SCH57404, an Antifungal Agent Possessing the Rare Sodaricin Skeleton and a Tricyclic Sugar Moiety

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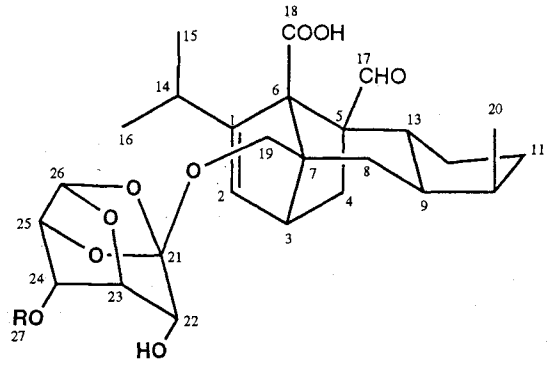
In the course of screening microbial fermentations for potential pharmaceutical activity, the extract from an unidentified fungus (Schering culture number SCF1082A) was found to inhibit *Candida albicans*. Various media and fermentation conditions were examined in an effort to maximize production of the antifungal agent. The conditions which proved to be the most productive are as follows. Stock cultures were maintained as frozen whole broths at −80°C in a final concentration of 10% glycerol. The inoculum medium for SCH57404 production contained (g/liter) proteus peptone 5, NaCl 5, KH_2PO_4 5, yeast extract 3, cerelose 20, soybean grits 5, antifoam 1 ml, tap H_2O to 1 liter. The pH was adjusted to 7.2 prior to autoclaving. A 250-ml erlenmeyer flask containing 50 ml of this medium was inoculated with 2.0 ml of the stock culture. The flasks were incubated for 96 hours at 24°C on a rotary shaker at 250 rpm. This seed culture (2.5 ml) was used to inoculate another 250-ml erlenmeyer flask containing 50 ml of the same seed medium and the flask was incubated as above.

Five percent of the second germination was used to inoculate the fermentation medium containing (g/liter) neopeptone 10, cerelose 40, $CaCO_3$ 4, and tap H_2O to 1 liter. The pH was adjusted to 7.4 prior to autoclaving.



1 R=CH_3
2 R=Ac

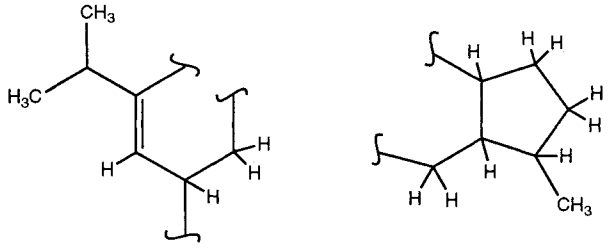
Table 1. NMR data for **1** and **2**.

	SCH 57404 (**1**)				**2**[2]
C-#	δ[a]	*m*	^{1}H δ[a,b]	Long range H to C correlations[c]	δ[d]
1	147.8	s	—	—	147.3
2	131.6	d	6.14	147.8, 72.0, 65.4, 59.2, 46.5, 28.1	131.2
3	46.5	d	2.81	147.8, 131.6, 72.0, 59.2	46.0
4	29.7	t	1.94, 1.27	204.8, 131.6, 65.4, 59.2, 46.5, 42.0	29.2
5	59.2	s	—	—	58.9
6	65.4	s	—	—	65.1
7	72.0	s	—	—	71.6
8	29.0	t	1.95, 1.70	na[e]	28.5
9	41.6	d	1.7	na	41.2
10	31.4	d	2.05	na	30.8
11	32.3	t	2.05, 1.20	na	31.9
12	26.5	t	1.85, 0.95	na	26.1
13	42.0	d	1.9	na	41.6
14	28.1	d	2.31	147.8, 131.6	27.6
15	22.5	q	0.95	147.8, 28.1	22.3
16	21.2	q	1.04	147.8, 28.1	21.0
17	204.8	d	9.66	59.2, 42.2	204.6
18	177.5	s	—	—	176.9
19	67.1	t	3.96	118.7, 67, 65.4, 46.5, 29.0	66.9
20	17.5	q	0.75	41.6, 31.4	17.2
21	118.7	s	—	—	118.4
22	78.5	d	4.28	118.7, 100.7, 74.7, 67.0	74.2
23	80.2	d	3.65	118.7, 78.5, 74.7, 67.0, 57.8	77.7
24	67.0	d	3.64	118.7, 78.5, 74.7, 67.0, 57.8	66.4
25	74.7	d	4.57	118.7, 100.7, 78.5	72.2
26	100.7	d	5.70	118.7, 78.5, 74.7	99.9
27	57.8	q	3.45	67.0	OAc

[a] in $CDCl_3$, [b] assigned by HETCOR, [c] observed by SINEPT, [d] in CD_2Cl_2, [e] not assigned.

† SCH 57404 (**1**): $[\alpha]_D$ −60° (MeOH); UV (EtOH) λ_{max} 204 (3460); IR (KBr) cm^{-1} 3410, 2955, 1710, 1275, 1065, 1029, 987; MS see discussion in text; ^{1}H and ^{13}C NMR see Table 1.

Scheme 1. Partial structures **a** and **b**.



a **b**

The fermentation was carried out in 500-ml erlenmeyer flasks containing 100 ml of the fermentation medium. The flasks were incubated for 120 hours at 24°C on a rotary shaker at 250 rpm.

Upon harvest, the fermentation broth was gravity filtered and extracted with ethyl acetate. Antifungal bioassay-guided fractionation of the extract, first using silica with gradient elution from CH_2Cl_2 to 30% MeOH - CH_2Cl_2, and then on Sephadex LH-20 with 1 : 1 CH_2Cl_2 - CH_3CN, gave pure SCH 57404 (**1**)† as the compound responsible for the antifungal activity (typical yield was 19 mg/liter).

The molecular formula of **1** was determined by high resolution FAB mass spectral peak matching. This analysis indicated the molecular formula $C_{27}H_{36}O_9$ ($(M+H)^+$ observed 505.2443 and calculated 505.2438 for $C_{27}H_{37}O_9$). The major fragment ion at 315.1960 was peak matched for $C_{20}H_{27}O_3$ (calculated 315.1960), which indicated the loss of $C_7H_9O_6$. These mass spectral data, in conjunction with the NMR data (Table 1), gave the first indication that SCH 57404 was a diterpene glycoside.

A preliminary analysis of the NMR data (Table 1)[1] showed the presence of an aldehyde, a carboxylic carbonyl, and a single olefinic double bond. Since all four carbons involved in these three unsaturated functions showed long range C-H correlations to non-sugar protons, it was apparent that these three double bonds were part of the aglycone. Based on the molecular formula indicated by the fragment at *m/z* 315.1960, the aglycone should possess seven double bond equivalents. Thus the aglycone must be tetracyclic. The three remaining double bond equivalents, required by molecular formula for the intact glycoside, can only be accounted for by a tricyclic sugar residue.

Further analysis of the NMR data (COSY, HETCOR, SINEPT) allowed the straight forward elucidation of the partial structures **a** and **b**. However, the correlations leading from **a** and **b** to the three contiguous quaternary carbons 5, 6 and 7 were so numerous that several plausible structures could be drawn. A survey of the literature revealed the compound BE31405 (**2**), an antifungal agent reported in a Japanese patent,[2] that is remarkably similar to SCH57404. A comparison of the ^{13}C NMR data for **1** and **2** is shown in Table 1. Careful analysis of all the spectral data for **1** and **2** indicated that SCH57404 differs from **2** by the presence of a methoxy in place of the acetate at C-4 of the tricyclic sugar moiety. Thus SCH57404 is only the second example of a fungal metabolite possessing this very unusual tricyclic sugar moiety. Both **1** and **2** are related to sordarin,[3] a rare class of tetracyclic diterpenes.

SCH57404 had narrow spectrum *in vitro* antifungal activity with a geometric mean MIC of 16 μg/ml against *Candida albicans*, but greater than 128 μg/ml against dermatophytes and *Aspergillus*.

Acknowledgment

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References

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3) HAUSER, D. & H. P. SIGG: Isolierung und Abbau von Sordarin. Helv. Chim. Acta 54: 1187~1190, 1971