Aranochlor A and Aranochlor B, Two New Metabolites from *Pseudoarachniotus roseus*: Production, Isolation, Structure Elucidation and Biological Properties

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During our screening program for secondary metabolites, we have isolated two new compounds named aranochlor A (**1**)[1] and aranochlor B (**2**)[1], as minor components from the fermented broth of the fungal strain *Pseudoarachniotus roseus* (HIL Y-30499). Earlier, we had reported the isolation and characterization of aranorosin[2,3], aranorosinol A[4] and aranorosinol B[4] from the same fungal strain. Herein, we report the isolation, structure elucidation and biological properties of aranochlor A (**1**) and aranochlor B (**2**), which are structurally related to aranorosin.

Fermentations were carried out in shake flasks as well as in laboratory fermenters using the methods and conditions reported earlier[2]. For the isolation of **1** and **2**, six batches of each 100 liters were processed. **1** and **2** were present in both the culture filtrate and the mycelium.

The culture filtrate (500 liters, pH 7) was extracted with ethyl acetate (2 × 180 liters). The mycelial cake (34.6 kg) was extracted with acetone (2 × 170 liters). The combined acetone extracts were concentrated under reduced pressure, diluted with water and extracted with ethyl acetate (2 × 30 liters). These ethyl acetate extracts were combined with those of the culture filtrate, concentrated under reduced pressure and lyophilized to get crude material (500 g). The crude material was chromatographed on silica gel (60~120 mesh, 1.5 kg, flow rate: 150 ml/minute) using 1%, 2%, 3% and 5% methanol in chloroform for elution. The active fractions, which eluted in 1~3% methanol, were combined and concentrated under reduced pressure to get 170 g of enriched material. This was subjected to a silica gel (60~120 mesh, 1.3 kg, flow rate: 100 ml/minute) column using a step-gradient of ethyl acetate in chloroform in 10% increments. A mixture of **1** and **2** and aranorosin eluted out with 40~50% ethyl acetate in chloroform. This mixture (28 g) was subjected to two successive chromatographies on MPLC (silica gel, 230~400 mesh, 900 g) using a step-gradient of methanol (increments of 0.5%) in chloroform at a flow rate of 35 ml/minute; a mixture of **1** and **2** (0.5 g) eluted in 1.5~2% methanol, while aranorosin eluted in 2~3% methanol. Finally, **1** and **2** were separated on a silica gel (70~230 mesh, 20 g) column using a step-gradient of ethyl acetate in dichloromethane in increments of 1% for elution at a flow rate of 8 ml/minute. Pure aranochlor A (**1**) (90 mg) eluted in 3~4% ethyl acetate and aranochlor A (**2**) (170 mg) in 5~6% ethyl acetate in dichloromethane. The purification was monitored by silica gel TLC (Article No. 5554, E. Merck) using CH_2Cl_2 - EtOAc (7 : 3) or Pet. ether - EtOAc (1 : 1) as mobile phase.

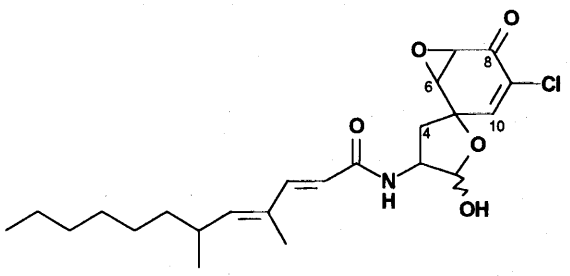
Aranochlor A (**1**) [MP 73~75°C; $[\alpha]_D$ +23.84° (*c* 0.26, MeOH); TLC Rf: 0.44 (70 : 30 CH_2Cl_2 - EtOAc) and 0.4 (50 : 50 Pet. ether - EtOAc); DCI-MS: 438 $(M+H)^+$; *Anal.* Found: C 63.4, H 7.2, N 3.0, Cl 9.1. Calcd for $C_{23}H_{32}ClNO_5$: C 63.1, H 7.3, N 3.2, Cl 8.1; UV (MeOH); 266 nm; IR (KBr): 3410, 3320, 1725, 1668, 1630, 1540, 970 and 865 cm^{-1}] and aranochlor B (**2**) [MP 69~71°C; $[\alpha]_D$ −102.5° (*c* 0.4, MeOH); TLC Rf: 0.39 (70 : 30 CH_2Cl_2 - EtOAc) and 0.35 (50 : 50 Pet. ether - EtOAc); DCI-MS: 438 $(M+H)^+$; *Anal.* Found: C 61.8, H 7.3, N 3.1, Cl 7.8. Calcd for $C_{23}H_{32}ClNO_5$ 0.5 H_2O: C 61.9, H 7.2, N 3.1, Cl 8.0; UV (MeOH): 266 nm; IR (KBr): 3400, 3300, 1720, 1665, 1625, 1545, 970 and 860 cm^{-1}] were obtained as white powders and were found to be soluble in CH_2Cl_2, $CHCl_3$, EtOAc, MeOH and DMSO.

The ^1H and ^{13}C NMR data of **1** and **2** are listed in Table 1. A comparison of the ^1H and ^{13}C NMR spectra of **1** and **2** with those of aranorosin[3] showed close structural similarity of the compounds. However, both **1** and **2** differ from aranorosin in having one chlorine atom each and the absence of one epoxide. Instead, both showed the presence of an additional double bond [**1**: δ_C: 128.0 (s), 143.4 (d); δ_H: 6.64 (d, 2.5 Hz); **2**: δ_C: 127.43 (s), 144.76 (d); δ_H: 6.75 (d, 2.5 Hz)]. The presence of only one additional olefinic proton suggested that the chlorine atom was on the double bond. The position of this double bond was established by NOE studies.

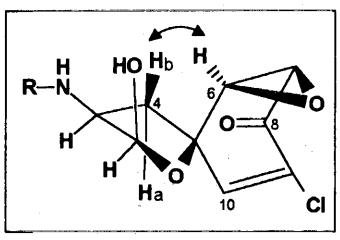
In the 2D NOESY spectrum of **1**, the correlation between the epoxy proton at δ 3.78 and 4-H_b proton at δ 2.14 suggested that the epoxide was present at C6-C7

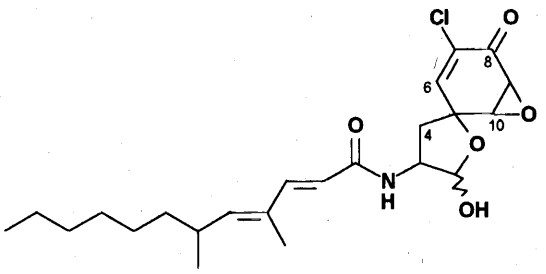
Table 1. ^{13}C and ^{1}H NMR data of aranochlor A (**1**) and aranochlor B (**2**) in $CDCl_3$.

Position	^{13}C (22.5 MHz)		^{1}H (300 MHz)	
	1	**2**	**1**	**2**
2	96.48 (d)	96.45 (d)	5.54 (d, 4.4 Hz)	5.55 (d, 4.3 Hz)
3	52.03 (d)	51.58 (d)	4.76 (m)	4.78 (m)
4	39.09 (t)	38.07 (t)	2.57 (dd, 13, 8.5 Hz)	2.64 (dd, 13, 10.6 Hz)
			2.14 (dd, 13, 10.6 Hz)	2.12 (dd, 13, 10.6 Hz)
5	80.03 (s)	78.94 (s)	—	—
6	58.34 (d)	144.76 (d)	3.78 (dd, 3.6, 2.5 Hz)	6.75 (d, 2.5 Hz)
7	53.03 (d)	127.43 (s)	3.60 (d, 3.6 Hz)	—
8	186.35 (s)	186.21 (s)	—	—
9	128.00 (s)	56.91 (d)	—	3.62 (m)
10	143.40 (d)	52.53 (d)	6.64 (d, 2.5 Hz)	3.62 (m)
1'	167.07 (s)	166.99 (s)	—	—
2'	116.82 (d)	116.70 (d)	5.74 (d, 15.2 Hz)	5.74 (d, 15.2 Hz)
3'	147.72 (d)	147.69 (d)	7.24 (d, 15.2 Hz)	7.22 (d, 15.2 Hz)
4'	130.83 (s)	130.74 (s)	—	—
5'	148.89 (d)	148.83 (d)	5.65 (d, 10 Hz)	5.65 (d, 10 Hz)
6'	33.29 (d)	33.20 (d)	2.52 (m)	2.56 (m)
7'	37.27 (t)	37.15 (t)	1.28 (br. s)	1.28 (br. s)
8'	27.52 (t)	27.42 (t)	1.28 (br. s)	1.28 (br. s)
9'	29.43 (t)	29.33 (t)	1.28 (br. s)	1.28 (br. s)
10'	31.88 (t)	31.76 (t)	1.28 (br. s)	1.28 (br. s)
11'	22.67 (t)	22.75 (t)	1.28 (br. s)	1.28 (br. s)
4'-CH_3	12.55 (q)	12.45 (q)	1.79 (d, 0.9 Hz)	1.78 (d, 0.9 Hz)
6'-CH_3	20.56 (q)	20.43 (q)	0.99 (d, 6.7 Hz)	0.99 (d, 6.5 Hz)
11'-CH_3	14.14 (q)	14.03 (q)	0.91 (t, 6.5 Hz)	0.91 (t, 6.8 Hz)
2-OH	—	—	Not seen	Not seen
NH	—	—	6.03 (d, 7.2 Hz)	6.02 (d, 7.2 Hz)



(1)





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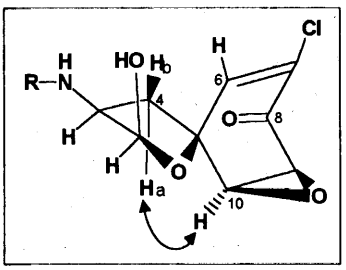

Table 2. *In vitro* antibacterial and antifungal activity (MIC) of aranochlor A (**1**) and aranochlor B (**2**).

Test Organism	MIC (mg/ml)	
	Aranochlor A	Aranochlor B
Staphylococcus aureus	3.12	1.56
Bacillus subtilis	3.12	3.12
Micrococcus luteus	0.39	0.39
Escherichia coli ESS 2231	50.00	25.00
Pseudomonas aeruginosa	>200	>200
Candida albicans	>200	>200
Saccharomyces cerevisiae	1.56	6.25
Aspergillus niger	>200	>200

position and hence, the double bond was located at C9-C10. Similar experiment with **2** showed correlation between the epoxy proton at δ 3.62 and 4-H_a proton at δ 2.64 indicating that the epoxide was present at C9-C10 position and therefore, the double bond at C6-C7.

Further, the position of the chlorine in **1** and **2** was considered to be α to the C8-carbonyl group by comparing the ^{13}C NMR chemical shifts of C10 in **1** and C6 in **2** with those reported for 2-cyclohexen-1-one and its chloro analogs[5,6]. The structures of aranochlors A and B were, thus, established as **1** and **2** respectively.

Aranochlor A (**1**) and aranochlor B (**2**) exhibited antibacterial and antifungal activity. The minimum inhibitory concentrations (MIC) of **1** and **2** required to inhibit a variety of bacterial and fungal strains are listed in Table 2.

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