NH_4OH–H_2O (2:2:1:1); IR 1702 cm^{-1}; ^1H NMR (D_2O) δ 2.10 (br, 10 H, 5 CH_2), 3.15 (s, 9 H, NMe_3), 3.83 (br, 1 H, CHN).

Anal. Calcd for $C_{10}H_{20}NOCl$: C, 58.39; H, 9.75; N, 6.81. Found: C, 58.31; H, 9.92; N, 6.58.

Hofmann Degradation of 4 (X = Cl). A 0.2-g sample of **4** (X = Cl) was dissolved in 30 mL of aqueous ethanolic KOH (1 N), the solution was refluxed on a water bath for 2 h in an N_2 atmosphere, and the outgoing gas was bubbled through an ethanolic solution of EtI. Removal of solvent from the bubbler furnished a white solid (0.08 g) which crystallized from EtOH as needles: mp 316–318 °C; ^1H NMR (D_2O) 3.19 (s, 9 H, NMe_3), 1.43 (tt, J = 7 and 2 Hz, 3 H, $N^+CH_2CH_3$), 3.50 (q, J = 7 Hz, 2 H, $N^+CH_2CH_3$). The nonbasic fraction left in ethanolic alkali was obtained as a gum which could not be purified for identification.

Conversion of *N,N*-Dimethylphysoperuvinium Iodide to Quaternary Alkaloid 4 (X = Cl). *N,N*-Dimethylphysoperuvinium iodide (0.3 g) was dissolved in ion-free water (30 mL), and the solution was stirred with anion-exchange resin (IRA 400, Cl^- form) for 4 h. The aqueous solution was filtered from the resin and evaporated to dryness under reduced pressure. The solid residue was crystallized from MeOH as colorless needles: 0.12 g; mp 255–258 °C; $[\alpha]_D$ +86.2° (*c* 1.0, H_2O); indistinguishable from the chloride salt of the natural quaternary base (melting point, mixture melting point, co-TLC, IR).

Acknowledgment. We are grateful to Professor H. Hikino of Tohoku University, Japan, for the ^{13}C NMR spectral analyses and to Dr. B. C. Das of the Institut de Chimie des Substances Naturelles, Gif-sur-Yvette, France, for the mass spectral analyses. A gift sample of 2-cycloheptenone from Dr. M. T. Shipchandler is gratefully acknowledged. Thanks are due to Dr. J. V. Juvarkar, College of Pharmacy, Ohio State University, for CD spectra and to Professor B. Dasgupta of our department for his kind interest in the problem. M.S. is grateful to CCRAS and CSIR, New Delhi, for financial assistance.

Registry No. (+)-**1**, 60723-27-5; (±)-**1**, 73744-99-7; (+)-**2**, 60723-28-6; (±)-**2**, 73745-00-3; (+)-**2a**, 60723-29-7; (+)-**2** oxime isomer 1, 73697-49-1; (+)-**2** oxime isomer 2, 73697-52-6; (+)-**4** (X = I), 73697-50-4; (+)-**4** (X = Cl), 73697-51-5; (±)-**4** (X = I), 73745-01-4; dimethylamine, 124-40-3; 2-cyclohepten-1-one, 1121-66-0; (+)-**2** oxime isomer 2, 73697-52-6.

Structure of Elasnin, a Novel Elastase Inhibitor Containing an α-Pyrone Ring

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Received December 3, 1979

Elasnin ($C_{24}H_{40}O_4$), isolated from the cultured broth of *Streptomyces noboritoensis* KM-2753, is a new specific inhibitor of human granulocyte elastase. Evidences based on spectral analyses, chemical degradations, and biosynthetic means using a ^{13}C-labeled precursor established its structure as **1**, having a highly alkylated 4-hydroxy-α-pyrone, rather than a 2-hydroxy-γ-pyrone, as the most likely skeletal structure. Structural comparison between elasnin and its methyl ether obtained by CH_2N_2 treatment was investigated, using UV data and ^{13}C–^{13}C couplings in both compounds biosynthetically enriched with [1,2-^{13}C]acetate. Structural change from α-pyrone to γ-pyrone occured in the methylation of elasnin with CH_2N_2 but not in the acetylation with acetic anhydride in pyridine.

In our screening program for inhibitors of human granulocyte elastase, a new inhibitor designated as elasnin was isolated from the culture filtrate of *Streptomyces noboritoensis* KM-2753.[1,2] Elasnin markedly inhibits human granulocyte elastase, but it is almost inactive against pancreatic elastase, chymotrypsin, and trypsin.[3,4] The present paper deals with the structural elucidation of elasnin on the basis of chemical degradations and a biosynthetic investigation using a ^{13}C-labeled precursor and ^{13}C NMR spectroscopy.[5]

Elasnin (**1**) (Chart I) is a lipophilic colorless and viscous oil: $C_{24}H_{40}O_4$, mass spectrum, m/z (M^+, 392); $n^{17}{}_D$ 1.4983; $[\alpha]^{18}{}_D$ –0.9° (*c* 1, EtOH); UV (EtOH) λ_{max} 291 nm (ϵ 7760). The IR (CCl_4) spectrum of **1** exhibited characteristic absorption bands due to methylenic and methyl groups at 2960 and 2860 cm^{-1}, a ketone carbonyl at 1715 cm^{-1}, a conjugated ester carbonyl at 1665 cm^{-1}, and a double bond at 1636 cm^{-1}. The 25.2-MHz ^{13}C NMR spectrum of **1** shows the presence of a ketone carbonyl at δ 207.0, an ester carbonyl at either δ 165.5 or 164.7, and four quaternary olefinic carbons at either δ 165.5 or 164.7, 153.8, 115.0, and 104.3, as shown in Figure 4 (A). The appearance of four methyl carbons completely overlapped at δ 13.9, thirteen methylenic carbons at δ 22.4–40.3, and a methine at δ 54.7 suggests the existence[6] of four linear C_4 and C_5 alkyl chains in **1**.

Acetylation of **1** with acetic anhydride and pyridine afforded monoacetyl elasnin (**2**): $C_{26}H_{42}O_5$, mass spectrum, m/z (M^+, 434); UV λ_{max} 306.5 nm (ϵ 7200). The IR spectrum of **2** exhibited the absorption band for enolic ester carbonyl at 1775 cm^{-1}, indicating the presence of an enolic hydroxyl group in **1**. This was also confirmed from the formation of the methyl ether **3**: $C_{25}H_{42}O_4$, mass spectrum, m/z (M^+, 406); UV λ_{max} 252 nm (ϵ 7890); ^1H NMR δ 3.8 (s, 3 H OCH_3). **3** is obtained by the treatment

(1) S. Ōmura, H. Ohno, T. Saheki, M. Yoshida, and A. Nakagawa, *Biochem. Biophys. Res. Commun.*, **83**, 704 (1978).
(2) H. Ohno, T. Saheki, J. Awaya, A. Nakagawa, and S Ōmura, *J. Antibiot.*, **31** (11), 1116 (1978).
(3) A. Janoff, *Annu. Rev. Med.*, **23**, 177 (1972).
(4) Studies on the protease inhibitors arising from *Streptomyces* have been summarized in the following reviews: (a) T. Aoyagi and H. Umezawa, *Cold Spring Harbor Conf. Cell Proliferation*, **2**, 429–454 (1975); (b) H. Umezawa, *Methods Enzymol.*, **45B**, 678–695 (1976); (c) H. Umezawa and T. Aoyagi, "Proteinases of Mammalian Cells and Tissues", A. J. Barrett, Ed., ASP Biological and Medical Press, Amsterdam, 1977, pp 637–662.
(5) S. Ōmura, A. Nakagawa, and H. Ohno, *J. Am. Chem. Soc.*, **101**, 4386 (1979).
(6) The assignment of each alkyl carbon in the ^{13}C NMR spectra of all compounds was mainly carried out on the basis of the data and references listed in: J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972, and L. F. Johnson and W. C. Jankowski, "Carbon-13 NMR Spectra", Wiley-Interscience, New York.

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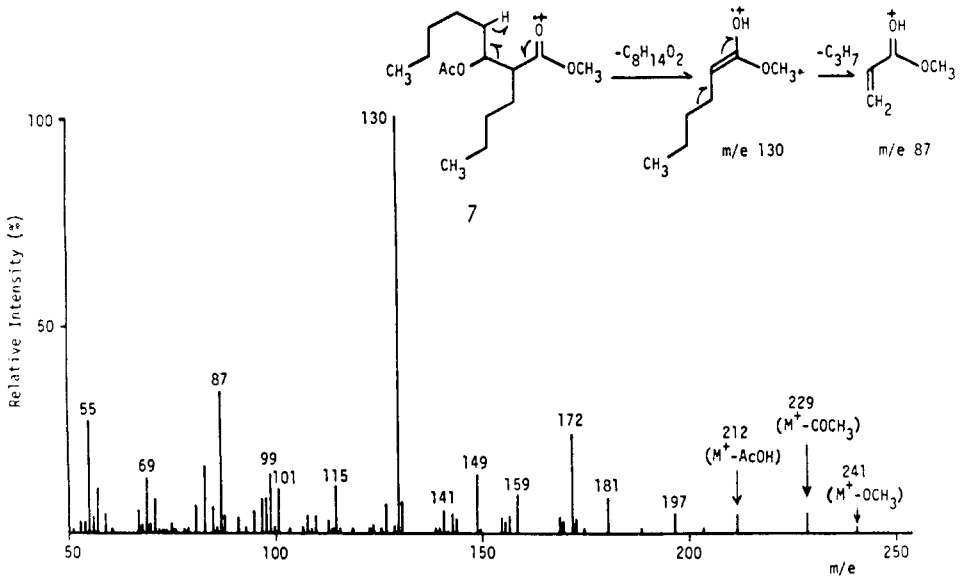
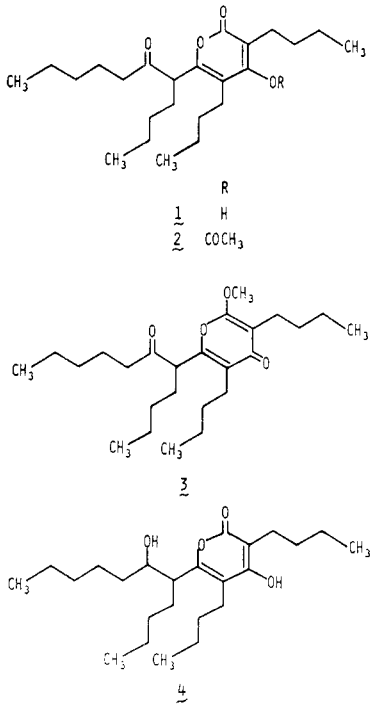


Figure 1. Mass spectrum of 2-butyl-3-acetoxyoctanoate (7).

Chart I



of 1 with CH_2N_2. However, the significant blue shift of **3**, relative to **1**, most explicitly suggests that the two compounds do not possess the same chromophore; therefore, treatment of **1** with CH_2N_2 has effected a rearrangement, as will be described later.

Reduction of **1** with $NaBH_4$ in EtOH afforded dihydro elasnin (**4**): $C_{24}H_{42}O_4$, mass spectrum, m/z (M^+, 394). The UV spectrum of **4** exhibits the absorption maximum at 292 nm (ϵ 6400), indicating the existence of a ketone carbonyl at the position isolated from the chromophore in **1**. Ozonolysis of **4** followed by oxidative degradation with 30% H_2O_2 and glacial CH_3COOH gave an acidic oil (**5**) in a low yield, which was then treated with CH_2N_2, giving a methyl ether (**6**): $C_{12}H_{24}O_3$; IR (CCl_4) 3520 (OH), 1730 cm^{-1} (CO). The ^{13}C NMR spectrum of **6** is compatible with the presence of two methyls (δ 13.9, unresolved), seven methylenes (δ 22.6–31.7, partially unresolved), a methoxy (δ 50.9), a methine (δ 51.4), an oxy carbon (δ 72.3), and an ester carbonyl (δ 176.1). The binding position of the hydroxyl and carboxyl groups to the hydrocarbon moiety in the structure of **5** was readily proved from the mass spectral analysis of the corresponding monoacetate (**7**), obtained by acetylation of **6** with acetic anhydride and pyridine. The mass spectrum indicated characteristic fragment ions[7] at m/z 241 ($M^+ - OCH_3$), 229 ($M^+ - COCH_3$), 212 ($M^+ - AcOH$), 130 ($CH_3CH_2CH_2CH_2CH{=}C(OH)(OCH_3)$), and 87 ($CH_2CH{=}C(OH)(OCH_3)$), as illustrated in Figure 1. These spectral evidences led to 2-butyl-3-hydroxyoctanoic acid as the structure of **5**. Since the hydroxyl carbon formed by $NaBH_4$ reduction in the structure of **4** should correspond to that of the isolated ketone carbonyl in **1**, the partial structure A must be present in **1**, as indicated in Chart III.

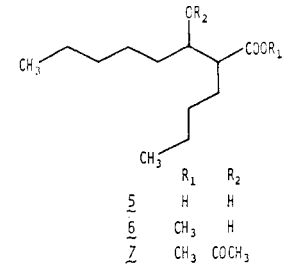
Similarly, an oily compound (**8**), $C_8H_{14}O_4$, was isolated from the ozonolysis of **3** followed by oxidative degradation. The structure of **8** was characterized by ^1H and ^{13}C NMR spectral analyses. The ^{13}C NMR spectrum clearly indicates the presence of an ester carbonyl (δ 174.5), a carboxy (δ 169.9), a methoxy (δ 52.6), a methine (δ 51.5), three methylenes (δ 22.3, 28.6, 29.4), and a methyl (δ 13.9), as assigned in Chart II. Furthermore, taking into consideration ^1H NMR spectral assignment [a carboxy proton (δ 6.70, s), a carboxy methyl (δ 3.72, s), a methine (δ 3.36, t, J = 8.0 Hz), a methylene (δ 1.87, q, J = 8.0 Hz), two methylenes, (δ 1.2–1.4, m), and a methyl (δ 0.90, t, J = 6.5 Hz)], the methyl ester of 2-carboxy-*n*-hexanoic acid was established for the structure of **8**. The chemical shift assignments in the ^{13}C NMR spectra of compounds **6** and **8** are shown in Chart II. The isolation of compound **8** by ozonolysis of **3** afford important evidences to validate the structural assignment for **3**, as will be mentioned later (cf. Scheme II).

A convenient proof of the substitution pattern of the chromophore of **1** was provided from the acidic hydrolysis of **1** and the transformation of **1** into an isoxazole derivative. Acidic degradation of **1** with 2.5 N HCl in CH_3CO-OH gave a colorless oil (**9**): $C_{23}H_{40}O_2$, mass spectrum, m/z (M^+, 348). The existence of an $\alpha,\beta,\alpha',\beta'$-alkyl-substituted γ-pyrone skeletone in structure **9** was suggested from the UV absorption maximum[8] at 258 nm (ϵ 7800) and IR ab-

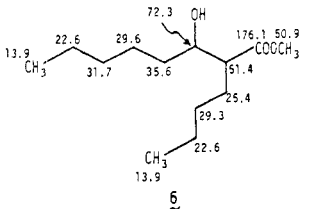
(7) Mass spectral analyses for **1** and its derivatives are based on "Mass Spectrometry of Organic Compounds", H. Budzikiewicz, C. Djerassi, and D. H. Widliams, Eds., Holden-Day, San Francisco.

(8) A. K. Ganguly, T. R. Govindachari, and P. A. Mohamed, *Tetrahedron*, **21**, 93 (1965).

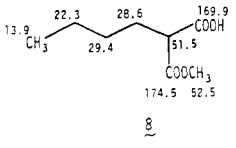
Chart II



	R_1	R_2
5	H	H
6	CH_3	H
7	CH_3	$COCH_3$



6



8

Scheme I

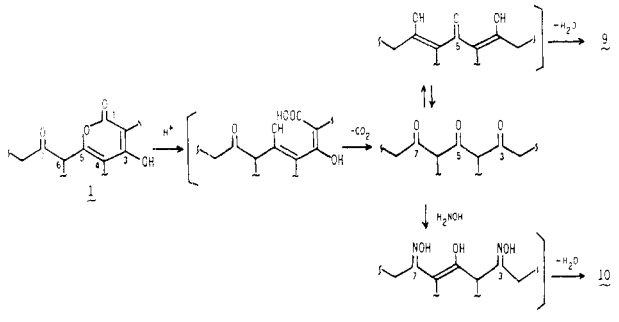
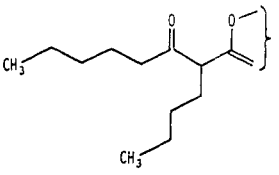
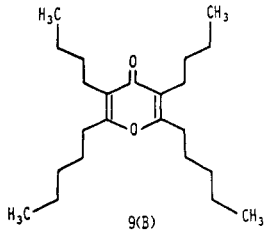


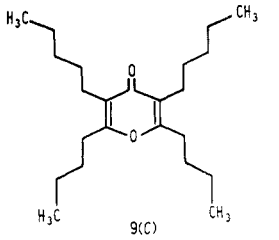
Chart III



A

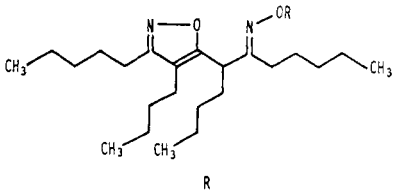


9(B)



9(C)

Chart IV



	R
10	H
11	$COCH_3$

sorption bands[9,10] due to an $\alpha,\beta,\alpha',\beta'$-unsaturated ketone carbonyl (γ-pyrone carbonyl) at 1645 cm^{-1} and a double bond at 1610 cm^{-1}. The ^{13}C NMR spectrum of **9** exhibits distinct signals based on the γ-pyrone carbonyl carbon at δ 179.0, two α carbons of the γ-pyrone at δ 163.6, and two β carbons of the γ-pyrone at δ 123.3, which are not coupled to any hydrogen, since they give singlet signals in ^{13}C–^{1}H spin-coupling multiplicity determination by the off-resonance technique. The high-field signals, accountable by the presence of four unresolved methyls at δ 13.9, five pairs of unresolved methylenes at δ 22.4, 23.0, 24.4, 27.2, and 31.0, and four unresolved methylenes at δ 31.4, imply the involvement of two linear C_4 and C_5 alkyl chains[6] in **9**. With regard to the substitution pattern of alkyl groups on the γ-pyrone nucleus, two symmetrical positional isomers, α,α'-*n*-pentyl-β,β'-*n*-butyl-γ-pyrone **9**(B) and α,α'-*n*-butyl-β,β'-*n*-pentyl-γ-pyrone **9**(C), for **9** were proposed on the basis of the number of ^{13}C NMR signals as well as the chemical shift of the alkyl carbons described above (Chart III). Since the β-carbon in the γ-pyrone of **9** should originate from the ketone carbonyl carbon of the previously established partial structure A in **1** (Chart III) (according to the reaction mechanism illustrated in Scheme I), the *n*-pentyl group must be substituted in the position of the γ-pyrone nucleus, and therefore, α,α'-*n*-pentyl-β,β'-*n*-butyl-γ-pyrone, **9**(B), was established as the structure of **9**.

This interesting transformation accompanied by the acid-catalyzed decarboxylation of **1** into **9** suggests the interpretation of "triketide" as an intermediate.[11] Furthermore, the substitution pattern of the pyrone nucleus and more explicit evidence for the existence of a "triketide intermediate" in the acid-catalytic reaction of **1** were obtained by the transformation of **1** into the isoxazole derivative **10** (Chart IV and Scheme I). Mild reflux of **1** with excess H_2NOH·HCl in CH_3COOH afforded **10**, a colorless oil ($C_{23}H_{42}N_2O_2$, mass spectrum, m/z (M^+, 378), in high yield.

The ^1H NMR spectrum of **10** exhibits the signal for an oxime which is exchangeable with D_2O at δ 9.0. Acetylation of **10** with acetic anhydride and pyridine gave a monoacetate (**11**): $C_{25}H_{44}N_2O_3$; mass spectrum, m/z (M^+, 420); IR 1768 cm^{-1} (=$NOCOCH_3$). The ^{13}C NMR spectral features of **10** indicate characteristic signals due to isoxazole carbons at δ 165.7, 163.4, and 116.0 and an oxime carbon at δ 160.6. More definitve information for the structure of **10** was obtained from its mass spectral analysis as shown in Figure 2. The mass spectrum indicates significant fragment peaks at m/z 194 (base peak, $C_{12}H_{20}NO$) and 264 ($C_{17}H_{30}NO$), corresponding to an isoxazole ring moiety containing alkyl chains, m/z 97 ($C_6H_{11}N$), 166 ($C_{11}H_{20}N$), and 322 ($C_{19}H_{34}N_2O_2$), which arise from McLafferty rearrangement of the *n*-pentyl group. On the

(9) J. D. Bu'Lock and H. G. Smith, *J. Chem. Soc.*, 502 (1960).
(10) D. Herbest, W. B. Mors, O. R. Gottlieb, and C. Djerassi, *J. Am. Chem. Soc.*, 81, 2427 (1959).
(11) L. C. Dorman, *J. Org. Chem.*, **32**, 4105 (1967).

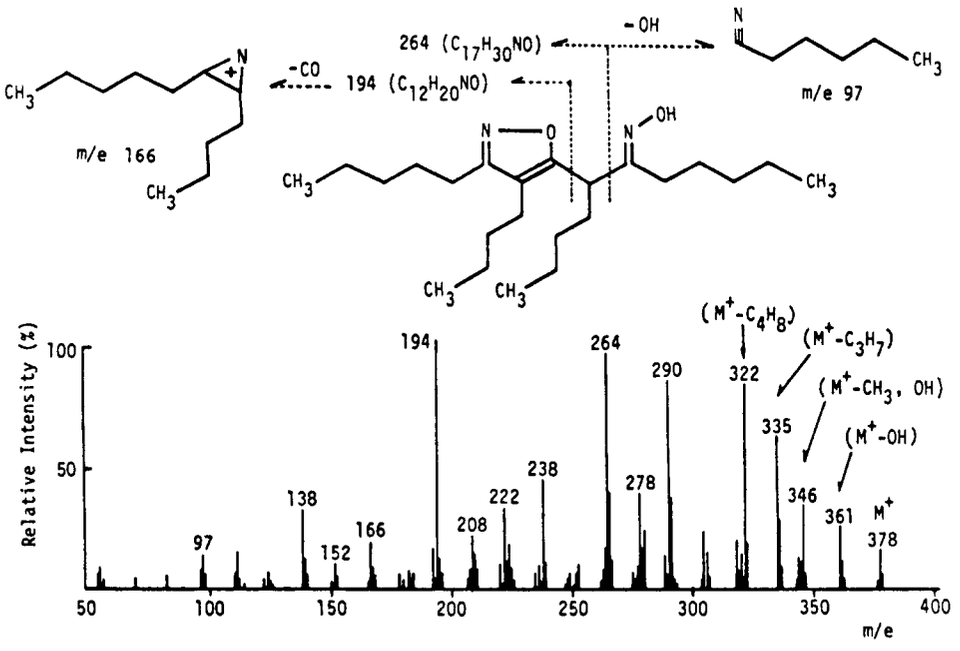


Figure 2. Mass spectrum of 10.

basis of the spectral evidences mentioned above, the structure **10** was established. Taking into consideration the formation of **9** and **10** based on the acid-catalyzed reactivities of **1** and the UV, IR, and NMR spectral evidences of **1**, it was concluded that 4-hydroxy-γ-pyrone, instead of 2-hydroxy-γ-pyrone, would be most suitable for the complete structure of **1**.

Confirmation for the structure of **1** was successfully accomplished through mass spectrometry, as shown in Figure 3. The appearance of the fragment peaks at m/z 99, representing the ion $C_6H_{11}O$, and m/z 294 ($M^+ - 98$), for the ion $C_{18}H_{30}O_3$, indicates the presence of an *n*-caproyl group in **1**. Furthermore, the ion peaks at m/z 251 ($C_{15}H_{23}O_3$) and 223 ($C_{13}H_{19}O_2$) are consistent with the substitution pattern of the alkyl groups on the γ-pyrone nucleus. Similar conclusions are also obtained from the mass spectral analysis of the corresponding derivatives **2**, **3**, and **4**.

Although the structure of the product **3**, obtained by CH_2N_2 treatment of **1**, could conceivably be a 4-methoxy-γ-pyrone[12] (**1**, R = OCH_3), such a structure can be ruled out from the significant hypsochromic shift[10] of the UV absorption maximum at 252 nm of **3** compared with that of **1**. It is well-known that the λ_{max} of α-pyrones (280–300 nm) is generally longer than that of γ-pyrones (240–260 nm). Consequently, a tautomeric isomer, 2-methoxy-γ-pyrone, was established for **3**. This was also supported by the appearance of the ^{13}C NMR signal at δ 179.8 due to the γ-pyrone carbonyl carbon and the IR band at 1658 cm^{-1} due to the conjugated carbonyl in **3**. In addition, the isolation of 2-carboxy-*n*-hexanoic acid methyl ether **8** from the ozonolysis of **3** conveniently explains the 2-methoxy-γ-pyrone structure of **3**. The reaction mechanism for the formation of **8** was assumed to involve the pathway via decomposition of the ozonide, followed by oxidative cleavage of the α-diketone, as depicted in Scheme II.

On the other hand, since monoacetate **2** shows a UV absorption maximum due to the α-pyrone at 306.5 nm and characteristic IR absorption bands[13] due to a lactone ester

Scheme II

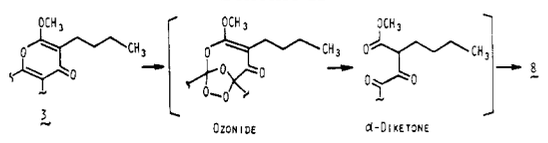
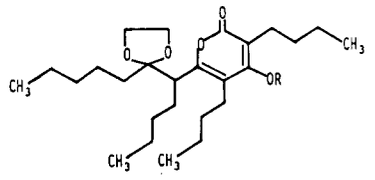
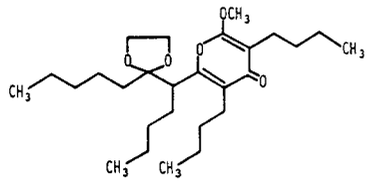


Chart V



	R
12	H
14	$COCH_3$



13

carbonyl at 1735 cm^{-1} and a double bond at 1640 cm^{-1}, its skeletal structure was concluded to be that of a 4-acetoxy-α-pyrone. The rearrangement of the α-pyrone to a γ-pyrone by methylation was also confirmed by the UV and IR spectral evidence for the methyl ether **13** and monoacetate **14** of the ketal (**12**) of **1** (Chart V). Namely, refluxing **1** with ethylene glycol and *p*-toluenesulfonic acid in benzene afforded ketal **12**: $C_{26}H_{44}O_5$; mass spectrum, m/z (M^+, 446). The ketal possesses the skeletal structure of a 4-hydroxy-α-pyrone, as evidenced by the UV absorption maximum at 284 nm. The methyl ether **13** obtained by treatment of **12** with CH_2N_2 exhibits an absorption maximum at 254.4 nm (ϵ 7825) and an IR band due to a γ-pyrone carbonyl at 1650 cm^{-1}, suggesting a skeletal change from an α-pyrone to a γ-pyrone. The monoacetate **14** which was obtained by acetylation of **12** with acetic anhydride in pyridine appears to possess a skeletal

(12) Y. Koyama, Y. Fukakusa, N. Kyomura, and S. Yamazaki, *Tetrahedron Lett.*, 355 (1969).
(13) E. A. Chandross and P. Yates, *Chem. Ind.*, 149 (1960).

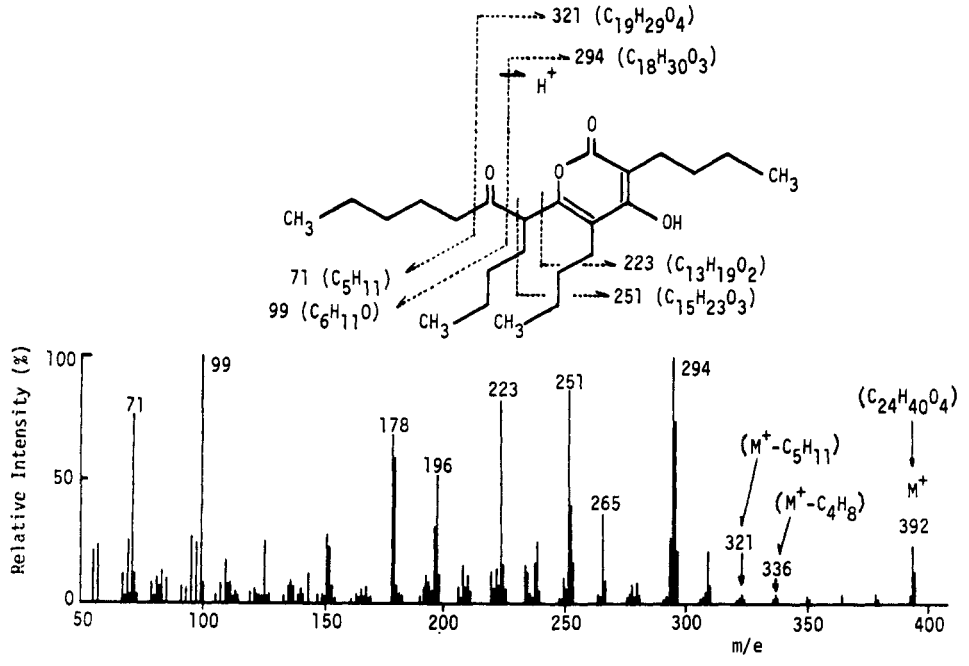


Figure 3. Mass spectrum of 1.

Table I. ^{13}C NMR Chemical Shifts and Coupling Constants ($J_{^{13}C-^{13}C}$) of [1,2-^{13}C]Acetate-Enriched Elasnin (1) and Its Methyl Ester 3

	chemical shift,[a] δ			$J_{^{13}C-^{13}C}$, Hz	
carbon no.	1	3	2	1	3
1	165.5	162.6	163.1	76.3	85.4
2	104.3	104.6	116.4	76.3	85.4
3	164.5	179.6	158.5	61.0	47.3
4	114.9	125.3	118.7	61.0	47.3
5	154.8	154.3	155.4	51.9	51.9
6	54.7	54.1	54.9	51.9	51.9
7	206.9	207.0	206.2	37.9	37.9
8	40.2	40.2	40.2	37.9	37.9
12, 16, 20, 24	13.9	13.9	13.9	33.6	33.6
$OCOCH_3$			167.0		

[a] Relative to internal Me_4Si.

structure of the 4-acetoxy-α-pyrone nucleus, from the absorption at 305 nm and the band due to an α-pyrone carbonyl at 1720 cm^{-1}. Therefore, it can be concluded that the structural change from an α-pyrone to a γ-pyrone occurs in the methylation with CH_2N_2 but not in the acetylation with acetic anhydride in pyridine.

This structural correlation between 1 and 3 was also supported by biosynthetic investigation using a ^{13}C-labeled precursor and Fourier transform (FT) ^{13}C NMR spectroscopy. *Streptomyces noboritoensis* KM-2753, a strain producing elasnin, was cultured in the following media: 2% glucose, 2% soybean meal, and 0.1% NaCl (pH 7.0). [1,2-^{13}C]Acetate (90% enriched in ^{13}C) was added to the media after 7 h of culture and the cultivation was continued at 27 °C for 48 h. ^{13}C-Labeled elasnin was isolated from the cultured broth. The ^{13}C-labeled elasnin was treated with CH_2N_2 to give the ^{13}C-labeled methyl ether 3. The ^{13}C NMR spectra of ^{13}C-labeled compounds 1 and 3 are shown in Figure 4 (B and C).

Both ^{13}C NMR spectra showed satellite resonances due to $^{13}C-^{13}C$ coupling. ^{13}C chemical shift and coupling constant ($J_{^{13}C-^{13}C}$) assignments of 1 and 3 are indicated in Table I. The coupling constants[14] of 76.3 Hz between C-1 at δ

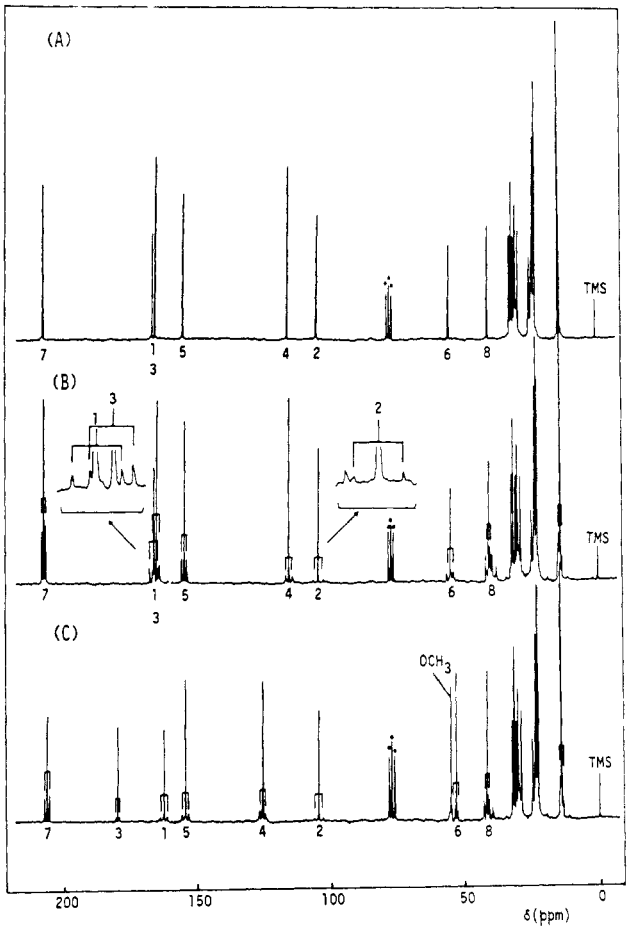


Figure 4. Proton noise-decoupled 25.2-MHz ^{13}C NMR spectra of elasnin (1) and its methyl ester (3); (A) natural abundance of 1, (B) 1 derived from [1,2-^{13}C]acetate, (C) 3 derived from [1,2-^{13}C]acetate.

165.6 and C-2 at δ 104.3 and 61.0 Hz between C-3 at δ 164.7 and C-4 at δ 115.0 in 1 altered to those of 85.4 Hz between C-1 at δ 162.6 and C-2 at δ 104.6 and 47.3 Hz between C-3 at δ 179.6 and C-4 at δ 125.0 in 3, respectively. The difference in the $^{13}C-^{13}C$ coupling constants and ^{13}C chemical shifts between 1 and 3 is highly consistent with the

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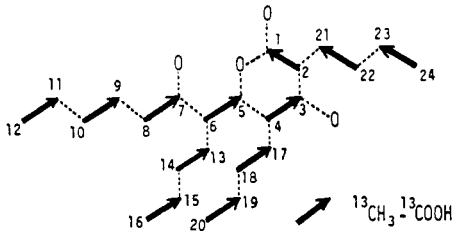
Scheme III. Plausible Biosynthetic Pattern of 1



structural change from an α-pyrone to a γ-pyrone, as suggested earlier by their UV and IR spectral data.

The incorporation pattern of [1,2-^{13}C]acetate into 1 satisfactorily explains that 1 is derived from 12 molecules of acetate through a hypothetical intermediate "polyketide" as depicted in Scheme III. Note that a large ^{13}C–^{13}C coupling constant, 85.4 Hz, was observed between C-1 and C-2 (sp^2–sp^2) of the γ-pyrone in **3**. This is the first report which clarifies the structural correlation of both tautomeric isomers through the detection of ^{13}C–^{13}C couplings in the ^{13}C NMR spectrum of a compound enriched with a ^{13}C-labeled precursor biosynthetically.

Experimental Section

General. Infrared (IR) spectra were recorded on a JASCO IRA-1 spectrophotometer. Samples were recorded in carbon tetrachloride or chloroform, using 0.10-mm sodium chloride cells. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-210 A spectrophotometer in 99.5% EtOH.

High-resolution mass spectra were recorded on a JMS-OISG mass spectrometer at 75 eV and 200 μA. Low-resolution mass spectra were performed on a JMS-D100 spectrometer at 20 eV and 300 μA. ^1H and ^{13}C NMR spectra were measured on JNM-PS-100 and JNM-PFT-100 spectrometers, respectively, with $CDCl_3$ as solvent. The following abbreviations are used to describe NMR spectral bands: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and δ (parts per million (ppm) downfield from tetramethylsilane).

Elasnin (1). Isolation from the fermentation broth of *Streptomyces noboritoensis* KM-2753 and many of the properties of this compound have been described previously.[1,2]

Monoacetylelasnin (2). Acetylation of 1 (300 mg) with Ac_2O (1 mL) and pyridine (0.4 mL) at room temperature for 24 h followed by the usual workup gave oily monoacetate **2**. Acetate **2** was purified by preparative TLC on silica gel GF_{254} (benzene–acetone, 40:1) to afford **2** (250 mg) as a colorless oil. The molecular formula, $C_{26}H_{42}O_5$, of **2** was obtained by high-resolution mass spectroscopy, m/e 434.300 (calcd for $C_{25}H_{42}O_5$, 434.303): mass spectrum, m/e 392 ($M^+ - COCH_3$), 336 ($M^+ - C_6H_{10}O$), 294, 251, 223, 178, 151, and 99; UV λ_{max} 306.5 nm (ϵ 7200); IR (CCl_4) 1775 (enolic ester CO), 1735 (ester and ketone CO), 1640 (double bond), 1565, 1455–1465 cm^{-1}; ^1H NMR δ 3.5 (t, J = 7.8 Hz, 1 H), 2.4 (t, 2 H); ^{13}C NMR δ 206.2 (ketone CO), 167.0 (ester CO), 163.1 (olefinic carbon), 158.5 (olefinic oxycarbon), 155.4, 118.7, 115.4, 54.9, 40.2, 28.6–31.8 (six methylenes), 20.4–25.2 (seven methylenes), 13.8 (four methyls).

Monomethylelasnin (3). Treatment of an ethereal solution of **1** (200 mg) with CH_2N_2 afforded oily monomethyl elasnin **3**, which was purified by preparative TLC on silica gel (benzene–acetone, 30:1): mass spectrum, m/e 406.304 (calcd for $C_{25}H_{42}O_4$, 406.304), 391 ($M^+ - CH_3$), 375 ($M^+ - OCH_3$), 363 ($M^+ - C_3H_7$), 307 ($M^+ - C_6H_{11}O$), 265, 237, 99; UV λ_{max} 252 nm (ϵ 7890); IR (CCl_4) 1735–1712 (ester and ketone CO), 1655 (γ-pyrone CO), 1625 and 1600 (double bond), 1460 cm^{-1}; ^1H NMR δ 3.8 (s, 3 H, OCH_3), 3.6 (t, J = 7.8 Hz, 1 H), 2.4 (t, 2 H); ^{13}C NMR δ 206.2 (ketone CO), 179.8 (γ-pyrone CO), 162.3, 154.5, 125.5, 104.7, 54.1, 53.7 (OCH_3), 41.7, 28.4–31.4 (five methylenes), 21.7–24.9 (seven methylenes), 13.9 (four methyls). Anal. Calcd for $C_{25}H_{42}O_4$: C, 73.85; H, 10.41; O, 15.74. Found: C, 73.77; H, 10.53; O, 15.70.

Dihydroelasnin (4). To a solution of 1 (1.00 g) in EtOH (10 mL) was added $NaBH_4$ (200 mg). The mixture was allowed to stir for 3 h at room temperature. A small amount of CH_3COOH was added to the reaction mixture to decompose any excess $NaBH_4$. The mixture was concentrated under reduced pressure. To the residue was added water (50 mL) and then ethyl acetate (50 mL). The ethyl acetate extract was dried over anhydrous Na_2SO_4 and then evaporated to dryness under reduced pressure to give an oily substance. The oil was chromatographed (Merck silica gel, 25 g, benzene–acetone, 20:1) to give **4** (750 mg) as a colorless oil: mass spectrum, m/e 394; UV λ_{max} 292 nm (ϵ 6400); IR (CCl_4) 3200 (OH), 1670, 1630, 1550, 1470 cm^{-1}; ^{13}C NMR δ 165.9, 164.7, 159.6, 114.6, 103.4, 72.9 (doublet in off-resonance experiment, oxy carbon), 46.3, 29.6–35.3 (six methylenes), 22.7–25.4 (seven methylenes), 13.9 (four methyls). Anal. Calcd for $C_{24}H_{42}O_4$: C, 73.04; H, 10.74; O, 16.22. Found: C, 72.95; H, 10.80; O 16.25.

2-Butyl-3-hydroxy-*n*-octanoic Acid (5), Methyl 2-Butyl-3-hydroxy-*n*-octanate (6) and Methyl 2-Butyl-3-acetoxy-*n*-octanate (7). A solution of **4** (1.20 g) in 90% CH_3OH containing H_2O (120 mL) was cooled to −10 °C (NaCl–ice water) and ozone was passed through the solution for 3 h. The solvent was then removed at 20 °C under vacuum. Acetic acid (24 mL) and 30% H_2O_2 (24 mL) were added to the residue and the mixture was kept at room temperature overnight. The CH_3COOH was then removed under vacuum below 40 °C, EtOAc (150 mL) was added, and the mixture was extracted with dilute NaOH to give an acidic material. Acidification with dilute HCl and EtOAc extraction, followed by washing with saturated NaCl solution, drying over $MgSO_4$, and evaporation, gave an acidic oil (180 mg). The oily substance was purified by preparative TLC on silica gel (diisopropyl ether–formic acid–water, 150:1:2; indicator, 0.1% bromocresol green in EtOH, R_f 0.30) to afford an acidic oil **5** (70 mg). The oil (50 mg) was then treated with CH_2N_2 in ethyl ether to afford the methyl ester **6** (51 mg) as a colorless oil. The mass spectrum of **6** indicates characteristic fragment peaks at m/e 213 ($M^+ - OH$), 199 ($M^+ - OCH_3$), 173 ($M^+ - C_4H_9$) and 159 ($M^+ - C_5H_{11}$): IR ($CHCl_3$) 3520 (OH), 1730 cm^{-1} (ester CO); ^1H NMR δ 3.70 (OCH_3), 2.43 (dt, 1 H), 2.38 (m, 1 H); ^{13}C NMR δ 176.1 (ester CO), 72.3 (oxy carbon), 51.4, 50.9 (OCH_3), 35.6, 31.7, 29.6, 29.3, 25.4, 22.6 (two methylenes), 13.9 (two methyls). Acetylation of **6** (30 mg) with acetic anhydride and pyridine for 24 h followed by the usual workup gave oily acetate **7**. The mass spectrum of **7** exhibits important fragment peaks at m/e 241 ($M^+ - OCH_3$), 229 ($M^+ - COCH_3$), 212 ($M^+ - CH_3COOH$), 130, and 87: IR (CCl_3) 1730 (ester CO), 1234 cm^{-1} (C–O–C); ^1H NMR δ 5.20 (m, 1 H, base proton of *O*-acetyl group), 3.77 (s, 3 H, $COOCH_3$), 2.70 (m, 1 H), 2.10 (s, 3 H, $OCOCH_3$).

Methyl 2-Carboxy-*n*-hexanoate (8). A solution of **3** (500 mg) in CH_3COOH (7 mL) was subjected to ozonolysis in a manner similar to that described above. The acidic oil was extracted with EtOAc from the reaction mixture and then purified by preparative TLC on silica gel (diisopropyl ether–formic acid–water, 150:2:3; indicator, 0.1% bromocresol green in EtOH, R_f 0.80) to give an oil, **8** (65 mg): ^1H NMR δ 6.70 (br s, 1 H, exchangeable with D_2O, COOH), 3.72 (s, 3 H, $COOCH_3$), 3.36 (t, J = 8.0 Hz, 1 H), 1.87 (q, 2 H, CH_2), 1.2–1.4 (m, 4 H, CH_2), 0.90 (t, J = 6.5 Hz, 3 H, CH_3).

α,α'-*n*-Pentyl-β,β'-*n*-butyl-γ-pyrone (9). A solution of 1 (300 mg) in CH_3COOH (5 mL) was refluxed with 2.5 N HCl (1 mL) for 3 h. The reaction mixture was poured into ice–water (70 mL). Extraction with EtOAc (50 mL), followed by washing with H_2O and then saturated $NaHCO_3$ solution and drying over anhydrous Na_2SO_4, gave a brown oil (270 mg). The oily substance was purified by preparative TLC on silica gel (developing solvent, benzene–acetone, 20:1) to give **9** (180 mg) as a colorless oil: mass spectrum, m/e 348.300 (M^+) (calcd for $C_{23}H_{40}O_2$, 348.302), 333 ($M^+ - CH_3$), 319 $C_{21}H_{35}O_2$ ($M^+ - C_2H_5$), 305 ($M^+ - C_3H_7$), 291 ($M^+ - C_4H_9$), 277 ($M^+ - C_5H_{11}$); UV λ_{max} 258 nm (ϵ 7800); IR (CCl_4) 1645 (γ-pyrone CO), 1610 cm^{-1} (double bond); ^{13}C NMR δ 179.0 (γ-pyrone CO), 163.6 (two olefinic oxy carbons), 123.3 (two olefinic carbons). Anal. Calcd for $C_{23}H_{40}O_2$: C, 79.31; H, 11.49; O, 9.20. Found: C, 79.27; H, 11.54; O, 9.19.

Isoxazole Derivative 10. A solution of 1 (500 mg) in CH_3COOH (5 mL) was refluxed with excess H_2NOH·HCl (100 mg) for 5 h. The reaction mixture was poured into ice–water and extracted with EtOAc (200 mL). The extract was washed with H_2O and then saturated $NaHCO_3$, dried over anhydrous Na_2SO_4, and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (benzene–ethanol, 50:1) to give an oil, **10** (320 mg): mass spectrum, m/e 378.322 (calcd for $C_{23}H_{42}N_2O_2$, 378.324), 361 ($M^+ - OH$), 322.262 (calcd

for $C_{19}H_{34}N_2O_2$, 322.262) ($M^+ - C_2H_4$), 264.229 (calcd for $C_{17}H_{30}NO$, 264.232), 194.152 (calcd for $C_{12}H_{20}NO$, 194.154); IR (CCl_4) 3250 (OH), 1620 cm^{-1} (double bond); ^{13}C NMR δ 165.7, 163.4, 160.6, 115.0.

Monoacetyl Isoxazole Derivative 11. Acetylation of **10** (100 mg) with acetic anhydride (0.8 mL) and pyridine (0.5 mL) at room temperature for 24 h gave an oily monoacetate, **11**: mass spectrum, m/e 420.333 (calcd for $C_{25}H_{44}N_2O_3$, 420.335); IR ($CHCl_3$) 1768 ($NCOCH_3$), 1620 cm^{-1} (double bond). Anal. Calcd for $C_{25}H_{44}N_2O_3$: C, 71.42; H, 10.48; N, 6.67; O, 11.43. Found: C, 71.50; H, 10.45; N, 6.63; O, 11.43.

Elasnin Ketal (12). A solution of **1** (400 mg) in benzene (7.2 mL) was refluxed with ethylene glycol (0.2 mL) and a trace amount of *p*-toluenesulfonic acid for 7 h. To the reaction mixture was added benzene (40 mL), and the mixture was washed with water and then 5% $NaHCO_3$ solution. The organic solvent layer was dried over anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was chromatographed over silica gel, using the solvent system benzene–acetone (60:1 to 15:1), to give ketal **12** (270 mg) as a colorless oil: mass spectrum, m/e 394 ($M^+ - C_3H_6$), 380 ($M^+ - C_4H_8$), 365 ($M^+ - C_5H_{11}$), 293 ($M^+ - C_8H_{15}O_2$), 223 ($M^+ - C_{13}H_{25}O_2$); UV (EtOH) λ_{max} 284 nm (ϵ 4000); IR (CCl_4) 1700 (α-pyrone CO), 1660 (γ-pyrone CO), 1630 cm^{-1} (double bond). Anal. Calcd for $C_{25}H_{44}O_5$: C, 71.56; H, 10.09; O, 18.35. Found: C, 72.05; H, 10.13; O, 17.82.

Methylelasnin Ketal (13). A solution of the ketal **12** (100 mg) in ethyl ether was treated with CH_2N_2 to give the methyl ether **13**: mass spectrum, m/e 450 ($C_{27}H_{46}O_5$), 408 ($M^+ - C_3H_6$), 379 ($M^+ - C_5H_{11}$), 265 ($M^+ - C_8H_{15}O_2 - C_3H_6$), ($CH_3$-$(CH_2)_3\overline{COCH_2CH_2O}$); UV λ_{max} 254.5 nm (ϵ 7450); IR (CCl_4) 1560 (α-pyrone CO), 1620 and 1600 cm^{-1} (enolic double bond).

Acetylelasnin Ketal (14). Ketal **13** (35 mg) was acetylated with acetic anhydride and pyridine at room temperature. The crude acetate was purified on silica gel preparative TLC (benzene–acetone, 25:1) to afford **14** (24 mg) as a colorless oil: mass spectrum, m/e 478 ($C_{28}H_{46}O_6$), 407 ($M^+ - 71$), 335 ($M^+ - C_8H_{15}O_2$), 293 (335 - C_3H_6), 292 (335 - $COCH_3$), 213 ($C_{13}H_{25}O_2$); UV λ_{max} 305 nm (ϵ 7400); IR (CCl_4) 1775 (enolic acetyl CO), 1720 (α-pyrone CO), 1190 cm^{-1} (C-O-C).

Incorporation of [1,2-^{13}C]Acetate. An elasnin-producing strain, *Streptomyces noboritoensis* KM-2753, was inoculated into a medium containing 2% dextrin, 0.2% glucose, 1.5% soybean meal, 0.3% yeast extract, and 0.3% $CaCO_3$ at pH 7.0 and cultivated at 27 °C. A 48-h culture was transferred into a producing medium containing 2% glucose, 2% soybean meal, and 0.1% NaCl in a reciprocal flask, and the initial pH was adjusted to 7.0. After cultivation for 7 h at 27 °C, [1,2-^{13}C]CH_3COONa as the ^{13}C-labeled precursor was added at a concentration of 0.1% to the culture, and the fermentation was continued for an additional 48 h. The culture filtrate (500 mL) was extracted with EtOAc (200 mL) and the extract was concentrated to dryness, affording a brownish oil (180 mg). The crude oil was chromatographed over a silica gel thin-layer plate, using benzene–ethanol (10:1) as the developing solvent, to isolate pure ^{13}C-labeled elasnin (70 mg). The methyl ether was obtained by treatment of ^{13}C-labeled elasnin with CH_2N_2.

Registry No. 1, 68112-21-0; 2, 73891-54-0; 3, 73891-55-1; 4, 73907-88-7; 5, 73891-56-2; 6, 73891-57-3; 7, 73891-58-4; 8, 73891-59-5; 9, 1168-16-7; 10, 73891-60-8; 11, 73891-61-9; 12, 73891-62-0; 13, 73891-63-1; 14, 73907-89-8.

Nucleosides. 115. Reaction of 3′-*O*-Mesylthymidine. Formation of 1-(3-Azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)thymine and Its Conversion into 6,3′-Imino-1-(2,3-dideoxy-β-D-*threo*-pentofuranosyl)thymine[1]

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Treatment of 5′-*O*-trityl-3′-*O*-mesylthymidine (**3**) with NaN_3 in DMF at reflux gave 5′-*O*-trityl-3′-azido-3′-deoxythymidine (**5**) and 6,3′-imino-1-(5-*O*-trityl-3-deoxy-β-D-*threo*-pentofuranosyl)thymine (**8**). Compound **8** was formed from the 3′-azido-*threo*-pentofuranosylthymine derivative **6**. A mechanism is proposed for the formation of **8** from **6**. Also, the 6,5′-imino-bridged thymidine **18** was prepared. Conformational features of these imino-bridged nucleosides are discussed.

3′-Amino-3′-deoxythymidine (**1**, Scheme I), synthesized in our laboratory in 1964,[2] was recently found to exhibit interesting biological activity against murine Sarcoma 180[3,4] and L1210[4,5] cells. For further biological evaluations, large amounts of **1** were required. **1** was prepared[2] originally by treatment of 5′-*O*-trityl-3′-*O*-mesylthymidine (**3**) with phthalimide followed by deprotection. The reaction was shown to proceed via the anhydronucleoside intermediate (**2**).[2] Subsequently, the synthesis of **1** was reported by reaction of NaN_3 with either **2**[6] or 5′-*O*-trityl-3′-*O*-mesyl-2′-deoxy-β-D-*threo*-pentofuranosylthymine (**4**).[4,7]

In an attempt to find an easier route to **1**, compound **3** was treated with NaN_3 in DMF at reflux temperature for 24 h. Two nucleosidic products were isolated from the reaction mixture. One of the products was identical with the known[6] 3′-azido-3′-deoxy-5′-*O*-tritylthymidine (**5**) from which 3′-amino-3′-deoxythymidine (**1**) was prepared. The formation of the "down" azido derivative **5** from **3** ap-

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