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Structure of a New Antimicrobial Unsaturated Fatty Acid from *Sm. kitasatoensis* NU-23-1

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Antimicrobial compound LA-1 (I) has been isolated from the culture broth of the leucomycin (LM) producing strain, *Sm. kitasatoensis* NU-23-1 and its structure determined. It has been found that the new compound I is transiently accumulated preceding the production of LM during fermentation process. I is extracted with organic solvents under acidic conditions from the culture filtrate of its highly accumulative mutant NU-4-4-2 after 27—30 hours incubation.

I is exceedingly unstable in its solid state. Its ultraviolet (UV) spectrum (UV λ_{max}^{MeOH} nm: 270) and infrared (IR) spectrum (IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3000-, 1925, 1700-, 1630—1610, 985) have suggested that I is an oxo-unsaturated fatty acid having a conjugated allene segment. Its proton magnetic resonance (PMR) and ^{13}Carbon-nuclear magnetic resonance (CMR) spectra and the spectroscopic data (IR, PMR, CMR, and mass spectrum) of its stable derivatives, octahydro LA-1 (II) and octahydro methyl LA-1 (III), have been fully compatible with the molecular formula, $C_{10}H_{10}O_3$ and with the molecular weight, 178. Its structure has been elucidated on the basis of these spectroscopic properties.

It has previously been found that *Streptomyces kitasatoensis* NU-23-1 produces leucomycin (LM),[2] one of the sixteen-membered macrolide antibiotics.[3] We now wish to report on the isolation of unsaturated fatty acid I from the variable strain NU-4-4-2 which was obtained by the nitrosoguanidine and ultraviolet (UV) treatment of the LM producing strain. This fat soluble compound possessing antimicrobial activity was transiently accumulated preceding the production of LM and disappeared rapidly with increasing amount of LM accumulation as fermentation proceeded.

LA-1 (I) was produced by cultivating the strain NU-4-4-2 on Waksman medium in a jar fermentor at 27° for 27—30 hours. The cultured broth was filtrated and the filtrate extracted first with chloroform under acidic conditions. It was then transferred into aqueous sodium bicarbonate, extracted again with chloroform under acidic conditions and concentrated. The concentrated solution was subsequently purified by silica gel column chromatography using a mixture of benzene and ethyl acetate as an eluent to provide the single component fractions identified as I.

I, isolated according to the procedure described earlier, was exceedingly labile under ordinary work up conditions as exemplified by the observation that sample of I decomposed rapidly in air into a dark brown resinous material.

Nevertheless, I was moderately stable in organic solvents, such as ethyl acetate and chloroform. We were therefore forced to manipulate I in solution throughout work up and to utilize its stable derivatives for structural elucidation.

The UV spectrum of I showed a maximum at 270 nm and its specific rotation in chloroform was $[\alpha]_D^{20}$ nm: +50° (589).

1) Location: *5-9-1 Shirokane, Minato-ku, Tokyo 108, Japan.*
2) S. Ōmura, M. Katagiri, and T. Hata, *J. Antibiotics*, **21**, 272 (1968); T. Hata, Y. Sano, H. Ohki, Y. Yokoyama, A. Matsumae, and S. Ito, *J. Antibiotics*, Ser. A, **6**, 87 (1953).
3) S. Omura, and A. Nakagawa, *J. Antibiotics*, **28**, 401 (1975); S. Ōmura, *Kagaku to Seibutsu*, **8**, 139 (1970).

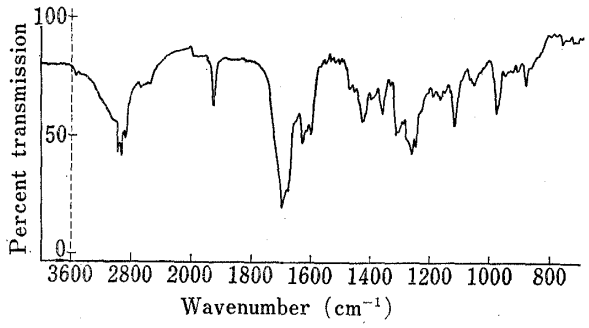

Fig. 1. IR Spectrum of I (CCl_4)

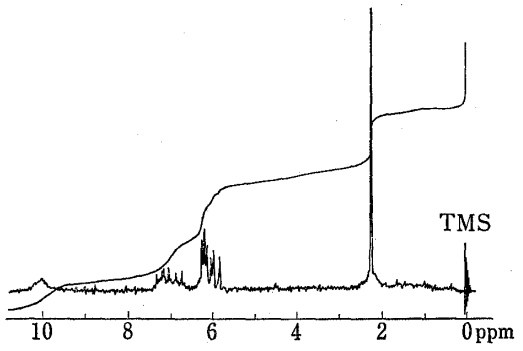

Fig. 2. PMR Spectrum of I (CCl_4, 100 MHz)

As shown in Fig. 1, its infrared (IR) spectrum suggested the presence of several distinct functional groups, such as a carboxyl group (3000 cm^{-1}), alkane group (2900—cm^{-1}), a conjugated allene segment (1925 cm^{-1}), an α,β-unsaturated carboxyl acid group (C=O, 1715—1680 cm^{-1}), an aliphatic α,β-unsaturated ketone (1690—1670 cm^{-1}), olefins (1630—1610 cm^{-1}) and *trans*-olefins (985 cm^{-1}).

Fig. 2 shows the 100 MHz proton magnetic resonance (PMR) spectrum of I taken in carbon tetrachloride. The total absorption of the spectrum integrated to ten protons; a methyl group adjacent to a carbonyl function at δ 2.20, two absorptions each corresponding to one proton at δ 5.92, and δ 6.12, two protons at δ 6.22, two absorptions each corresponding to one proton at δ 6.88 and δ 7.20 and one low-field proton at δ 10- due to the hydroxylic proton of the carboxyl group.

The ^{13}Carbon-nuclear magnetic resonance (CMR) spectrum of I in chloroform, as shown in Fig. 3, indicated a total of ten magnetically distinguishable carbons; the methyl group adjacent to the ketonic carbonyl group at δ 27.1, six olefin carbons at δ 94.4, 95.0, 121.6, 131.3, 137.7 and 140.2, the carboxyl carbon at δ 171.1, the ketone carbonyl carbon at δ 197.7 and the allene carbon at δ 218.6. On the basis of these spectroscopic data, it was suggested that I is an oxo-unsaturated fatty acid which consists of ten carbons and ten hydrogens containing a conjugated allene segment.

Since I itself was not particularly suitable for a number of instrumental analysis because of its unusual lability, it was derived into more stable compounds, that is, the hydrogenated derivative and its methyl ester. The concentrated solution of I was catalytically hydrogenated in methanol in the presence of platinum oxide and the product purified by silica gel column chromatography to afford octahydro LA-1 (II); its specific rotation in chloroform was $[\alpha]_D^{23}$ nm:

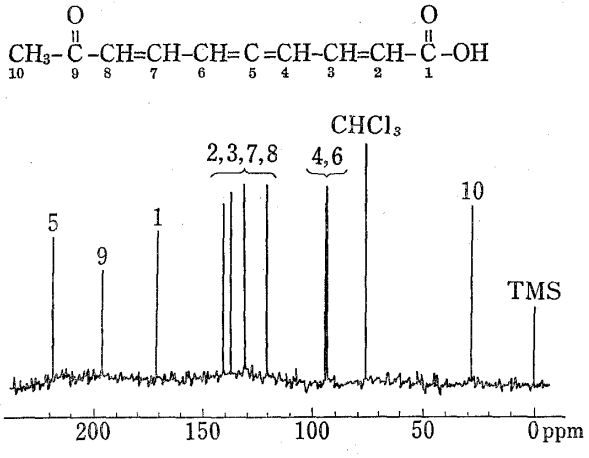

Fig. 3. CMR Spectrum of I ($CHCl_3$, 25.15 MHz)

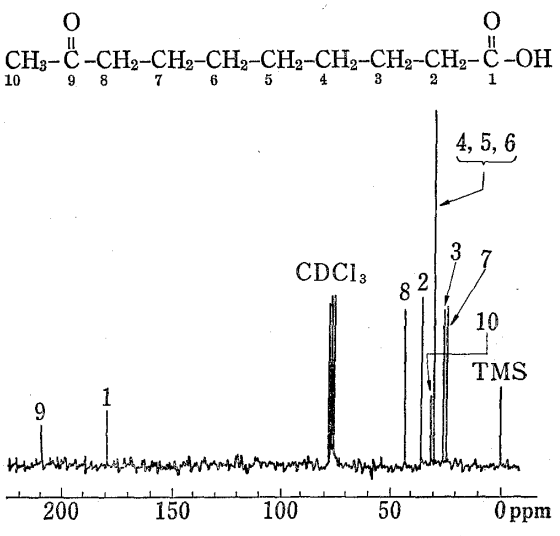

Fig. 4. CMR Spectrum of II ($CDCl_3$, 25.15 MHz)

$-24°$ (589), mp 42—45°. The disappearance of the absorption at 270 nm which had been seen in I was apparent in the UV spectrum of II. The IR spectrum of II also showed no absorptions due to the conjugated allene (1925 cm^{-1}) and the olefins (1630—1610 cm^{-1}, 985 cm^{-1}) that were present in I, whereas new absorptions emerged at 2900—, 1400—, and 1300—1100 cm^{-1} presumably due to methylene protons. The two α,β-conjugated carbonyl absorptions of I at 1715—1680 cm^{-1} and 1690—1670 cm^{-1} shifted forward 1730 and 1710 cm^{-1}, respectively. The PMR spectrum of II exhibited absorptions at δ 1.2—1.8 (10 H) due to methylene protons, δ 2.08 of a methyl group, δ 2.30 and δ 2.36 due to the methylene protons adjacent to the ketonic carbonyl and the carboxyl groups and δ 10.8 of the hydroxylic proton of the carboxyl group.

As indicated in Fig. 4, the CMR spectrum of II showed absorptions due to the two methylene carbons at δ 24.0, δ 24.8 and an overlapping absorption corresponding to three methylene carbons at δ 29.2. In addition to these, resonance peaks due to the remaining five carbons were observed the methyl group adjacent to the ketonic carbonyl group at δ 30.2, methylene carbon adjacent to the carboxyl group at δ 34.0, to ketonic carbonyl group at δ 43.9, the carboxyl carbon at δ 179.6 and ketone carbonyl carbon at δ 209.4. All of these CMR peaks were fully consistent with the assigned structure of an oxo-fatty acid. The assignment of these ^{13}C peaks were achieved by comparing with the CMR spectral data of model compounds[4] and by the use of off-resonance decoupling.

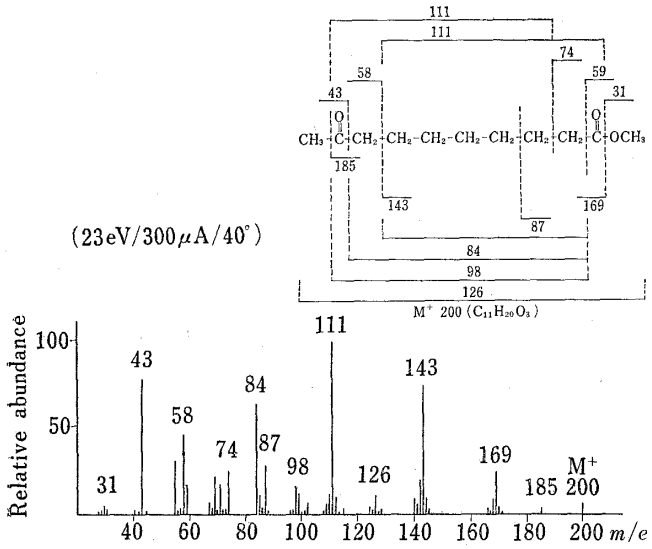

Fig. 5. Mass Spectrum of III

A(I): $CH_3-\overset{O}{\overset{\|}{C}}-CH=CH-CH=C=CH-CH=CH-\overset{O}{\overset{\|}{C}}-OH$ (10 9 8 7 6 5 4 3 2 1)

B: $CH_3-\overset{O}{\overset{\|}{C}}-CH=CH-CH=CH-CH=C=CH-\overset{O}{\overset{\|}{C}}-OH$ (4 3 2)

C: $CH_3-\overset{O}{\overset{\|}{C}}-CH=C=CH-CH=CH-CH=CH-\overset{O}{\overset{\|}{C}}-OH$ (8 7 6)

Chart 1

In order to obtain further supporting evidence for the proposed structure, II was methylated with diazomethane in ether. Purification of octahydro methyl LA-1 (III) thus obtained was carried out by silica gel column chromatography. The high-resolution mass spectrum of III exhibited the fragmentation pattern as depicted in Fig. 5. The parent peak M^+ of III appeared at *m/e*: 200.1412 in agreement with the calculated value of 200.1411 for $C_{11}H_{20}O_3$. Other eminent peaks showed up at *m/e*: 185 (M^+-CH_3), 169 (M^+-CH_3O), 157 ($M^+-C_2H_3O$), 143 ($M^+-C_3H_5O$), 126 (M^+-CH_3, $C_2H_3O_2$), 111 (M^+-CH_3, $C_3H_5O_2$ or CH_3O, C_3H_5O) base peak, 98 ($M^+-C_2H_3O_2$, C_2H_3O), 87 ($M^+-C_7H_{13}O$), 84 ($M^+-C_3H_5O$, $C_2H_3O_2$), 74 ($M^+-C_8H_{14}O$), 58 ($M^+-C_8H_{14}O_2$), 43 ($M^+-C_9H_{17}O_2$) and 31 ($M^+-C_{10}H_{17}O_2$). All of these mass spectral peaks rationalized the molecular formula, $C_{11}H_{20}O_3$. Accordingly, the molecular formula of I and II were found to be $C_{10}H_{10}O_3$ (mol. wt., 178) and $C_{10}H_{18}O_3$ (mol. wt., 186), respectively. From the spectroscopic data of I, II and III, three structures, A, B, and C illustrated in Chart 1,

4) L.F. Johnson and W.C. Jankowski, "Carbon-13 NMR Spectra," A Wiley-Interscience Publication, Canada, 1972.

are suggested as most likely positional isomers for I. However, the similarity of the ^{13}C chemical shifts due to the two allenic carbons[5] (δ 94.4 and δ 95.0) adjacent to the central allenic carbon (δ 218.6) precluded both possibilities B and C, in each of which the chemical shift of one of the end allenic carbons should differ from that of the other carbons because of its proximity to one of the carbonyl groups. On the other hand, the two allenic carbons at 4- and 6-positions are located in closely similar magnetic environment, suggesting that their chemical shifts should be approximately identical. Thus it was concluded that structure A would be the most suitable for I.

I showed barely detectable antimicrobial activity against Gram positive bacteria. Although there are several examples of naturally occurring antibiotics containing allene segment, such as Mycomycin,[6] Nemotin[7] and Marasin,[8] it would be particularly intriguing to note that such an unsaturated fatty acid was isolated from the macrolide producing strain.

Experimental

Melting point was taken on the Mitamura Riken melting point apparatus. The specific rotation was determined on a JASCO DIP-SL spectrometer. IR spectra were taken on a JASCO DS-403G spectrometer. 100 MHz PMR spectra were recorded on a JEOL PS-100 or a JEOL JNM-MH-100 nuclear magnetic resonance (NMR) spectrometer. All proton chemical shifts were reported in ppm relative to internal tetramethylsilane at δ: 0.00 ppm. CMR spectra were obtained on a JEOL PFT-100 spectrometer equipped with a NMR Fourier transformer. All carbon chemical shifts were also reported in ppm relative to internal tetramethylsilane at δ: 0.00 ppm. UV spectra were determined on a Hitachi EPS-032 spectrophotometer and mass spectrum was obtained on a JEOL JMS-D100 spectrometer.

Fermentation——Production of I was preformed on Waksman medium containing glucose 2.0%, peptone 0.5%, yeast extracts 0.3%, meat extracts 0.5%, NaCl 0.5% and $CaCO_3$ 0.3%. The pH was adjusted to 7. 100 ml of the medium was a 500 ml Sakaguchi flask and sterilized. *Sm. kitasatoensis* NU-4-4-2 was seeded to this medium which was then incubated for 48 hours at 27°. In jar fermentation, the culture filtrate for the extraction I was incubated with 2% (v/v) of the seed for 27—30 hours at 27°, whereas in tank fermentation, it was incubated for 27—30 hours at 27° in the presence of 2% (v/v) of the seed which was obtained by jar fermentation. The production of I was followed by the paper disk method using *Bacillus subtilis* PCI-219 as a test organism.

LA-1 (I)——40 liter of the culture filtrate incubated for 27—30 hours was acidified to pH 2 with 1N hydrochloric acid and extracted twice with 8 liter chlofororm. The combined organic extract was transferred into 2 liter of 1% aqueous sodium bicarbonate, acidified again to pH 2 with 1N hydrochloric acid and then extracted twice with 1 liter chloroform. The extracts were combined, concentrated, and dissolved benzene. The benzene solution was concentrated and the concentrated material again dissolved in benzene. Insolved material in benzene was removed. The concentrated benzene solution was then chromatographically separated (Silica gel treated with 2% sulfuric acid, eluting with benzene–ethyl acetate=4: 1) to provide the single component fraction of I. $C_{10}H_{10}O_3$; mol. wt., 178. $[\alpha]_D^{20}$ nm: +50° (589). UV λ_{max}^{MeOH} nm: 270. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3000- (-COOH), 2900- (alkane), 1925 (=C=), 1700- (C=O), 1630—1610 (>C=C<), 985 (*trans* >C=C<). PMR (CCl_4) δ: 2.20 (3H, s., CH_3), 5.92 (1H, d, J=16 Hz, CH), 6.12 (1H, d., J=16 Hz, CH), 6.22 (1H, d., J=11 Hz, CH×2), 6.88 (1H, d.d., J=16 Hz, CH), 7.20 (1H, d.d., J=16 Hz, CH), 10- (1H, broad s., OH). CMR ($CHCl_3$) δ: 27.1 (q., CH_3), 94.4 (d., CH), 95.0 (d., CH), 121.6 (d., CH), 131.3 (d., CH), 137.7 (d., CH), 140.2 (d., CH), 171.1 (s., C=O), 197.7 (s., C=O), 218.6 (s., =C=).

Octahydro LA-1 (II)——A solution of I in benzene was concentrated and the solvent replaced by methanol. The methanol solution of I was then hydrogenated in the presence of 30 mg platinum oxide. As soon as the UV absorption maximum at 270 nm had disappeared, the reaction mixture was work up in the usual manner. The crude product was then purified by silica gel (treated with 2% sulfuric acid) column chromatography using benzene–ethyl acetate (20: 1) as eluent to provide 300 mg of the octahydro-derivative II as colorless needles. *Anal.* Calcd. for $C_{10}H_{18}O_3$: C, 64.53; H, 9.67; Found: C, 64.22; H, 9.51. $[\alpha]_D^{23}$ nm: −24°

5) R.A. Friedel and H.L. Retocofsky, *J. Am. Chem. Soc.*, **85**, 1300 (1963); G.B. Sovitsky and K. Namikawa, *J. Phys. Chem.*, **68**, 1956 (1964); R. Steur, J. Van Dongen, M. Debie, W. Drenth, *Tetrahedron Letters*, **1971**, 3307.
6) W.D. Celmer and I.A. Solomons, *J. Am. Chem. Soc.*, **74**, 3838 (1952); **75**, 1372 (1953).
7) J.D. Bu'lock, E.R.H. Hones, *J. Chem. Soc.*, **1957**, 1075; M. Anchel and M.P. Cohen, *J. Biol. Chem.*, **208**, 319 (1954).
8) Bendz: *Ark. Kerui.*, **14**, 475 (1959); R.C. Cambie, A. Hirschbery, E.R.H. Jones, and G. Lowe, *J. Chem. Soc.*, **1963**, 4120.

(589). mp 42—45°. UV λ_{max}^{MeOH} nm: end absorption. IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3000- (–COOH), 2900- (alkane), 1730 (C=O), 1710 (C=O), 1470—1420 (CH_2), 1300—1100 (CH_2). PMR (CCl_4) δ: 1.2—1.8 (10H, m., CH_2), 2.08 (3H, s., CH_3), 2.30 (2H, t., J=6 Hz, CH_2), 2.36 (2H, t., J=6 Hz, CH_2), 10.8- (1H, broad s., OH). CMR ($CDCl_3$) δ: 24.0 (t., CH_2), 24.8 (t., CH_2), 29.2 (t., $CH_2\times3$), 30.2 (q., CH_3), 34.0 (t., CH_2), 43.9 (t., CH_2), 179.6 (s., C=O), 209.4 (s., C=O).

Octahydro Methyl LA-1 (III)——The octahydro-derivative II (200 mg) was treated with diazomethane in ethyl ether. The product was purified by silica gel (treated with 2% sulfuric acid) column chromatography eluting with benzene–ethyl acetate (40: 1) mixed solvent to afford 180 mg of III as a colorless oil. $C_{11}H_{20}O_3$; mol. wt., 200.1411, *m/e*: 200.1412 (M^+). PMR ($CDCl_3$) δ: 1.2—1.8 (10H, m., CH_2), 2.14 (3H, s., CH_3), 2.32 (2H, t., J=6 Hz., CH_2), 2.43 (2H, t., J=6 Hz, CH_2), 3.68 (3H, s., CH_3). CMR ($CDCl_3$) δ: 23.6 (t., CH_2), 24.7 (t., CH_2), 28.8 (t., $CH_2\times3$), 29.7 (q., CH_3), 33.9 (t., CH_2). 43.6 (t., CH_2), 51.3 (q., CH_3), 173.9 (s., C=O), 208.9 (s., C=O).