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A NOVEL CLASS OF ANTITUMOR METABOLITES FROM THE FUNGUS *NATTRASSIA MANGIFERAE*

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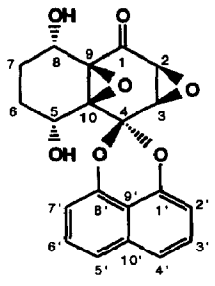
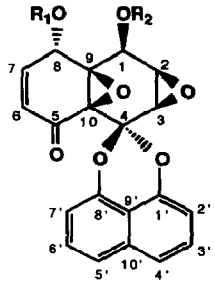
Abstract: *Sch 49210, Sch 53514 and Sch 53516 have been isolated from a fungal culture, and identified by analysis of 2D NMR data. Two diacetylation derivatives Sch 53515 and Sch 53517 have been synthesized. These compounds demonstrate inhibitory activity of phospholipase D (PLD), and display potent activity in the antitumor invasion chamber assay.*

During the course of searching for new antitumor metabolites from microorganisms, a novel keto-diepoxide family of compounds Sch 49210 (**1**), Sch 53514 (**2**) and Sch 53516 (**3**), have been isolated in our natural products program. The three antitumor agents were produced in the fermentation broth of a culture[1] identified as *Nattrassia mangiferae*[2]. The structure elucidation of these compounds indicated an unusual highly functionalized ketoepoxide decalone with a spiro-ketal linkage through a naphthalene moiety. In this paper we describe the isolation, structure and bioactivity of **1**, **2** and **3**.

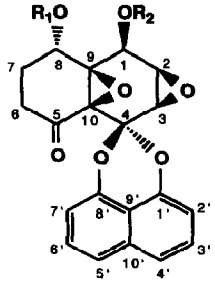
The fermentation broth (20 L) was extracted with ethyl acetate at harvest pH. The EtOAc extract was chromatographed on two consecutive flash silica gel columns with 2-15% EtOAc and 2-5% MeOH in CH_2Cl_2, respectively. Pure **1** (15 mg, mp = 140-143°C) and a mixture of **2** and **3** (20 mg) were obtained. The separation of **2** and **3** was accomplished by utilizing a polyvinyl alcohol coated silica gel column with a methanol: 1-chlorobutane solvent system (YMC, semi-preparative PVA-SIL column 20 x 250 mm, S-5, 5-10% MeOH in n-BuCl with a linear gradient in 20 min, 8 mL/min. flow rate, UV detection at 230 nm). Pure **2** (8 mg) and **3** (6 mg) were obtained as white solids with mp = 152-154°C (dec.) and 270-272°C (dec.), respectively.



Sch 49210(1)



Sch 53514(**2**) $R_1=R_2=H$
Sch 53515(**4**) $R_1=R_2=Ac$



Sch 53516(**3**) $R_1=R_2=H$
Sch 53517(**5**) $R_1=R_2=Ac$

The molecular formula of **1** was established as $C_{20}H_{16}O_7$ on the basis of HREIMS (Calcd: 368.0896, Found: 368.0886) and ^{13}C NMR data. UV absorptions at 225 and 298 nm suggested the presence of a naphthalene moiety. In the IR spectrum, hydroxyl and carbonyl functionalities (3409 and 1715 cm^{-1}) were observed. As shown in Table 1, ^1H NMR data of **1** revealed the presence of six naphthalene proton multiplets at δ 7.05 -7.67. Two multiplets at δ 4.65 and 4.85 were assigned to methine protons of secondary hydroxyl groups. Two doublets at δ 3.44 and 3.80 (J=4.1 Hz) represented a cis-disubstituted epoxide as well as two

multiplets at δ 1.60 and 1.95 showed two methylene groups. ^{13}C NMR data (Table 2) were consistent with ^{1}H NMR interpretations. Two methylene carbons at δ 20.55 and 23.06 together with two oxymethine signals at δ 61.81 and 62.72 were assigned to a saturated 1,4-diol system. The observation of two oxy-quaternary and two oxy-methine carbons revealed the presence of a tetra, as well as a disubstituted epoxide. The resonances at δ 95.09 and 199.6 indicated a ketal and a carbonyl carbon, respectively. The remaining ten aromatic carbons were assigned to a 1,8-disubstituted naphthalene.

Table 1. ^{1}H NMR data of **1**, **2** and **3**[a]

	1	2	3
1	--	5.37 (br.s)	4.99 (br.s)
2	3.45 (d, 4.1)[b]	3.63 (m)	3.58 (m)
3	3.80 (d, 4.1)	3.64 (m)	3.58 (m)
5	4.85 (m)	--	--
6	1.52-1.92 (m)	6.05(d, 10.6)	1.84-2.22 (m)
7	1.45-2.15 (m)	6.77 (dd, 4.8, 10.6)	2.41-2.73 (m)
8	4.66 (m)	5.15 (m)	4.88 (m)
2'	7.58 (d, 8.0)[c]	7.58 (d, 8.0)[c]	7.58 (d, 8.0)[c]
3'	7.48 (t, 8.0)	7.47 (t, 8.0)	7.46 (t, 8.0)
4'	7.05 (d, 7.2)	7.00 (d, 7.5)	7.00 (d, 7.3)
5'	7.24 (d, 7.2)	7.19 (d, 7.5)	7.17 (d, 7.3)
6'	7.52 (t, 8.0)	7.52 (t, 8.0)	7.51 (t, 8.0)
7'	7.60 (d, 8.0)	7.60 (d, 8.0)	7.60 (d, 8.0)
OH	3.12[d] (br.s)	4.40[d] (br.s)	2.84[d] (br.s)
OH	2.51[d] (d, 4.1)	3.77[d] (br.d)	1.70[d] (br.s)

a. Recorded at 300 MHz in $CDCl_3$, chemical shifts in ppm from TMS.
b. Multiplicity and coupling constant (Hz) in parentheses.
c. The assignments of naphthalene protons between 2' and 7', 3' and 6', as well as 4' and 5' are interchangeable.
d. Exchangeable with D_2O.

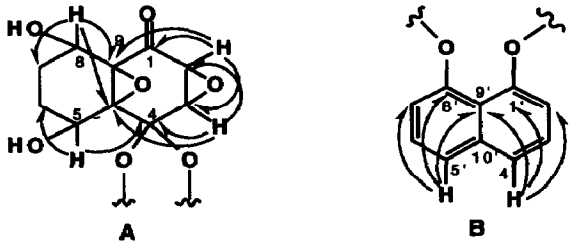
Table 2. ^{13}C NMR data of **1**, **2** and **3**[a,b]

Carbon	1	2	3
1	199.6 s	61.44 s	61.73 d
2	54.49 d	54.40 d	54.03 d
3	56.24 d	55.58 d	55.49 d
4	95.09 s	94.58 s	94.74 s
5	61.81 d	187.6 s	198.3 s
6	20.55 t	126.9 d	24.35 t
7	23.06 t	142.8 d	32.60 t
8	62.72 d	61.44 d	62.50 d
9	64.30 s	63.28 s	63.91 s
10	70.85 s	70.86 s	69.70 s
1'	144.7 s	145.4 s	145.5 s
2'	121.4 d	121.3 d	121.1 d
3'	127.1 d	127.5 d	127.4 d
4'	109.3 d	109.1 d	108.9 d
5'	110.1 d	109.9 d	109.6 d
6'	127.3 d	127.9 d	127.8 d
7'	121.3 d	121.2 d	121.0 d
8'	144.9 s	145.6 s	145.7 s
9'	112.0 s	112.2 s	112.2 s
10'	133.6 s	134.4 s	134.3 s

a. Recorded at 75 MHz in $CDCl_3$, chemical shifts in ppm from TMS.
b. Multiplicity was determined by APT or DEPT data.

Analysis of 2D-NMR data, including COSY, HETCOR and SINEPT experiments, permitted the establishment of partial structures A and B. As shown in Fig. 1, long range NMR correlations between A and B were not observed in SINEPT experiments. Therefore, the connection between decalone A and naphthalene B must be linked through two oxygen forming a spiro-ketal ring.

Fig. 1 Structure of 1 as revealed by SINEPT experiments.
Arrows indicate ^{1}H-^{13}C long range couplings



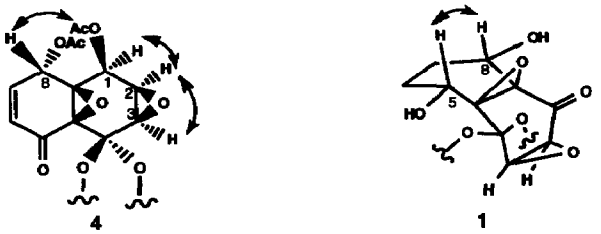
A B

Compound 3 was found to be analogues of 1 due to a striking resemblance of UV and IR spectral data. The molecular weight of 3 was identical to 1 based on CI-MS data (m/z 369, $M+H^{+}$). Analysis of ^{1}H and ^{13}C NMR data of 3 (Table 1 and 2) indicated a position change of the carbonyl and hydroxyl groups in comparison with 1. Correlation between H-1 and H-2 observed in the COSY experiment led to the assignment of a carbonyl at position-5 and a hydroxyl at position-1. The remaining structure of 3 was determined to be the same as 1.

The molecular weight of 2 was determined to be 366 based on CI-MS data (m/z 367, $M+H^{+}$) indicating additional unsaturation in comparison to 3. The IR spectral data of 2 further suggested the presence of a conjugated ketone functionality (1694 cm^{-1}). The assignment of the double bond at position-6,7 was supported by the observation of vinyl carbons at δ 126.9 and 142.8, as well as olefinic protons at δ 6.05 and 6.77 in the ^{13}C and ^{1}H NMR spectra, respectively.

In addition, 2 and 3 were acetylated with Ac_2O/pyridine to afford Sch 53515 (4)[3] and Sch 53517 (5)[4] for the purpose of stereochemical analysis. As shown in Fig. 2, NOESY experiment on 4 demonstrated a *cis* arrangement for H-1, H-2 and H-3 as well as *trans* configuration for H-1 and H-8. Determination of the relative stereochemistry of the two epoxide groups for this family of compounds, however, can not be accomplished by NMR techniques due to the lack of protons on the ring junction at position-9,10. The *cis* configuration of the two epoxide oxygens was unambiguously established by single crystal X-ray diffraction analysis on the major component.[5] Therefore, all these diepoxides 1, 2 and 3 were assigned to be the same *cis* configuration as the major component based on analysis of the X-ray data.

Fig. 2 Important NOESY data for 4 and 1



4 1

The stereochemistry of **1** was determined based on NOESY data (see Figure 2) by the observation of a weak correlation from H-8 to H-5 due to the 1,4-diaxial interaction between these two protons. The configuration of H-8 was presumed to be analogous to that of **2**.

Biological evaluation of these five compounds exhibited potent *in vitro* inhibitory activity in phospholipase D (PLD) assay, and anti-invasive activity against various tumor cells in the antitumor invasion chamber assay. The most potent compounds, **1** and **2**, showed IC_{50} values of 1.6 and 0.2 μM in PLD assay, as well as 0.26 and 0.37 μM against HT 1080 human fibrosarcoma in invasion assay, respectively. Preliminary *in vivo* study of **1** displayed encouraging results indicating a reduction of primary and metastatic tumors. Detailed investigations will be published elsewhere.[6]

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References:

1. The fungus was supplied by Dr. B. Katz from MYCOsearch.
2. Sutton, B.C. and Dyko, B.J., *Mycol. Res.* **93** (4), 466 (1989).
3. Sch 53515 (**4**): mp = 225-227°C (dec.), ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.24 (s, 3H, $COCH_3$), 2.27 (s, 3H, $COCH_3$), 3.49 (dd, 1H, J=2.9, 4.3 Hz, H-2), 3.59 (d, 1H, J=4.3 Hz, H-3), 5.95 (d, 1H, J=4.9 Hz, H-8), 6.12 (d, 1H, J=10.9 Hz, H-6), 6.18 (d, 1H, J=2.9 Hz, H-1), 6.54 (dd, 1H, J=4.9, 10.9 Hz, H-7), 7.02 (d, 1H, J=7.6 Hz, H-4'), 7.18 (d, 1H, J=7.6 Hz, H-5'), 7.48 (t, 1H, J=8.0 Hz, H-3'), 7.52 (t, 1H, J=8.0 Hz, H-6'), 7.58 (br.d, 2H, J=8.0 Hz, H-2' and H-7'), ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.37, 20.37, 52.47, 52.93, 61.80, 62.98, 66.07, 66.07, 94.45, 109.0, 110.1, 112.1, 121.2, 121.4, 127.4, 128.0, 129.1, 134.4, 137.1, 145.3, 145.6, 169.8, 170.8, 186.5. EI-MS (relative intensity) m/z 450 (18, M^+), 160 (26), 115 (48), 60 (100).
4. Sch 53517 (**5**): mp = 120-122°C, ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.91-2.25 (m, 2H, CH_2-7), 2.24 (s, 3H, $COCH_3$), 2.28 (s, 3H, $COCH_3$), 2.50 (dd, 2H, J=4.5, 8.9 Hz, CH_2-6), 3.49 (t, 1H, J=2.9 Hz, H-2), 3.53 (d, 1H, J=4.3 Hz, H-3), 5.67 (t, 1H, J=3.2 Hz, H-8), 5.94 (d, 1H, J=2.9 Hz, H-1), 7.00 (d, 1H, J=7.5 Hz, H-4'), 7.15 (d, 1H, J=7.5 Hz, H-5'), 7.46 (t, 1H, J=8.0 Hz, H-3'), 7.50 (t, 1H, J=8.0 Hz, H-6'), 7.56 (br.d, 2H, J=8.0 Hz, H-2' and H-7'). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.39, 20.73, 21.47, 32.71, 52.41, 52.92, 62.69, 63.58, 64.95, 65.30, 94.54, 109.0, 110.0, 112.2, 121.1, 121.3, 127.3, 128.0, 134.4, 145.4, 145.6, 170.1, 170.8, 195.9. EI-MS (relative intensity) m/z 452 (51, M^+), 211 (21), 160 (67), 115 (100), 55 (67).
5. The paper which contains X-ray crystallographic data for the major component of keto-diepoxide family of compounds is in press, and will be published in *J. Org. Chem.*
6. A manuscript providing details of taxonomy, fermentation, isolation and biological evaluation is in preparation.

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