ANTIVIRAL ACTIVITIES OF PENTALENOLACTONES

Sir:

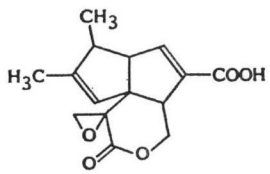
During the course of screening for antiviral antibiotics from microorganisms isolated from soil samples we found that pentalenolactones isolated from the fermentation broth of *Streptomyces* sp. OM-2718 possess potent antiviral activities. In this paper, we wish to describe the production, isolation and selective antiviral activities of pentalenolactones, especially the specific antiviral activity of pentalenolactone O.

To obtain antiviral substances specifically active against DNA viruses, we have picked out substances which exhibit antiviral activities against herpes simplex virus-1 (HSV-1) but not against vesiqular stomatitis virus (VSV) in Vero cells. *Streptomyces* sp. OM-2718 was obtained in this program. The seed culture was inoculated to a production medium (35 liters, glucose 0.1%, starch 2.4%, peptone 0.3%, meat extract 0.3%, yeast extract 0.5%, agar 0.1%, $CoCl_2 \cdot 6H_2O$ 20 mg/liter, $CaCO_3$ 0.4%, pH 7.0) in a 50-liter jar fermentor and incubated at 27°C for four days under aerobic conditions. The broth filtrate (30 liters) was extracted with EtOAc under an acidic condition. The extract was evaporated to afford a brown oil (3.8 g). The oil was chromatographed on silica gel column (solvent system: $CHCl_3$ - MeOH, 15: 1 ~ 5: 1). The active eluate containing antiviral principles was evaporated to give a powder (210 mg). The crude powder was further purified by Prep. HPLC [YMC PAK A-324, ODS Type, UV: 210 nm, solvent system: 25% CH_3CN - 10 mM KH_2PO_4 buffer] to provide two antiviral substances **1** (45 mg) and **2** (30 mg), and a very weakly active substance **3** (23 mg). From the IR and NMR spectral data of these substance, **1** ($C_{15}H_{16}O_5$, M^+ *m/z* 276), **2** ($C_{15}H_{18}O_6$, M^+ *m/z* 294) and **3** ($C_5H_5NO_2$, M^+ *m/z* 111) were assigned as pentalenolactone, pentalenolactone O (=arenaemycin D) and pyrrole-2-carboxylic acid, respectively.

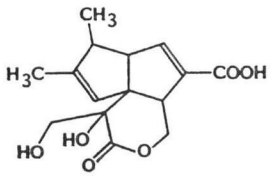
Pentalenolactones have been isolated as antibacterial antibiotics from *Streptomyces* sp., No. 8403-MC_1 by TAKEUCHI *et al.*[1] and from *Streptomyces arenae* by SCHIERLEIN *et al.*[2], independently. Pentalenolactone **1** showed a strong inhibitory activity against *Bacteroides fragilis* KB 169 (ATCC 23745) (MIC: 0.1 μg/ml on heart infusion agar) and *Treponema hyodysenteriae* B 234. However, pentalenolactone O (**2**), in which an epoxy group in **1** has been converted to a diol, lacked substantial antimicrobial activity. Pyrrole-2-carboxylic acid was isolated as an inhibitor of proline racemase (Dr. INOUE of Meiji Seika, unpublished data) and as an inhibitor of platelet aggregation induced by tumor cells from *Streptomyces* (KOMIYAMA *et al.*[3]).

The antiviral activities of pentalenolactone (**1**), pentalenolactone O (**2**) and pyrrole-2-carboxylic acid (**3**) against HF strain of HSV-1 were assayed as described by MCLAREN *et al.*[4] with some modification. Drug-treated and virus-infected Vero cells [a continuous line of African green monkey kidney cells in microtiter plates were stained with methylrosaniline according to the method of ARMSTRONG[5]]. A viral cytopathic effect (CPE) was colorimetrically measured by a microplate photometer (Colona Electric). ED_{50} values of **1** and **2** for production of a 50% reduction in viral CPE compared with cell controls (0%) and virus controls (100%) were 0.05 and 0.025 μg/ml, respectively. The antiviral activity of **3** was very weak (MIC > 100 μg/ml). At the same time the concentration inhibiting the growth of Vero cells by 50% (ID_{50}) was measured. The values of **1** and **2** were 0.27 and 56 μg/ml, respectively. The selectivity index (ID_{50}/ED_{50}) showed very high selectivity for the diol derivative **2** (2,240, *cf.* 5.4 for **1**).

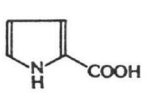
The antiviral activities of **1** and **2** against several viruses were assayed by a plaque reduc-



Pentalenolactone (**1**)



Pentalenolactone O (**2**)



Pyrrole-2-carboxylic acid (**3**)

Table 1. Antiviral activity of pentalenolactone and pentalenolactone O against several DNA viruses.

Virus	ED_{50}* (μg/ml)		
	Pentalenolactone	Pentalenolactone O	Ara A
DNA virus			
Vac-IHD	1.0	1.0	1.3
Vac-DIE	0.5	>10	3.2
HSV-1	0.4	2.0	1.0
HSV-2	<0.1	0.4	0.6
RNA virus			
NDV	>10	>10	<0.1
VSV	>10	>10	10
WEE	>10	>10	>10

* 50% Effective dose, measured by plaque reduction method.

tion test. The following seven animal viruses were used: Indiana strain of VSV, McMillan strain of Western equine encephalitis virus (WEE), Miyadera strain of Newcastle disease virus (NDV), DIE strain of vaccinia virus (Vac-DIE), IHD strain of vaccinia virus (Vac-IHD), HSV-1, UW strain of herpes simplex virus type 2 (HSV-2). For testing, primary chick embryonic cells were prepared by trypsinization of 9-day chick embryos and cultivated with 3 ml of minimum essential medium supplemented with 10% calf serum (MEM-CS 10%) in 30 mm plastic dishes. Monolayers were inoculated with 100 μl of 50 plaque forming unit (PFU) of virus diluted with MEM-CS 2% and after 2 hours overlayed 2 ml of MEM containing 1% agar and the antibiotics (10~0.1 μg/ml). The concentration showing 50% reduction of plaque count of a challenge virus (ED_{50}) is shown in Table 1. All DNA viruses (except Vac-DIE for **2**) examined were inhibited by the antibiotics. However RNA viruses were not inhibited even at the concentration of 10 μg/ml. These results demonstrated that the antibiotics **1** and **2** appear to inhibit the replication of DNA viruses specifically.

It has been reported that pentalenolactone shows antibacterial activity by selective inhibition of glyceraldehyde-3-phosphate dehydrogenase[6]. On the contrary, antibiotic **2** shows no antimicrobial activity. This indicates that the action mechanism of the antibiotics for antiviral activity is different from that for antimicrobial activity. The mechanism of action and the evaluation of **1** and **2** in the *in vivo* system are being investigated.

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