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SCH 53823 AND SCH 53825, NOVEL FUNGAL METABOLITES WITH PHOSPHOLIPASE D INHIBITORY ACTIVITY

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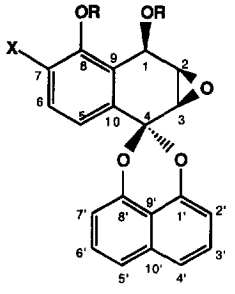
Abstract: Two novel phospholipase D (PLD) inhibitors, Sch 53823 (**1**) and Sch 53825 (**2**), were isolated from a fungal culture. The acetylated derivatives, Sch 53827 (**3**) and Sch 53829 (**4**), were prepared for the purpose of structure determination. The extensive NMR studies of **3** and **4** including DEPT, COSY, NOESY, HETCOR, and selective INEPT experiments permitted the establishment of their structures, as well as relative stereochemistries. Among these compounds, **3** exhibited the most potent inhibitory activity in the phospholipase D assay with the IC_{50} = 17 μM.

A number of novel fungal metabolites, containing a highly strained ketoepoxy decalone with a spiroketal linkage to a naphthalene moiety, have been recently discovered by our laboratory[1-3] as well as other groups.[4-8] This class of polycyclic ketoepoxides demonstrated a wide range of biological activities including antimicrobial, antitumor, and inhibitory activity in various receptor-binding assays. However, the mechanism of action for their different biological effects remain unknown. In the course of screening for new natural products, two phospholipase D (PLD) inhibitors, Sch 53823 (**1**) and Sch 53825 (**2**), were isolated from the fermentation broth of a unidentified fungus which was collected from the dead leaves of *Ruercus virginiana* Miller growing in Tamalupas, Mexico.[9] Compounds **1** and **2** were characterized as new members of the ketoepoxide family of compounds reported previously.[10] The acetylated derivatives, Sch 52827 (**3**) and Sch 53829 (**4**) were synthesized and chosen for the structure elucidation study. We report herein the isolation, structure determination, and biological activity of these ketoepoxides.



Sch 53823 (**1**) R=H X=H
Sch 53825 (**2**) R=H X=Cl
Sch 53827 (**3**) R=Ac X=H
Sch 53829 (**4**) R=Ac X=Cl

The fermentation broth (6 L) was extracted with ethyl acetate at harvest pH (6.5). The EtOAc extract was precipitated with methanol. The PLD active precipitate was purified by semi-preparative reversed phase HPLC (YMC-ODS 50 × 500 mm column, irregular 15 μ particles, mobile phase: linear gradient 80–90%

aqueous methanol in 20 min, flow rate: 20 mL/min, detection: UV at 208 nm, injection: 30 mg in 1 mL of DMSO). Two pure fungal metabolites, compound **1**[11] (42 mg, Rt = 20.5 min) and compound **2**[12] (33 mg, Rt = 23.5 min), were obtained as a white solid and a light yellow amorphous powders after lyophilization, respectively.

The molecular weight of **1** was determined to be 334 based on CIMS data that showed a molecular ion at m/z 335 $(M+H)^+$. The molecular formula, $C_{20}H_{14}O_5$, was derived from the HRFAB-MS (Calcd: 334.0841. Found: 334.0845) and ^{13}C NMR data. The UV absorptions at 224 and 300 nm suggested the presence of a naphthalene moiety. In the IR spectrum, absorption at 3520 and 1270 cm^{-1} were indicative of hydroxyl and

Table 1. ^1H (300 MHz) and ^{13}C (75 MHz) NMR Data for 1 and 2

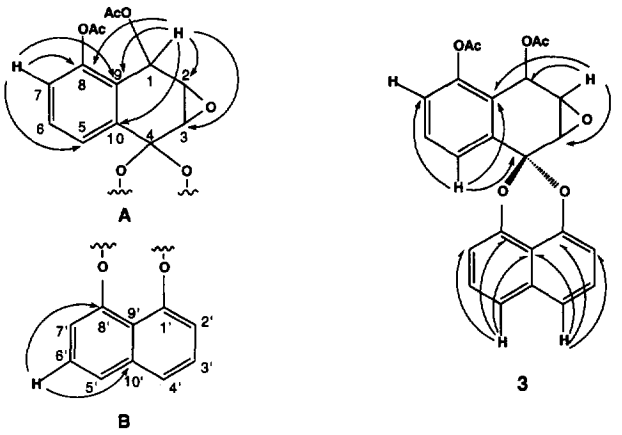
	1 (DMSO-d_6)		**2** ($CDCl_3$)	
Position	^1H	^{13}C	^1H	^{13}C
1	5.35 (br. d, 2.0)[a]	58.47 d[b]	5.66 (br. d, 2.3)	61.49 d
2	3.53 (dd, 2.0, 4.0)	52.69 d	3.72 (dd, 2.3, 4.0)	52.93 d
3	3.64(d, 4.0)	49.16 d	3.81 (d, 4.0)	50.46 d
4	---	97.03 s	---	97.46 s
5	7.17 (d, 7.8)	120.2 d	7.44 - 7.48 (m)	120.1 d
6	7.29 (t, 7.8)	128.4 d	7.44 - 7.48 (m)	130.0 d
7	7.18 (d, 7.8)	120.0 d	---	123.4 s
8	---	155.1 s	---	150.2 s
9	---	122.0 s	---	122.3 s
10	---	131.4 s	---	131.3 s
1′	---	146.6 s	---	147.3 s
2′	7.66 (d, 7.6)	115.7 d	7.42 - 7.63 (m)	121.2 d
3′	7.55 (t, 7.6)	127.2 d	7.42 - 7.63 (m)	127.6 d
4′	7.00 (d, 7.6)	108.3 d	6.97 (d, 7.3)	109.2 d
5′	7.00 (d, 7.6)	108.8 d	7.18 (d, 7.3)	110.0 d
6′	7.60 (t, 7.6)	127.3 d	7.42 - 7.63 (m)	127.9 d
7′	7.69 (d, 7.6)	116.6 d	7.42 - 7.63 (m)	121.2 d
8′	---	146.7 d	---	147.4 s
9′	---	111.8 s	---	112.9 s
10′	---	133.2 s	---	134.3 s

[a]Multiplicity and coupling constant (Hz) in parentheses
[b]Multiplicity was determined by APT or DEPT data

epoxy functionalities. The ^1H NMR spectral data (Table 1) revealed the presence of a total of nine aromatic protons. Six naphthalene at δ 7.00–7.69 and three benzene protons at δ 7.17, 7.18, and 7.29, were assigned, respectively, based on the connections observed in the COSY experiment. Two oxygenated methine protons at

δ 3.53 and 3.64 displayed at 1,2-disubstituted epoxide. A doublet of doublet oxygenated proton at δ 5.35 represented a secondary hydroxyl group. Furthermore, the COSY data showed the correlation of these oxygenated protons indicating that the secondary hydroxyl group is adjacent to the epoxide. The ^{13}C NMR data (Table 1) supported the above proton assignments by the observation of 16 aromatic carbons for a 1′,8′-disubstituted naphthalene and a phenolic moiety, respectively. Three oxy-methine carbons were also observed to confirm the presence of a secondary hydroxyl and a 1,2-disubstituted epoxide. One remaining quaternary carbon at δ 97.03 clearly indicated a ketal functional group.

Figure 1 Structure of 3 as Revealed by SINEPT Experiments
Arrows Indicate ^{1}H-^{13}C Long Range Couplings

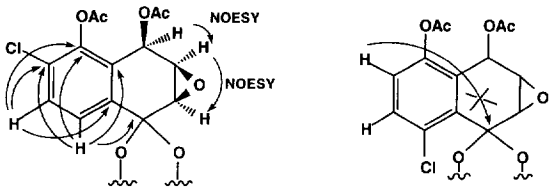


In order to further evaluate its bioactivity, and also provide better quality of NMR spectrum for the purpose of extensive studies, **1** was treated with acetic anhydride/pyridine to afford diacetylated derivative, Sch 53827 (**3**).[13] Detailed 2D NMR studies, including HETCOR and SINEPT experiments, were focused on **3**. As shown in Figure 1, partial structures A and B were established by analysis of SINEPT data. However, long range correlations between A and B were not observed. This evidence strongly suggested that the connection between bicyclic epoxide A and naphthalene B is linked through two oxygens to form a spiro-ketal ring.

The molecular weight of **2** was found to be 368 based on CIMS data that showed a molecular ion at m/z 369 $(M+H)^+$. The characteristic chlorine containing ion clusters was observed in the mass spectrum. The molecular formula was established as $C_{20}H_{13}O_5Cl$ by means of the HRFAB-MS (Calcd: 368.0452. Found: 368,0454) and ^{13}C NMR data. Elemental analysis of chlorine content for **2** provided a confirmatory evidence for the above formula (Calcd: Cl 9.51. Found: Cl 9.35). The structure of **2** closely resembled **1** by comparison of UV and IR spectra.[11,12] Both ^{1}H and ^{13}C NMR spectral data further confirmed the similarity by matching

the proton and carbon signals in compound **1** and **2** (see Table 1). The only difference was the absence of one aromatic proton which was replaced by a chlorine atom in **2**. As illustrated in Figure 2, there are only two possible positions (position-5 or -7) for the chlorine to replace a proton, because the two protons on phenyl ring couple to each other based on results of coupling constant measurements and COSY experiments. To further complete the structure elucidation, SINEPT experiments were performed on the diacetylated compound, Sch 53829 (**4**),[14] derivatized from **2**. A long range coupling of proton-5 and carbon-4 clearly suggested that the chlorine located position-7. If the chlorine is located in position-5, a correlation of proton-7 and carbon-4 can not be detected in SINEPT experiments because a long range coupling will not go beyound a four-bond range. As a typical example of SINEPT data for **3**, shown in Figure 1, the proton-7 only correlates carbon-5, -8, and -9 (two and three-bond correlations). Therefore, this evidence provides a quite convincible support for the assignment of chlorine at position-7.

Figure 2 Structure of 4 Proposed Based on COSY, SINEPT & NOESY Experiments



Most favorable assignment for 4 Least favorable assignment for 4

The relative stereochemistry of **4** was determined by means of NOESY experiments. The NOESY correlation signals between proton-1, -2, and -3 indicated the *cis* configuration for these three protons as epicted in Figure 2. In addition, the stereochemistry of **3** was presumed to be analogous to that of **4**.

Like other compounds in this class, biological evaluation of compounds **1–3** exhibited in vitro inhibitory activity in the *f*MLP-stimulated phospholipase (PLD) assay.[15] The IC_{50} values of **1**, **2**, and **3** were found to be 24, 19, and 17 μM, respectively. However, **4** was much less active (IC_{50} > 50 μM) in the PLD assay.

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9. The fungus was supplied by Dr. B. Katz from MYCOsearch.
10. In the course of preparation of this manuscript, a recent article [*Cf.* Krohn, K.; Michel, A.; Florke, U.; Aust, H.-J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1994**, *11*, 1099] reported an antimicrobial compound, palmarumycin C_{11}, which appears to have the same gross structure as **1**. However, the melting point and optical rotation of palmarumycin C_{11} {mp 170 °C (dec.), $[\alpha]^{22}_D$ -153° (*c* = 0.24, CH_2Cl_2)} are different in comparison with **1** {mp 235–240 °C (dec.), $[\alpha]^{22}_D$ + 227.0° (*c* = 0.1, DMSO)}. Furthermore, the solubility of **1** is found to be very poor in CH_2Cl_2 and $CHCl_3$. These evidences suggest that palmarumycin C_{11} and **1** are different stereoisomer.
11. Sch 53823 (**1**): mp 235–240 °C (dec.), $[\alpha]^{22}_D$ + 227.0° (*c* = 0.1, DMSO); CIMS m/z (relative intensity) 335 [48, $(M+H)^+$], 334 (52, M^+), 317(100), 289 (16), 175 (7.6), 160 (21), 159 (15), 147 (12); UV (MeOH) λmax nm (ε) 224 (49, 350), 288 (6,240), 300 (5,560), 314 (4,510), 328 (3,930); IR (KBr) νmax cm^{-1} 3520, 3280, 1605, 1410, 1380, 1270, 1115, 1030, 980, 960, 810, 780, 720.
12. Sch 53825 (**2**): mp 110–112 °C, $[\alpha]^{22}_D$ + 167.0° (*c* = 0.1, $CHCl_3$); CIMS m/z (relative intensity) 371 [18, $(M+3H)^+$], 370 [32, $(M+2H)^+$], 369 [53, $(M+H)^+$], 368 (65, M^+), 353(39), 351 (100), 323 (12), 181 (12), 160 (27) 159 (17); UV (MeOH) λmax nm (ε) 225 (48, 560), 289 (6, 130), 300 (5, 710), 314 (4, 320), 328 (3, 810); IR (KBr) νmax cm^{-1} 3430, 3310, 1610, 1415, 1370, 1280, 1110, 1040, 980, 955, 815, 770.
13. To a solution of compound **1** (20 mg, 0.06 mmol.) and pyridine (6 drops, excess) in CH_2Cl_2-THF (10:5, 15 mL), was added acetic anhydride (6 drops) at rt. The reaction solution was stirred overnight, and the solvents were evaporated in vacuo. The residue containing crude product was purified by flash silica gel chromatography with 1~2% EtOAc in CH_2Cl_2 to obtain pure diacetylated compound **3** (20 mg, 80%

yield) as a white solid. By analogous methods, compound **4** was prepared. Sch 53827 (**3**): $[\alpha]^{22}_D$ + 74.0° (*c* = 0.1, $CHCl_3$); mp 182–183 °C; FAB-MS m/z (relative intensity) 419 [58, $(M+H)^+$], 418 (76, M^+), 359 (65), 346 (68), 311 (100), 245 (69); UV (MeOH) λmax nm (ε) 225 (46, 850), 289 (6, 210), 300 (4, 950), 313 (4, 290), 378 (3, 780); IR (KBr) nmax cm^{-1} 3430, 1760, 1610, 1415, 1380, 1270, 1120, 1060, 818, 750; ^1H NMR (400 MHz, $CDCl_3$) δ 2.16 (s, 3H), 2.34 (s, 3H), 3.54 (t, J = 3.9 Hz, 1H), 3.76 (d, J = 3.9 Hz, 1H), 6.73 (d, br, J = 2.4 Hz, 1H), 6.96 (d, J = 7.3 Hz, 1H), 7.14 (d, J = 7.3 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.46 (t, J = 8.0 Hz, 1H), 7.52 (t, J = 8.0 Hz, 1H), 7.54 (t, J = 8.0 Hz, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.58 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 169.1, 149.3, 147.3, 147.2 134.1, 133.8, 130.8, 127.7, 127.5, 125.3, 124.6, 123.1, 121.1, 121.0, 112.9, 110.0, 109.1, 97.0, 61.8, 50.6, 50.0, 20.9, 20.9.

14. Sch 53829 (**4**): $[\alpha]^{22}_D$ + 146.0° (*c* = 0.1, $CHCl_3$); mp 108–109 °C; FAB-MS m/z (relative intensity) 455 [37, $(M+3H)^+$], 454 [72, $(M+2H)^+$], 453 [82, $(M+H)^+$], 452 (100 M^+), 395 (32), 393 (73), 353 (28), 351 (58), 183 (42), 181 (75); UV (MeOH) λmax nm (ε) 225 (48, 750), 290 (6, 500), 299 (4, 650), 313 (4, 110), 328 (3, 690); IR (KBr) vmax cm^{-1} 3410, 1755, 1605, 1410, 1385, 1275, 1090, 1060, 819, 745; ^1H NMR (400 MHz, $CDCl_3$) δ 2.18 (s, 3H), 2.40 (s, 3H), 3.51 (dd, J = 2.6, 3.9 Hz, 1H), 3.74 (d, J = 3.9 Hz, 1H), 6.68 (s, br, 1H), 6.95 (d, J = 7.6 Hz, 1H), 7.12 (d, J = 7.6Hz, 1H), 7.44 (t, J = 8.0 Hz, 1H), 7.50 (t, J = 8.0 Hz, 1H), 7.54 (d, J = 8.0 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.79 (d, J = 8.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 167.8, 147.2, 147.0, 134.1, 132.4, 131.5, 129.9, 127.7, 127.5, 126.3, 125.8, 121.2, 121.1, 112.8, 110.0, 109.2, 96.8, 61.8, 50.6, 49.9, 29.7, 20.8, 20.4.

15. Procedures for the labeling of the cells were as described previously [*Cf.* Pai, J-K.; Frank, E. A.; Blood C.; Chu, M. *Anti-Cancer Drug Design* **1994**, *9*, 363]. The assay mixtures, containing 10^7 prelabeled cell, 1.5 mM $CaCl_2$ and 5 μM cytochalasin B in a total volume of 450 μL Hepes-saline BSA buffer, were incubated (37 °C) for 5 min before initiating the reaction by adding *f*MLP (100 nM) in a volume of 50 μL. After 2 min incubation, the reaction was stopped by adding chloroform:methanol:acetic acid mixture (100:200:40, by volume). The phases were separated by the procedure of Bligh and Dyer [*Cf.* Bligh, E. G.; Dyer, W. J. *Canadian J. of Biochemistry and Physiology,* **1959**, *37*, 911]. One mL of water phase was transferred to vials and counted for radioactivity. Recovery of $[^3H]$-choline in the water phase indicates the induction of PLD activity. *f*MLP and testing compounds were initially dissolved in dimethylsulfoxide and then diluted with buffer to appropriate concentrations. The final concentration of dimethylsulfoxide in assay did not exceed 0.1%, which had no discernible effects on the parameter measured.

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