Sch 38518 and Sch 39185: Two Novel Macrolactam Antifungals

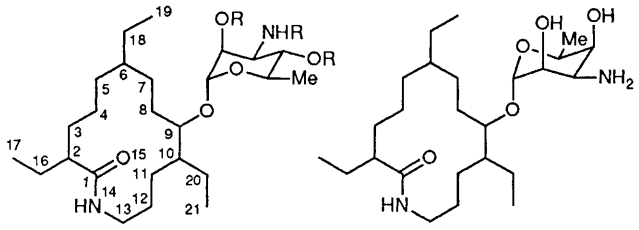
Vinod R. Hegde,*[a] Mahesh G. Patel,[a] Vincent P. Gullo[a] and Mohindar S. Puar[b]

[a] Microbial Products Department and [b] Molecular Spectroscopy Division, Schering–Plough Research, Bloomfield, NJ 07003, USA

The structures of Sch 38518 and Sch 39185, two antifungals isolated from the fermentation broth of an *Actinomadura vulgaris* subsp. *vulgaris*, have been assigned as macrocyclic lactam structures based on chemical transformations and spectroscopic data.

During the course of our continued research on novel antifungals using a galactose permeation assay,[1] we have isolated a novel family of antifungal macrolactams,[2] from various species of *Actinomadura*. The culture broth of one of the subspecies of actinomadura[3] namely *Actinomadura vulgaris* subsp. *vulgaris*[4] produced two novel compounds, Sch 38518 and Sch 39185. Both belong to the macrolactam family but differ by having isomeric sugars.[2] We herein report the structure determination of these novel antifungals. Fermentation, isolation and biological properties will be published elsewhere.[5] Sch 38518 and Sch 39185 have minimum inhibitory concentrations (MICs) of 1.8, 2.0 and >40.3, ≥101.6 μg ml^{-1} against *Candida* sp. and dermatophytes, respectively.[6]

Sch 38518 **1** is a white amorphous solid (base), ninhydrin positive; m.p. 220 °C; R_f (SiO_2) 0.25 (chloroform–methanol 8:2); $[\alpha]_D^{26}$ +9.7° (*c*, 0.5, MeOH); FAB mass spectrum (glycerol + thioglycerol matrix) *m/z*, 457, 312, 294, 163, 146; high resolution FAB (fast atom bombardment) mass spectrum *m/z* 457.3674 $(M + H)^+$ for $C_{25}H_{49}N_2O_5$ (calcd. 457.3641); UV(MeOH) end absorption; IR(KBr) ν_{max}: 3420, 3300, 2930, 1640, 1550, 1455 and 1045 cm^{-1}; ^1H NMR ($CDCl_3$: CD_3OD): three primary methyls at δ 0.9, one secondary methyl at δ



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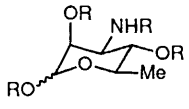
7 R = Ac

2



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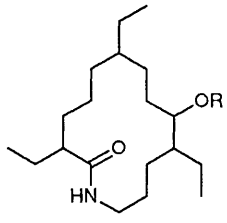

4 R = H
6 R = Ac

1.25, several saturated methylene and methine proton signals, eight O–CH– and N–CH–type proton signals and one anomeric proton at δ 4.8; ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 178.2 (C-1), 50.7 (C-2), 33.9 (C-3), 25.5 (C-4), 32.5 (C-5), 39.0 (C-6), 22.7 (C-7), 21.7 (C-8), 76.9 (C-9), 41.2 (C-10), 25.5 (C-11), 28.1 (C-12), 39.2 (C-13), 26.9 (C-16), 12.3 (C-17), 27.6 (C-18), 12.6 (C-19), 21.5 (C-20), 8.9 (C-21), 97.5 (C-1′), 72.9 (C-2′), 53.8 (C-3′), 71.0 (C-4′), 69.8 (C-5′) and 17.7 (C-6′).

For **1**, the IR absorption at 1640 and 1550 cm^{-1} suggested the presence of an amide and the proton signal at δ 4.8 indicated the presence of a sugar in the molecule. The ^{13}C NMR showed all 25 carbon signals and also confirmed the presence of a sugar. The attached proton test (APT) ^{13}C NMR experiment identified the carbon signals as being due to four methyls, eleven methylenes, nine methines and a quaternary carbon. Evaluation of all the physicochemical data revealed this antifungal to be related to the macrolactam, Sch 38516.[7]

Upon hydrolysis with 6 mol dm^{-3} hydrochloric acid for 3 h, **1** yielded two products: a sugar **3** and an aglycone, **4**. Both were purified as their acetates **5** and **6**† respectively. The sugar acetate was identified to be the tetraacetate of 3-amino-3,6-dideoxyhexose, by the evaluation of its spectral data and 2D (^{1}H–^{1}H) correlation studies. Further, it was confirmed to be mycosamine tetraacetate by its comparison with mycosamine tetraacetate obtained from amphotericin B. However, mycosamine tetraacetate from amphotericin B is the (+)-isomer {[α] + 39.3°} but that from Sch 38518 is (−)-mycosamine {$[\alpha]_D^{26}$ −34.2°}. Evaluation of the spectral data of the aglycone monoacetate revealed that it has no other functionality besides an amide and a hydroxy group (attached to sugar). Also analysis of its molecular formula suggested a ring system. However, to arrive at the correct structure was difficult owing to the lack of functionalities and the presence of many saturated carbons.

The antifungal, **1**, on acetylation with Ac_2O–pyridine gave a triacetate **7**.‡ The structure of this triacetate was determined by 2D (^{13}C–^{13}C) chemical shift correlation studies[8] as shown in Fig. 1. Analogous to macrolactones, Sch 38518 contains a 14-membered lactam ring. It has three ethyl groups attached to the 2, 6, 10 ring carbons. The sugar, mycosamine, is connected to this ring at carbon 9. It was also apparent from the chemical shift of the anomeric carbon (δ 97.5) that the sugar is α-linked to the aglycone.[9]

Sch 39185 has similar physicochemical properties,§ and mass spectrum but a different optical rotation. These data suggested Sch 39185 must be an isomer of Sch 38518. Upon hydrolysis with 6 mol dm^{-3} hydrochloric acid for 3 h, **2**, like **1** gave an aglycone and a sugar. This aglycone proved to be identical with that of Sch 38518. However, the sugar was found to be an isomer of mycosamine. Comparison of this sugar with other 3-amino sugars revealed it to be identical with the sugar from Sch 38516,[7] *i.e.* 3-amino-3,6-dideoxy-L-talose.§

In summary, the antifungals **1** and **2** both differ from Sch 38516[7] by containing a higher homologue of the aglycone. This aglycone has an ethyl group instead of methyl group on carbon 6. The absolute stereochemistry at carbon centres 2, 6, 9 and 10 was determined to be 2*R*, 6*S*, 9*R* and 10*R* based on its similarity with that of Sch 38516.[7] Also **1** contains a different sugar, mycosamine, instead of L-talose. These two antifungals represent two series of compounds one containing mycosamine and other 3-amino-3,6-dideoxy L-talose.[10]

Though many macrocyclic mixed lactone–lactams[11] and polyenic lactams[12] are known in the literature, to our knowledge this family of compounds are the first examples of pure macrocyclic lactams. Isolation of these compounds thus

† Sugar tetraacetate **5** (α-isomer): IR (KBr) 3230, 2920, 1740, 1650, 1550, 1470, 1225 and 1050 cm^{-1}; FAB mass spectrum 332 (M + H)$^+$; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, *J* 7 Hz, 3H), 1.96 (s, 3H), 2.13 (s, 3H), 2.18 (s, 3H), 2.22 (s, 3H), 3.76 (m, 1H), 4.44 (ddd, *J* 10, 9 and 3 Hz, 1H), 4.82 (dd, *J* 10 and 3 Hz, 1H), 5.4 (dd, *J* 1 and 3 Hz, 1H), 5.67 (brd, *J* 9 Hz, 1H) and 5.86 (d, 1H).

Aglycone monoacetate **6**: IR (KBr) 3320, 2930, 1725, 1630, 1545, 1350 and 1250 cm^{-1}; FAB mass spectrum: 354 (M + H)$^+$; ^{1}H NMR ($CDCl_3$) δ 0.82 (t, *J* 7 Hz, 3H), 0.84 (t, *J* 7 Hz, 3H), 0.90 (t, *J* 7 Hz, 3H), 1.0–1.7 (–CH_2– and –CH's, 22H), 1.96 (m, 1H), 2.95 (m, 1H), 3.74 (m, 1H), 4.82 (m, 1H) and 5.62 (br, 1H); ^{13}C NMR ($CDCl_3$–MeOH) δ 175.95 (C-1), 50.43 (C-2), 33.69 (C-3), 26.10 (C-4), 31.94 (C-5), 38.47 (C-6), 24.56 (C-7), 21.46 (C-8), 76.10 (C-9), 41.06 (C-10), 25.16 (C-11), 27.50 (C-12), 39.02 (C-13), 26.78 (C-16), 12.4 (C-17), 27.00 (C-18), 12.55 (C-19), 21.68 (C-20), 10.24 (C-21), 24.05 ($COCH_3$) and 170.96 (*C*OMe).

‡ Triacetate of Sch 38518 **7**: IR (KBr) 3320, 2930, 1750, 1680, 1640, 1550, 1380, 1240 and 1050 cm^{-1}; FAB mass spectrum 583 M + H)$^+$; ^{1}H NMR [$CDCl_3$) δ 0.82 (t, *J* 7 Hz, 3H), 0.84 (t, *J* 7 Hz, 3H), 0.87 (t, *J* 7 Hz, 3H), 1.18 (d, *J* 7 Hz, 3H), 1.0–1.8 (–CH_2– and –CH, 22H), 1.9 (m, 1H), 1.92 (s, 3H), 2.05 (s, 3H), 2.15 (s, 3H), 2.98 (dd, *J* 12, 2 Hz, 1H), 3.55 (br, 2H), 3.73 (dt, *J* 12, 12 Hz, 1H), 4.0 (dt, *J* 4, 7 Hz, 1H), 4.6 (dt, *J* 8, 10, 4 Hz, 1H), 4.75 (t, *J* 8, 8 Hz, 1H), 4.82 (d, *J* 1 Hz, 1H), 5.58 (m, 1H) and 5.60 (d, *J* 8 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 176.04 (C-1), 50.74 (C-2), 33.92 (C-3), 24.92 (C-4), 31.69 (C-5), 38.53 (C-6), 22.69 (C-7), 21.64 (C-8), 78.75 (C-9), 40.91 (C-10), 25.09 (C-11), 28.36 (C-12), 38.69 (C-13), 26.59 (C-16), 12.31 (C-17), 27.11 (C-18), 12.41 (C-19), 21.06 (C-20), 8.91 (C-21), 94.28 (C-1′), 73.14 (C-2′), 48.50 (C-3′), 72.35 (C-4′), 66.66 (C-5′), 17.52 (C-6′), 20.90 ($COCH_3$), 23.09 ($COCH_3$), 23.31 ($COCH_3$), 169.76 (*C*OMe), 170.03 (*C*OMe) and 171.59 (*C*OMe).

§ *Selected spectral data* for Sch 39185: white amorphous solid, ninhydrin positive, m.p.: 216 °C R_f (SiO_2) 0.30 (Analtech silica gel plates) (chloroform–methanol 8:2); $[\alpha]_D^{26}$ −5.8° (*c*, 0.5, MeOH); FAB high res. mass spec. *m/z* 457.3663 (M + H)$^+$ for $C_{25}H_{49}N_2O_5$ (calcd. 457.3641); UV (MeOH), end absorption; IR (KBr) ν_{max} 3420, 3300, 2920, 1640, 1555, 1385 and 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$–CD_3OD) δ 0.85 (t, 3-CH_3), 1.25 (d, –CH_3), 1.0–1.7 (–CH_2– and –CH) 2.05 (m, 1H), 3.05 (dd, CH), 3.3 (m, CH), 3.4–3.7 (5–CH) and 4.9 (1H); ^{13}C NMR ($CDCl_3$ + CD_3OD) δ 178.2 (C-1), 50.9 (C-2), 33.9 (C-3), 25.5 (C-4), 32.6 (C-5), 39.2 (C-6), 22.7 (C-7), 21.8 (C-8), 77.6 (C-9), 41.2 (C-10), 25.6 (C-11), 28.11 (C-12), 39.2 (C-13), 26.9 (C-16), 12.3 (C-17), 27.7 (C-18), 12.6 (C-19), 21.6 (C-20), 9.0 (C-21), 97.8 (C-1′) 70.3 (C-2′), 48.7 (C-3′), 69.3 (C-4′), 67.5 (C-5′) and 16.7 (C-6′). Sugar from Sch 39185 and Sch 38516 identified as methyl glycoside triacetate of 3-amino-3,6-dihydroxy-L-talopyranoside: ^{1}H NMR ($CDCl_3$) 1.17 (d, *J* 7 Hz, 5-Me) 1.97 (s, COMe), 2.18 (s, COMe), 2.20 (s, COMe), 3.4 (s, –OMe), 4.13 (dq, *J* 7, 1 Hz, 5-H), 4.69 (dt, *J* 8, 4, 4 Hz, 3-H), 4.76 (d, *J* 1 Hz, 1-H), 4.82 (dd, *J* 4, 1 Hz, 2-H), 5.12 (dd, *J* 4, 1 Hz, 4-H) and 5.76 (brd, *J* 8 Hz, NH); ^{13}C NMR ($CDCl_3$) δ, 98.20 (C-1), 70.23 (C-2), 44.93 (C-3), 69.26 (C-4), 64.92 (C-5), 16.42 (C-6), 20.84 (–$COCH_3$), 21.18 (–$COCH_3$), 23.21 ($COCH_3$), 169.47 ($NHCOCH_3$) 170.55 (*C*OMe) and 170.74 (*C*OMe).

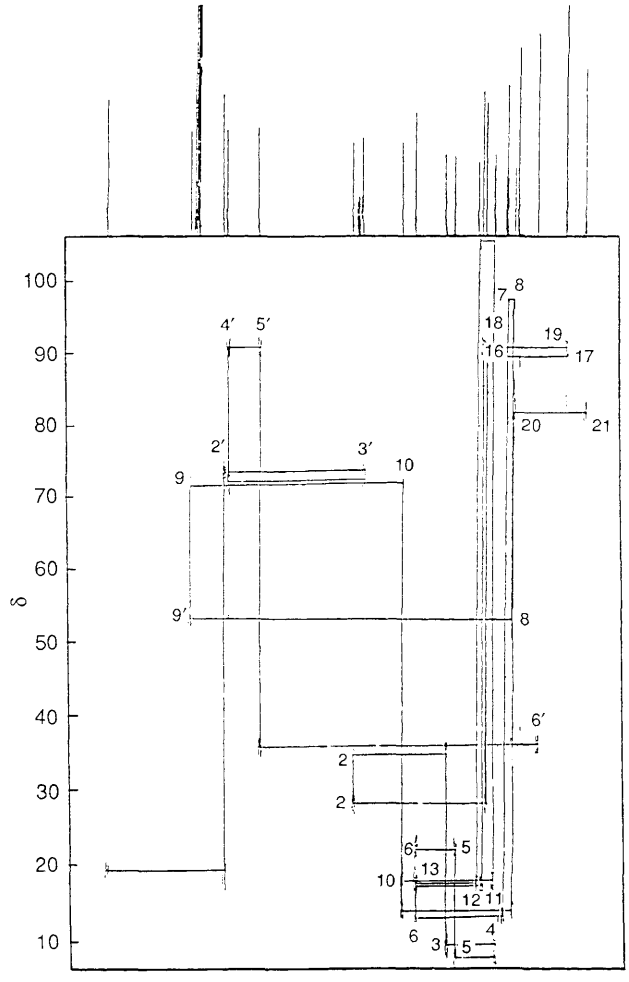

Fig. 1 2D (^{13}C–^{13}C) Chemical shift correlation of **7**

represents a new class of antifungal agents. Since fungal diseases are a major medical concern, this structural class will be an important target for structure modification. Structure modification as well as complete biological evaluation of these compounds are in progress.

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2 Four papers were presented on these macrolactams at the 28th ICAAC meeting held in Los Angeles, Paper Nos. 305–308, October 1988.
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4 A manuscript detailing the taxonomy and subspecies classification is to be published in *J. Antibiot.*
5 A manuscript detailing fermentation, isolation and structure of Sch 38518 and Sch 39185 is in preparation.
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