STRUCTURE OF LACTACYSTIN, A NEW MICROBIAL METABOLITE WHICH INDUCES DIFFERENTIATION OF NEUROBLASTOMA CELLS

Sir:

During the course of screening for microbial metabolites which induce differentiation of Neuro 2A cells, a mouse neuroblastoma cell line, a novel compound designated lactacystin was isolated from the cultured broth of *Streptomyces* sp. OM-6519.[1] Lactacystin causes a transient increase of the intracellular cAMP level in Neuro 2A cells and morphological changes including neuritogenesis. In this paper we wish to report the structure elucidation of lactacystin by NMR spectroscopic and X-ray crystallographic analyses.

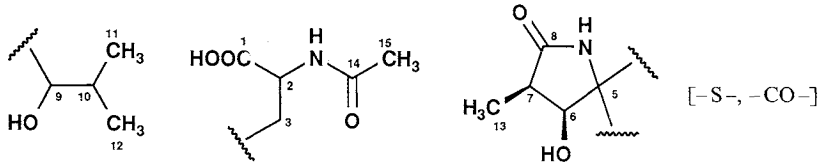
The molecular formula of lactacystin (mp 237~238°C (dec), $[\alpha]_D^{25}$ +71.3° (*c* 0.5, methanol)) was determined to be $C_{15}H_{24}N_2O_7S$ by elemental analysis and HRFAB-MS (*m/z* 377.13 $(M+H)^+$). Detailed ^1H and ^{13}C NMR studies of lactacystin revealed the presence of three structural moieties [A], [B], and [C] (Fig. 1). The cross peaks (11-H (δ 1.14)–12-H (δ 1.07)–10-H (δ 2.14)–9-H (δ 4.48)) observed in the ^1H-^1H COSY spectrum (400 MHz, pyridine-d_5) of lactacystin indicated the presence of a hydroxyisobutyl group, [A]. The existence of an *N*-acetylalanyl moiety, [B], was evidenced from the following spectral data; (i) the isotope shift observed in the signals of a methine (C-2, δ 53.1) and an amide carbon (C-14, δ 170.5) by the exchange of an amide proton (δ 8.71) with a deuterium atom, (ii) the appearance of cross peaks (3-H (δ 3.73 and 3.94)–2-H (δ 5.30)), and (iii) the observation of a NOE between the amide proton and *N*-acetyl proton (δ 1.94). The presence of a γ-lactam moiety, [C] in lactacystin was confirmed from clear cross peaks of an amide proton (δ 9.78) with a quarternary carbon (C-5, δ 81.5), an oxymethine (C-6, δ 76.2), and a methine (C-7, δ 42.0) in the heteronuclear multiple-bond connectivity spectrum. The γ-lactam structure was also supported from an isotope shift observed in the signals of an amide carbon (C-8, δ 181.5) and a quarternary carbon (C-5) in the ^{13}C NMR spectrum after addition of D_2O.

The connectivity of C-9 in partial structure [A] to C-5 in [C] was confirmed from the observation of a clear NOE between 6-H (δ 5.23) and 9-H (δ 4.48) and ^{13}C-^1H long range couplings of 6-H with C-9 (δ 80.1) and 10-H (δ 2.14) with C-5. It was considered that the remaining functions, a carbonyl group (δ 203.1) and a sulfur atom, were part of a thioester group whose location was proved by determining the structure of a degradation product ($C_7H_{13}O_3NS$) obtained by the treatment of lactacystin with diazomethane. The structure of the product was easily assigned as *N*-acetyl-*S*-methyl-L-cysteine methyl ester from the NMR data ($-CH_3 \times 3$; δ 2.06 (s), 2.11 (s), 3.78 (s), CH_2; δ_H 2.98 (dd), (CH); δ_H 4.84 (t)) and by comparing the CD spectrum with that of L-cysteine. These results show that the moieties [B] and [C] must be connected through a thioester group. This connectivity was

Table 1. ^1H and ^{13}C chemical shift assignments of lactacystin (δ ppm, in pyridine-d_5).

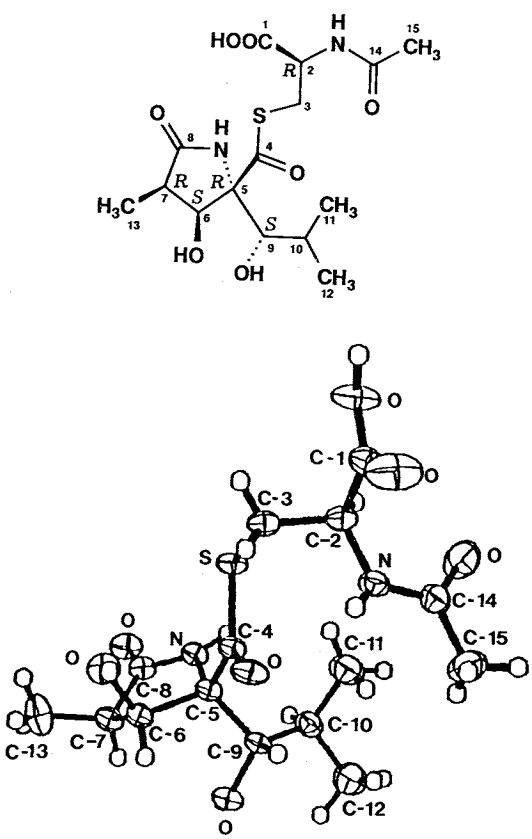
Carbon No.	δ_H	δ_C
1		173.8
2	5.30 m	53.1
3	3.94 dd, 3.73 dd	31.5
4		203.1
5		81.5
6	5.23 d	76.2
7	3.36 m	42.0
8		181.5
9	4.48 d	80.1
10	2.14 ddd	32.2
11	1.14 d	21.6
12	1.07 d	20.1
13	1.50 d	10.4
14		170.5
15	1.94 s	23.2
NH	9.78 s	
NH	8.71 d	

Fig. 1. Partial structures of lactacystin.



[A] [B] [C]

Fig. 2. Structure and absolute configuration of lactacystin.



also supported from the cross peaks between methylene protons (3-H, (δ 3.73 and 3.94)) and an ester carbonyl carbon through a sulfur atom. Thus, the novel structure for lactacystin, containing γ-lactam, cysteinyl, and hydroxyisobutyl groups, was established. Table 1 shows ^{1}H and ^{13}C chemical shift values for lactacystin.

For confirmation of the proposed structure of lactacystin and determination of the absolute configuration, an X-ray crystallographic analysis was performed. Diffraction data were collected with graphite-monochromated CuKα radiation (λ= 1.54184 Å). The crystal data are as follows: chemical formula $C_{15}H_{24}N_2O_7S \cdot H_2O$, orthorhombic space group $P2_12_12_1$, a=10.680 (1), b=31.930 (1), c=5,627 (1) A, z=4, D_{calc}=1.365 gcm^{-3}. Of 1,819 independent reflections with $2\theta < 125°$, 1,675 were used for structure determination. The structure was solved by direct methods using program SHELX 86[2] and the structural parameters were refined by block-diagonal least-squares methods. The final R factor, based on the 1,675 reflections, was 0.049. The absolute configuration was determined from measurements of Bijvoet pairs exhibiting the greatest effect of anomalous scattering due to the sulfur atom with Cu radiation. The ratios of $|F_{calc}(hkl)|/|F_{calc}(h\bar{k}\bar{l})|$ for an enantiomer were in agreement with the observed values.† Consequently, the absolute configuration of lactacystin was determined to be 2*R*,5*R*,6*S*,7*R*,9*S*, as shown in Fig. 2. A detailed description of the structure elucidation will be described elsewhere.

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References

1) ŌMURA, S.; T. FUJIMOTO, K. OTOGURO, K. MATSUZAKI, R. MORIGUCHI, H. TANAKA & Y. SASAKI: Lactacystin, a novel microbial metabolite, induces neuritogenesis of neuroblastoma cells. J. Antibiotics 44: 113~116, 1991

2) SHELDRICK, G. M.: SHELX 86. *In* Crystallographic computing 3. *Ed.*, G. M. SHELDRICK *et al.*, pp. 175~189, Oxford Univ. Press, 1985

† The ratios $|F_{obs}(hkl)|/|F_{obs}(h\bar{k}\bar{l})|$ for 9 selected observed reflections (1,2,2), (4,8,1), (3,11,1), (1,6,1), (2,1,2), (1,15,3), (3,1,3), (3,7,2), (5,2,2): 0.91, 1.08, 1.09, 0.95, 0.95, 0.96, 1.05, 1.06, 1.06, correspond to ratios $|F_c(hkl)|/|F_c(h\bar{k}\bar{l})|$ for the chosen enantiomer: 0.92, 1.08, 0.94, 0.93, 0.94, 1.05, 1.05, 1.05.

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