Notes

DEMALONYL DERIVATIVES OF AZALOMYCIN F_4 AND SCOPAFUNGIN

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Azalomycins F_3, F_4 (**1**) and F_5,[1,2] scopafungin (niphimycin) (**2**)[3,4] (Fig. 1), copiamycin, neocopiamycin A[5,6] and guanidylfungins A and B[7] have similar chemical structures and antimicrobial spectra. These antibiotics are active against fungi and Gram-positive bacteria, and the activity is fungistatic even at high concentrations. Their structures are characterized by a macrocyclic polyhydroxyl lactone ring, an intramolecular six-membered hemiketal, a malonyl monoester, and a side chain with a terminal guanidine. Demalonyl derivatives of methylated guanidylfungin A and copiamycin have much stronger antimicrobial activity than the native compounds, and have fungicidal action.[8] This paper describes the synthesis, structure, and antimicrobial activity of methylation-demalonylation products of azalomycin (AZL) F_4 and scopafungin (SCPF) shown in Fig. 1.

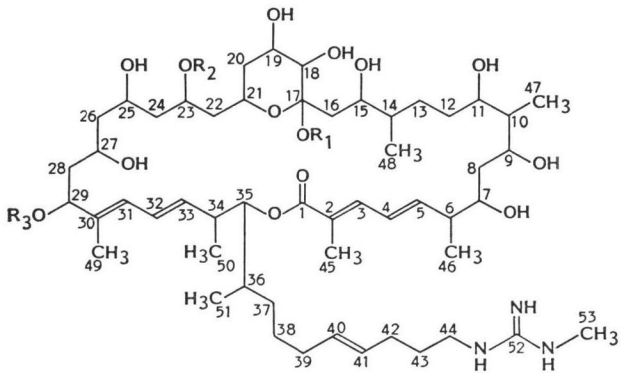
AZL F_4 and SCPF were isolated from the culture broths of *Streptomyces hygroscopicus* var. *azalomyceticus* ATCC 13810 and *S. hygroscopicus* var. *enhygrus* NRRL 3664, respectively, by successive silica gel column chromatography with $CHCl_3$ - MeOH - H_2O and with 2-butanol - H_2O followed by precipitation from aq acetone or aq MeOH.

AZL F_4 (MW 1,081) was treated with methanolic hydrogen chloride (MeOH - HCl) for about 20 minutes to give a mixture of methylated products (SI-MS *m/z* 1,110 (M+H)). The mixture was then treated with 0.5 N KOH overnight in MeOH - H_2O (1:1) to give a mixture of demalonyl products, **3** (yield 35%, mp 121~126°C, SI-MS *m/z* 1,024 (M+H)), which was separated into **3a** and **3b** by preparative ODS-silica gel HPLC. **3a**: ^{1}H NMR (400 MHz, CD_3OD) δ 7.10 (1H, d, J=11 Hz, H-3), 6.45 (1H, dd, J=11, 14 Hz, H-4), 6.25 (1H, dd, J=11, 14 Hz, H-32), 6.10 (1H, dd, J=14, 8 Hz, H-5), 5.95 (1H, d, J=11 Hz, H-31), 5.5~5.35 (3H, H-33, 40, 41), 4.81 (1H, m, H-35), 4.2~3.65 (10H), 3.52 (1H, d, J=9 Hz, H-18), 3.25 (3H, s, OCH_3), 3.17 (2H, t, J=6 Hz, H_2-44), 3.14 (3H, s, OCH_3), 2.84 (3H, s, NCH_3), 2.58 (1H, m), 2.46 (1H, m), 1.90 (3H, s, H_3-45), 1.59 (3H, s, H_3-49), 2.1~1.1 (31H), 1.12 (3H, d, J=7 Hz), 1.03 (3H, d, J=7 Hz), 0.96 (3H, d, J=7 Hz), 0.92 (3H, d, J=7 Hz), 0.89 (3H, d, J=7 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 169.2 (C-1), 157.5 (C=N), 145.0, 139.3, 137.0, 135.3, 132.0, 129.4, 128.6, 127.4, 127.0, 126.3, 102.1 (C-17), 86.4, 80.3, 76.5, 75.3, 74.8, 72.4, 71.5, 69.0, 67.4, 66.3, 65.8, 65.7, 55.9, 49.6, 47.7, 47.1, 46.1, 44.7, 44.1, 42.7, 41.8, 41.1, 41.0, 40.6, 39.3, 35.4, 35.0, 34.5, 33.5, 33.2, 30.3, 30.1, 29.7, 28.2, 27.8, 17.6, 16.9, 14.8, 14.0, 12.8, 11.3, 10.7. Product **3b** was found to be a mixture of some isomers at its methylated positions and other positions from its ^{1}H and ^{13}C NMR.

As indicated by the spectral data described above, the methylated AZL F_4 and **3** should have been methylated at two positions, one of which was at C-17. To determine the other methylated position, **3** was decomposed by ozonolysis followed by $NaBH_4$ reduction. Then the product was subjected to $NaIO_4$ oxidation, and further $NaBH_4$ reduction. The mixture of degradation products obtained was acetylated with acetic anhydride in the presence of pyridine and 4-dimethylaminopyridine, and separated by preparative HPLC using ODS-silica gel and silica gel to give compounds **5**~**9** (Fig. 2).

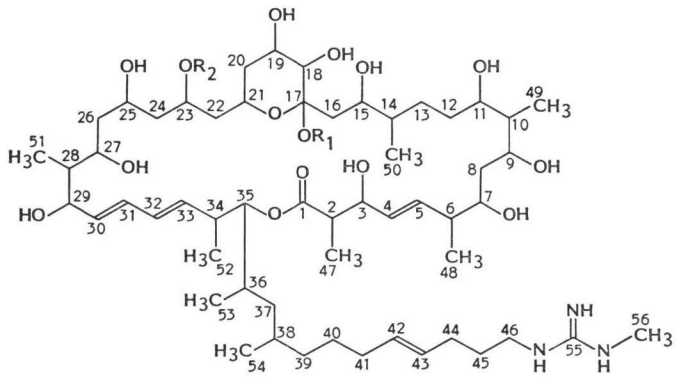
Compounds **5** and **6** were identified spectroscopically and chromatographically as the same degradation products obtained from AZL

Fig. 1. Structures of AZL F_4, SCPF and their demalonyl derivatives.



AZL F_4 (**1**) $R_1 = H$, $R_2 = COCH_2COOH$, $R_3 = H$

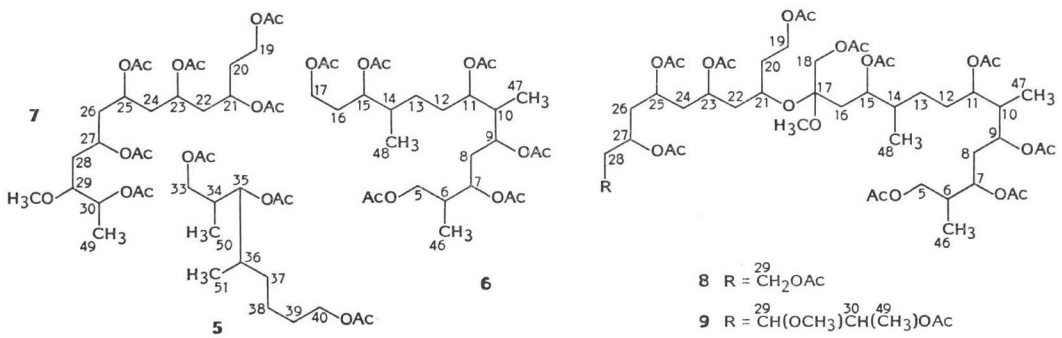
3 $R_1 = CH_3$, $R_2 = H$, $R_3 = CH_3$



SCPF (**2**) $R_1 = H$, $R_2 = COCH_2COOH$

4 $R_1 = CH_3$, $R_2 = H$

Fig. 2. Degradation products of **3** by O_3 - $NaBH_4$ - $NaIO_4$ - $NaBH_4$.



8 R = $^{29}CH_2OAc$

9 R = $^{29}CH(OCH_3)^{30}CH(^{49}CH_3)OAc$

Table 1. ^{1}H NMR of **7** (400 MHz in $CDCl_3$).

Chemical shift (ppm)	Intensity, multiplicity, J (Hz)	Assignment
5.05 (5.02)	1H, m, $J_{30,49}$=7 (7), $J_{30,29}$=3 (5)	H-30
5.12~4.9	4H	H-21, 23, 25, 27
4.08	2H, t, $J_{19,20}$=7	H_2-19
3.39 (3.41, 3.38, 3.37)	3H, s	OCH_3
3.15 (3.30)	1H, m, $J_{29,30}$=3 (5)	H-29
2.06, 2.05, 2.04, 2.01	18H, 6s	Acetyl methyls
2.1~1.5	10H	
1.17 (1.20, 1.19, 1.18)	3H, d, $J_{49,30}$=7 (7)	H_3-49

Signals in parentheses were split due to stereo-isomerisms at C-29 and 30.

Table 2. Antifungal activity of AZL F_4, SCPF and their demalonyl derivatives against *Candida albicans* Yu 1200 and *Aspergillus fumigatus* IAM 2046.

Compounds	MIC (μg/ml)		MCC (μg/ml)	
	C. albicans	*A. fumigatus*	*C. albicans*	*A. fumigatus*
AZL F_4 (**1**)	12.5	25	>200	>200
3	12.5	12.5	25	50
SCPF (**2**)	12.5	25	>200	>200
4	6.25	6.25	12.5	12.5

MIC and MCC were determined by the method described in ref 8.

F_{4a} (compounds **8a** and **16a**, respectively, in ref 9). The structure of **7** was elucidated spectroscopically. Its ^{1}H NMR (Table 1) indicated that the other methylated position was the hydroxyl group at C-29. Product **7** was a mixture of stereo-isomers at C-29 and 30, which should be brought about in the methoxyl group substitution at C-29 with MeOH - HCl and in ozonolysis of Δ^{30} followed by $NaBH_4$ treatment. The structures of **8** and **9** were elucidated by comparison of their ^{1}H NMR spectra with those of **6** and **7**. **8**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.2~4.7 (7H), 4.08 (2H, t, H_2-29), 4.00 (2H, t, H_2-5), 4.25~3.8 (5H), 3.35 (3.30)[a] (3H, s, OCH_3), 2.07, 2.06, 2.05, 2.03, 2.01 and 2.00 (33H, 11s, acetyl methyls), 2.2~1.0 (21H), 0.95 (6H, 2d, J=7 Hz, H_3-46, 47), 0.82 (3H, d, J=7 Hz, H_3-48). **9**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 5.2~4.7 (8H), 4.00 (2H, H_2-5), 4.25~3.65 (5H), 3.38 (3.41)[a] (3H, s, C-29-OCH_3), 3.35 (3.30)[a] (3H, s, C-17-OCH_3), 3.15 (1H, H-29), 2.07, 2.06, 2.04, 2.03, 2.01 and 2.00 (33H, 11s, acetyl methyls), 2.2~1.0 (21H), 1.17 (1.18)[a] (3H, d, J=7 Hz, H_3-49), 0.95 (6H, 2d, J=7 Hz, H_3-46, 47), 0.82 (3H, d, J=7 Hz, H_3-48).

SCPF (MW 1,141) was similarly treated with MeOH - HCl and then with KOH to obtain **4** yield 65%: mp 123~126°C; SI-MS m/z 1,070 (M+H); ^{13}C NMR (25 MHz, CD_3OD) δ 176.4 (C-1), 158.1 (C=N), 137.0, 135.0, 132.8, 132.1, 129.8, 102.5 (C-17), 79.5, 76.6, 76.0, 75.2, 72.1, 69.3, 68.9, 66.2, 66.1, 45.3, 45.0, 43.5, 42.6, 41.9, 41.0, 40.8, 39.0, 37.5, 35.4, 33.8, 32.4, 30.5, 29.7, 28.3, 27.8, 20.3, 17.7, 16.9, 14.9, 14.5, 10.9, 10.3.

The allylic hydroxyl groups at C-29 of AZL F_3 and F_5 were also methylated in addition to the hemiketal hydroxyl group at C-17 (data not shown), whereas such allylic hydroxyl groups of SCPF (at C-3 and C-29) and of the other related antibiotics were not. The difference in the reactivities of these allylic hydroxyl groups toward MeOH - HCl treatment may be caused by steric factors at adjacent carbon atoms (C-28 of AZL Fs, C-2 and C-28 of SCPF and of guanidylfungins, and C-26 of copiamycins) on which only AZL Fs are not substituted by a methyl group.

The demalonyl derivatives thus obtained were soluble in H_2O (10~30 mg/ml). The antimicrobial activities of these derivatives are shown in Table 2. Compounds **3** and **4** were slightly more active than their native compounds in their MICs. However, these demalonyl derivatives were highly fungicidal, though the mother compounds were fungistatic even at high con-

[a] The signals in parentheses were split because of stereo-isomerisms at C-17, 29 or 30.

centrations, as in the case of guanidylfungin A and copiamycin.[8]

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