Structure-activity Relationships of New Aranorosin Analogs to Antifungal Activity

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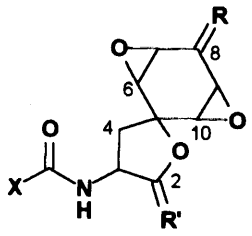
Aranorosin (**1**) is a novel antifungal antibiotic isolated from the fungal strain *Pseudoarachniotus roseus* (HIL Y-30499)[1~3]. The antifungal activity of **1** was comparable to ketoconazole in the *in vitro* assays. Also isolated from the same species were aranorosinol A (**2**) and aranorosinol B (**3**)[4], which were the reduced forms of **1** at C8-position and aranochlor A (**4**) and aranochlor B (**5**)[5], which were the monoepoxy chloro compounds. The antifungal activity was found to be reduced considerably in the case of **2**~**5** suggesting that the cyclohexanone bisoxirane moiety may have a critical role for the antifungal activity of aranorosin (**1**). This prompted us to initiate a semisynthetic program to study the structure-activity relationships and to prepare more potent analogues. Herein, we report the preparation of a series of derivatives and their biological properties.

Esters **7**~**10** were prepared by treating aranorosin (**1**) with the corresponding anhydride (2.5 equiv.) in pyridine at room temperature for 24 hours. Esters **11**~**13** were prepared by stirring a mixture of **1** with the corresponding carboxylic acid (1.1 equiv.) in the presence of dicyclohexylcarbodiimide (DCC) (1.1 equiv.) and 4-dimethylaminopyridine (DMAP) (0.1 equiv.) in CH_2Cl_2 at room temperature for 24 hours. The crude products, obtained after usual work up, were purified by preparative TLC on silica gel (Article No. 13794, E. Merck) using $CHCl_3$-MeOH (9:1) as the developing solvent.

Ethers **14**~**21** were prepared by refluxing **1** with the corresponding alcohol (4 equiv.) and *p*-TsOH (1 equiv.) in dry THF for 15 hours. The products were purified as above.

Attempts to modify the C-8 carbonyl of **1** by Wittig reaction[6] resulted in a very complex reaction mixture. However, the same reaction on ketolactone (**6**)[2,3] proceeded cleanly to give compounds **22**~**23** which are the two geometrical isomers. The two compounds differed in their TLC, Rf and melting point; however, the configuration of the individual isomers could not be established. Thus, **6** was stirred with phosphorane (1.1 equiv.) (prepared from triphenyl phosphine and ethyl bromoacetate) in CH_2Cl_2 at room temperature for 3 hours. The mixture was purified by silica gel (200~400 mesh) column chromatography using petroleum ether-



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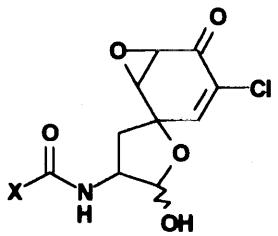

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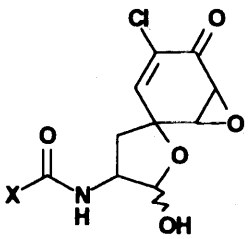

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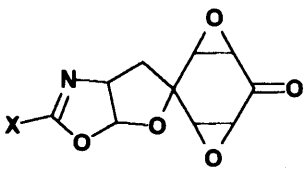

X=–CH=CH–C(CH$_3$)=CH–CH(CH$_3$)–(CH$_2$)$_5$–CH$_3$

1: R=O; R′=H, OH
2: R=R′=H, OH
3: R=OH, CH_2COCH_3; R′=H, OH
6: R=R′=O
7: R=O; R′=H, acetyloxy
8: R=O; R′=H, propionyloxy
9: R=O; R′=H, *iso*-butyryloxy
10: R=O; R′=H, valeryloxy
11: R=O; R′=H, octanoyloxy
12: R=O; R′=H, decanoyloxy
13: R=O; R′=H, myristoyloxy
14: R=O; R′=H, methoxy
15: R=O; R′=H, ethoxy
16: R=O; R′=H, propoxy
17: R=O; R′=H, butoxy
18: R=O; R′=H, pentyloxy
19: R=O; R′=H, hexyloxy
20: R=O; R′=H, octanyloxy
21: R=O; R′=H, decanyloxy
22: R=CHCOOC$_2$H$_5$; R′=O (Isomer 1)
23: R=CHCOOC$_2$H$_5$; R′=O (Isomer 2)

Table 1. Physico-chemical characteristics of compounds **7**~**24**.

Compound	Yield (%)	MP (°C)	MS $(M+H)^+$	Mol. formula	Antimicrobial activity[a]		
					Bacillus subtilis	*Aspergillus niger*	*Candida albicans*
1[2,3]	—	150 (dec)	419	$C_{23}H_{33}NO_6$	24	28	17
6[2,3]	—	175~178	417	$C_{23}H_{31}NO_6$	18	26	Sl
7[2,3]	68	108~110	462	$C_{25}H_{35}NO_7$	19	22	11
8	56	119~121	476	$C_{26}H_{37}NO_7$	21	21h	NA
9	79	179~180	490	$C_{27}H_{39}NO_7$	18	20h	NA
10	59	167~169	504	$C_{28}H_{41}NO_7$	14	16h	NA
11	87	166~168	546	$C_{31}H_{47}NO_7$	NA	NA	NA
12	55	145~147	574	$C_{33}H_{51}NO_7$	NA	NA	NA
13	60	126~128	630	$C_{37}H_{59}NO_7$	NA	NA	NA
14	64	128~129	434	$C_{24}H_{35}NO_6$	21	20h	NA
15	59	67~69	448	$C_{25}H_{37}NO_6$	19	21h	NA
16	42	68~70	462	$C_{26}H_{39}NO_6$	16	16h	NA
17	63	60~61	476	$C_{27}H_{41}NO_6$	15	15h	NA
18	46	57~59	490	$C_{28}H_{43}NO_6$	14	13h	NA
19	55	77~78	504	$C_{29}H_{45}NO_6$	NA	NA	NA
20	52	[b]	532	$C_{31}H_{49}NO_6$	NA	NA	NA
21	52	[b]	560	$C_{33}H_{53}NO_6$	NA	NA	NA
22	8	71~73	488	$C_{27}H_{37}NO_7$	NA	NA	NA
23	14	180~183	488	$C_{27}H_{37}NO_7$	NA	NA	NA
24	60	117~118	402	$C_{33}H_{31}NO_5$	30	21	NT

[a] Inhibition zone size in mm at 1 mg/ml concentration. [b] Low melting solids.
NA: Not active; NT: Not tested; h=hazy; Sl: Slight.

ethyl acetate mixtures followed by preparative TLC on silica gel (Article No. 13794, E. Merck) using petroleum ether-ethyl acetate (35:65) as the developing solvent.

Further, in order to study the effect of side chain on the bioactivity, attempts were made to hydrolyze the amide linkage and introduce different side chains. Due to the isomeric and unstable nature of aranorosin (**1**), the hydrolysis of the amide was tried under mild conditions by stirring a solution of aranorosin in CH_2Cl_2 with triethyl oxonium fluoborate[7] (3 equiv.) at room temperature for 5 hours. The crude product was purified by chromatography on silica gel (70~230 mesh) using petroleum ether-ethyl acetate (10:90) for elution. The compound **24**, thus obtained, was not however the expected product.

All the compounds **7**~**24** were obtained as white solids and their physico-chemical properties and the *in vitro* antibacterial and antifungal activities are summarized in Table 1. The compounds were characterized by DCI-MS and ^1H NMR (90 and 300 MHz) spectra recorded in $CDCl_3$.

It was evident from the data that the antifungal activity reduced gradually with increasing lipophilicity (increasing the chain length of the esters and ethers) at the C-2 position. The modification of the C8-carbonyl group as in the case of **23**~**24** as well as in the naturally occurring compounds **2**~**3** suggest that the carbonyl group is necessary for the antifungal activity. All the modifications so far indicate that the cyclohexanone bisoxirane moiety is essential for the antifungal activity of aranorosin (**1**). The influence of side chain on the activity profile could not be evaluated.

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