Isolation and Structure of an Antifungal, Sch 40873

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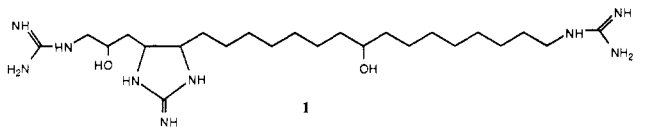
The structure of Sch 40873 is assigned as 1. Elucidation of the structure is based primarily on two-dimensional NMR experiments, high-resolution mass measurements, and selective degradation studies. This antifungal contains an imidazoline ring attached to a 3-guanidino-2-hydroxypropyl group at the C_4 position and an 8-hydroxy aliphatic chain at the C_5 position with a terminal guanidino group.

Naturally occurring compounds possessing three guanidino functions are not very common. Recently during our search for new antifungals from soil microorganisms, we have isolated a novel antifungal compound having three



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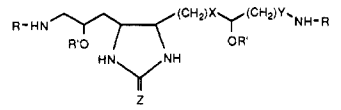
guanidino groups, produced by an *Actinomadura* sp.[1] This compound possesses a unique structure with one cyclic and two terminal guanidino groups and exhibits antifungal activity against yeast and dermatophytes.

The antifungal, 1, was isolated as a white solid $[\alpha]^{26}_D$ = −1.5° (MeOH, *c* 0.5). The fast atom bombardment (FAB) mass spectrum displayed an intense protonated $(M + H)^+$ peak at 498 and a sodiated species at 520 (M + Na). High-resolution mass measurements showed the elemental composition $C_{24}H_{51}N_9O_2$ (obsd *m/z* 497.4240, calcd 497.4239). The UV spectrum of this compound showed only end absorption, and the IR (KBr) spectrum displayed peaks at 3430, 2930, 2850, 1670, 1650, 1460, 1140, 1110, and 620 cm^{-1}. The ^{13}C NMR spectrum, measured in D_2O at 100 MHz, revealed the presence of 24 carbon atoms, and ^{15}N NMR indicated nine nitrogen atoms, supporting the above elemental composition. The most valuable structural information came from NMR investigations. The ^{13}C NMR measurements revealed the presence of 17 methylene, four methine, and three quaternary carbons. Chemical shifts of two methylene carbons, at 43.64 and 49.75 ppm, and four methine carbon signals at 56.93, 60.90, 68.89, and 74.07 ppm revealed that the first four carbons were attached to nitrogen and the latter two must be attached to oxygens, respectively. Chemical shifts of quaternary carbons (159.07, 159.72, and 161.38 ppm) indicated that guanidino, ureido, or oxime types of functionalities were present.

The 300-MHz ^1H NMR spectrum in D_2O showed eight downfield protons [δ 4.1 (1 H), 3.95 (1 H), 3.8 (1 H), 3.55 (1 H), 3.25 (1 H, J_{gem} = 15 Hz), 3.1 (1 H, J_{gem} = 15 Hz), 3.05 (2 H)] fairly separated from the wide band of protons (δ 1.0–1.6) due to methylene groups. It was evident from these data that the four protons at δ 3.0–3.3 were due to two methylene groups attached to nitrogen, the protons of one of which show geminal coupling (Figure 1). Expansion of the peaks at δ 4.1 and 3.95 also revealed that they were two protons of the type X–C*H*–C*H*X–CH_2–. Further information about the structure of 1 was obtained by correlation experiments. A 2D(^1H–^1H) chemcial shift correlation experiment in D_2O revealed that the protons at δ 4.1 and 3.95 are coupled, and this observation established the structural fragments as shown in Figure 1. 2D(^1H–^1H) chemical shift correlation in DMSO-d_6 showed that the vicinal methines are connected to an –NH. This observation was consistent with the 2D(^1H–^{13}C) chemical shift correlation studies which also showed that the protons with signals at δ 4.1 and 3.95 are attached to carbons at 56.93 and 60.90 ppm. Further analyses of this study showed that the δ 3.8 and 3.55 proton signals correlated to carbon signals at 68.89 and 74.07 ppm, respectively.

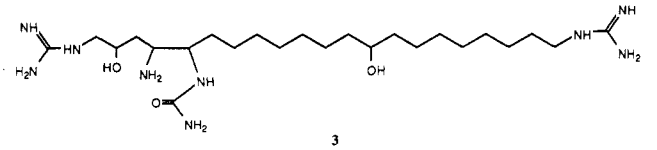
The strong basic nature of 1, coupled with its base instability, positive test against Sakaguchi reagent, and a multiple number of nitrogens in the molecule, suggested the presence of guanidino functions. This was confirmed by a strong absorption band in the 1700–1600-cm^{-1} region of the IR spectrum[2] and the three quaternary carbons in the ^{13}C NMR spectrum. On the basis of this information, this antifungal was assigned partial structure 2.



2 X+Y = 15, R =  , R' = H, Z = NH

4 X = 7, Y = 8, R =  , R' = H, Z = O

6 X = 7, Y = 8, R = R' = Ac, Z = O



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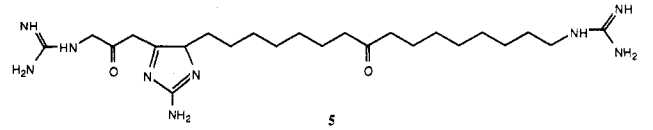

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This antifungal is stable to dilute mineral acids; however, when refluxed with 6 N HCl in a sealed tube, it gave a single hydrolysis product (3) with the addition of a molecule of water. This compound was very unstable to base and decomposed when refluxed with 2 N NH_4OH to give 4. These experiments confirmed the presence of a cyclic guanidino group.

Establishing the position of the isolated hydroxyl group on a saturated side chain was rather challenging. Oxida-

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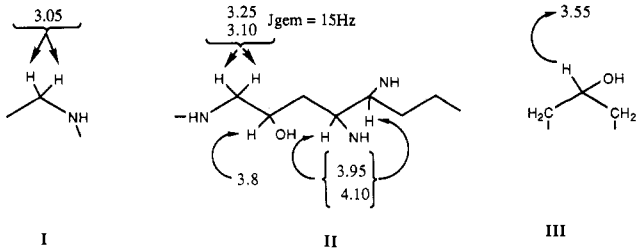

Figure 1.

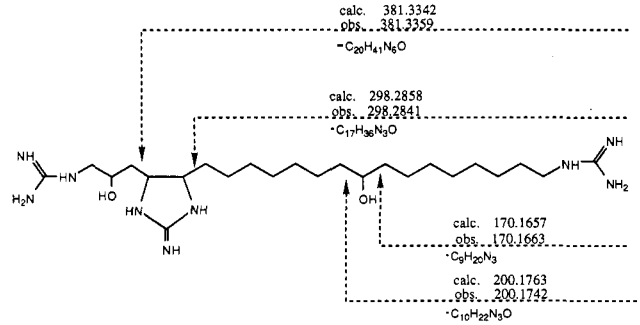

Figure 2.

tion of 1 by Jones reagent gave 5, which only strengthened the above partial structure. However, oxidation with concentrated HNO_3 yielded three dicarboxylic acids, pimelic acid [$(CH_2)_5(COOH)_2$], suberic acid [$(CH_2)_6(COOH)_2$], and azelaic acid [$(CH_2)_7(COOH)_2$]. This experiment indicated that the position of the hydroxyl group was in the middle of the aliphatic chain. The FAB mass spectrum of Sch 40873 displayed an intense molecular-ion peak (498) and other fragment peaks at m/z 381, 298, 200, and 170. Determination of their elemental composition by high-resolution mass measurements (Figure 2) confirmed the partial structure as well as providing information for the placement of the hydroxyl function at C_8 in the hydrocarbon chain.

The hydrolysis of Sch 40873 by barium hydroxide[3] also further added evidence for the above structure. Sch 40873, when refluxed with 0.5 M $Ba(OH)_2$ in a sealed tube for 20 h, gave a hydrolysis product [FAB, 415 (M + H)] which on acetylation afforded a tetraacetate 6. The spectral data of this compound are consistent with the proposed structure.

This compound showed antifungal activity[1,4] against dermatophytes (MIC < 0.13 μg/mL) and yeasts (MIC < 1.4 μg/mL) and potent activity against the mycelial phase of *Candida albicans* (MIC < 0.013 μg/mL).[5] Antibacterial activity against *Staphylococcus aureus* (MIC < 2.0 μg/mL) was also observed.

Experimental Section

General Procedures. Solvents employed for chromatography were obtained from Mallinckrodt Inc., Paris, KY 40361. Polymeric adsorbent resin XAD-16 was supplied by Rohm and Haas, Philadelphia, PA 19105. The cellulose-based weak cation-exchange resin, CM-Sephadex C-25(Na^+) (40–120 μm), was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Silica gel C-18 based reverse phase chromatographic support, LRP-2 (37–53 μm), was obtained from Whatman Inc., Clifton, NJ 07014.

All FAB mass spectra were obtained by using a glycerol–thioglycerol matrix. ^1H and ^{13}C NMR were run at 300 and 75.5 MHz, respectively, and ^{15}N NMR, 2D(^1H–^1H), and 2D(^1H–^{13}C) were performed at 400, 100.64, and 40.52 MHz for ^1H, ^{13}C, and ^{15}N NMR, respectively.

Isolation of 1. A 100-L portion of fermentation broth[4] was filtered, and the filtrate was passed through a column (2.5 × 30 in.) packed with XAD-16 resin. The column was washed with water (5 L), 50% methanol–water (5 L), and acidic methanol (0.25 mL of concentrated HCl/L). The antibiotic complex which elutes with acidic methanol (5 L) was dried, dissolved in water, and loaded on a CM-Sephadex C-25(Na^+) weak cation-exchange column (2.5 × 8 in.). The resin was washed with water and eluted with 1.0 M sodium chloride (3 L). The antifungal active fractions (monitored by agar diffusion assay against *C. albicans*) were combined and passed through a reverse-phase LRP-2 column (C-18 silica, 1 × 4 in.). The column was washed with water (2 L) and eluted with 25%, 50%, and 75% methanol in water. The antifungal eluted with 50% methanol. Evaporation of methanol from the active fractions and acidified (HCl, pH 2.0) aqueous solution freeze drying afforded 101.5 mg of Sch 40873. The spectral data of the hydrochloride salt are as follows: mp > 270 °C; $[\alpha]^{26}_D$ = −1.5 (MeOH, *c* 0.5); UV (MeOH) end absorption; IR (KBr) 3430, 2930, 2850, 1670, 1650, 1460, 1140, 1110, and 620 cm^{-1}. FABMS 498 (M + H); HRFABMS $C_{24}H_{52}N_9O_2$ (M + H)$^+$ (obsd 498.4240, calcd 498.4239); ^1H NMR (D_2O) δ 4.1 (dt, *J* = 2, 10 Hz, 1 H), 3.95 (dt, *J* = 2, 8 Hz, 1 H), 3.8 (m, 1 H), 3.55 (m, 1 H), 3.25 (dd, *J* = 4, 15 Hz, 1 H), 3.1 (dd, *J* = 7, 15 Hz, 1 H), 3.05 (t, *J* = 7 Hz, 2 H), 1.6 (dt, *J* = 2, 8 Hz, 1 H), 1.55 (dt, *J* = 2, 8 Hz, 1 H), 1.1–1.5 (br, 28 H); ^{13}C NMR (D_2O) (ppm) 27.05 (t), 27.07 (t), 27.76 (t), 28.22 (t), 30.28 (t), 30.67 (t), 30.81 (t), 30.87 (t), 30.91 (t), 30.95 (t), 31.08 (t), 31.11 (t), 34.95 (t), 38.21 (t), 38.26 (t), 43.64 (t), 49.75 (t), 56.93 (d), 60.90 (d), 68.89 (d), 74.07 (d), 159.07 (s), 159.72 (s), 161.38 (s); ^{15}N NMR (D_2O) (ppm) 142.94 (1 N), 146.22 (4 N), 155.83 (1 N), 161.88 (1 N), 169.86 (2 N). Elemental Anal. Found: C, 54.93; H, 9.29; N, 23.26; Cl, 6.41. Calcd for $C_{24}H_{52}N_9O_2Cl$: C, 53.98; H, 9.75; N, 23.62; Cl, 6.65.

Acid Hydrolysis of 1. 1 (10 mg) was refluxed with 6 N hydrochloric acid in a sealed tube at 100 °C, overnight, diluted with water, and freeze-dried to yield 9.5 mg (oil, yield 92%, 95% pure by ^{13}C NMR) of 3: FABMS 516 (M + H); IR (KBr) 3320, 2930, 1670, 1650, 1470, 1100 cm^{-1}; ^1H NMR (D_2O) δ 4.0 (dt, *J* = 2, 7 Hz), 3.9 (m, 1 H), 3.8 (m, 1 H), 3.3 (m, 1 H), 3.25 (dd, *J* = 4, 15 Hz, 1 H), 3.1 (dd, *J* = 7, 15 Hz, 1 H), 3.05 (t, *J* = 7 Hz, 2 H), 1.01–1.7 (br, 30 H); ^{13}C NMR (D_2O) (ppm) 27.44, 27.55, 27.87, 28.23, 30.47, 30.62, 30.72, 30.81, 31.11, 31.29, 31.43, 31.60, 34.82, 43.38, 43.44, 44.58, 49.53, 56.68, 60.66, 68.56, 68.60, 158.83, 159.48, 161.14. (Open-chain structure 3 obtained by ring opening was confirmed by correlation studies in DMSO.)

Base Hydrolysis of 1. 1 (10 mg) was dissolved in 25 mL of 2 N NH_4OH, stirred for 4 h, and then refluxed overnight. The contents of the reaction mixture on freeze drying gave 8.8 mg (oil, yield 88%, 90% by ^{13}C NMR) of 4: FABMS 499 (M + H); IR (KBr) 3300, 2930, 1680, 1380, 980 cm^{-1}; ^1H NMR (D_2O) δ 4.08 (dt, *J* = 2, 10 Hz, 1 H), 3.93 (dt, *J* = 2, 8 Hz, 1 H), 3.65 (m, 1 H), 3.5 (m, 1 H), 2.9–3.15 (m, 4 H), 1.1–1.8 (m, 30 H); ^{13}C NMR (D_2O) (ppm) 27.04, 27.67, 27.71, 28.19, 30.14, 30.20, 30.61, 30.71, 30.82, 30.98, 31.05, 31.71, 34.94, 38.22, 38.27, 43.42, 48.17, 56.85, 60.66, 69.36, 73.71, 158.86, 161.20, 163.90.

Jones Oxidation. To a solution of 30 mg (0.06 mM) of 1 in 25 mL of acetone was added 25 mL of 1.0 M chromic acid in 3 N H_2SO_4, and the solution was stirred overnight. The solution was poured into 200 g of ice, and the aqueous solution was brought to pH 7.0 and filtered. The filtrate was loaded on a CM-Sephadex C-25(Na^+) column (1 × 2 in.). The column was washed with water and then eluted with 1.0 M sodium chloride. The oxidized product was separated from the active fraction by reverse-phase chromatography on an LRP-2 (Whatman, C-18 silica gel) column (1 × 2 in.). The oxidation product was eluted with 50% methanol–water, evaporated, and freeze-dried to afford 17.2 mg (yield 58%; TLC silica gel, system $CHCl_3$–MeOH–water, 1:1:1, LP (lower phase)) of 5: mp 220 °C dec; FABMS 492 (M + H); IR (KBr) 3300, 2930, 1675, 1400, 1070 cm^{-1}; ^1H NMR (D_2O) δ 4.15 (d, *J* = 10 Hz, exchangeable, 2 H), 3.9 (m, 1 H), 3.05 (m, 2 H), 3.02 (t, *J* = 7 Hz, 2 H), 2.4 (t, *J* = 7 Hz, 4 H), 1.1–1.5 (br, 22 H); ^{13}C NMR (D_2O) (ppm) 25.49, 25.56, 27.40, 27.79, 29.93, 30.09, 30.15, 30.20,

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30.25, 30.34, 30.46, 30.50, 35.02, 38.93, 38.93, 43.29, 44.46, 87.05, 115.62, 138.79, 159.61, 163.56, 223.2, 223.2.

Concentrated HNO_3 Oxidation. A 25-mg sample of 1 was dissolved in about 2 mL of ice-cold concentrated nitric acid. The solution was stirred for 2 h at room temperature and then heated at 100 °C for 24 h. Contents of the cooled solution were diluted with water (25 mL) and freeze-dried to yield 15 mg of crystalline solid. Thermospray mass spectrum revealed the presence of three dicarboxylic acids, namely, pimelic, suberic, and azelaic acids [(M + 1) 161, 175, and 189 and intensity 60%, 100%, and 38%, respectively]. This was confirmed by TLC [Analtech, silica gel plates, solvent system 1-butanol-xylene-phenol-formic acid-water, 10:70:30:8:2 (v/v), showed *Rf* values 0.3, 0.4, and 0.45, respectively][6] and paper chromatographic comparisons (Whatman No. 1 paper, solvent system 1-propanol-2 N ammonia, 70:30, R_f 0.32, 0.38, and 0.44, respectively),[7] with authentic samples.

Hydrolysis with Barium Hydroxide. A solution of 50 mg (0.1 mM) of 1 in 15 mL of 0.5 N barium hydroxide was refluxed for 20 h. The aqueous hydrolysate was filtered and acidified to pH 5.0 with sulfuric acid, the precipitated barium sulfate was removed, and the filtrate was lyophilized to give 36.7 mg of crude solid [FABMS 415 (M + H)].

This crude solid was acetylated by stirring with 10 mL of a mixture of acetic anhydride and pyridine (1:1.5), overnight, at room temperature and under anhydrous conditions. The reaction was quenched by pouring the reaction mixture into ice. The aqueous solution was extracted with ethyl acetate, and the organic layer was washed with dilute HCl and brine and dried (Na_2SO_4). Solvent was removed to give a gummy solid. This product was purified by silica gel chromatography using chloroform-methanol (95:5) as the eluting solvent. The pure acetate derivative **6** (17.6 mg, yield 31%; oil, pure by ^{13}C NMR and TLC on silica gel $CHCl_3$-MeOH, 9:1) was obtained: FABMS 583 (M + H); IR (KBr) 3300, 2930, 1735, 1660, 1380, 980 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.3 (br, 1 H), 5.63 (br, 1 H), 5.57 (br s, 1 H), 5.03 (br s, 1 H), 5.0 (m, 1 H), 4.85 (dt, J = 2, 7 Hz, 1 H), 3.75 (m, 1 H), 3.65 (m, 1 H), 3.45 (t, J = 7 Hz, 2 H), 3.23 (dd, J = 7, 15 Hz, 2 H), 2.13 (s, 3 H), 2.08 (s, 3 H), 2.01 (s, 3 H), 1.95 (s, 3 H), 1.2-1.7 (br, 30 H); ^{13}C NMR ($CDCl_3$) (ppm) 21.03, 21.33, 23.14, 23.34, 26.82, 27.25, 27.32, 29.00, 29.12, 29.27, 29.29, 29.32, 29.36, 29.55, 31.93, 32.14, 34.05, 39.88, 39.88, 42.32, 43.33, 52.10, 56.22, 70.66, 74.35, 164.75, 168.15, 169.25, 170.96, 171.24.

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Registry No. 1·HCl, 119948-40-2; **3**, 119948-41-3; **4**, 119948-42-4; **5**, 119948-43-5; **6**, 119948-44-6.

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Sulfoxide Analogues of Dihydro- and Tetrahydroprephenate as Inhibitors of Prephenate Dehydratase

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The sulfoxide derivatives **4-7** were prepared as analogues of tetrahydro- and dihydroprephenate, respectively, and a synthesis was attempted for **8**, the prephenate mimic itself. As expected, the saturated analogues **4** and **5** were modest, reversible inhibitors (IC_{50}/K_m = 16 and 27, respectively) of prephenate dehydratase, the enzyme responsible for the Grob-type fragmentation of prephenic acid to phenylpyruvic acid. The unsaturated analogues **6** and **7** were envisaged as potential suicide substrates of the enzyme, if they could undergo an enzyme-induced Pummerer-type fragmentation. However, these compounds also proved to be modest, reversible inhibitors (IC_{50}/K_m = 29 and 21, respectively), and the synthesis of **8** failed because of apparent instability of this compound.

The shikimic acid pathway is a key biosynthetic sequence in plants and microorganisms, leading to the production of the aromatic amino acids as well as cofactor precursors and isoprenoid quinones.[1] This sequence has been a fertile area for investigation since it is replete with enzymatic transformations of unusual or unique mechanisms. In addition, the absence of this pathway in mammals and the success of the inhibitor glyphosate have made it an attractive target for herbicide development.[2]

Among the final steps in the biosynthesis of phenylalanine are the formal Claisen rearrangement of chorismic acid (**1**) to prephenic acid (**2**) and the Grob-type fragmentation of the latter to phenylpyruvic acid (**3**). In *Escherichia coli*, both of these steps are catalyzed by the bifunctional enzyme chorismate mutase/prephenate dehydratase. Chemical modification,[3] mutation,[4] and kinetic studies[5] all suggest that the active sites for the two activities are separate.

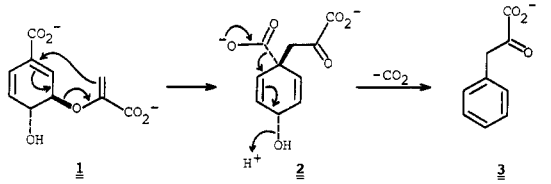

Table I. Structural Comparison of Carbinols and Sulfoxides[7]

	Bond Lengths (Å)		
C—C	1.51-1.52	C—S	1.78-1.80
C—OH	1.44-1.46	S=O	1.45-1.49
	Bond Angles (deg)		
C—C—C	111-112	C—S—C	96-100
C—C—OH	107-111	C—S=O	105-108
	pK_a		
$C—OH_2^+$	-2 to -5	$>S^+—OH$	-1 to -3

The structure of prephenate and the presumed mechanism of the dehydratase reaction presented an opportunity to test an idea for inhibition of enzymes that promote the

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