PAXISTEROL, A NEW ANALGESIC STEROL WITHOUT ANTI-INFLAMMATION ACTIVITY FROM *PENICILLIUM*

Sir:

We have screened fungi isolated from soils and plants for their ability to produce new steroids which inhibit growth of a strain of *Saccharomyces cerevisiae* in a medium without supplement of ergosterol. We now have isolated a new sterol from a culture broth of a *Penicillium* and found that this new sterol, named paxisterol, has analgesic activity in the mouse AcOH writhing assay[1]. In contrast to corticosteroid, paxisterol does not have anti-inflammatory activity. These results suggest that paxisterol is a new class of analgesic sterol and is of interest to use in modifying pain which has been difficult to relieve.

We have isolated a new fungal steroid from a fermentation broth of a *Penicillium* KAC 1843. Strain KAC 1843 was isolated from fallen leaves of a *Saghalien spruce* growing in Onuma Park, Hokkaido, Japan, and has been identified as *Penicillium paxilli*. Fermentation was carried out at 25°C for 5 days under aeration and agitation in a 30-liter jar fermentor containing 18 liters of a culture medium consisting of sucrose 5%, corn steep liquor 2%, KH_2PO_4 0.05%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCO_3$ 0.5%, pH 7.0. Paxisterol accumulated in both mycelium and extra cellular medium and so was extracted with EtOAc from the whole culture broth. The extract was chromatographed on a silica gel column using a mixture of $CHCl_3$ - EtOAc to give a crude precipitate. The crude solid product was recrystallized from EtOAc to give white-needled crystals of paxisterol: Yield 1.4 g. The physico-chemical properties of paxisterol are: $C_{28}H_{42}O_4$ (found: 442 (M^+), C 75.97, H

Fig. 1. Structure of paxisterol.

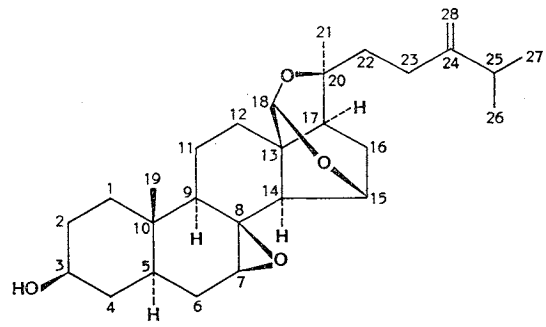
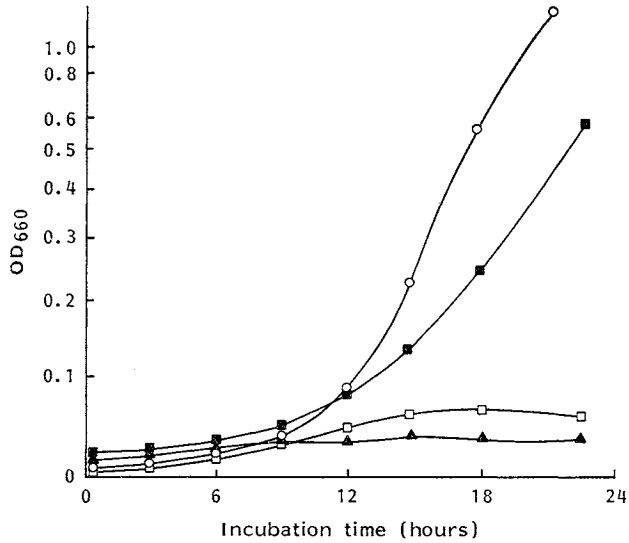


Fig. 2. Inhibition by paxisterol of growth of *Saccharomyces cerevisiae* in culture with and without ergosterol.



Strain AM3-4B was grown in YPG medium (yeast extract 1%, peptone 2% and glucose 2%) at 30°C.

○ Without drug (control), □ paxisterol 5 μg/ml, ■ paxisterol 5 μg/ml and ergosterol 80 μg/ml, ▲ paxisterol 50 μg/ml and ergosterol 80 μg/ml.

9.56, N 0. $C_{28}H_{42}O_4$ requires M, 442 (M) C 75.978, H 9.564); mp 198.9°C; $[\alpha]_D^{25}$ −60.8° (*c* 1.0, $CHCl_3$). The structure (Fig. 1) was assigned by NMR spectroscopic studies and by chemical degradation which will be reported elsewhere[2].

Paxisterol is weakly active against *S. cerevisiae* but does not show antimicrobial activity against the following bacteria and phyto-pathogenic fungi; *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Candida albicans*, *Aspergillus fumigatus*, *Trichophyton rubrum*. The addition of paxisterol at 5 μg/ml to liquid medium inhibited the growth of *S. cerevisiae* AM3-4B[3], and ergosterol (80 μg/ml) restored cell growth as shown in Fig. 2.

Paxisterol shows analgesic activity in the AcOH induced writhing assay in a concentration-dependent pattern up to 100 mg/kg after intraperitoneal administration. ED_{50} value of paxisterol is 17 mg/kg. The lack of paxisterol anti-inflammation activity suggests that the mechanism of analgesic action of paxisterol is different from that of corticosteroids. Clinically, corticosteroids are widely used and highly effective agents in the relief of pain. The rationale generally given is that the primary therapeutic action of corticosteroids is their anti-inflammatory effects, for example by reducing the concentration of arachidonic acid metabolites, and the reduced inflammation has a secondary effect on nerve excitability[4]. Recent evidence also suggests that, in addition to this well-documented mode of action, steroids also interact directly on abnormally excitable nerve fibers[5,6]. Although the mechanism of analgesic action of paxisterol remains to be investigated, the discovery of paxisterol may contribute to develop a new class of analgesic agents without anti-inflammatory activity.

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