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

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Chemical structures of the minor components obtained from the culture filtrate of *Streptomyces rochei* var. *volubilis*, lankacyclinol A and iso-lankacidinol, and the antimicrobially active metabolites, lankacyclinol, were proposed by chemical degradation studies and spectral analyses. Lankacyclinol A and lankacyclinol were the decarboxylated compounds of lankacidinol A and lankacidinol, respectively. Iso-lankacidinol was assumed to be 16-epimer of lankacidinol. These compounds are belong to lankacidin-group (T-2636) antibiotics whose chemical structures are shown in Chart 1.

Lankacidin-group (T-2636) antibiotics, lankacidin A (T-2636 A, I), lankacidin C (T-2636 C, II), lankacidinol A (T-2636 D, III), lankacidinol (T-2636 F, IV) and lankacyclinol A (T-2636 E, V) have been isolated from the culture filtrate of *Streptomyces rochei* var. *volubilis*.[3-5] Their chemical structures except for V were determined as shown in Chart 1.[1,6,7]

These antibiotics and their derivatives are active against Gram-positive bacteria including resistant strains of staphylococci, mycoplasma and some of Gram-negative bacteria.[3,5,8] In protecting experiments of mice infection challenged with *Staph. aureus* 308 A-1, they show strong effect by oral or intraperitoneal administration and have low toxicity.[8,9]

In metabolic studies of lankacidin C 14-propionate-^{14}C, II, IV, lankacidinol 14-propionate, lankacyclinol (T-2636 G, VI) and T-2636 H (VII) were obtained from the rat bile as the antimicrobially active metabolites.[10]

While, new components of iso-lankacidinol (T-2636 I, VIII) and iso-lankacidinol O (T-2636 J, IX) were isolated from the culture filtrate of this organism.

This paper deals with the structural elucidation studies of V, VI and VIII by chemical degradation and spectral analyses as shown in Chart 1.

Isolation and Characterization of Iso-lankacidinol (VIII) and Iso-lankacidinol O (IX)

The culture filtrate of *Str. rochei* var. *volubilis* was extracted with methyl isobutyl ketone (MIBK) at pH 7. The extract was washed with water and concentrated *in vacuo*. The concentrate was purified with column chromatography on silica gel and followed by preferential crystallization of ethyl acetate–methanol to give VIII and IX.

These new components are distinguishable from other known components of lankacidin-group (T-2636) antibiotics by the *Rf* values of thin-layer chromatography (TLC).[9]

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2) Location: *Juso, Yodogawa-ku, Osaka, 532, Japan.*
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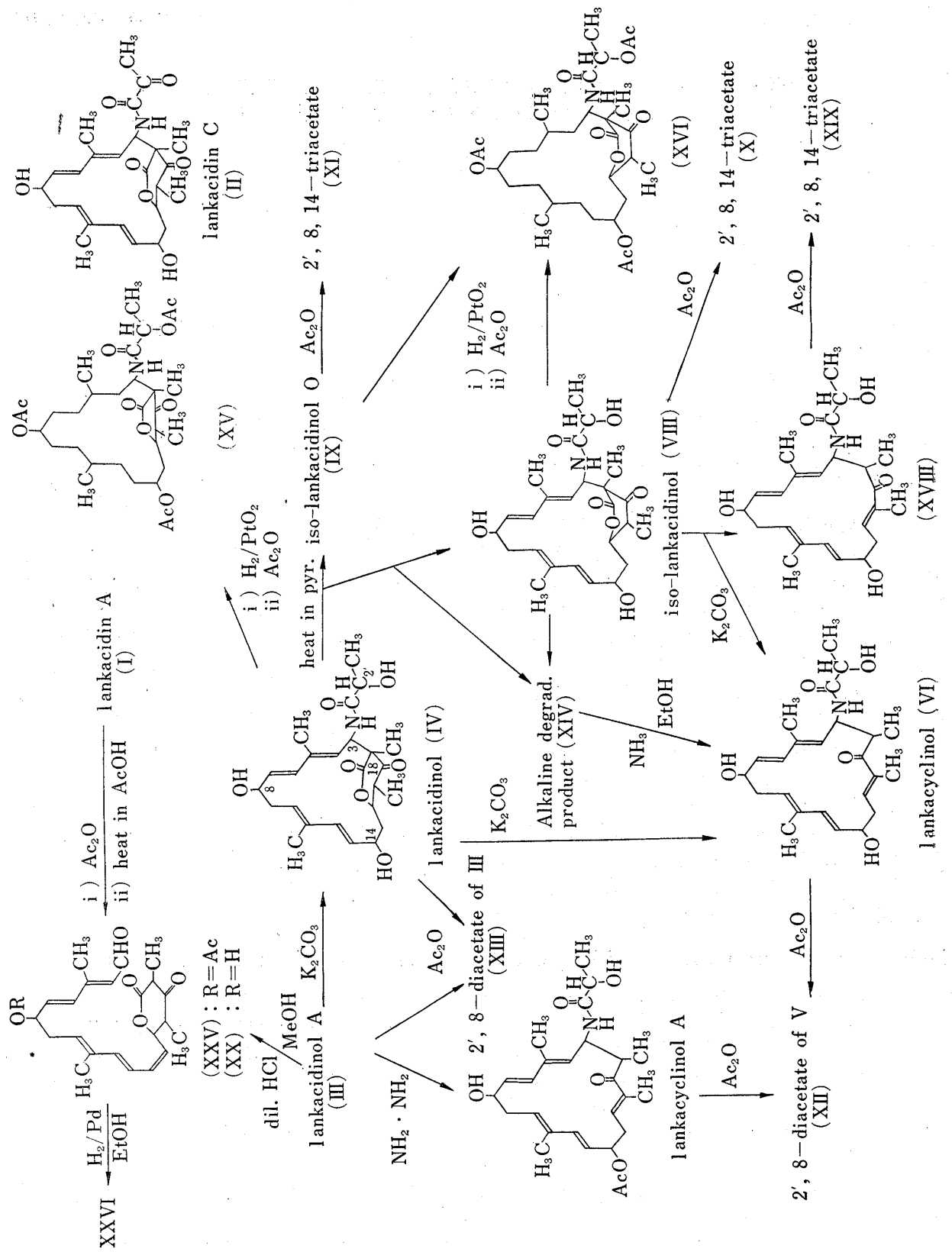


Chart 1

VIII was obtained from ethyl acetate–methanol as colorless prisms, mp 193° (decomp.), $[\alpha]_D^{25}$ −190° (c=0.53 in ethanol). This antibiotic shows violet-blue color with conc. sulfuric acid on TLC as same as known lankacidin-group antibiotics.[4] It is slightly soluble in diethyl ether, moderately soluble in ethyl acetate and soluble in acetone, methanol or

pyridine. Its molecular weight was measured with vapor pressure osmometry (V.P.O.) to be 490 in ethanol. VIII was acetylated to iso-lankacidinol 2′,8,14-triacetate (X). Its mass spectrum shows the peaks at *m/e* 587 (M^+), 543 (M^+-44 (CO_2)) and 527 (M^+-60 (AcOH)). These data and the elemental analysis of VIII indicate that the molecular formula is assumed to be $C_{25}H_{35}O_7N$. The ultraviolet (UV) spectrum of VIII shows a maximum at 228 nm in ethanol. The infrared (IR) and nuclear magnetic resonance (NMR) spectra are shown in Figs. 1 and 2, respectively.

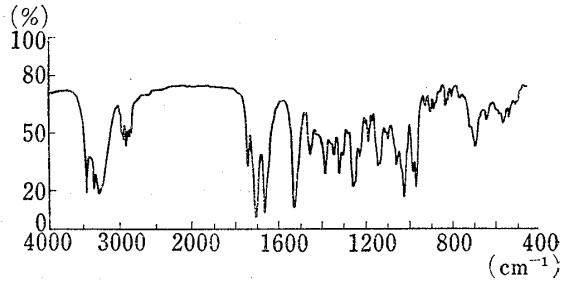


Fig. 1. IR Spectrum of Iso-lankacidinol (T-2636 I)
(KBr)

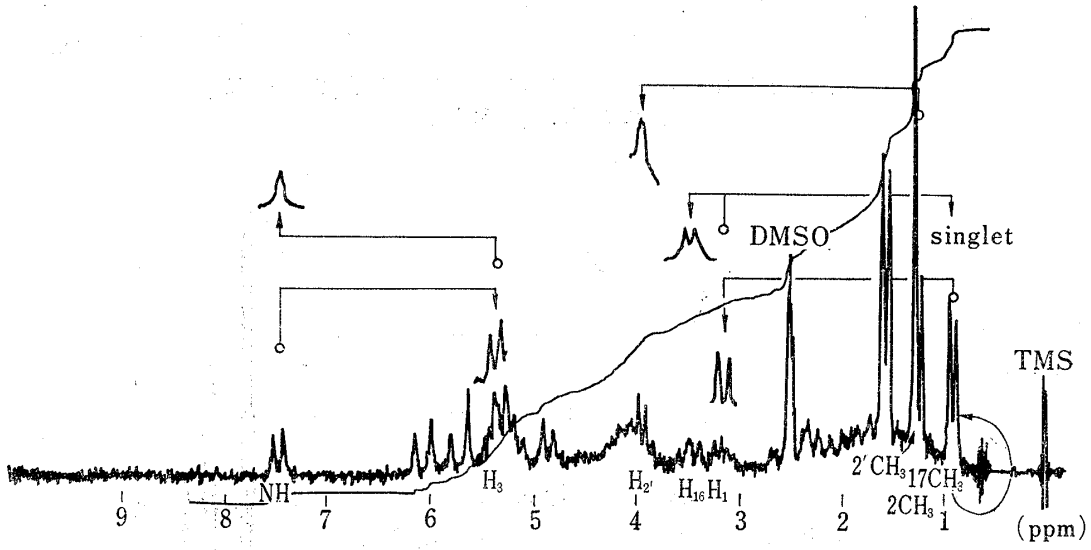


Fig. 2. NMR Spectrum of Iso-lankacidinol (T-2636 I)
(100 MHz, d_6-DMSO)
TMS: tetramethylsilane

IX was obtained from ethyl acetate–methanol as colorless prisms, mp 167° $[\alpha]_D^{26}$ +90.1° (c=0.52 in ethanol). It is more soluble in ethyl acetate than VIII, but solubility in other solvent is almost the same as VIII. The molecular weight is measured from V.P.O. to be 462 in ethanol. Acetylation of IX gave iso-lankacidinol O 2′ ,8,14-triacetate (XI) and its mass spectrum shows the peaks at *m/e* 603 (M^+), 587 (M^+-16 (O)), 543 (587−44 (CO_2)) and 527 (587−60 (AcOH)). From these data and the elemental analysis of IX, the molecular formula of IX is assumed to be $C_{25}H_{35}O_8N$. The UV spectrum of IX shows a maximum at 228 nm in ethanol. The NMR spectrum is shown in Fig. 3.

Chemical Strucrures of Lankacyclinol A (V) and Lankacyclinol (VI)

In the mass spectrum of V, the peaks assigned are *m/e* 459 (M^+), 399 (M^+-60 (AcOH)), 310 (399−89 ($H_2NCOCHOHCH_3$)) and 292 (310−18 (H_2O)). Its analytical data indicate the molecular formula of V as $C_{26}H_{37}O_6N$.[4] The subtraction value of the UV spectrum of III from that of V (ε=*ca.* 18000 at 228 nm) and the IR spectrum of V[4] suggest the presence of α,β-unsaturated ketone. In the NMR spectrum of V in d_6-DMSO (dimethylsulfoxide), newly occurred signals present at 0.84 (3H, d, J=6.0 Hz, 2-CH_3) and 1.65 ppm (3H, d, J=1.5 Hz, 17-CH_3) in comparison with that of III.

V was acetylated to lankacyclinol A 2′,8-diacetate (XII) whose mass spectrum showed the peaks at *m/e* 543 (M^+) and 483 (M^+-60 (AcOH)). The NMR spin-decoupling data of XII

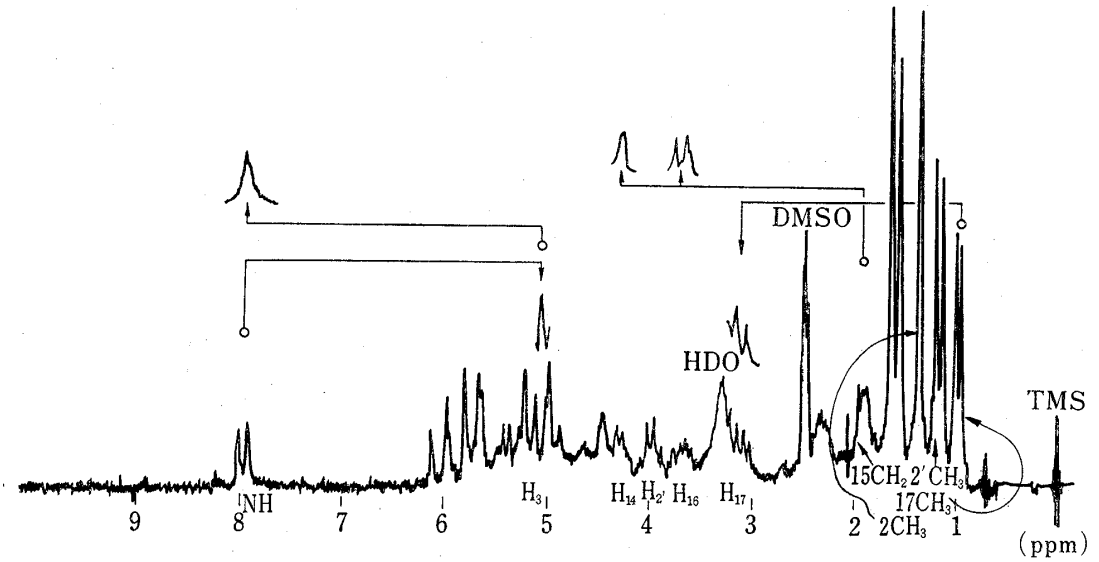


Fig. 3. NMR Spectrum of Iso-lankacidinol O (T-2636 J)
(100 MHz, d_6-DMSO)

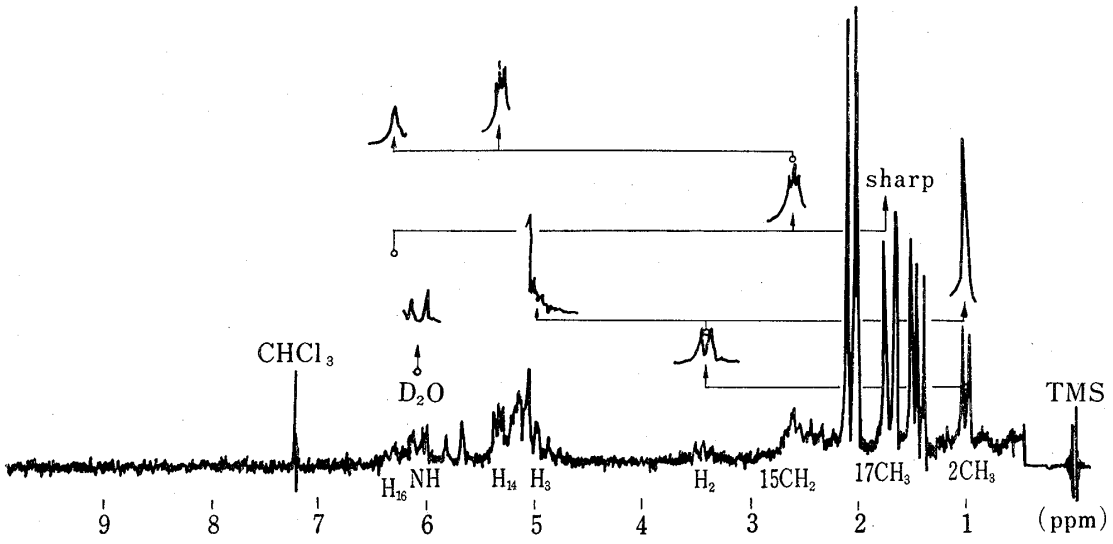


Fig. 4. NMR Spectrum of Lankacyclinol 2′,8,14-Triacetate (100 MHz, $CDCl_3$)

Table I. Chemical Shifts of the Compounds XII and XIII

Proton	15-CH_2	16-H	17-H	17-CH_3	2-CH_3	2-H	3-H	NH
$\delta_{ppm}^{CDCl_3}$	2.3	4.33	2.40	1.29	1.40	···	5.4	7.3 (XIII)
J (Hz)	3	11	6				9	
$\delta_{ppm}^{CDCl_3}$	2.6	6.34	···	1.78	1.02	3.44	5.0	6.1 (XII)
J (Hz)	7	1.5			6	9	8	

(Fig. 4) supports the partial structure as shown in Table I comparing with lankacidinol A 2′,8-diacetate (XIII).[4]

The chemical shifts of 2-H, 16-H and 17-CH_3 are strongly affected by anisotoropy of carbonyl function at 18-position. These findings suggest the cleavage of the lactone in III,

dehydration at newly occurred 16-hydroxyl group and followed by decarboxylation to give structure V (Chart 1).

While, the IR absorptions at 1720 and 1240 cm^{-1} (OAc) and the NMR signals in d_6-DMSO at 1.99 ppm (3H, s, OAc) in V are disappeared in the IR and NMR spectra of VI.

VI was acetylated to lankacyclinol 2′,8,14-triacetate which was identical with XII. The chemical structure of VI is assumed to be the deacetoxy compound of V.

The syntheses of V and VI were performed in order to confirm the above assumption. On basic hydrolysis of III with potassium carbonate, three products, V, IV and VI, were obtained and their acetates were in accord with XII, XIII and XII, respectively. Furthermore, when III or IV was treated more vigorously with potassium carbonate, VI was only obtained in good yield.

And also, III was converted to V by reaction with hydrazine hydrate. This fact suggests that the basicity of hydrazine suited well for decarboxylation of III without deacetylation at 14-position.

Chemical Structure of Iso-lankacidinol (VIII)

Iso-lankacidinol 2′,8,14-triacetate (X) was slightly different with lankacidinol A 2′,8-diacetate (XIII)[4] in the *Rf* values of TLC and IR spectrum in $CHCl_3$, but similar to XIII in the elemental analysis, UV and mass spectra (*m/e* 587 (M^+), 543 (M^+-44 (CO_2)) and 527 (M^+-60 (AcOH)). In the NMR spectrum of X all signals presented in that of XIII are observed. From these spectral data, VIII was suggested to be a stereoisomer of IV, but X was not identical with 2′,8,14-triacetate of 2′-epimer of IV (2′-D type)[1] from their NMR spectra. However, the signals of 17-CH_3 ($\delta^{CDCl_3}_{ppm}$ 1.31 (in XIII)→1.05 (in X) and 16-H (4.37→3.69)) are shifted to up-field and the signal of 17-H (2.37→2.72) is shifted to down-field. The similar shifts are observed in 6,7,12,13-tetrahydro lankacidin C[1] ($\delta^{CDCl_3}_{ppm}$ 2.35 (17-H in II)→2.98 (17-H in this reduction product)). These phenomena might be accounted that the effect of the anisotropy of a 18-carbonyl function was derived from the varied conformation in the six-membered ring.

When VIII was treated with potassium carbonate, VI was obtained as a main product, therefore, VIII was clearly distinct with IV only in the six-membered ring. On reflux in pyridine or 50% acetone–water, IV gave VIII together with IX and undermined alkaline degradation product which was converted to VI with dry NH_3/EtOH (XIV), but only XIV was obtained when VIII was treated as well. On hydrogenation of IV and VIII with platinic oxide, octahydro derivatives were obtained and they gave 2′,8,14-triacetate (XV and XVI, respectively). Their mass spectra show the same peaks at *m/e* 595 (M^+), 551 (M^+-44 (CO_2)), 535 (M^+-60 (AcOH)), 491 (535−44) and 431 (491−60), however the NMR spectra of them were apparently different with one another in 16-H signals ($\delta^{CDCl_3}_{ppm}$ 4.40 (1H, d like, in XV) and 4.09 (1H, t like, in XVI)). The optical rotatory dispersion (ORD) curves of XV, XVI and 4,5,6,7,10,11,12,13-octahydro lankacidin A 2′,18-diol (XVII)[1] showed very weak positive Cotton effect, negative Cotton effect and the plain curve, respectively, as shown in Fig. 5 and therefore, it might be deduced that the 18-carbonyl function did not contribute to the Cotton effect of these compounds but the lactone group contributed remarkably. From the result

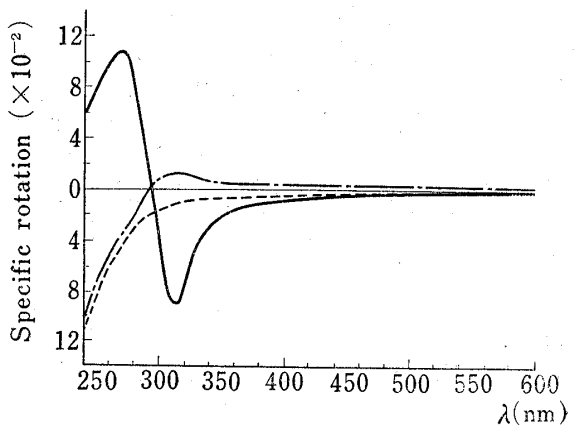
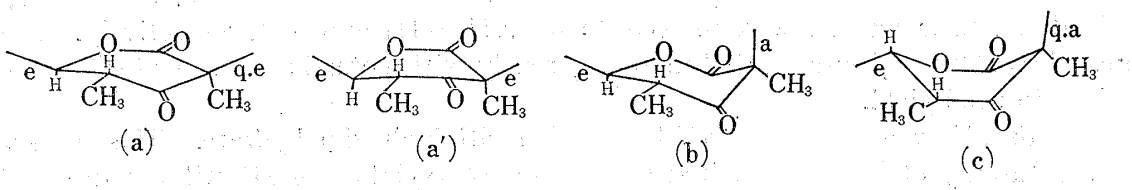


Fig. 5. ORD Curves of Hydrogenated Compounds, XV, XVI, and XVII

·—·— : XV
—— : XVI
------ : XVII

of the NMR spin-decoupling of VIII, relation with the protons at 16- and 17-positions should be diaxial since the J value of them was 11 Hz as same as IV.

From X-ray analysis of I[7] the conformation of the six-membered ring of IV is found to be formula (a) and the hydrogenated derivatives may be formula (a′) from their down-field shifts of 17-H proton. All findings described above, the possible stereostructure of VIII may be postulated partial structure (b) or (c) as shown in Chart 2. Formula (b) should be



(a) lankacidinol (a′) hydrogenated products of II or IV (b) chair form (conformer of IV) (c) boat form (16-epimer of IV)
a: axial, e: equatorial, q: quasi-

Chart 2

denied from the following data, 1) interconversion of XV and XVI whose ring strain were loosed in comparison with the starting material from disappearance of four trans double bonds was not observed in any non-polar solvents, 2) it is unstable because the bulky group of C_2-C_3 bond is rigid axial to the six-membered ring, 3) the δ-lactone is normally in the boat form with a planar lactone group and interaction of the substituents may force the molecule into the half-chair or half-boat conformation[11] and 4) it is known that the six-membered ring having the carbonyl group is often consisted of boat form by the presence of bulky substituents.[12] On the other hand, formula (c) which was derived from the attack by anion at 16-position is the boat form, however, it may be stable since the bulky group of C_2-C_3 bond is flexible quasi-axial and it is able to be accounted for the above-mentioned phenomena. According to the reports,[11,13] it is predicted that formula (a) is positive Cotton effect and (c) is negative.

The obtained data was in good accordance with them. From these findings, the structure of VIII was proposed as shown in Chart 1.

When III or VIII was treated with potassium carbonate, a by-product (XVIII) was obtained together with VI. XVIII shows almost the same data in the elemental analysis, mass and IR spectra with those of VI, but it is different in the *Rf* values of TLC. XVIII was acetylated to 2′,8,14-triacetate (XIX). It is also similar to XII in the IR and mass spectra (*m/e* 543 (M^+) and 483 (M^+—60 (AcOH)). In α,β-unsaturated ketone, it is known that UV extinction coefficient of *S-cis* isomer is in all cases considerably smaller than that of *S-trans* isomer.[14] And also, nuclear Overhauser enhancement (NOE) is usually existed between an allylic methyl and a vinyl proton in relation of *cis* form. Extinction of XVIII and VI are 69300 and 62100 at 228 nm, respectively. The NOE of 17-methyl group and 16-proton could not be detected in either XIX or XII. VIII was more rapidly decarboxylated than III or IV, and XVIII was yielded better in treatment of VIII (VI: XVIII=4.4: 1) than in that of III (VI: XVIII= 46: 1). From these data, it may be the most ascribable to be assigned that XVIII is *S-trans* conformation and VI is *S-cis* as shown in Chart 3.

11) H. Wolf, *Tetrahedron Letters*, **1966**, 5151.
12) E.L. Eliel, N.L. Alliger, S.J. Angyal, and G.A. Morrison, "Conformational Analysis," John Wiley & Sons, Inc., New York, 1965, pp. 469—486.
13) O. Korver, *Tetrahedron*, **26**, 2391 (1970).
14) E.L. Eliel, "Stereochemistry of Carbon Compounds," MacGraw-Hill Book Co., New York, 1962, p. 329.

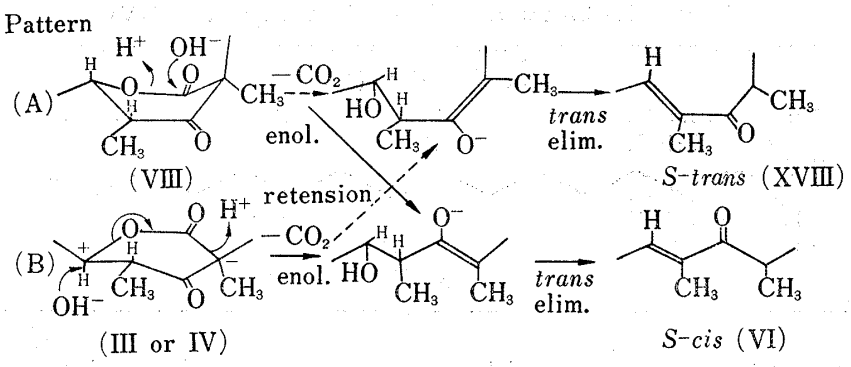


Chart 3

When III was moderately hydrolyzed with potassium carbonate, only IV was afforded by easy deacetylation at 14-position. However, in the case of hydrazine the reaction pathway may be proceeded through the pattern (B) since only V was obtained without deacetylation.

Acidic Decomposition Studies

On acidic hydrolysis of III with 50% acetic acid–water or 70% methanol–1/4N hydrochloric acid, the unstable lipophilic material (XX) containing no nitrogen from its elemental analysis was obtained together with L-lactamide.[1] It is colorlized to be pink with conc. sulfuric acid on TLC and have the same *Rf* values of I. The mass peak is observed at *m/e* 372 (M^+). In the IR spectrum three absorptions in carbonyl region are found at 1770 (lactone), 1710 (18-CO) and 1680 ($\alpha,\beta,\gamma,\delta$-unsaturated aldehyde) cm^{-1}. The NMR spectrum in $CDCl_3$ shows new signal at 10.08 ppm (1H, d, J=8 Hz, –CHO) instead of disappearance of carbinol, acid amide and acetyl groups presented in that of III.

Furthermore, the mass spectrum of monomethoxy compound (XXI) obtained from methanolysis of III exhibits the peaks at *m/e* 386 (M^+), 368 (M^+—18 (H_2O)), 354 (M^+—32 (MeOH)) and 324 (368-44 (CO_2)).

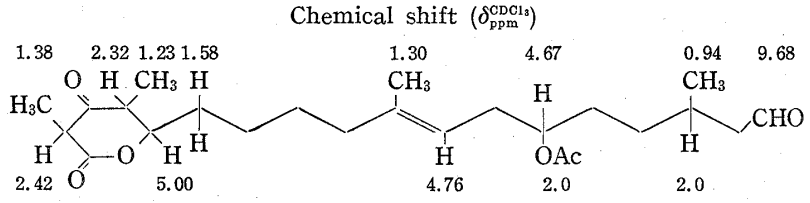
The UV spectrum in EtOH shows two maxima at 235 and 277 nm. The NMR spectrum of the compound in $CDCl_3$ shows that the aldehydic doublet proton centered at 10.1 ppm is coupled to the vinyl proton at 5.96 ppm with $J_{3H,4H}$=8 Hz.

On methanolysis of I or II, 8,14-dimethyl lankacidin C (XXII) and 2′,2′,8,14-tetramethyl lankacidin C (XXIII) were obtained in both cases contrary to expectation. The mass spectra of XXII and XXIII show the apparent peaks at *m/e* 487 (M^+), 455 (M^+—32 (MeOH)), 423 (455—32) and *m/e* 533 (M^+), 501 (M^+—32), 469 (501—32), respectively. No absorption of hydroxyl group observes in the IR spectra. The NMR spectrum of XXIII in $CDCl_3$ shows signals at 1.45 (3H, s, 3′-CH_3), 3.20 (3H×2, s, 8,14-OCH_3) and 3.27 ppm (3H×2, s, 2′,2′-OCH_3).

On acidic degradation of lankacidin C 8,14-diacetate (XXIV),[4] a pale yellow product (XXV) having the same *Rf* values of XXIV was obtained which had an acetyl and no hydroxyl group determined from the IR and NMR spectra and gave a dimethone derivative. XXV was treated with potassium carbonate/methanol–water to afford the acidic compound. As a result of these facts, it is appreciated that the functional groups of an aldehyde, an acetyl and a β-keto-δ-lactone present in XXV. XXV was hydrogenated with palladium-charcoal in ethanol to absorb 3—4 molar hydrogen. The IR spectrum of the obtained oily compound (XXVI) in film exhibits some absorptions at 2700 (–CHO), 1760 (lactone) and 1720 cm^{-1} (OAc, –CHO and 18-CO), but no absorption of acid amide and hydroxyl groups. In the mass spectrum, the peaks are observed at *m/e* 394 (M^+—28 (CO)), 378 (M^+—44 (CO_2)), 350 (394—44), 334 (394—60 (AcOH)), 318 (378—60) and 290 (318—28). From the result of the NMR spin-decoupling of XXVI, the signals assigned to the formula shown in Table II were found.

On acidic hydrolysis of 6,7,12,13-tetrahydro lankacidin C or lankacidin C 2′,18-diol[1] gave a pyruvamide or a lactamide, respectively. However, 4,5,6,7,10,11,12,13-octahydro lankacidin

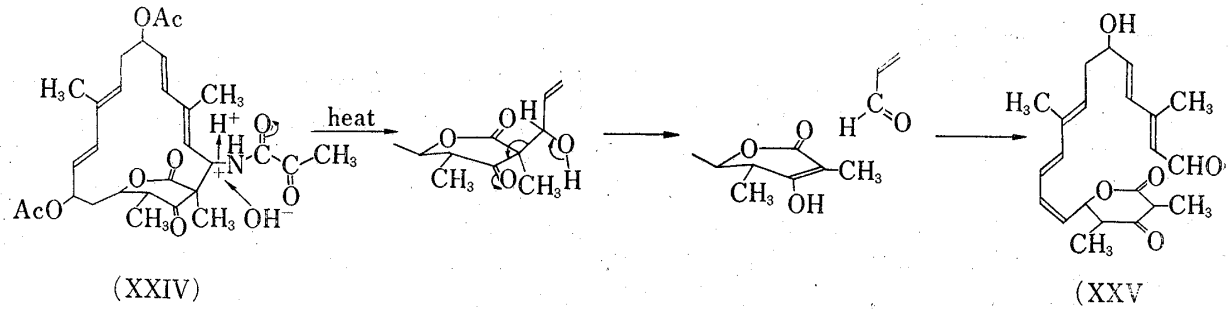
Table II. Chemical Shifts of the Compound XXVI



XXVI

A 2′,18-diol could not be hydrolyzed with the same acidic condition. XX was also obtained from II in Krebs-Ringer bicarbonate or potassium carbonate/methanol–water.

All of these data and the findings reveal the chemical structure of (acidic) hydrolytic products as shown in Chart 1 and decomposition mechanism shown in Chart 4 is very ascribable.



(XXIV) (XXV

Chart 4

Experimental

All melting points are with decomposition and measured with Mettler FP 5 (3°/min). Optical rotations and UV spectra are determined in EtOH unless otherwise stated. The absorbents of TLC and column chromatography were used SiO_2 HF_{254} and SiO_2 (0.05—0.2 mm) purchased from Merck Co. Ltd., respectively.

Isolation of Iso-lankacidinol (VIII) and Iso-lankacidinol O (IX)——The culture filtrate of *Streptomyces rochei* var. *volubilis* (70 liters) was extracted with MIBK (1/2 volume × 2) at pH 7 and the aqueous layer was reextracted with BuOH. Two extracts was separately washed with 2% $NaHCO_3$ and H_2O, and concentrated *in vacuo*. MIBK extract was applied to chromatography by a column (50 g) and eluted with C_6H_6–AcOEt–acetone. II (11.5 g), mixture of VIII and IX(6.0 g) and IV (4.5 g) were obtained from C_6H_6: AcOEt (6—4: 4—6), (2: 8) and AcOEt: acetone (10—8: 0—2), respectively. And then VIII was preferentially recrystallized from AcOEt–MeOH as colorless prisms (2.2 g) and its mother liquor gave IX (2.3 g) from AcOEt as colorless prisms. BuOH extract was crystallized from MeOH to give IV (5.6 g). VIII; mp 193°, $[\alpha]_D^{25}$ −190° (c=0.53), UV λ_{max} nm (ε): 228 (47400). *Anal.* Calcd. for $C_{25}H_{35}O_7N$: C, 65.06; H, 7.64; N, 3.03. Found: C, 65.06; H, 7.79; N, 3.01. IX; mp 167°, $[\alpha]_D^{26}$ +90.1° (c=0.52), UV λ_{max} nm (ε): 228 (38200). *Anal.* Calcd. for $C_{25}H_{35}O_8N$: C, 62.88; H, 7.39; N, 2.93. Found: C, 63.01; H, 7.84; N, 2.71.

Iso-lankacidinol 2′,8,14-Triacetate (X)——A solution of VIII (200 mg) in Ac_2O (2 ml) and pyridine (2 ml) was allowed to stand at 25° overnight. The reaction solution was poured into iced-water and extracted with AcOEt.

The extract was washed with 1/10 N HCl, 1% $NaHCO_3$ and H_2O. The concentrate of the extract was precipitated from ether–hexane to give white powder of X (210 mg). $[\alpha]_D^{28}$ −128° (c=0.5), UV λ_{max} nm (ε): 228 (40700). *Anal.* Calcd. for $C_{31}H_{41}O_{10}N$: C, 63.36; H, 7.03; N, 2.38. Found: C, 63.20; H, 7.13; N, 2.40.

Iso-lankacidinol O 2′,8,14-Triacetate (XI)——IX (200 mg) was acetylated with Ac_2O (2 ml) and pyridine (2 ml) at 5—10° for 5 day. The reaction solution was treated with the almost same procedure and the concentrate was applied to TLC of C_6H_6: AcOEt (1: 1). The purified acetate was precipitated from ether–hexane to give white powder of XI (100 mg). $[\alpha]_D^{25}$ +9.3° (c=0.5), UV λ_{max} nm (ε): 228 (37600). *Anal.* Calcd. for $C_{31}H_{41}O_{11}N$: C, 61.68; H, 6.85; N, 2.32. Found: C, 62.15; H, 7.01; N, 2.23.

Lankacyclinol (VI)——1) A solution of III (5 g) in MeOH (400 ml) and 1% K_2CO_3 (84 ml) was stirred at 25° for 1 hr. After adjusting at pH 2.5 with 1N HCl, the reaction solution was concentrated under reduced pressure and extracted with BuOH after addition of H_2O.

The organic solvent layer was washed with H_2O and removed off. The residue was applied to chromatography by a column (100 g).

The eluates of AcOEt, AcOEt: acetone (9: 1) and (8: 2) were separately concentrated, which were crystallized from MeOH to yield IV (0.6 g), V (0.3 g) and VI (0.3 g).

2) To a solution of III (10 g) in MeOH (500 ml) was added 2% K_2CO_3 (500 ml), which was stirred at 25° for 1 hr. The basic solution was acidified with 1N HCl to pH 2.0 and allowed to stand at 0—5° overnight. The solution was extracted with BuOH and the extract was washed with H_2O and followed by concentration *in vacuo*. The concentrate was applied to XAD-2 (500 ml, Rohm & Haas Co.) and eluted with 50% MeOH–H_2O. Crystallization from MeOH–H_2O gave VI (5 g) as colorless plates.

3) To a mixture of IV (960 mg) in MeOH (100 ml) and 10% K_2CO_3 (3 ml) solution was added and the whole was stirred at 25° for 24 hr. After IV was completely dissolved, the reaction solution was further stirred for 1 hr and extracted with BuOH. The concentrate of the extract was crystallized from MeOH–AcOEt to afford VI (320 mg) as fine crystals. mp 221°, $[\alpha]_D^{25}$ −373° (c=0.5), UV λ_{max} nm (ε): 227 (62100). *Anal.* Calcd. for $C_{24}H_{35}O_5N$: C, 69.04; H, 8.45; N, 3.35. Found: C, 68.58; H, 8.55; N, 3.30.

Lankacyclinol A (V)——To a solution of III (5 g) in MeOH (500 ml) hydrazine hydrate (2.5 ml) was added and the whole was refluxed for 1 hr. After addition of H_2O the reaction mixture was extracted with BuOH and the extract was washed with H_2O and concentrated *in vacuo*. The concentrate was applied to chromatography by a column and eluted with C_6H_6: AcOEt (1—0: 9—10). The concentrate of the eluate was crystallized from MeOH–AcOEt to give crystals of V (650 mg). mp 235°, $[\alpha]_D^{23}$ −338° (c=0.48, EtOH: DMSO (1: 1)), UV λ_{max} nm (ε): 227 (61200). *Anal.* Calcd. for $C_{26}H_{37}O_6N$: C, 67.95; H, 8.11; N, 3.05. Found: C, 67.66; H, 8.29; N, 3.06.

This synthetic sample was identified with the natural product[4] in the mixedmp (233°), IR, NMR, and mass spectra.

Lankacyclinol A 2′,8-Diacetate (XII)——V (naturally obtained, 100 mg) or VI (synthetic, 100 mg) was acetylated with Ac_2O (2 ml) and pyridine (2 ml) at 25° overnight. After addition of H_2O the reaction mixture was extracted with AcOEt. The extract was washed with 1/10N HCl and 2% $NaHCO_3$, dehydrated and concentrated. The concentrate was precipitated with ether–hexane to give colorless crystalline powder of XII (103 mg). $[\alpha]_D^{23}$ −268° (c=0.52), UV λ_{max} nm (ε): 227 (54300). *Anal.* Calcd. for $C_{30}H_{41}O_8N$: C, 66.28; H, 7.60; N, 2.58. Found: C, 65.89; H, 7.74; N, 2.70.

Decarboxylation of Iso-lankadicinol (VIII)——To a solution of VIII (1 g) in MeOH (50 ml) was added 2% K_2CO_3 (50 ml) and the whole was stirred at 25° for 1 hr. After acidification with 1N HCl to pH 2, the reaction solution was extracted with BuOH. The extract was washed with H_2O and concentrated *in vacuo*. The residual oil was applied to TLC and developed with C_6H_6: acetone (1: 1) VI (365 mg) and XVIII (83 mg) were obtained from MeOH–H_2O and MeOH–AcOEt as colorless plates and colorless needles, respectively. mp 249°, $[\alpha]_D^{30}$ −28.6° (c=0.36), UV λ_{max} nm (ε): 227 (69300). *Anal.* Calcd. for $C_{24}H_{35}O_5N$: C, 69.04; H, 8.45; N, 3.35. Found: C, 68.85; H, 8.39; N, 3.50.

XVIII (50 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (0.5 ml) to give colorless crystals of XIX (54 mg) from AcOEt–hexane. mp 138°, $[\alpha]_D^{30}$ −50.1° (c=0.46), UV λ_{max} nm (ε): 227 (48200). *Anal.* Calcd. for $C_{30}H_{41}O_8N$: C, 66.28; H, 7.60; N, 2.58. Found: C, 65.91; H, 7.89; N, 2.55.

Transformation of Lankacidinol (IV) and Iso-lankacidinol (VIII)——1) A solution of VIII (1 g) in pyridine (20 ml) (or 50% acetone–H_2O (120 ml)) was refluxed for 2 hr (or 6 hr) and concentrated *in vacuo*.

After the concentrate was suspended in H_2O (1 liter), the aqueous solution was extracted with BuOH (1 liter × 2) at pH 2. The extract was evaporated under reduced pressure to obtain crude residual oil (*ca.* 0.9 g). The residue was separated by TLC with C_6H_6: acetone (1: 1). The main component was precipitated with ether as pale yellow powder of XIV (324 mg) and the starting material was recovered (356 mg).

2) A solution of IV (5 g) in 50% acetone–H_2O (600 ml) (or pyridine (100 ml)) was refluxed for 3 hr (or 30 min). The reaction solution was concentrated *in vacuo* and the residue was crystallized from MeOH to give IV (1.9 g). The concentrate of the mother liquor (*ca.* 3 g) was partitioned with the solvent system of hexane: AcOEt: acetone: H_2O (1: 4: 1: 4). The concentrate of the upper layer was purified with chromatography by a column (60 g) and afforded mixture of VIII and IX from the eluate of C_6H_6: AcOEt (2: 8). The mixture was preferrentially crystallized from AcOEt to give VIII (280 mg) and IX (200 mg). The lower layer was extracted with BuOH and the extract was washed and concentrated. The concentrate was purified by TLC of C_6H_6: acetone (1: 1) to give XIV (360 mg). XIV; $[\alpha]_D^{26}$ +75.0° (c=0.5), UV λ_{max} nm (ε): 231 (38500), 239 (40200), 278 (6230). *Anal.* Calcd. for $C_{25}H_{35}O_7N \cdot H_2O$: C, 62.61; H, 7.78; N, 2.92. Found: C, 62.71; H, 7.89; N, 2.71.

VIII and IX were identified with the natural products in the *Rf* values of TLC, mixedmp (VIII: 193°, IX: 167°), IR and NMR spectra and antimicrobial spectra.

Decomposition of XIV with Ammonia——A solution of XIV (700 mg) in 10% dry NH_3/EtOH solution was allowed to stand at 25° for 15 hr. The reaction solution was evaporated to a small extent of volume and extracted with BuOH (250 ml × 3) after addition of H_2O (500 ml). The extract was concentrated and followed by purification of TLC with C_6H_6: acetone (1: 1). The obtained main product was crystallized from MeOH–

AcOEt to give VI (175 mg) which was identical with the authentic sample in mmp (220°), $[\alpha]_D$, UV, IR, and NMR spectra.

Hydrogenation of Lankacidinol (IV), Iso-lankacidinol (VIII) and Iso-lankacidinol O (IX)——IV (920 mg), VIII (920 mg) or IX (954 mg) was hydrogenated with PtO_2 (150 mg, Wako Pure Chem.) for 5 hr.

Approximately 4—4.5 molar hydrogen was absorbed in three compounds. The reaction mixtures were filtered and concentrated.

The dried residues were acetylated with Ac_2O (2 ml) and pyridine (2 ml). The crude acetates were separately purified with chromatography by a column (40 g) and eluted with C_6H_6: AcOEt (85—80: 15—20). The concentrates gave XV (540 mg), XVI (460 mg) or XVI (480 mg) as colorless powder, respectively. XV; $[\alpha]_D^{25}$ +4.6° (c=0.52), UV; end absorption. *Anal.* Calcd. for $C_{31}H_{49}O_{10}N$: C, 62.50; H, 8.29; N, 2.35. Found: C, 62.65; H, 8.33; N, 2.37.

And also, XV was identical with 4,5,6,7,10,11,12,13-octahydro lankacidinol A 2′,8-diacetate[1] in the *Rf* values of TLC, mass, IR, and NMR spectra. XVI; $[\alpha]_D^{25}$ −11.0° (c=0.49), UV; end absorption. *Anal.* Calcd. for $C_{31}H_{49}O_{10}N$: C, 62.50; H, 8.29; N, 2.35. Found: C, 62.93; H, 8.18; N, 2.24.

Acidic Hydrolysis of Lankacidinol A (III)——A solution of III (240 mg) in 50% AcOH–H_2O (20 ml) was warmed at 80° for 2 hr and allowed to stand at 25° overnight. The reaction mixture was extracted with AcOEt and the extract was applied to TLC with C_6H_6: AcOEt (1: 2). The extract of the product with AcOEt was concentrated *in vacuo* and precipitated with AcOEt–hexane to obtain pale yellow powder of XX (40 mg). UV λ_{max} nm (ε): 235 (23800), 278 (18900). *Anal.* Calcd. for $C_{22}H_{28}O_5 \cdot H_2O$: C, 67.67; H, 7.74; N, 0.0. Found: C, 67.80; H, 7.26; N, 0.16.

Methanolysis of Lankacidinol A (III)——The solution dissolving III (1 g) in MeOH (50 ml) was held at 25° overnight after addition of conc. HCl (1.5 ml). The reaction solution poured into iced water was extracted with AcOEt and ether twice. The extract was washed with H_2O, 2% $NaHCO_3$ and H_2O. The crude powder was applied to column chromatography (20 g). The eluate of C_6H_6: AcOEt (8: 2) was concentrated *in vacuo* and precipitated with ether to obtain pale yellow powder (XXI, 40 mg). UV λ_{max} nm (ε): 235 (25600), 277 (23400). *Anal.* Calcd. for $C_{23}H_{30}O_5 \cdot H_2O$: C, 68.29; H, 7.97; N, 0.0. Found: C, 68.78; H, 7.53; N, 0.0.

8,14-Dimethyl Lankacidin C (XXII) and 2′,2′,8,14-Tetramethyl Lankacidin C (XXIII)——To a solution of I (500 mg) (or II (460 mg)) in MeOH (50 mg) was added conc. HCl (3 ml) and the solution was allowed to stand at 25° for 20 hr. The AcOEt extract was washed, dehydrated and concentrated. Two components were separated by TLC with C_6H_6: AcOEt (1: 1) and precipitated from petroleum ether to yield XXII (78 mg) and XXIII (124 mg). XXII; $[\alpha]_D^{23}$ +1.8° (c=0.5), UV λ_{max} nm (ε): 228 (42500). *Anal.* Calcd. for $C_{27}H_{37}O_7N$: C, 66.51; H, 7.65; N, 2.87. Found: C, 66.82; H, 8.06; N, 2.92. XXIII; $[\alpha]_D^{23}$ +5.6° (c=0.5), UV λ_{max} nm (ε): 230 (43800). *Anal.* Calcd. for $C_{29}H_{43}O_8N$: C, 65.27; H, 8.12; N, 2.62. Found: C, 65.08; H, 7.80; N, 2.73.

Thermal Decomposition of Lankacidin C 8,14-Diacetate (XXIV) in Acetic Acid——A solution dissolving XXIV (10 g) in AcOH (200 ml) was refluxed for 30 min. After air cooling the reaction solution was concentrated and the residue was chromatographed by a column. The eluate of C_6H_6: AcOEt (95: 5) was concentrated and precipitated from petroleum ether to yield a pale yellow powder (XXV, 1 g). XXV (460 mg) in EtOH (110 ml) was hydrogenated with 10% Pd-C (120 mg, Engelhard Co.) and hydrogen (3—4 mmole) was consumed during 2 hr. After filtration the reaction solution was evaporated *in vacuo* and the concentrate was applied to chromatography by a column. The main product was afforded from the eluate of C_6H_6: AcOEt (9: 1) as a colorless oily compound (XXVI, 150 mg). $[\alpha]_D^{22}$ −3.8° (c=0.47), UV: End absorption. *Anal.* Calcd. for $C_{24}H_{38}O_6$: C, 68.22; H, 9.07; N, 0.0. Found: C, 68.17; H, 8.97; N, 0.0.

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