obtained. In the IR spectrum of XXVII, absorptions at ν_{max}^{KBr} cm^{-1} 1750 (lactone), 1710 (18 carbonyl) were observed. From the NMR spectrum and the results of its spin-decoupling study only one signal of a methylene at 15-position was observed in down-field ($\delta_{ppm}^{CD_3OD}$ 2.06 (2H, triplet like, $J_{16H,15CH_2}$=3 Hz, $J_{16H,17H}$=11 Hz)) and the other signals originated from methylenes were observed in up-field ($\delta_{ppm}^{CD_3OD}$ 1.2—1.5). These spectral data are attributable to determine the structure XXVII as shown in Chart 4.

Experimental

All melting points are uncorrected and show decomposition points. Optical rotations and UV spectra are measured in EtOH unless otherwise stated.

Lankacidin C 8,14-Diacetate (VI), C 14-Acetate (I) and C 8-Acetate (V)——To a solution of II (230 mg) in pyridine (0.5 ml) and THF (2 ml) was dropwise added AcCl (0.12 ml) and the whole was stirred at 0—5° for 1.5 hr. The reaction mixture was poured into ice water and extracted with AcOEt. The concentrate of the extract was separated with preparative thin-layer chromatography (TLC) on Silica gel HF_{254} (Merck Co.) with AcOEt: benzene (1: 2) to obtain VI (40 mg), I (32 mg) and V (34 mg). VI and I were in good accord with the standard samples[4] in *Rf* values of TLC, mp, $[\alpha]_D$, UV, IR, MS and NMR spectra. V was recrystallized from AcOEt–ether mp 203—204°, $[\alpha]_D^{22}$ −218° (*c*=0.5), UV λ_{max}nm (ε): 226 (50600). *Anal.* Calcd. for $C_{27}H_{35}O_8N$: C, 64.65; H, 7.03; N, 2.79. Found: C, 64.62; H, 7.31; N, 2.83.

2′-Oxo Lankacidinol A (I)——A solution dissolving III (1 g) in pyridine (10 ml) was added dropwise into a complex prepared from CrO_3 (1 g) and pyridine (10 ml). The mixture was stirred at 25° for 5 min, poured into ice water, neutralized with dil. HCl and extracted with AcOEt. The concentrate of the extract was purified by chromatography on silica gel (0.05—0.2 mm, Merck Co.). After evaporation of the eluate of benzene: AcOEt (7: 3), the obtained crude crystals were recrystallized from AcOEt–ether to give colorless prisms (20 mg). This compound was identified with I in *Rf* values of TLC, mp, IR, MS and NMR spectra, and antimicrobial activities.

Lankacidinol (IV) and 2′-Stereoisomer of IV (XXI)——$NaBH_4$ (162 mg) in MeOH (18 ml) was on a portion added into a solution of II (6.9 g) in MeOH (300 ml) at 0—5° for 1 hr with stirring. After stirring at 25° for 3 hr, AcOH (7.5 ml) and H_2O (150 ml) were added to the reaction mixture and the solution was concentrated *in vacuo* to give crystallized mass (4.0 g). Recrystallization from MeOH afforded IV (3.7 g) which

was identified with the standard sample[4] in *Rf* values of TLC, IR and NMR spectra, mp 178—179°, $[\alpha]_D^{23}$ −202° (c=0.5, DMF), UV λ_{max} nm (ε): 228 (49300). *Anal.* Calcd. for $C_{25}H_{35}O_7N \cdot MeOH$: C, 63.27; H, 7.96; N, 2.84. Found: C, 63.03; H, 8.03; N, 2.55.

Mother liquor after filtration was cooled at 0—5° overnight to give crystals (1.4 g). Recrystallization from MeOH gave XXI (0.75 g) as colorless plates, mp 169—171°, $[\alpha]_D^{26}$ −192° (c=0.5, DMF), UV λ_{max} nm (ε): 228 (50000). *Anal.* Calcd. for $C_{25}H_{35}O_7N \cdot MeOH$: C, 63.27; H, 7.96; N, 2.84. Found: C, 63.03; H, 8.02; N, 2.59.

Lankacidinol 2′,8,14-Triacetate (VII)——A solution of synthesized IV (250 mg) in Ac_2O (1 ml) and pyridine (2 ml) was held at 25° overnight and then, poured into ice water. Crude crystals were filtered and recrystallized from ether to give pure crystals (217 mg). The obtained acetate was identified with lankacidinol A 2′,8-diacetate[4] in *Rf* values of TLC, mp, $[\alpha]_D$, *Anal.*, UV, IR, MS and NMR spectra.

2′-Stereoisomer of VII (XXII)——A solution of XXI (150 mg) in Ac_2O (1 ml) and pyridine (2 ml) was allowed to stand at 25° overnight and then poured into ice water. The crude acetate collected by filtration and recrystallized from hexane–ether to afford colorless needles (137 mg). mp 122—124°, $[\alpha]_D^{25}$ −166° (c=0.5), UV λ_{max} nm (ε): 228 (51400). *Anal.* Calcd. for $C_{31}H_{41}O_{10}N$: C, 63.36; H, 7.03; N, 2.38. Found: C, 63.13; H, 7.01; N, 2.05.

Lankacidinol 8,14-Diacetate (XXIII) and Its 2′-Stereoisomer (XXIV)——To a solution of VI (5.5 g) in MeOH (250 ml) a suspension of $NaBH_4$ (120 mg) in MeOH (15 ml) was added by portions at 5—10° with stirring and the whole was stirred at 25° for 3 hr. After addition of H_2O, the reaction mixture was extracted with AcOEt and followed by washing with H_2O. The concentrated residue was applied to silica gel (100 g) on a column and each eluate of benzene: AcOEt (70—65: 30—35), (60: 40) and (55—50: 45—50) was separately evaporated. Each concentrate was crystallized from AcOEt–ether to give XXIII (780 mg), the mixture of XXIII and XXIV (2.3 g) and XXIV (875 mg), respectively. XXIII; mp 181°, $[\alpha]_D^{19}$ −218° (c=0.48), UV λ_{max} nm (ε): 228 (45200). *Anal.* Calcd. for $C_{29}H_{39}O_9N$: C, 63.83; H, 7.21; N, 2.57. Found: C, 63.38; H, 7.28; N, 2.70. XXIV; mp 145°, $[\alpha]_D^{19}$ −188° (c=0.57). UV λ_{max} nm (ε): 229 (44100). *Anal.* Calcd. for $C_{29}H_{39}NO_9$: C, 63.83; H, 7.21; N, 2.57. Found: C, 63.15; H, 7.41; N, 2.49.

Lankacidin A 2′,18-Diol (XVIII)——$NaBH_4$ (925 mg) in MeOH (10 ml) was added dropwise into a solution of I (5 g) dissolved in MeOH (100 ml) at 0—5° for 1 hr with stirring. After stirring at room temperature for 1 hr, AcOH (10 ml) was added in order to decompose the adduct. After addition of H_2O (100 ml), the reaction mixture was evaporated *in vacuo* and extracted with AcOEt.

The extract was concentrated after washing with H_2O and dehydration. The concentrate was precipitated with ether to give colorless powder (4 g); $[\alpha]_D^{23}$ −165° (c=0.5), UV λ_{max} nm (ε): 231 (49600). *Anal.* Calcd. for $C_{27}H_{39}O_8N$: C, 64.14; H, 7.78; N, 2.77. Found: C, 64.21; H, 7.84; N, 2.74.

Lankacidin A 2′,18-Diol 2′,8,18-Triacetate (XIX)——A solution of XVIII (500 mg) in Ac_2O (2.5 ml) and pyridine (5 ml) was allowed to stand at 25° for 24 hr. The reaction mixture was added into ice water and the mass was filtered. The crude mass was applied by prep. TLC of silica gel and developed with benzene: AcOEt (1: 1).

The main product scratched from silica gel plate was extracted with AcOEt and the extract was concentrated after washing with H_2O. The residue was recrystallized from ether to obtain colorless prisms (310 mg); mp 145—149°, $[\alpha]_D^{22}$ −180° (c=0.43), UV λ_{max} nm (ε): 230.5 (55100). *Anal.* Calcd. for $C_{33}H_{45}O_{11}N$: C, 62.74; H, 7.18; N, 2.22. Found: C, 62.25; H, 7.15; N, 2.35.

Lankacidin C 2′,18-Diol (VIII)——$NaBH_4$ (110 mg) in MeOH (10 ml) was added dropwise into a solution of II (460 mg) dissolved in MeOH (20 ml) at 0—5° with stirring and the reaction mixture was stirred at 25° for 2 hr. After addition of AcOH (1 ml) and H_2O (10 ml), the solution was concentrated *in vacuo* to remove MeOH. The concentrate was extracted with BuOH after addition of H_2O and the extract was concentrated *in vacuo*. The residue was precipitated with ether to give colorless powder (450 mg); $[\alpha]_D^{24}$ −35.1° (c=0.5, DMF). UV λ_{max} nm (ε): 229 (44600). *Anal.* Calcd. for $C_{25}H_{37}O_7N$: C, 64.77; H, 8.05; N, 3.02. Found: C, 64.90; H, 8.17; N, 2.74.

4,5,6,7,10,11,12,13-Octahydro Lankacidin A 2′,18-Diol (XVII)——To a solution of XVIII (1 g) in EtOH (100 ml) was added PtO_2 (200 mg) in order to hydrogenate by shaking for several hr. The solution absorbed 4 molar hydrogen was filtered, concentrated and crystallized from AcOEt to yield colorless prisms (500 mg); mp 216—220°, $[\alpha]_D^{22}$ −3.5° (c=1.0), UV; end absorption (ε<10).

6,7-Dihydro Lankacidin C (XIV)——To a solution of II (460 mg) in EtOH (50 ml) was added 5% Pd/$BaSO_4$ (100 mg) in order to perform on contact hydrogenation and the mixed reaction solution was shaken at room temperature for 40 min to absorb *ca.* 1.2 molar hydrogen. After filtration of the reaction solution, the concentrate of the filtrate was separated by prep. TLC of silica gel. This compound had a very few higher *Rf* value than that of starting material and colorized light blue with conc. H_2SO_4. The absorption-band detected with UV lamp was extracted with AcOEt. The extract was washed with H_2O, concentrated *in vacuo* and precipitated with hexane–AcOEt to afford colorless powder (160 mg). $[\alpha]_D^{22}$ −113° (c=0.5), UV λ_{max} nm (ε): 234 (21600). *Anal.* Calcd. for $C_{25}H_{35}O_7N$: C, 65.06; H, 7.64; N, 3.03. Found: C, 64.54; H, 8.19; N, 2.85.

6,7,12,13-Tetrahydro Lankacidin C (X)——A solution of II (920 mg) in EtOH (100 ml) was hydrogenated over 5% Pd-C (200 mg) to absorb *ca.* 2.5 molar hydrogen during 2 hr. After filtration the reaction mixture

was concentrated and crystallized from AcOEt to give colorless plates (400 mg); mp 170—174°, $[\alpha]_D^{22}$ −127° (c=0.5), UV λ_{max} nm (ε): 245 (3140). *Anal.* Calcd. for $C_{25}H_{37}O_7N$: C, 64.77; H, 8.05; N, 3.02. Found: C, 64.80; H, 8.09; N, 2.98.

4,5,6,7,10,11,12,13-Octahydro Lankacidinol A (XI)——A solution of III (1 g) was hydrogenated in EtOH (150 ml) over PtO_2 (150 mg) for 6 hr to absorb *ca.* 4.2 molar hydrogen at which time the hydrogen uptake had ceased. The filtrate of the reaction solution was concentrated *in vacuo* and recrystallized from AcOEt toafford colorless needles (580 mg); mp 172—175°, $[\alpha]_D^{22}$ +3.8° (c=1.0), UV λ_{max} nm (ε): 285 (107). *Anal.* Calcd. for $C_{27}H_{45}O_8N$: C, 63.38; H, 8.87; N, 2.74. Found: C, 63.34; H, 8.84; N. 2.92.

4,5,6,7,10,11,12,13-Octahydro Lankacidinol A (XI′, 2′-D,L-mixture)——A solution of I (1 g) in EtOH (150 ml) was hydrogenated over PtO_2 (150 mg) at 25° for 8 hr to absorb 4.8 molar hydrogen. The filtrate of the reaction mixture was concentrated and purified by column chromatography on silica gel (30 g). The eluate of benzene: AcOEt (2: 8) gave colorless crystalline powder (480 mg) which was crystallized from ether–AcOEt; mp 125—128°, $[\alpha]_D^{23}$ +5.2° (c=1.0). *Anal.* Calcd. for $C_{27}H_{45}O_8N$: C, 63.38; H, 8.87; N, 2.74. Found: C, 63.69; H, 8.92; N, 2.85.

4,5,6,7,10,11,12,13-Octahydro Lankacidinol A 2′,8-Diacetate (XXVIII)——A solution of XI (2′-L-type, 97 mg) in Ac_2O (0.5 ml) and pyridine (1 ml) was allowed to stand at 25° for 40 hr. After treatment with the usual method, the concentrated residue was purified by column chromatography on silica gel (3 g) with benzene: AcOEt (8: 2).

The concentrated fraction was precipitated with ether–hexane to give a white powder (50 mg). This diacetate was almost in accord with the diacetate of XI′ (2′-D,L-mixture) in *Rf* values of TLC, IR and MS spectra except for NMR spectrum.

4,5,6,7,10,11,12,13-Octahydro Lankacidin C 2′-ol (XXVI) and 8-Deoxy 4,5,6,7,10,11,12,13-Octahydro Lankacidin C 2′-ol (XXVII)——A solution of II (4.6 g) was hydrogenated in AcOH (500 ml) over PtO_2 (1.5 g) for 8 hr. The reaction solution which absorbed 6.2 molar hydrogen was concentrated *in vacuo* and the residue was purified by chromatography on silica gel (130 g). Evaporation of the eluate of AcOEt afforded colorless needles (2.6 g). mp 158—160°, $[\alpha]_D^{22}$ +29.6° (c=1.0), UV λ_{max} nm (ε): 292 (52). *Anal.* Calcd. for $C_{25}H_{43}O_7N$: C, 63.94; H, 9.23; N, 2.98. Found: C, 63.70; H, 9.46; N, 2.76.

Furthermore, from the eluate of benzene: AcOEt (1: 1) a deoxy product was obtained as colorless needles (210 mg); mp 185—187°, $[\alpha]_D^{21}$ +3.6° (c=0.5), UV λ_{max} nm (ε): 300 (47). *Anal.* Calcd. for $C_{25}H_{43}O_6N$: C, 66.19; H, 9.56; N, 3.09. Found: C, 66.40; H, 9.68; N, 3.69.

8-Oxo Lankacidinol A (XXIX), Lankacidin A (I) and 8-Oxo Lankacidin A (XV)——III (2 g) in pyridine (20 ml) was added to CrO_3 (1 g) in pyridine (10 ml) at 0—5° with stirring and held at 0° for 13 hr.

After addition into ice water, the reaction mixture was extracted with AcOEt and the extract was washed with dil. HCl, H_2O and 2% $NaHCO_3$, dehydrated and concentrated *in vacuo*. The concentrate was purified by chromatography on silica gel (30 g).

The eluate of benzene: AcOEt (7: 3) was again separated with prep. TLC of silica gel with benzene: AcOEt (1: 1) and crystallized from AcOEt, ether and ether–AcOEt to give XXIX (10 mg), I (30 mg) and XV (5 mg), respectively. XXIX; mp 187—188°, UV λ_{max}nm (ε): 237 (29400), 275 (19900). *Anal.* Calcd. for $C_{27}H_{35}O_8N$: C, 64.65; H, 7.03; N, 2.79. Found: C, 64.21; H, 6.95; N, 3.01.

8-Oxo Lankacidin A (XV) and Its 14-Deacetoxy Compound (XXV)——The CrO_3-pyridine oxidation of I (5 g) was carried out by the method as described above and furnished XV (70 mg) as colorless plates and XXV (40 mg) as pale yellow plates. XV; mp 199—203°, $[\alpha]_D^{22}$ −206° (c=1.0), UV λ_{max} nm (ε): 237 (34900), 275 (17800). *Anal.* Calcd. for $C_{27}H_{33}O_8N$: C, 64.92; H, 6.66; N, 2.80. Found: C, 64.71; H, 6.65; N, 2.80. XXV; mp 178—180°, $[\alpha]_D^{22}$ −892° (c=0.5), UV λ_{max} nm (ε): 233 (23600), 260 (20000), 340 (12000). *Anal.* Calcd. for $C_{25}H_{29}O_6N$: C, 68.32; H, 6.65; N, 3.19. Found: C, 68.61; H, 6.64; N, 3.18.

14-Oxo Lankacidin C (XXX)——A solution of II (10 g) in pyridine (100 ml) was added at 0—5° into the complex prepared from CrO_3 (10 g) with pyridine (100 ml). After stirring for 3 hr, H_2O (1 liter) and AcOEt (1 liter) were added into the reaction mixture, filtered with Hyflo-Super Cel and the organic layer was concentrated. The residual oil was applied by chromatography of silica gel (200 mg) and eluted with benzene: AcOEt (7: 3). The concentrate of the eluate was crystallized from ether–AcOEt to afford colorless plates (170 mg). mp 198—203°, $[\alpha]_D^{23}$ −328° (c=0.5), UV λ_{max} nm (ε): 234 (30600), 292 (15500), $\lambda_{max}^{dioxane}$ nm (ε): 232 (28600), 292 (16400). *Anal.* Calcd. for $C_{25}H_{31}O_7N$: C, 65.63; H, 6.83; N, 3.06. Found: C, 65.47; H, 6.83; N, 2.95.

14-Oxo Lankacidin C 8-Acetate (XVI)——1) Ac_2O (0.4 ml) was added into a solution of XXX (100 mg) in pyridine (0.8 ml), which was allowed to stand at 25° for 13 hr. The reaction mixture was poured into ice water. The precipitates were filtered, washed with H_2O and crystallized from ether to obtain colorless plates (63 mg).

2) To a solution of V (1 g) in $CHCl_3$ (30 ml) was added activated MnO_2 (7 g), which was stirred at 25° for 3 hr. The precipitate was extracted well with $CHCl_3$ and the extract was concentrated after filtration. The concentrate was purified by chromatography on silica gel (30 g). The eluate of benzene: AcOEt (6: 4) was concentrated and crystallized from ether–AcOEt to yield colorless plates (200 mg); mp 197—200°, $[\alpha]_D^{22}$ −340° (c=0.366), UV λ_{max} nm (ε): 233 (29600), 288 (16000). *Anal.* Calcd. for $C_{27}H_{33}O_8N$: C, 64.92; H, 6.66; N, 2.80. Found: C, 64.67; H, 6.90; N, 2.54.

Pyruvamide, Acid Hydrolysate of II (XII)——A solution of II (10 g) in MeOH (230 ml), 1N HCl (55 ml) and H_2O (130 ml) was refluxed for 2 hr. The reaction mixture was extracted with $CHCl_3$ after addition of H_2O and its aqueous layer was concentrated *in vacuo* after neutralization with 1N NaOH. The oily residue was dissolved in EtOH and the soluble part was concentrated *in vacuo*, and then, these treatments were repeated twice. The concentrate was recrystallized from AcOEt–EtOH to yield colorless sublime prisms (70 mg); mp 104° (softened), 122° (melted). *Anal.* Calcd. for $C_3H_5NO_2$: C, 41.38; H, 5.79; N, 16.09. Found: C, 41.78; H, 5.74; N, 15.88), IR, NMR and MS spectra of this compound were in good accord with those of pyruvamide.[10] And also, NH_4Cl and pyruvic acid in the reaction mixture were detected with paper chromatography, NH_4Cl: Whatmann No. 1, BuOH: AcOH: H_2O (4: 1: 5), Rf=0.21 (ninhydrin reagent), $CH_3COCOOH$: Whatmann No. 1, EtOH: H_2O: NH_3 (20: 15: 1), Rf=0.67 (bromo phenol blue reagent).

Lactamide Benzoate from Hydrolysate of III (XIII)——A solution of III (10 g) dissolved in MeOH (300 ml) was refluxed for 1 hr after addition of 1N HCl (60 ml) and H_2O (100 ml). The reaction mixture neutralized with 2% $NaHCO_3$ was extracted with AcOEt and the aqueous layer was concentrated *in vacuo*. The soluble part in EtOH was concentrated *in vacuo* to give oily residue (*ca.* 1 g). This residue coincided in Rf value on paper chromatogram of BuOH: AcOH: H_2O (4: 1: 5) with D,L-lactamide.

This crude lactamide in pyridine (10 ml) was held at 25° for 15 hr after addition of Bz_2O (2 g). The reaction mixture added in ice water was extracted with AcOEt. The extract was washed with dil. HCl and 2% $NaHCO_3$, dried and concentrated *in vacuo*. The concentrate was purified by chromatography on silica gel (10 g) with benzene: AcOEt (7: 3). The main eluate was concentrated *in vacuo* and the residue was crystallized from ether–hexane to yield colorless needles (250 mg) which were recrystallized twice from ether; mp 94—101° (after melting of the needles, the prisms were newly appeared and followed slowly to decomposition), $[\alpha]_D^{23}$ +40° (c=0.5), UV λ_{max} nm (ε): 229 (14800), 274 (976) and 281 (761). *Anal.* Calcd. for $C_{10}H_{11}O_3N$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.57; H, 5.64; N, 7.61. These data were in good accord with those of L-lactamide benzoate.[11] And also, the IR ($CHCl_3$), MS and NMR spectra of XIII were identical with those of synthesized D,L-lactamide benzoate.

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