Pergamon

Tetrahedron Letters, Vol. 35, No. 28, pp. 5009-5012, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)01012-9

Biosynthesis of Lactacystin. Origin of the Carbons and Stereospecific NMR Assignment of the Two Diastereotopic Methyl Groups

Akira Nakagawa,* Senji Takahashi and Kenichi Uchida

Department of Biosciences, Teikyo University, 1-1 Toyosatodai, Utsunomiya 320, Japan.

Keiichi Matsuzaki and Satoshi Ōmura

School of Pharmaceutical Sciences, Kitasato University and The Kitasato Institute,

5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan.

Asao Nakamura, Noboru Kurihara and Tsuyoshi Nakamatsu

Central Research Laboratories, Ajinomoto Co. Inc., 1-1 Suzuki-cho, Kawasaki 210, Japan.

Yoko Miyake, Kanako Take and Masatsune Kainosho

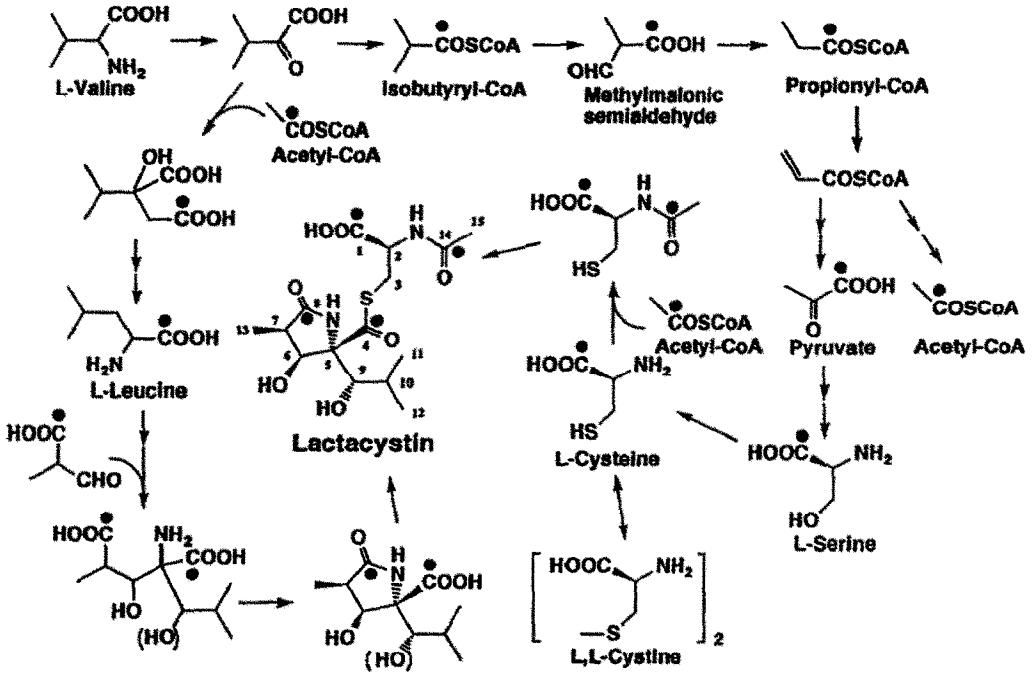
Faculty of Science, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji, Tokyo 192, Japan.

Abstract. Biosynthetic pathway of lactacystin and its stereochemical aspect were investigated by feeding experiments of ^{13}C enriched compounds.

Lactacystin [1], a novel compound which induces differentiation of Neuro 2a cells, a mouse neuroblastoma cell line, has been isolated from the cultured broth of *Streptomyces* sp. OM-6519. A unique γ-lactam structure for lactacystin has been determined by NMR spectroscopy and X-ray crystallographic analysis [2]. The γ-lactam skeleton of lactacystin containing hydroxyisobutyl and cysteinylthioester moieties led us to study its biosynthesis. In this communication we describe the biosynthetic origin and stereochemical assignment of methyl groups, C-11 and C-12 of lactacystin by feeding experiments with ^{13}C enriched compounds.

^{13}C Labeled precursors, L-[2-^{13}C] leucine (90% ^{13}C, 0.05% w/v), sodium [1-^{13}C] isobutyrate (90% ^{13}C, 0.04% w/v), sodium [1-^{13}C] propionate (99% ^{13}C, 0.04% w/v), L,L-[1,1'-$^{13}C_2$] cystine (99% ^{13}C, 0.03% w/v), ^{13}C-labeled L-valine (preparation see below, average 33% ^{13}C per carbon, 0.03% w/v), ^{13}C-labeled L-leucine (preparation see below, average 33% ^{13}C per carbon, 0.06% w/v), DL-[2-^{13}C,4-^{2}H] leucine (99% ^{13}C, 80% ^{2}H, 0.07% w/v) were fed to 36 hrours old cultures of *Streptomyces* sp. OM- 6519 grown in 10 ml of oatmeal medium in test tubes (27 °C, 232 rpm, shaking), and fermentations were harvested 96 hours later. Each ^{13}C enriched lactacystin (2 - 5 mg) was isolated as a white powder from each broth filtrate (total volume ; 500 - 900 ml). The ^{13}C NMR spectra were acquired in C_5D_5N at 60 °C.

The feeding of L-[2 -^{13}C] leucine gave lactacystin which showed a very intense signal (13.2 times the relative ^{13}C abundance of C-3) for C-5, indicating that the C_6-segment (C-4, C-5, C-9, C-10, C-11, and C-12) is derived from L-leucine. The feeding experiment with sodium [1-^{13}C] isobutyrate revealed equal levels of enrichment (each 2.0 - 3.1 times the relative ^{13}C abundance of C-3) for C-1, C-4, C-8, and C-14. The incorporation at C-8, especially, provided unequivocal evidence that the γ-lactam ring is formed by



Scheme 1. Incorporation pattern of [1-^{13}C] isobutyrate to lactacystin.

condensation of the C_6 unit arising from L-leucine and a Schiff base of methylmalonic semialdehyde in the presence of pyridoxal phosphate cofactor, followed by intramolecular cyclization, as shown in Scheme 1. The additional incorporation of [1-^{13}C] isobutyrate at C-1, C-4 and C-14 also indicates the presence of metabolic pathways from isobutyrate *via* propionyl-CoA to acetyl-CoA and to cysteine. Enrichment at C-4 implies that the β-hydroxyleucine moiety was formed by condensation of 2-ketoisovalerate from valine with acetyl-CoA derived from [1-^{13}C] isobutyrate, followed by hydroxylation. This notion was further supported by some level of enrichment at the C-1, C-4 and C-14 positions observed in the feeding experiment with sodium [1-^{13}C] propionate. Feeding of L,L-[1,1'-$^{13}C_2$] cystine [3] resulted in a very high enrichment at C-1, indicating that the C_3 unit (C-1, C-2 and C-3) is derived from L-cysteine, itself formed by an enzymatic reduction of the labeled cystine. Thus, the ^{13}C distribution indicates that lactacystin is biosynthesized from the following three units, L-leucine, isobutyrate (and / or L-valine) and L-cysteine, respectively.

It has been reported that two nonequivalent methyl groups of L-valine and L-leucine give rise to separate signals in the ^{13}C NMR spectra. This stereospecific correlation of the two heterotopic methyl groups of L-valine has been applied to the biosynthesis of β-lactam antibiotics [4-6] by feeding experiment with valine carrying a stereospecific ^{13}C label in one of methyl groups. On the other hand, Gould *et al.* [7] and other groups have reported the usefulness of feedings of [U-$^{13}C_6$] glucose followed by analysis of ^{13}C-^{13}C coupling patterns in biosynthetic studies of microbial secondary metabolites. For confirmation of the

biosynthetic origin of the above C_6-segment and stereospecific NMR assignment of the two diastereotopic methyl groups, C-11 and C-12 of lactacystin, we carried out a feeding experiment with ^{13}C-labeled L-leucine which was obtained by fermentation of a leucine-producing microorganism, *Brevibacterium lactofermentum* AJ 3918, on a mixture (1 : 2) of 98% [U-$^{13}C_6$] glucose and glucose with no isotope label [8] as a carbon sourse. The isopropyl group in ^{13}C-labeled L-leucine is stereospecifically build up of a two-carbon fragment from one pyruvate unit, while the second methyl group is migrated from another pyruvate unit during the metabolism from glucose. The ^{13}C NMR spectrum of lactacystin obtained by feeding experiment of ^{13}C-labeled L-leucine exhibited satellite peaks, Jcc = 34.3 Hz, based on intacts ^{13}C-^{13}C coupling between C-10 and C-11 and Jcc = 51.1 Hz between C-4 and C-5 and singlet peaks for C-9 and C-12, as shown in Fig. 1. This corresponds to the spectral pattern of the precursor, leucine ; it clearly demonstrates that L-leucine is an intact precursor of the C_6-segment and no racemization at C-10 has occurred in the formation of this segment from leucine. The feeding experiment of DL-[2-^{13}C,4-^{2}H] leucine which was synthesized by condensation of [2-^{2}H]1-bromo-2-methylpropane with [2-^{13}C] ethyl N-(diphenyl methylene) glycinate, carried out to determine if C-10 has undergone retention or inversion of configuration. Appearance of two singlet signals

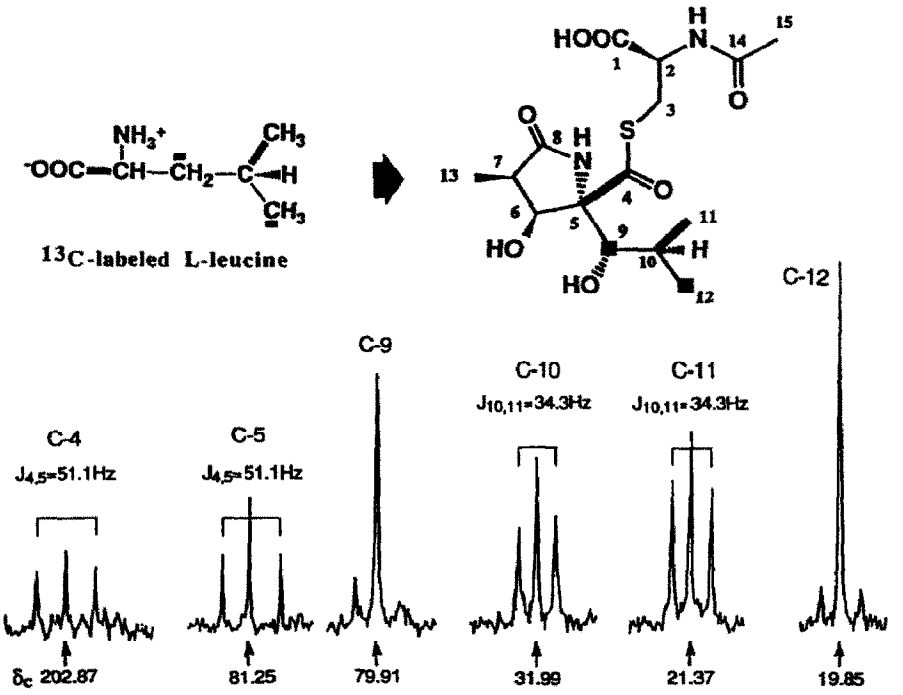


Fig.1 ^{13}C NMR Spectral pattern of lactacystin enriched with ^{13}C-labeled L-leucine.

for the diastereotopic methyl groups, C-11 and C-12 (δ 0.86 and 0.71, respectively, in DMSO-d_6) based on incorporation of the deuterium atom to C-10 in the ^{1}H-NMR spectrum of the labeled lactacystin, accompanied by a high ^{13}C enrichement at C-5 indicated retention of configuration at C-10. Therefore, diastereotopic methyl groups, C-11 and C-12 are assignable as *pro*-R and *pro*-S, respectively. The unequivocal outcome of this feeding experiment with ^{13}C-labeled L-leucine sheds light on the stereospecific formation of lactacystin. Such experiments in general are valuable means to contribute to the understanding of the biosynthesis of secondary metabolites containing valine and leucine moieties.

Acknowledgement. We wish to thank Prof. Heinz G. Floss, Department of Chemistry, University of Washington, for stimulating discussions. This research was supported in part by a Grant-in-Aid for Scientific Reseach from the Ministry of Education, Science and Culture of Japan, the Special Coordinate Founds of the Science and Technology Agency to MK and The Fujisawa Foundation.

References

1. Ōmura, S.; Fujimoto, T.; Otoguro, K.; Matuszaki, K.; Moriguchi, R.; Tanaka, H.; Sasaki, Y. *J. Antibiot.* **1991**, 44, 113-116.
2. Ōmura, S.; Matuszaki, K.; Fujimoto, T.; Kosuge, K.; Furuya, T.; Fujita, S.; Nakagawa, A. *J. Antibiot.* **1991**, 44, 117-118.
3. Uchida, K.; Kainosho, M. *J. Labelled Comp. Radiopharm.* **1991**, 29, 867-874.
4. Baldwin, J. E.; Löliger, J.; Rastetter, W.; Neuss, N.; Huckstep, L. L.; De La Higuera, N. *J. Am. Chem. Soc.* **1973**, 95, 3796-3797.
5. Neuss, N.; Nash, C. H.; Baldwin, J. E.; Lemke, P. A.; Grutzner, J. B. *J. Am. Chem. Soc.* **1973**, 95, 3797-3798.
6. Kluender, H.; Bradley, C. H.; Sih, C. J.; Fawcett, P.; Abraham, E. P. *J. Am. Chem. Soc.* **1973**, 95, 6149-6150.
7. Gould, S. J.; Cane, D. E. *J. Am. Chem. Soc.* **1982**, 104, 343-346.
8. Kainosho, M. et al., to be published.

(*Received in Japan* 16 *March* 1994; *accepted* 13 *May* 1994)