Structure of Sch 49209: A Novel Antitumor Agent from the Fungus *Nattrassia mangiferae*

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Received November 30, 1993

In the course of our search for new pharmacologically active compounds from microbial sources, a novel antitumor metabolite, Sch 49209, has been discovered from the fermentation of a fungal culture, SCF-0642, *Nattrassia mangiferae*[1] (ATCC 74078). We report herein on the structure elucidation and biological activity of this new compound, which contains a unique, highly-strained keto diepoxide decalone with a spiroketal linkage through a naphthalene moiety.

Sch 49209 was isolated from the fermentation of *N. mangiferae* as a major active component with mp 144–146 °C, $[\alpha]^{22}_D$ +79.1° (*c* 0.2, $CHCl_3$).[2] Its molecular weight was determined to be 364 based on CI-MS data that showed a molecular ion at *m/z* 365 $(M + H)^+$. The molecular formula, $C_{20}H_{12}O_7$, was deduced from HREIMS (found 364.0583, calcd 364.0578) and ^{13}C NMR spectroscopy (20 carbons) and reflected a high degree of unsaturation (15). UV absorptions at 225 and 299 nm clearly revealed the presence of a naphthalene moiety. Absorptions at 3425, 1723, and 1697 cm^{-1} in the IR spectrum were indicative of OH, C=O, and conjugated C=O, respectively; two carbonyl signals at 197.1 and 185.6 ppm in the ^{13}C NMR spectrum (Table 1) supported the IR data. The ^{13}C NMR spectrum also contained signals for 12 aromatic or vinylic carbons (eight of which were attached to protons), while eight resonances in the ^1H NMR spectrum were ascribed to six aromatic and two olefinic protons. One distinctive quaternary carbon signal at 93.5 ppm obviously indicated a ketal carbon. The remaining five carbons, three methine and two quaternary, were all bonded to oxygen atoms; the two oxygenated quaternary carbons were assigned to a tetrasubstituted epoxide. Two oxygenated methine proton doublets at 3.52 and 3.87 ppm displayed chemical shifts typical of a 1,2-disubstituted epoxide. Treatment of Sch 49209 with acetic anhydride/pyridine afforded monoacetylated Sch 49209 acetate. One oxygenated methine proton signal at 5.19 in the former was shifted to 6.28 ppm in the latter (Table 1) indicating that this proton was associated with a secondary hydroxyl group in Sch 49209. All evidence pointed to the presence of a 1′,8′-disubstituted naphthalene and a secondary hydroxyl group adjacent to a cis-disubstituted double bond which was conjugated with a carbonyl group.

Detailed 2D-NMR studies, including COSY, HETCOR, and SINEPT experiments, were all focused on Sch 49209 acetate since it provided more information. 2D-NMR spectral data permitted the establishment of two partial structures, A and B, as shown in Figure 1. That the keto diepoxide decalone A and naphthalene B must be connected through two oxygens to form a spiroketal six-membered ring between the two units was indicated by the absence of any long-range NMR correlations between A and B in SINEPT experiments. NMR experiments were unable to reveal the stereochemistry of Sch 49209, and crystals of it as well as its acetate proved to be unsuitable for an X-ray diffraction analysis. In order to obtain a suitable single crystal, an acetylated triepoxide derivative, Sch 50674, was synthesized from Sch 49209 as shown in Scheme 1. X-ray crystallographic analysis of Sch 50674 not only confirmed the proposed structure of Sch 49209 but also established the relative stereochemistry.[3] An ORTEP drawing of Sch 50674 is provided in Figure 2.

Polycyclic compounds possessing two aromatic units connected through a bis-ketal have been reported.[4] Sch

Table 1. ^1H and ^{13}C NMR Chemical Shift Assignments for Sch 49209 and Sch 49209 Acetate[a]

	Sch 49209		Sch 49209 Acetate	
C No.	^1H	^{13}C	^1H	^{13}C
1		197.1s[c]		192.2s
2	3.53 (d, 3.9)[b]	54.6d	3.49 (d, 3.9)	54.4d
3	3.87 (d, 3.9)	57.9d	3.85 (d, 3.9)	57.3d
4		93.5s		93.6s
5		185.6s		185.5s
6	6.05 (d, 10.5)	127.8d	6.10 (d, 10.7)	129.2d
7	6.66 (dd, 4.5, 10.5)	140.1d	6.66 (dd, 5.5, 10.7)	136.9d
8	5.19 (t, 4.3)	61.7d	6.20 (d, 5.5)	57.3d
9		65.4s		61.5s
10		66.3s		62.9s
1′		144.9s		144.9s
2′	7.56 (d, 8.5)	121.4d	7.56 (d, 8.2)	121.4d
3′	7.45 (t, 8.0)	127.4d	7.47 (d, 8.2)	127.4d
4′	6.95 (d, 7.5)	109.2d	7.04 (d, 7.4)	109.3d
5′	7.18 (d, 7.5)	109.9d	7.18 (d, 7.4)	110.1d
6′	7.51 (t, 8.0)	127.7d	7.52 (d, 8.4)	127.9d
7′	7.59 (d, 8.5)	121.5d	7.59 (d, 8.2)	121.6d
8′		144.9s		145.0s
9′		111.9s		112.0s
10′		134.2s		134.3s
C=O				169.3s
CH_3			2.17 (s)	20.6 q

[a] Recorded in $CDCl_3$ at 300 and 75 MHz, respectively. [b] Multiplicity and coupling constant (Hz) in parentheses. [c] Multiplicity based on DEPT data.

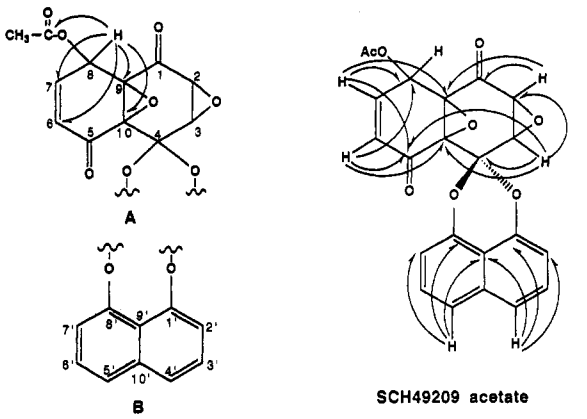

Figure 1. Structure of Sch 49202 acetate as revealed by SINEPT experiments. Arrows indicate ^1H–^{13}C long-range couplings.

(1) The fungus substrate was collected in Guatemala and supplied by Dr. B. Katz from MYCOsearch.

(2) A manuscript providing details of the taxonomy, fermentation, isolation, and biological evaluation is in preparation.

(3) Attempts to determine the absolute stereochemistry by use of the anomalous scattering of X-rays were inconclusive.

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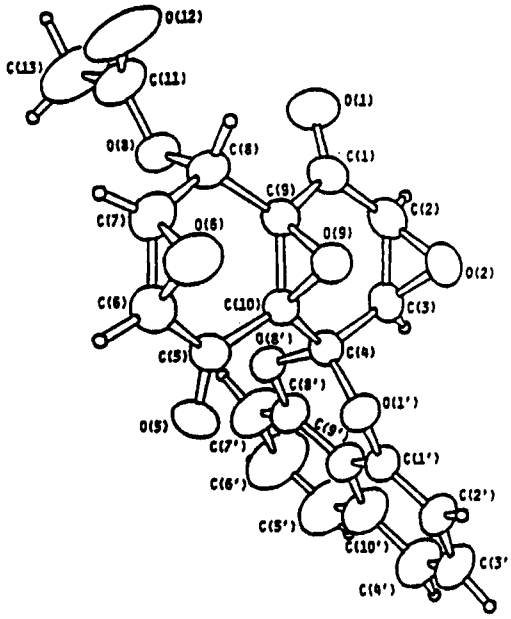
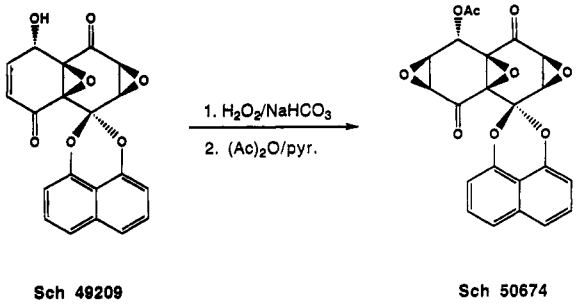


Figure 2. ORTEP diagram showing the structure and solid-state conformation of Sch 50674. Small circles represent hydrogen atoms.

Scheme 1



Sch 49209 Sch 50674

49209, however, and its related compounds, with a novel keto diepoxide polycyclic ring system, have not been previously reported from natural sources. As a lead substance for cancer research, Sch 49209 is a representative of a novel class of compounds structurally differing from other types of promising chemotherapeutic agents, such as the enediyne[5] and indolocarbazole[6] family of compounds.

Preliminary biological evaluation of Sch 49209 and its acetate derivative revealed potent *in vitro* inhibitory activity against the invasion of HT1080 human fibrosarcoma cells through a matrigel membrane (IC_{50} = 0.75 and 0.25 μM, respectively) in the invasion chamber assay. Furthermore, *in vivo* these compounds demonstrated a significant reduction in the size of primary tumors and the number of metastases. The results of detailed investigations of the potential antitumor therapeutic activity of Sch 49209, its derivatives, and other minor components from *N. mangiferae* will be published separately.[2]

Experimental Section

Isolation. The culture broth (40 L) was extracted with ethyl acetate at harvest pH. The EtOAc extract was evaporated in vacuo. The residue was dissolved in MeOH and absorbed on a small amount of XAD-16 resin, in order to prepare a plug impregnated with the active components. The plug was loaded onto an XAD-16 column and eluted with a 0–100% aqueous MeOH gradient. The 80–100% MeOH fractions were combined as the active complex. The complex was sequentially chromatographed on HP-20 column with an aqueous MeOH gradient (0–100%) and then an aqueous CH_3CN (20–40%) to obtain pure Sch 49209 (120 mg) as a white solid.

Derivatization. To a mixture of Sch 49209 (240 mg, 0.66 mmol), sodium bicarbonate (1.4 g), water (10 mL), THF (10 mL), and methanol (10 mL) at 0 °C was added a 30% aqueous hydrogen peroxide solution (3 mL). The reaction mixture was stirred for 2 h at 0 °C and allowed to warm to room temperature overnight. Additional water (10 mL) was added to quench the reaction, forming a clear solution. The solution was extracted with ether (3 × 30 mL), following which the ether solution was dried and concentrated. The residue was purified by flash silica gel chromatography using 10% ether in CH_2Cl_2 to afford 117 mg (47% yield) of pure triepoxide (*cf.* Hijfte, L. V.; Little, R. D.; Petersen, J. L.; Moeller, K. D. *J. Org Chem.* **1987**, *52*, 4647). The triepoxide was acetylated with Ac_2O/pyridine. Purification of the crude product by flash silica gel column chromatography using 2% EtOAc in CH_2Cl_2 gave 17 mg (80% yield) of pure Sch 50674. X-ray diffraction-quality crystals of Sch 50674 were grown from a $CHCl_3$/hexane (1:1) solution: mp 245–247 °C dec; $[\alpha]^{25}_D$ +33.1° (*c* 0.2, $CHCl_3$); HRSIMS *m/z* 422.0653 (calcd 422.0637); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.26 (s, 3H), 3.45 (d, J = 4.0 Hz, 1H), 3.49 (d, J = 3.8 Hz, 1H), 3.68 (dd, J = 2.1, 3.8 Hz, 1H), 3.81 (d, J = 4.0 Hz, 1H), 6.34 (d, J = 2.1 Hz, 1H), 7.05 (d, J = 7.5 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H), 7.62 (d, br. J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.6, 190.5, 169.3, 145.1, 145.0, 134.4, 128.0, 127.5, 121.7, 121.6, 111.9, 110.2, 109.2, 93.4, 67.2, 66.5, 60.1, 56.9, 56.4, 54.2, 53.9, 20.4.

X-ray Crystallographic Analysis. Crystallographic data for Sch 50674: $C_{22}H_{14}O_9$, M = 422.35, monoclinic, space group $C2(C_2^3)$, a = 27.552(2) Å, b = 8.623(1) Å, c = 8.447(1) Å, β = 97.91(1)° (from 25 accurately-centered reflections, 37° < θ < 40°), V = 1987.8(7) Å^3, Z = 4, D_{calcd} = 1.411 g cm^{-3}, μ(Cu Kα radiation, λ = 1.5418 Å) = 9.1 cm^{-1}. Crystal dimensions: 0.08 × 0.18 × 0.60 mm. Intensity data ($+h,+k,\pm l$, 2183 reflections, θ_{max} = 75°) were recorded on an Enraf-Nonius CAD-4 diffractometer (Cu Kα radiation, graphite monochromator; $\omega - 2\theta$ scans). The crystal structure was solved by direct methods (MULTAN 11/82). Full-matrix least-squares refinement of atomic positional and thermal parameters (anisotropic C, O; isotropic H, other than acetyl methyl hydrogens which were included at calculated positions) converged (maximum shift 0.02σ) at R = 0.033 (R_w = 0.046, GOF = 1.37) over 1945 reflections with $I > 3.0\sigma(I)$. Crystallographic calculations were performed on PDP11/44 and MicroVAX computers by use of the Enraf-Nonius Structure Determination Package (SDP 4.0). The author has deposited atomic coordinates for Sch 50674 with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

Acknowledgment. Authors thank Drs. J. Pai, W. R. Bishop, C. Blood, and I. King for biological data and Dr. P. Das for mass spectral data.

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