STUDIES ON THE IONOPHOROUS ANTIBIOTICS. XI[1]
THE ARTIFACTS AND THE DEGRADATION PRODUCTS OF LYSOCELLIN*

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Lysocellin is a new polyether antibiotic produced by *Streptomyces cacaoi* var. *asoensis* and is characterized as a broad spectrum ionophore having a higher complexation affinity for divalent cations than for monovalents and also having an ability to transport biological amines. The structures of two artifacts designated L_1 and M_1 have been elucidated based on spectral evidence, and the formation mechanism of these compounds was discussed with respect to the reactivity of the antibiotic. In addition, a number of degradation and modified products were prepared in order to examine their biological activity and to utilize as the model compounds for ^{13}C-NMR assignment.

Lysocellin[2] (**I**) is a new antibiotic, produced by *Streptomyces cacaoi* var. *asoensis,* which shows both antibacterial and anticoccidial activities.

The structure of **I** has been established by a three-dimensional X-ray analysis of the silver salt[3,4], which contains the functions of a carboxylic acid, a β-hydroxyketone, and two five-membered and a six-membered ether rings as depicted in Scheme 1+.

A noticeable feature of its biological activity is that the antibiotic shows rather stronger complexation affinity for the divalent metal cations than for the monovalent, and it is also able to transport the biological amines such as serotonin, histamine and β-norepinephrine[5]. In addition, the effect of lysocellin on aggregation of blood platelets was reported in the previous paper[6].

In the course of our search for the concurrent minor components of **I** in the culture broth or mycelium of the producing organism, we have succeeded in isolating two structural analogs, designated L_1 and M_1. Originally, L_1 was isolated along with lysocellin methylester (**II**) from a reaction mixture of **I** with diazomethane, whereas M_1 was isolated from the methanolic mother liquors of **I**. Evidently, M_1 is an artifact deriving from **I** in the isolation work-up, because it could not be detected in the culture broth. Presumably, L_1 is also an artifact, since neither its sodium salt nor its free acid could be detected in the broths or in the crude extracts of the antibiotic.

This paper concerns with the characterization and structural elucidation of a number of the degradation and modified products, including L_1 and M_1.

For convenience, the degradation studies and the spectral data of these degradation products will be discussed first, followed by structural elucidation of the artifacts and the characterization of the

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* Abbreviations: TLC, thin-layer chromatography; TMS; tetramethylsilane; s, singlet; d, doublet; t, triplet; dd, doublet of doublets; bdd, broad doublet of doublets; q, quartet; m, multiplet.

\+ To simplify understanding, the same numbering of carbons as with the parent antibiotic was used to indicate the structural analogs and the degradation products.

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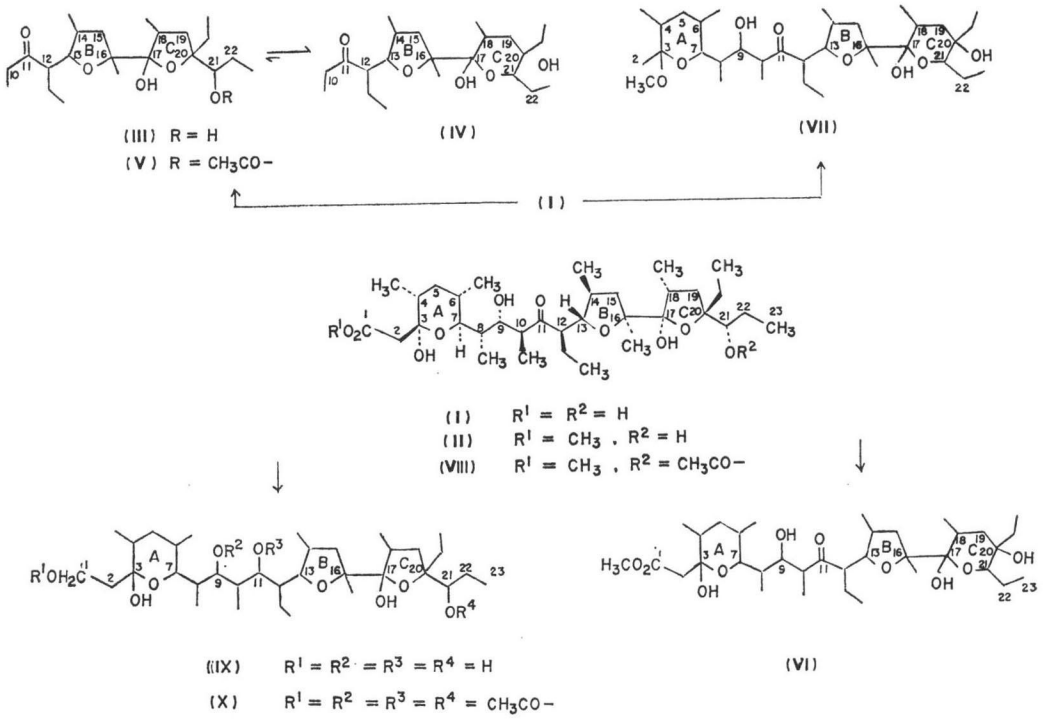
Scheme 1. The structures of lysocellin and its degradation products and artifacts.

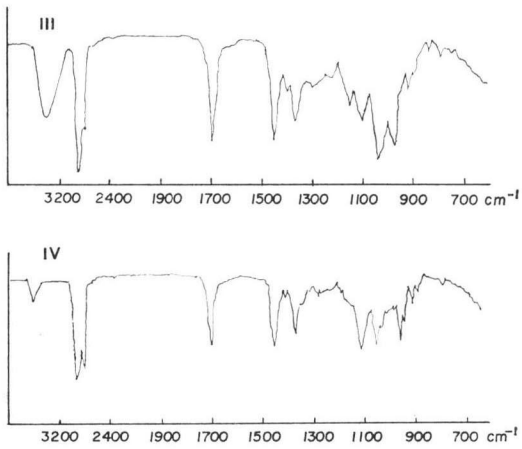


modified products. The structures of the compounds discussed herein are presented in Scheme 1.

Retro-Aldol Cleavage and Characterization of the Degradation Products

For the sake of utilizing the spectral data of simplified degradation products in structural elucidation of L_1 and M_1, a degradation study of **I** by retro-aldol cleavage was undertaken. By analogy with lasalocid[7] it would be expected that **I** could be cleaved by alkali at the β-hydroxyketone portion into two moieties: those of ring *A*, containing the functions of an aldehyde and a carboxylic acid side chain, and those of a neutral ketone containing the rings *B* and *C*, respectively. Only a neutral compound designated N_2 (**III**) was obtained as an oil.

Fig. 1. The IR spectra of N_2 (**III**) and N_3 (**IV**).



The molecular formula $C_{22}H_{40}O_5$ was established by high-resolution mass spectrometry of the dehydration peak at *m/e* 366.2764 ($C_{22}H_{38}O_4$, Δ 0.3).

It was observed that **III** was further gradually transformed into an isomeric compound designated N_3 (**IV**), which could be separated by preparative TLC as an oil. The same molecular formula $C_{20}H_{40}O_5$

was established analogously by high-resolution mass spectrometry.

A comparison of the absorption peaks appearing in the IR spectra of **III** and **IV** as presented in Fig. 1 indicates that essentially the two compounds show close resemblance except for the bands at 900~1,200 cm^{-1} corresponding to the region of C-O-C stretching vibration, suggesting a possible difference between the two compounds in the structure of the cyclic ether moieties (ring *B* or *C*) as was the case of the retro-aldol cleavage products of lasalocid and *iso*-lasalocid[8].

On acetylation with acetic anhydride in pyridine, **III** afforded an oily monoacetate (**V**), $C_{24}H_{42}O_{26}$, but **IV** remained unchanged. This indicates that an apparent difference between these compounds is the presence of an acetylatable hydroxyl group in the molecule.

Inspection of the PMR spectra of **III** and **V** reveals that the overlapped signal centered at δ 3.60 (1H, t) in the spectrum of **III** has shifted downfield to δ 4.90 (dd, J=3.50 Hz and 10.0 Hz) in that of **V**. Thus, this signal is assigned to the proton linked on the hydroxyl-bearing carbon at C_{21}. This proton is further coupled with the methylene protons of C_{22} at δ 1.55 (J=3.50 Hz) and δ 1.80 (J=10.0 Hz), respectively.

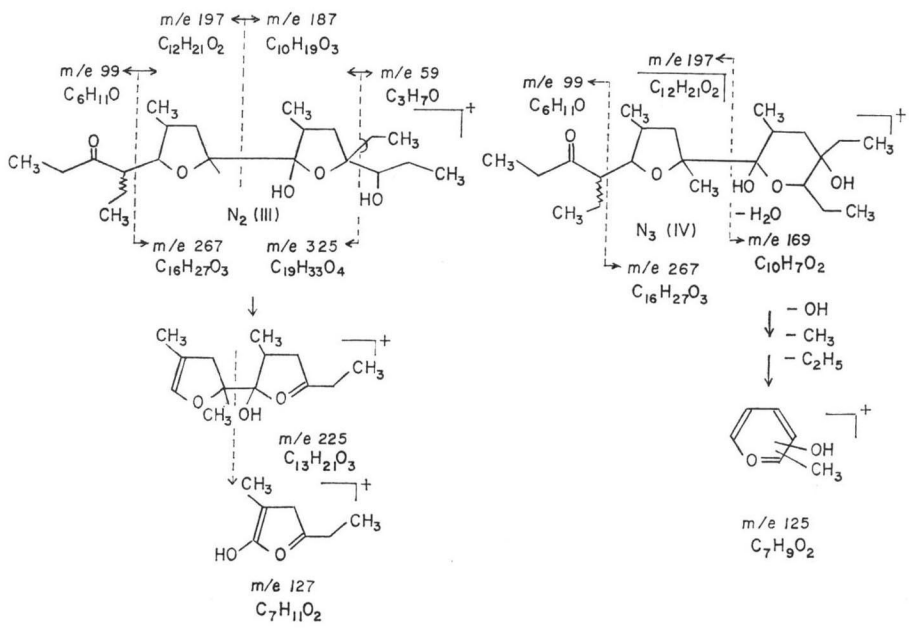
Furthermore, a broadened signal at δ 2.55 (bdd), which in turn is coupled with the methine proton of C_{13} at δ 3.50 (J=2.50 Hz) and to the methylene protons at δ 1.85 (J=10.0 Hz), respectively, is assigned to that of C_{12}, and this signal broadening may be explained by the presence of an epimeric mixture.

It is noteworthy that both the chemical shifts and the coupling pattern of **III** show close similarity with those arising from the respective moieties containing rings *B* and *C* in the PMR spectrum of **I**.

The sum of these arguments leaves very little doubt that **III** is derived from a retro-aldol cleavage of the C_9–C_{10} bond of β-hydroxy ketone group of the parent antibiotic; hence, the structure **III** is assigned to the degradation product N_2.

On the other hand, the chemical shifts and coupling patterns in the PMR of **IV** differ distinctly

Fig. 2. Fragmentation patterns of N_2 (**III**) and N_3 (**IV**).



from those of **III**, particularly at the signals belonging to the ring C moiety. Namely, a proton which appears at δ 3.65 (m), and which is coupled with two methine protons at δ 2.00 (m) and δ 2.43 (m), is assigned to that of C_{13}; therefore, the neighbouring protons must be those at C_{12} and C_{14}, since this coupling pattern is very similar to those observed in the spectra of both **I** and **III**.

A proton signal which splits into doublet of doublets centered at δ 3.50 is coupled with the methylene protons at δ 1.45 (J=3.50 Hz) and δ 1.64 (J=7.50 Hz), respectively. Based on the chemical shift together with its coupling pattern, this proton is assigned to that of C_{21} adjacent to the oxygen of ether ring. Accordingly, this indicates that the ring recyclization involving the hydroxyl group at C_{21} took place during the course of transformation from **III** to **IV**. A comparison of the chemical shift as well as the coupling pattern of this diagnostically important C_{21} proton leads to the assignment of the structure **IV** for N_3. The stereochemistry of ring *C* has not been determined.

Support for the structures **III** and **IV** is provided by the mass spectral evidence obtained by high-resolution mass spectrometry. The fragmentation patterns of these two compounds are compared in Fig. 2; the formation of the peaks observed in the spectrum of **III** at *m/e* 59.0442 ($C_3H_7O^+$, Δ-0.4), *m/e* 187.1344 ($C_{10}H_{19}O_3^+$, Δ 1.1), *m/e* 225.1498 ($C_{13}H_{21}O^+$; M-$C_3H_7O^+$-$C_6H_{11}O^+$, Δ 0.8) and *m/e* 325.2385 (M-$C_3H_7O^+$, Δ 0.8)[8] may result from cleavage at the α-position of the ether rings[9,10].

Of special significance is the fact that a set of these ions has a diagnostic importance thereafter for distinction of the structural analogs having the five-membered ring *C*; therefore, these are totally designated the "ions A". In contrast, a characteristic peak at *m/e* 125.0607 ($C_7H_9O_2^+$, Δ 0.5) which is observed with an appreciable intensity in the mass spectrum of **IV** is assigned to that derived from the moiety of ring *C* having a six-membered hemiketal; therefore, it is designated the "ion B".

A major fragmentation route for formation of this ion involved cleavage of C_{16}–C_{17} bond and simultaneous loss of a molecule of water generating the ion at *m/e* 169.1256 ($C_{10}H_{17}O_2^+$, Δ 2.8) which subsequently lost the substituents of methyl and ethyl groups.

It is of great importance that the "ions A" and "ion B" can be used henceforth as the diagnostic peaks to distinguish the framework of ring *C* moiety in the structural analogs of this series.

The Structure of L_1

Originally, L_1 (**VI**) was isolated from the mother liquors of **II** as an artifact; it was also obtainable in good quantities by transformation of **II** in boiling methanol under catalysis of acetic acid. The identity of this transformed compound with **II** was confirmed by comparison of the IR and the NMR spectra. The molecular formula $C_{35}H_{62}O_{10}$ was established by both elementary analyses and high-resolution mass spectrometry. The peak corresponding to the dehydration ion was observed at *m/e* 624.4278 ($C_{35}H_{60}O_9$, Δ-4.8).

The PMR parameters of **VI** are similar in many respects to those of **II** except for the presence of a methine proton at δ 3.50 (1H, dd, J=3.50 Hz and J=7.50 Hz) which is coupled in turn with the methylene protons at δ 1.45 (J=3.50 Hz) and δ 1.64 (J=7.50 Hz), respectively. It is noteworthy that this coupling pattern shows close similarity to that observed in the PMR spectrum of **IV** as aforementioned.

In addition, it is of great importance that **VI** failed to be acetylated by acetic anhydride in pyridine; in contrast, **II** readily yielded the corresponding monoacetate, $C_{37}H_{64}O_{11}$ (**VIII**).

These results lead to the view that the ring *C* portion in **VI** is similarly isomerized to the six-mem-

bered framework, as is the case for **IV**. This inference is strongly supported by comparison of the mass spectra of the isomeric compounds **II** and **VI**, namely, a distinct peak with an appreciable intensity of the "ion B" which is observed at *m/e* 125.0598 ($C_7H_9O_2^+$, Δ 0.52) and lack of the "ion A" in the mass spectrum of **VI**.

Based on the foregoing evidence, the structure **VI** is assigned to L_1, which, however, contains the ring system of six-membered hemiketal in the moiety of ring *C*.

The Structure of M_1

The artifact M_1 (**VII**) was isolated as an oil from the methanolic mother liquors of the free acid of **I**. It is observed that the free acid of **I** is not stable enough, as it is readily transformed to **VII** in boiling methanol, and that this transformation is markedly enhanced by catalysis of acetic acid. The identity of this transformed compound with **VII** was confirmed by comparison of the IR and the NMR spectra.

The molecular formula $C_{34}H_{62}O_8$ was established by high-resolution mass spectrometry based on the corresponding dehydration ion at *m/e* 580.4346 ($M^+ - H_2O$, $C_{34}H_{60}O_7$, Δ 1.0).

Comparison of both the IR and PMR spectral parameters of **VII** with those of the parent antibiotic discloses fundamental differences in many respects, *e.g.*, a lack of the absorption band corresponding to the carboxyl group at 1716 cm^{-1} in the IR spectrum and a new appearance of the signals at δ 3.20 (3H, s, $-OCH_3$) and at δ 1.26 (3H, s, $-CH_3$) in the PMR spectrum of the former.

Furthermore, a comparison of the molecular formula indicates a loss of two oxygens and an increase of two hydrogens in **VII** from the parent antibiotic.

These data are consistent with the view that the appearance of the methyl and methoxy groups in the molecule of the artifact **VII** is one of the major changes during this transformation, and that, obviously, the introduction of these two functional groups takes place at the environment of ring *A*, probably by decarboxylation of C_1-carboxylic acid and substitution of C_3-hemiketal group.

It is well established that the decarboxylation of β-keto acid occurs with great ease[11], however, it is not certain whether the decarboxylation of C_1-carboxylic acid is concerted with the introduction of methoxyl group at C_3-hemiketal group.

With regard to the structure of ring *C* moiety in the molecule, the coupling pattern of C_{21}-proton appearing in the PMR spectrum of **VII** has great significance. This proton, however, is observed in the neighborhood of δ 3.50 as the overlapped signals of doublet of doublets (J = 3.50 Hz and J = 7.50 Hz) which is coupled with the methylene protons at δ 1.45 and δ 1.64, respectively. This pattern is similar to those of the aforementioned compounds **IV** and **VI** having the six-membered hemiketal ring system in the ring *C* moiety.

In addition, **VII** also failed to be acetylated like **IV** and **VI**.

These data are all consistent with the view that the ring *C* portion of **VII** is transformed into the six-membered hemiketal; hence, the structure **VII** is assigned to the artifact M_1.

Support for this structure is provided by the evidence obtained with high-resolution mass spectrometry. The diagnostic peak of the "ion B" is distinctly observed at *m/e* 125.0620 ($C_7H_9O_2^+$, Δ 1.8) in the mass spectrum of **VII**, indicating the probable presence of the six-membered hemiketal of ring *C* by analogy with the cases of **IV** and **VI**.

Ultimately, unambiguous evidence was obtained by direct characterization of **IV** in the degradation

products of **VII** by retro-aldol cleavage.

In spite of the quite stable nature of the antibiotic in the form of sodium salt, the free acid was found to be rather unstable, and a marked depression of biological activity was observed in the aqueous methanolic solution left overnight at room temperature. This fact is in good agreement with the observed biological activity of **VII**, as will be discussed below. It is of interest that the five-membered hemiketal of ring *C* is not stable enough; therefore, presumably, the rearrangement in a six-membered framework takes place with great ease when the molecule is not in the salt complex form.

Preparation of Lysocellin Hexol

In order to elucidate the structure-activity relationship of the antibiotic in connection with its interesting behaviours as an ionophore, the contribution of carboxylic acid and the β-hydroxyketone group to the biological and metal-complexation activities should be clarified.

Lysocellin methyl ester was reduced with sodium borohydride in dioxane to afford an oily reduction product which was designated lysocellin hexol (**IX**). The IR spectrum of **IX** showed a complete absence of an absorption band at the carbonyl region. **IX** was further characterized as lysocellin hexol tetraacetate (**X**), $C_{42}H_{72}O_{13}$ (M^+, *m/e* 784) whose PMR spectrum showed a new appearance of four acetoxyl peaks at δ 2.05 to δ 2.20, two methylene protons adjacent to an acetoxyl group at δ 4.08 (2H, t, J=6.5 Hz) and three methine protons bonded to the carbons bearing an acetoxyl group at δ 4.7~δ 5.3 as the unsplit broad signals, respectively.

Fig. 3. Fragmentation pattern of lysocellin hexol tetraacetate (**X**).

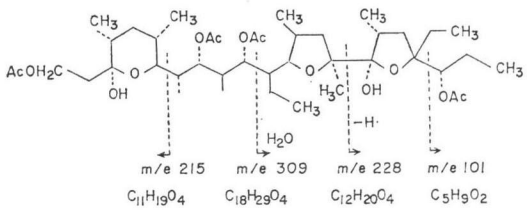


Table 1. Relative antibacterial activity of lysocellin and its structural analogs*

Compounds	Relative antibacterial activity (%)
Lysocellin sodium salt (**I**)	100
Lysocellin methyl ester (**II**)	2.8
Degradation product (**III**)	inactive
Degradation product (**IV**)	inactive
Acetate (**V**)	inactive
L_1 (**VI**)	0.56
M_1 (**VII**)	3.3
Lysocellin methyl ester monoacetate (**VIII**)	inactive
Lysocellin hexol (**IX**)	3.3

* *Bacillus subtilis* PCI 209P was used as test-organism and antibacterial activity was measured by agar disk method. Relative antibacterial activity is given as percentage of that of the parent antibiotic.

In contrast, two methine protons at δ 3.4 (1H, m) and δ 3.6 (1H, m), which were observed inherent in the parent antibiotics as well as commonly in a number of the structural analogs, were assigned to those bonded to C_7 and C_{13} adjacent to the ring oxygens, respectively. This indicated that rings *A* and *B* are left unchanged through the sodium borohydride reduction.

With respect to ring *C*, the presence of an inherent five-member framework is analogously confirmed based on the NMR evidence; the signal centered at δ 5.00 which appears as a broadening multiplet is assigned to the methine proton at C_{21} adjacent to an acetoxyl substituent. Likewise, in the PMR spectra of **V** and **VIII**, this proton appears as distinct peaks at δ 4.95 and δ 5.05, respectively.

This evidence leads to the conclusion that the acetoxyl groups were introduced to the carbons of C_1, C_9, C_{11} and C_{21}, thereby allowing the assignment of structures **IX** to lysocellin hexol and **X** to the corresponding tetraacetate, respectively.

Support for the structural assignment of **X** was further evidenced by the fragmentation pattern of the mass spectrum as illustrated in Fig. 3.

Antibacterial Activity of I to IX

Relative antibacterial activity of the compounds **I** to **IX** against *Bacillus subtilis* PCI 209P is presented in Table 1.

As was reported for lasalocid[12], the degradation products of lysocellin, **III**, **IV**, **V** and **VIII** were also found inactive even at a concentration of 200 mcg/ml. However **II**, **VI** and **VII** retained a weak antibacterial activity (0.5~3.3% relative to lysocellin).

The biological activity of these artifacts and degradation products has been investigated using organelles and is reported in the following paper[13].

Experimental

General methods

Melting points were determined on a Yanagimoto microheating stage and were uncorrected. PMR spectra were recorded on a JEOL JNM 4H-100 spectrometer in $CDCl_3$ with TMS as an internal standard. IR spectra were measured with a JASCO IR-S spectrometer. Optical rotations were measured in MeOH on a JASCO DIP-4 polarimeter. The mass spectra were determined on a single focus Hitachi RMH-2 and a double focus Hitachi RMU-6L mass spectrometer equipped with a Hitachi Datalyzer for high resolution mass spectrometry. Errors (Δ) of high resolution mass spectra are represented by millimass units (mmu) compared with the calculated values. Kieselgel GF_{254} was used for preparative TLC through this work.

Lysocellin (I)

The sodium salt of **I** was isolated from the cultured mycelia of *S. cacaoi* var. *asoensis* K-9 Met$^-$ according to the procedure described by EHATA *et al.*[2] The sodium salt was recrystallized from methanol as needles of mp. 158~160°C. PMR (δ); 0.65~2.90 (>50 H, developed), 1.21 (3H, s, C_{16}-CH_3), 3.46 (1H, bdd), 3.87 (1H, dd, J=3.50, J=10 Hz, C_{21}-H), 3.98 (1H, s), 5.04 (1H, s), 7.76 (1H, bs). IR (nujol, cm^{-1}); 3600, 3420, 3140, 1702, 1587, 1452, 1380, 1115, 1096, 1030.

Lysocellin methyl ester (II)

A solution of **I** (100 mg) dissolved in ethyl acetate (20 ml) was shaken twice with 0.1 N hydrochloric acid (each 5 ml), then the solvent layer was washed thoroughly with water to remove hydrochloric acid. To this solution an excess of ethereal diazomethane was added, and the mixture was left 3 hours at room temperature. After removal of the solvents *in vacuo*, the residue was chromatographed on a column of silicic acid (Wako gel, 2×15 cm) using benzene - ethyl acetate (4: 1) to afford **II** (95 mg). Recrystallization from ethyl acetate gave crystalline needles of mp 101~102°C. Anal. calcd for $C_{35}H_{62}O_{10}$: C, 65.39; H, 9.72; O, 24.89. Found: C, 65.28; H, 9.65; O, 25.00. MS; *m/e* 624 (M^+-H_2O) ,565, 437, 366, 325, 225, 187, 169, 127, 59. PMR (δ); 0.65~2.95 (48 H, developed), 3.04 (1H, s), 3.50~4.10 (4H, overlapped), 3.70 (3H, s, $-COOCH_3$), 3.89 (1H, s), 4.00 (1H, s), 4.55 (1H, s). IR ($CHCl_3$, cm^{-1}); 3500, 2950, 1710, 1460, 1112, 1037, 988.

Retro-Aldol cleavage of I and isolation of N_2 (III)

To a solution of **I** (2 g) in dioxane (50 ml) was added 10% aqueous NaOH (50 ml) and the mixture was stirred at room temperature for 48 hours. The reaction mixture was extracted twice with 200 ml of ether, and the combined extracts were washed with water and dried over anhydrous sodium sulfate, followed by evaporation *in vacuo* to yield a viscous oil (1.2 g). The oily residues were then subjected to silicic acid column chromatography (300 g, Mallinckrodt) using a solvent system of *n*-hexane - methanol - ethyl acetate (20: 1: 1) to give **III** (1.07 g) as an oil. MS; *m/e* 366.2764 (M-H_2O, $C_{22}H_{38}O_4$, Δ-0.3), 325.2385 (M-C_3H_7O, $C_{19}H_{33}O_4$, Δ 0.8), 225.1498 ($C_{13}H_{21}O_3$, Δ 0.8 mmu), 207.1379 ($C_{13}H_{19}O_2$, Δ-0.4

mmu), 197.1552 ($C_{12}H_{21}O_2$, Δ 1.2 mmu), 187.1344 ($C_{10}H_{19}O_3$, Δ 1.1 mmu), 169.1236 ($C_{10}H_{17}O_2$, Δ 0.8 mmu), 127.0750 ($C_7H_{11}O_2$, Δ-0.8 mmu), 59.0442 (C_3H_7O, Δ-0.4 mmu), 57.0324 (C_3H_5O, Δ-1.5 mmu). PMR (δ); 0.65 1.85 (>3 OH, developed), 2.05 (2H, m), 2.55 (1H, bdd, J=2.50 Hz, J=10.0 Hz, C_{12}–H), 3.50 (1H, dd, J=2.50 Hz, C_{13}–H), 3.60 (1H, t, C_{21}–H). IR (neat, cm^{-1}); 3400, 2920, 1696, 1452, 1370, 1104 and 1049.

N_2 monoacetate (**V**)

III (300 mg) was dissolved in dry pyridine (2 ml) and acetic anhydride (1 ml) was added under ice-cooling; the mixture was kept at room temperature for 17 hours, then was poured into ice water. This mixture was evaporated under reduced pressure and the residues were further purified by preparative TLC using a solvent system of benzene - ethyl acetate - methanol (100: 5: 3) to yield **V** (150 mg) as an oil. MS; *m/e* 408 ($M^+ - HO$), 325 ($M^+ - C_3H_7O$), 243, 197, 169 and 57. PMR (δ); 1.18 (3H, s, CH_3CO–), 2.05 (3H, s, CH_3COO–), 3.50 (1H, bdd, J=10 Hz, C_{21}–H). IR ($CHCl_3$, cm^{-1}); 3400, 2920, 1735, 1709, 1460, 1104 and 1050.

Transformation of **III** into N_3 (**IV**)

A solution of **III** (1.0 g) dissolved in methanol (5 ml) was kept for a month at room temperature and then methanol was removed by evaporation to yield an oily mixture of **III** (Rf 0.12) and **IV** (Rf 0.68), which was separated by a preparative TLC using *n*-hexane - ethyl acetate (10: 1) to give pure **IV** (450 mg) as an oil.

Alternatively, to **III** (100 mg) in methanol (10 ml) was added one drop of acetic acid, and the mixture was refluxed for 3 hours. After removal of the solvents by evaporation, the residues were purified as described above to obtain **IV** in almost quantitative yield. MS; *m/e* 366.2790 ($M - H_2O$, $C_{23}H_{38}O_4$, Δ 2.2 mmu), 267.1981 ($C_{16}H_{27}O_3$, Δ 2.2 mmu), 197.1552 ($C_{12}H_{21}O_2$, Δ 1.2 mmu), 169.1256 ($C_{10}H_{17}O_2$, Δ 2.8 mmu), 151.1103 ($C_{10}H_{15}O$, Δ-1.8 mmu), 125.0607 ($C_7H_9O_2$, Δ 0.5 mmu), 57.0537 (C_3H_5O, Δ-1.2 mmu). PMR (δ); 0.65 ~ 1.35 (>30 H, developed), 1.45 ~ 1.64 (2H, m), 2.05 (1H, m, C_{14}–H), 2.43 (1H, m, C_{12}–H), 3.50 (1H, dd, J=3.5 Hz, C_{21}–H), 3.65 (1H, m, C_{13}–H). IR (neat, cm^{-1}); 3400, 2920, 1702, 1452, 1370, 1104, 1052, 955.

Isolation of L_1 (**VI**)

To a solution of **I** (200 mg) dissolved in methanol (10 ml) was added an excess of ethereal diazomethane, and the mixture was kept overnight at room temperature, then the solvents were evaporated *in vacuo* to yield an oily mixture which was dissolved in methanol (200 ml). TLC analysis of this mixture using *n*-hexane - ethyl acetate (4: 1) revealed a gradual transformation of **II** (Rf 0.35) into **VI** (Rf 0.13) as distinct spots. After two weeks, the solvent was removed by evaporation and the residues were separated by preparative TLC using the same solvent to yield **VI** (90 mg) as crystalline needles. mp. 124.5 ~ 127°C.

This transformation was markedly enhanced by addition of a drop of acetic acid to boiling methanol. Anal. Calcd. for $C_{35}H_{62}O_{10}$: C, 65.39; H, 9.72; O, 24.88. Found: C, 65.30; H, 9.85; O, 24.60. MS; *m/e* 624.4278 ($M - H_2O$, $C_{35}H_{60}O_9$, Δ-4.8 mmu), 606.3991 ($M - 2H_2O$, $C_{35}H_{58}O_8$, Δ-3.6 mmu), 437.2814 ($C_{25}H_{41}O_6$, Δ-4.8 mmu), 366.2758 ($C_{22}H_{38}O_4$, Δ-0.9 mmu), 267.1954 ($C_{16}H_{27}O_3$, Δ-0.4 mmu), 225.1491 ($C_{13}H_{21}O_3$, Δ 0.1 mmu), 197.1539 ($C_{12}H_{21}O_2$, Δ-0.1 mmu), 169.1179 ($C_{10}H_{17}O_2$, Δ-4.8 mmu), 125.0598 ($C_7H_9O_2$, Δ 4.1 mmu). PMR (δ); 0.65 ~ 2.80 (50H, developed), 2.98 (1H, s), 3.0 ~ 3.50 (3H, overlapped), 3.50 (1H, dd, J=3.5 Hz, J=7.5 Hz), 3.67 (3H, s, $-COOCH_3$), 4.55 (1H, s, OH). IR ($CHCl_3$, cm^{-1}); 3500, 2920, 1700, 1452, 1110, 1030, 990.

Isolation of M_1 (**VII**)

A solution of the free acid of **I** (200 mg) in methanol (100 ml) was kept at room temperature for a month, then methanol was evaporated to give a mixture of **VII** (Rf 0.78), **I** (Rf 0.22) and some impurities. Separation by a preparative TLC of this mixture using a solvent system of *n*-hexane - ethyl acetate (4: 1) yielded **VII** (60 mg) as an oil.

Alternatively, **VII** was obtained in almost quantitative yield by refluxing a methanol solution of **I** (free acid) for 3 hours under catalysis of acetic acid (one drop). MS; *m/e* 580.4346 ($M - H_2O$, $C_{34}H_{60}O_7$, Δ 1.0 mmu), 379.2676 ($C_{23}H_{39}O_4$, Δ-1.7 mmu), 366.2886 ($C_{22}H_{38}O_4$, Δ-4.8 mmu), 281.2023 ($C_{17}H_{29}O_3$,

Δ-3.1 mmu), 267.1958 ($C_{16}H_{27}O_3$, Δ 0 mmu), 211.1712 ($C_{13}H_{23}O_2$, Δ 1.5 mmu), 183.1368 ($C_{11}H_{19}O_2$, Δ 4.4 mmu), 169.1199 ($C_{10}H_{17}O_2$, Δ-2.7 mmu), 151.1113 ($C_{10}H_{15}O$, Δ-0.9 mmu), 125.0943 ($C_8H_{13}O$, Δ-2.2 mmu), 125.0620 ($C_7H_9O_2$, Δ 1.8 mmu). PMR (δ); 0.65~3.10 (47H, developed), 3.20 (3H, s, $-OCH_3$), 3.3~4.1 (6H, overlapped). IR ($CHCl_3$, cm^{-1}); 3480, 2900, 1702, 1455, 1375, 1114, 982.

Lysocellin methyl ester monoacetate (**VIII**)

To a solution of **II** (200 mg) in pyridine (2 ml) was added acetic anhydride (1 ml), and the mixture was kept overnight at room temperature, then water (5 ml) was added, followed by evaporation *in vacuo*. The residue was purified by preparative TLC to yield **VIII** (190 mg) as an oil. MS; *m/e* 666 ($M-H_2O$). PMR (δ); 0.65~3.0 (48H, developed), 3.70 (3H, s, $-COOCH_3$), 3.7~4.0 (3H, overlapped), 5.05 (1H, dd, J=3.0 Hz, J=10 Hz, C_{21}–H). IR ($CHCl_3$, cm^{-1}); 3500, 2950, 1710, 1460.

Lysocellin hexol (**IX**) and lysocellin hexol tetraacetate (**X**)

A mixture of **II** (400 mg) and sodium borohydride (200 mg) in dioxane (50 ml) was kept at room temperature overnight. After removal of dioxane, the residue was extracted with ether. The ether extract was washed twice with water and dried over anhydrous sodium sulfate, followed by evaporation *in vacuo* to yield an oily residue which was further purified by a preparative TLC using ethyl acetate to afford hexol (**IX**, 120 mg). IR (nujol, cm^{-1}); 3450, 2960, 1460, 1382, 1100 and 970.

To a solution of **IX** (120 mg) in pyridine (2 ml) was added acetic anhydride (1.0 ml) and the mixture was kept overnight at room temperature. This was evaporated twice *in vacuo* with the addition of water to remove an excess of acetic acid to yield an oily residue which was further purified by preparative TLC using *n*-hexane - ethyl acetate (3: 1) to yield **X** (95 mg). $C_{42}H_{72}O_{13}$ (M^+, *m/e* 784). MS; *m/e* 309.2082 ($C_{18}H_{29}O_4$, Δ 1.8 mmu), 241.1394 ($C_{13}H_{21}O_4$, Δ-4.4 mmu), 228.1323 ($C_{12}H_{20}O_4$, Δ-4.4 mmu), 215.1307 ($C_{11}H_{12}O_4$, Δ 2.5 mmu). PMR (δ); 0.5~2.4 (>40H, developed, 2.05~2.20 (12H, 4×CH_3CO–), 3.35 (1H, bm), 3.60 (1H, m), 4.10 (2H, t), 4.7~5.3 (3H, overlapped). IR ($CHCl_3$, cm^{-1}); 3500, 2900, 1725, 1460, 1370, 1105 and 1040.

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