Isolation, Characterization and Structure of a New Allenic Polyine Antibiotic Produced by Fungus LL-07F275

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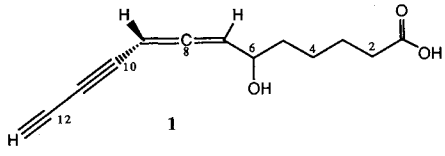
Antibiotic 07F275 (**1**), produced by submerged fermentations of fungal culture LL-07F275, was isolated and characterized despite its inherent instability. Its UV spectrum was identical with that of nemotin, a member of the allenic polyacetylene family, but a molecular weight of 218 daltons indicated a new compound. Structure **1** was determined on the basis of spectroscopic evidence, particularly NMR. Since **1** is a thirteen carbon-containing allenic diyne, it is closely related to mycomycin.

In the search for novel antimicrobial compounds produced by microorganisms, a filamentous, non-sporulating fungus, LL-07F275 (NRRL 21081), isolated from a tree bark sample collected in Panama, was found to produce two types of antibiotics. The first are the diepoxins[1], a novel class of compounds containing two 6,6-fused ring systems joined through a spiroketal bridge; and the second is the new allenic diyne (**1**) described in this report.

The UV spectrum of **1** suggested possible identity with one of the polyacetylenic allene antibiotics[2,3] (*e.g.* nemotin or nemotinic acid[4,5]). However, thermospray LC/MS analysis indicated a molecular weight of 218 which is different from that of any reported allenic diyne. We subsequently isolated and characterized antibiotic 07F275 (**1**) and recognized it to be a new compound, related to mycomycin[6~8] (Fig. 4), to which the name 3,4,5,6-tetrahydro-6-hydroxymycomycin can be assigned.

Fig. 1. Antibiotic 07F275 (3,4,5,6-tetrahydro-6-hydroxy-mycomycin).



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Results and Discussion

Isolation

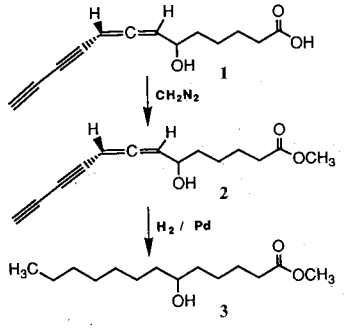
The antibiotic was readily extracted from fermentation broths with ethyl acetate under mildly acidic conditions. Concentration of the organic phase produced a dark-brown, viscous oil from which **1** was isolated by a succession of chromatographic steps on silica gel and/or reverse phase media. For spectroscopic purposes, the antibiotic was purified after conversion to the methyl ester (**2**). Alternatively, the antibiotic could be recovered from the broth by adsorption onto Amberlite XAD-2 and subsequent elution with methanol-water mixtures. The latter procedure offers the advantage of fractionation during the elution steps.

Structure Elucidation

Structure **1** was assigned to the isolated antibiotic on the basis of spectroscopic analysis of **1** and its methyl ester derivative **2**. Treatment of **1** with diazomethane yielded a single, less polar reaction product (**2**, see Fig. 2) with a molecular weight of 232, 14 mass units higher than that of **1**. New signals in both the ^{1}H and ^{13}C NMR spectra at 3.68 and 51.5 ppm, indicative of a methoxyl group, together with a concomitant shift of one ^{13}C-signal from 179.0 to 174.1 ppm, indicated the conversion of **1** to **2**. The remaining signals for **2** in both the ^{13}C NMR and ^{1}H NMR spectra were virtually identical with those of **1**.

The structure of the remaining portion of **2** was derived *via* the following correlations. The empirical formula

Fig. 2. Methylation of **1** and reduction of **2**.

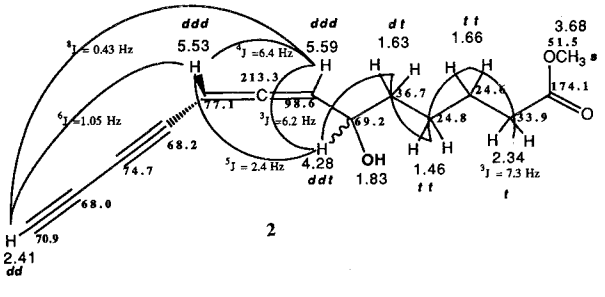


$C_{14}H_{16}O_3$ was deduced from 14 signals for carbons in the ^{13}C NMR spectrum and signals for 15 protons in the ^1H NMR spectrum. Three oxygen atoms were accounted for by one carboxyl and one hydroxyl group. Both functional groups were evident from IR signals and ^{13}C-chemical shifts. The ^1H NMR spectrum combined with DEPT and HETCOR experiments indicated the presence of four methylene groups, two apparently olefinic protons (δ_H 5.53 and 5.59), bonded to carbons with the uncharacteristic chemical shifts of 77.1 and 98.6 ppm, and one methine proton (δ_H 4.28) located on an oxygen-bearing carbon (δ_C 69.2). The NMR spectra provided no evidence for a terminal methyl group, but instead revealed the presence of a single weakly-coupled proton (δ_H 2.41) bonded to a carbon resonating at 70.9 ppm. One terminus of the molecule was therefore suspected to be an allenic diyne moiety. This hypothesis was supported by the IR spectrum of **1** (evaporated onto KBr), which provided evidence for an allene (ν_{max} 1951 cm^{-1}), an acetylene (ν_{max} 2210 cm^{-1}), and a terminal acetylene (ν_{max} 2065 cm^{-1}) function. The presence of an allenic diyne group was also deduced from the UV spectra of **1** and **2** which are identical with that of nemotinic acid[4].

Analysis of ^1H-^1H COSY data in conjunction with ^1H-^{13}C connectivities established by HETCOR measurements enabled the assembly of the core structure as shown in Fig. 3. The four catenated CH_2-groups link the carboxyl group with the carbinol function, the methine proton of which was shown to strongly couple with the olefinic proton resonating at 5.59 ppm. Therefore, the hydroxymethyl group is located adjacent to the allene function. This leaves the two acetylene groups to be placed at the end of the carbon chain. Observed long range couplings of the single alkyne proton (δ_H 2.41) to both allene protons support this arrangement.

Unambiguous assignment of the acetylenic carbon resonances and corroboration of the proposed structure was accomplished by HMBC measurements. The terminal alkyne proton (δ_H 2.41) couples to two carbons (δ_C 74.7 and 68.0) of the diyne group; and one of the allene protons (δ_H 5.53) showed connectivity to two acetylenic carbons (δ_C 74.7 and 68.2). Thus, the carbon resonating at 74.7 ppm, located half way between both protons can be assigned to C-11, since only it can correlate to these protons *via* three-bond couplings. The remaining correlations must consequently span two bonds and hence lead to the assignments for C-10 (δ_C 68.2) and C-12 (δ_C 68.0). Assignment of the resonance at 213.3 ppm to the central allene carbon (C-8) is evident from its three bond correlation to the carbinol proton and its two bond correlations to each of the olefinic protons. Correlations within the aliphatic region of the molecule *via* two or three bonds were straightforward and corroborated the previous assignments. The above assignments are similar to those published for scorodonin[9], the only other acetylenic allene whose structure was solved by NMR spectroscopy.

Fig. 3. Structure of Antibiotic 07F275 methyl ester (**2**) as established by 1-D and 2-D (COSY, HETCOR) NMR measurements.

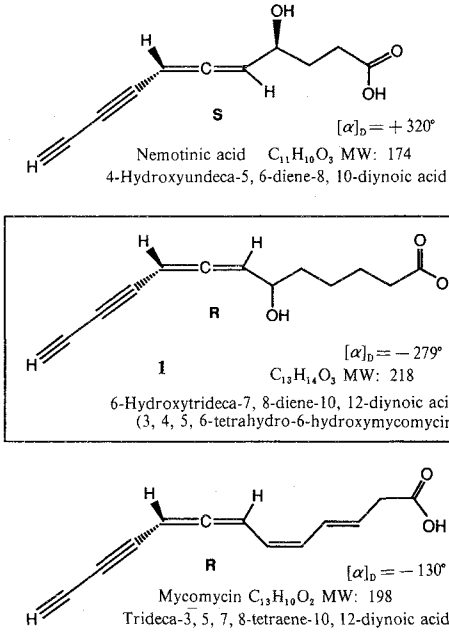


Absolute Configuration

The absolute configuration of allenic diynes is readily deduced from the sign of their optical rotations in accordance with *Lowe*'s rule[10]. The antibiotic's strongly negative rotation, owing to the asymmetric arrangement of ligands at the allene, allows the *R* assignment as illustrated by the structure diagrams in Fig. 4. Naturally occurring allenes have been reported to exist in either the *R* or the *S* form as determined from their optical rotations. Thus, odyssin and nemotin[5] are strongly dextrorotatory which indicates the *S* configuration, whereas cepacin[11] and mycomycin[6~8] are strongly levorotatory and therefore have the *R* configuration. Since **1** also has the *R* configuration, its similarity to mycomycin becomes even more obvious. This relationship earns further support from the fact that the original producer of mycomycin, thought to be a strain of *Nocardia*, was reported to be a probable *basidiomycete*[12].

In an attempt to assign also the absolute configuration of the hydroxyl bearing carbon (C-6), **2** was catalytically

Fig. 4. Structures and optical activities of three naturally occurring allenic diynes.



hydrogenated to produce 6-hydroxytridecanoic acid (**3**), see Fig. 3. Optical rotation measurement of **3** was to provide evidence for the absolute configuration at the only asymmetric center of this molecule according to BREWSTER's Rule, but the reduced product showed no specific rotation at 589 nm (Na D-line). The CD spectrum, however, recorded on the same material (9.4 mg), displayed a slightly negative curve and could possibly imply the *S*-configuration for **3** or *R*-configuration for **1** or **2**. The failure of compounds with a single asymmetric center such as **3** to show optical activity is not uncommon, since their expected rotation is predictably small[13], and often insufficient amounts of material are available. Natural products are rarely racemic however, and for this reason should not be optically inactive.

The structure of **3** was readily deduced from well dispersed ^{13}C-signals, confirming that the reduction product was indeed the expected saturated 6-hydroxytridecanoic acid methyl ester. Although a racemic form of **3** (6-hydroxytridecanoic acid) has been synthesized[4], NMR data are not available for a direct comparison.

Stability

Alkali-induced rearrangement is one of the most characteristic properties of allenic diynes[2,15]. On treatment with 1 M NaOH, **1** underwent an irreversible reaction as evidenced by dramatic changes in its UV absorption spectrum. This base-catalysed isomerization caused the disappearance of the typical four-finger

Fig. 5. Alkaline conversion of **2** to **4** as followed by UV.

—— 0 minute, – – – 5 minutes, —··— 10 minutes, ------ 15 minutes, —— 30 minutes.

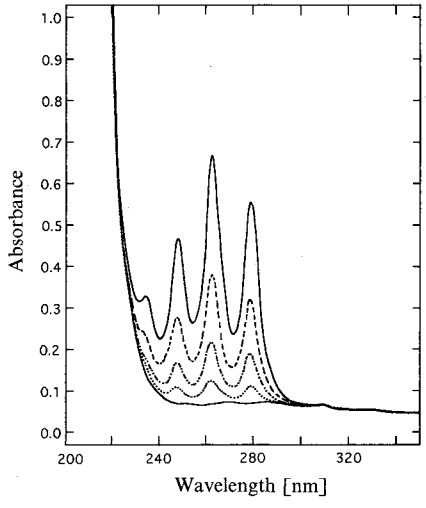
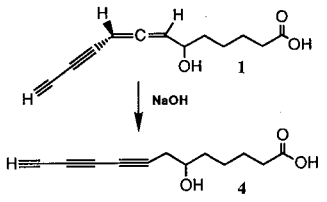


Fig. 6. Base-catalysed isomerisation of **1** yielding **4**.



spectrum and produced a stronger UV absorbance at 207 nm as shown in Fig. 5. The NMR spectra of the isomerized product revealed a lack of olefinic protons associated with the disappearance of carbon signals assigned to the allene group. A terminal acetylene, however, was still represented by a signal at 2.50 ppm in the proton spectrum. Seven ^{13}C-signals were observed between 60 to 80 ppm suggesting that the allene isomerized to an acetylene group under basic conditions as formulated in Fig. 6.

Product **4** as well as the original compounds **1** and **2** are unstable in the condensed phase and rapidly decompose, particularly on exposure to light or heat. This instability is most marked for **1**. Evaporation of solutions of these compounds under reduced pressure, inert gas (nitrogen), in the dark, and freeze-drying, gives gums which cannot be completely redissolved. Overnight freeze-drying of **1** (40 mg) from *t*-BuOH produced an insoluble black tar. Because of the intractable nature of the compounds, concentration to a residue was circumvented during the preparation of these compounds whenever possible.

Structure-Activity Relationship

The antimicrobial activity of **1** or **2** appears to be associated with the allene function since the isomerized molecule **4** is essentially inactive. It is however possible, that some other biological activities are retained or even invoked, since compounds with structures similar to that of **4** have been reported to possess certain activities. Examples include the antifungal activity and squalene epoxidase inhibition of EV-22[16] also known as L-660,631[17], the HMG-CoA reductase inhibition of xerulin and dihydroxerulin[18], and the activity of caryoynecins[19] against plant pathogenic bacteria.

Biological Activities

Exact MIC values for **1** or **2** are difficult to determine given that both compounds are too unstable to concentrate sufficiently or to prepare in solid form. All allene-diyne antibiotics are expected to have very similar antimicrobial activities because their mode of action relates to the same functional group. MIC's have been reported for nemotin and nemotinic acid[4], scorodonin[9], cepacin[11], and PA 789/80[20].

1 and **2** were assayed by serial dilution on seeded agar plates. Marginal zones of growth inhibition were observed against *Staphylococcus aureus* at 2~4 μg/ml, *Bacillus subtilis* at 2 μg/ml, *Candida albicans* at 10 μg/ml, *Ustilago maydis* at 250 μg/ml, and *Rhodotorula rubra* at 250 μg/ml, when 20 μl aliquots were tested.

Materials and Methods

Instrumental

A Hewlett-Packard 1090M LC system with diode array detection employing a Zorbax RX (C8) reverse phase column (5 μ, 4.6 × 250 mm), eluted isocratically with 45% aqueous methanol at 45°C, or, alternatively, with 30%

aqueous acetonitrile, was used for analysis of fractions or to check the purity of isolated components. NMR spectra were obtained on either a Bruker AMX 300, a GE QE+ 300 MHz, or a GE Omega 500 MHz NMR instrument at ambient temperature. Chemical shifts of ^{1}H and ^{13}C NMR signals were determined in parts per million relative to TMS or referenced to the solvent signals of deuterochloroform at δ_H 7.26 ppm and δ_C 77.0 ppm, or deuteromethanol at δ_H 3.30 ppm and δ_C 49.0 ppm.

UV spectra were recorded using a Hewlett-Packard Model 8450A spectrometer, or obtained "on the fly" with a HP 1090M LC system with diode array detection. IR spectra were obtained with a Nicolet 20AXB FT-IR spectrometer. Thermospray LC-mass spectra were obtained on a Finnigan TSP 46 single quadrupole mass spectrometer with injecting flow from a Waters 600MS HPLC system.

Microbiology

Fungal culture LL-07F275 was obtained from Dr. BARRY KATZ at MYCOsearch, Durham, NC. This culture has been characterized as a non-sporulating fungus and has been deposited with the NRRL (21081). The culture was maintained on a slant of 3% malt extract agar. Following inoculation of the slant, the culture was grown at 22°C for approximately one week and then stored at room temperature. For the development of seed growth, an agar plug of this culture was added to a tube containing 10 ml of potato dextrose broth and incubated under aeration at 22°C and 170 rpm. After a period of between 3 ~ 5 days, vigorous growth in the form of black spheres, about 2 mm in diameter, was observed. An aliquot (2.5 ml) of this seed inoculum was transferred to a 250-ml Erlenmeyer flask containing 50 ml of potato dextrose broth and incubated at 22°C with aeration at 200 rpm with a two inch throw until vigorous growth was observed, usually after 3 days incubation. This procedure was repeated a second time, except that the flasks were incubated for 5 to 7 days, then assayed for bioactivity. The following zones of inhibition were observed when 25 μl of whole fermentation broth was applied to the surface of seeded agar plates; *S. aureus* 44 mm, *E. coli* 14 mm, *B. subtilis* 26 mm and *C. albicans* 31 mm.

Isolation and Purification of Antibiotic 07F275 (1)

To 100 ml of fermentation broth (pH adjusted to 3) of culture LL-07F275 was added 70 ml ethyl acetate and the mixture was vigorously shaken. The organic phase was separated after centrifugation and concentrated to yield approximately 150 mg of oily material. This was immediately dissolved in 10 ml CH_2Cl_2 and applied to a small silica gel column (1 × 5 cm). This column was eluted with 10 ml of each of the following eluents, CH_2Cl_2, 5% MeOH-CH_2Cl_2, 20% MeOH-CH_2Cl_2 and 20 ml MeOH. The MeOH fraction, containing **1**, was applied to a preparative TLC plate (Whatman PLK5F) and developed with ether containing 1% acetic acid. The most polar UV-active zone (Rf 0.12) yielded 7.3 mg of **1** which was subsequently dissolved in $CDCl_3$ for NMR analysis.

Esterification of 1 and Purification of 2

Compound **1** (80 mg), prepared as described above, was dissolved in 20 ml CH_2Cl_2. Diazomethane, previously prepared as an ethereal solution, was then added to generate the ester **2**. The reaction was followed by TLC (Whatman K5F, 10% MeOH in CH_2Cl_2). When the reaction appeared to be complete, excess diazomethane and ether together with some CH_2Cl_2 were removed by distillation under reduced pressure. Crude ester **2** was then chromatographed isocratically on a MODCol C18 column using 50% aqueous MeOH as eluent. The column effluent was monitored at 280 nm and collected in fractions of 50 ml. One major UV-absorbing peak was detected in fractions 7 and 8 yielding 24 mg of pure **2**. Under the standard analytical HPLC conditions (see above), **1** has a retention time of 4.3 minutes and **2** elutes at 33.1 minutes.

Catalytic Reduction of 2 to Yield 3

A mixture of 50 mg of 10% Pd/C and 15 ml methanol was gassed with H_2 (1 atm, 22°C) for 30 minutes. Ester **2** (24 mg), dissolved in 1 ml MeOH was then added and gassing with H_2 continued for 15 minutes, at which time the catalyst was removed by filtration. Concentration of the solvent yielded 27 mg of an oil which was analysed without further purification. GC analysis revealed that the reaction product contained 68% of the 13:0 hydroxy fatty acid, methyl 6-hydroxytridecanoate (**3**) and 22% of the 13:0 fatty acid, methyl tridecanoate. This composition was also discernible in the ^{13}C NMR spectrum. The carbon resonances of the hydroxyl carbon (δ_C 71.7) as well as the neighboring carbons (δ_C 37.0 and 37.5) were not accompanied by small satellite signals as the signals of the aliphatic region were, due to the presence of the totally hydrogenated product, methyl tridecanoiate.

Antibiotic 07F275 (1) (6-Hydroxytrideca-7,8-diene-10,12-diynoic acid)

$C_{13}H_{14}O_3$ MW 218; UV (MeOH) λ_{max} nm (ε) 208 (38,000), 236 (6,300), 249 (10,200), 263 (14,600), 278 (12,500); IR (KBr) 3540 (sh), 3380 (s) 3281 (s), 2210 (s), 2065 (s), 1951 (s), 1708 (s), 1629, 1610 (s), 1586 (s), 1457 (s), 1411 (s), 1380 (s), 1330 (s), 1274, 1165, 1084 cm^{-1}; ^{1}H NMR ($CDCl_3$): 5.59 (H-7, ddd, ^{3}J=6.2 Hz, ^{4}J=6.4 Hz), 5.53 (H-9, ddd, ^{4}J=6.4 Hz, ^{5}J=2.4 Hz), 4.28 (H-6, ddt, ^{3}J=6.3 Hz, ^{3}J=6.2 Hz, ^{5}J=2.4 Hz,), 2.41 (H-13, s), 2.39 (2 H-2, t, ^{3}J=7.3 Hz), 1.66 (2 H-3, m, ^{3}J=7.3 Hz), 1.63 (2 H-5, m), 1.46 (2 H-4, m); ^{13}C NMR ($CDCl_3$): 213.4 (C-8), 179.0 (C-1), 98.5 (C-7), 77.2 (C-9), 74.7 (C-11), 71.0 (C-13), 69.2 (C-6), 68.2 (C-10), 68.0 (C-12), 36.6 (C-5), 33.8 (C-2), 24.6 (C-4), 24.4 (C-3); MS (nTSP) $[M]^- = m/z$ 218;

2 (Methyl 6-hydroxytrideca-7,8-diene-10,12-diynoate): $C_{14}H_{16}O_3$ MW 232; $[\alpha]_D^{26} = -279 \pm 2$ (*c* 0.47% in CH_3OH); UV (MeOH) λ_{max} nm (ε) 208 (38,280), 236 (6,340), 249 (10,190), 263 (14,670), 278 (12,530); ($CHCl_3$) λ_{max} nm (ε) 238 (6,270), 250 (9,850), 264 (14,290), 280 (12,240); ^1H NMR ($CDCl_3$): (see Fig. 2); ^{13}C NMR ($CDCl_3$): (see Fig. 2); ^1H NMR (CD_3OD): 5.59 (H-9, H-7, m), 4.15 (H-6, ddt, $^3J = 6.3$ Hz, $^5J = 3.0$ Hz), 3.65 (OCH_3, s), 3.02 (H-13, s), 2.34 (2 H-2, t, $^3J = 7.4$ Hz), 1.62 (2 H-3, m, $^3J = 7.4$ Hz), 1.58 (2 H-5, m), 1.44 (2 H-4, m); ^{13}C NMR (CD_3OD): 214.7 (C-8), 175.9 (C-1), 99.1 (C-7), 76.7 (C-9), 75.0 (C-11), 72.8 (C-13), 70.3 (C-6), 68.9 (C-10), 68.6 (C-12), 52.0 (OMe), 37.8 (C-5), 34.7 (C-2), 26.0 (C-4), 25.8 (C-3); MS(nTSP) $[M]^- = m/z$ 232;

3 (Methyl 6-hydroxytridecanoate): $C_{14}H_{28}O_3$ MW 244; ^1H NMR ($CDCl_3$): 3.67 (3 H, s), 3.60 (1 H-6, m, $^3J = 6.3$ Hz), 2.33 (2 H-2, t, $^3J = 7.3$ Hz), 1.66 (2 H-3, m), 1.63 (2 H-5, m), 1.46 (2 H-4, m), 1.22~1.26 (12 H, m), 0.88 (3 H-13, t, $^3J = 7.3$ Hz); ^{13}C NMR ($CDCl_3$): 174.2 (C-1), 71.7 (C-6), 51.5 (OMe), 37.5 (C-7), 37.0 (C-5), 34.0 (C-2), 31.8 (C-11), 29.6 (C-10), 29.3 (C-9), 25.7 (C-8), 25.2 (C-4), 24.9 (C-3), 22.6 (C-12), 14.1 (C-13)

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References

1) SCHLINGMANN, G. R. R. WEST, L. MILNE, C.P. PEARCE & G.T. CARTER: Diepoxins, Novel Fungal Metabolites with Antibiotic Activity. Tetrahedron Lett. 34(45): 7225~7228, 1993
2) BEW, R. E.; J. R. CHAPMAN, E. R. H. JONES, B. E. LOWE & G. LOWE: Natural Acetylenes. Part XVIII. Some Allenic Polyacetylenes from *Basidiomycetes*. J. Chem. Soc. (C): 129~135, 1966
3) BEW, R. E.; R. C. CAMBIE, E. R. H. JONES & G. LOWE: Natural Acetylenes. Part XIX. Metabolites from Some Poria Species. J. Chem. Soc. (C): 135~138, 1966
4) KAVANAGH, F.; A. HERVEY & W. J. ROBINS: Antibiotic Substances from *Basidiomycetes*. V. *Poria corticola, Poria tenuis* and an unidentified *Basidiomycete*. Proc. Nat. Acad. Sci. 36: 1, 1950
5) BU'LOCK, J. D.; E. R. H. JONES & P. R. LEEMING: Chemistry of Higher Fungi. Part V. The Structures of Nemotinic Acid and Nemotin. J. Chem. Soc. 4270~4276, 1955
6) CELMER, W. D. & I. A. SOLOMONS: Mycomycin. I. Isolation, Crystallization and Chemical Characterization. J. Am. Chem. Soc. 74: 2245~2248, 1952
7) CELMER, W. D. & I. A. SOLOMONS: The Structure of the Antibiotic Mycomycin. J. Am. Chem. Soc. 74: 1870~1871, 1952
8) CELMER, W. D. & I. A. SOLOMONS: Mycomycin. III. The Structure of Mycomycin, an Antibiotic Containing Allene, Diacetylene, and *cis, trans*-Diene Groupings. J. Am. Chem. Soc. 75: 1372~1376, 1953
9) ANKE, T.; J. KUPKA, G. SCHRAMM & W. STEGLICH: Antibiotics from *Basidiomycetes*. X. Scorodonin, A New Antibacterial and Antifungal Metabolite from *Marasmius scorodonius* (*Fr.*) Fr. J. Antibiotics 33: 463~467, 1980
10) LOWE, G.: The absolute configuration of allenes. J. Chem. Soc. Chem. Comm.: 411~413, 1965
11) PARKER, W. L.; M. L. RATHNUM, V. SEINER, W. H. TREJO, P. A. PRINCIPE & R. B. SYKES: Cepacin A and Cepacin B, Two New Antibiotics Produced by *Pseudomonas cepacia*, J. Antibiotics 37: 431~440, 1984
12) PRIDHAM, T. G. & A. J. LYONS: *Nocardia acidophilus*—Not an *Actinomycetales*. Dev. Ind. Microbiol. 14: 200~204, 1973
13) MCGAHREN, W. J.; K. J. SAX, M. P. KUNSTMANN & G. E. ELLESTAD: Enzymatic and Chemical Resolution of 1-Octyn-4-ol. J. Org. Chem. 42: 1659~1660, 1977
14) TANCHUK, Y. V.; S. I. KOTENKO & T. P. VOLOSCHUK: Synthesis of 6-hydroxy acids and 4-alkylbutyrolactones Ukr Khim. zh (Russ. Ed.). 46: Vol. 7, 763~767, 1980
15) BU'LOCK, J. D.; E. R. H. JONES, P. R. LEEMING & J. M. THOMSON: Chemistry of Higher Fungi. Part VI. Isomerization reactions of naturally occurring allenes. J. Chem. Soc. 3767~3771, 1956
16) WRIGHT, J. J.; MOHINDAR S. PUAR, B. PRAMANIK & A. FISHMAN: EV-22, a Novel Antifungal Triacetylenic Dioxolone. J. Chem. Soc. Chem. Comm.: 413~414, 1988
17) LEWIS, M. D. & R. MENES: Absolute and Relative Configuration of L-660,631. Tetrahedron Lett. 28: 5129~5132, 1987
18) KUHNT, D.; T. ANKE, H. BESL, M. BROSS, R. HERRMANN, U. MOCEK, B. STEFFAN & W. STEGLICH: Antibiotics from Basidiomycetes. XXXVII. New Inhibitors of Cholesterol Biosynthesis from Cultures of *Xerula melanotricha* Dörfelt. J. Antibiotics 43: 1413~1420, 1990
19) KUSUMI, T.; I. OHTANI, K. NISHIYAMA & H. KAKISAWA: Caryoynencins, Potent Antibiotics from A Plant Pathogen *Pseudomonas caryophylli*. Tetrahedron Lett. 28: 3981~3984, 1987
20) PALMA, N. & F. KNAUSEDER: PA789/80, A Polyacetylenic Antibiotic from *Marasmius alliaceus* (*Jacq. ex Fr.*) Fr. J. Antibiotics 33: 1556~1559, 1980