Notes

A NEW ANTIFUNGAL ANTIBIOTIC, 3′-*O*-DECARBAMOYLIRUMAMYCIN

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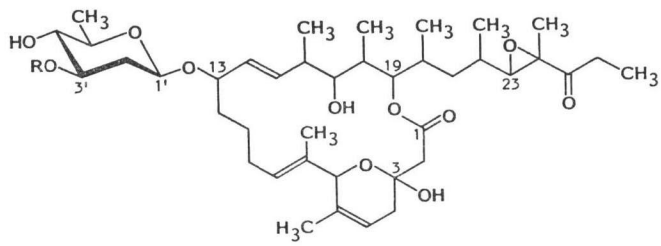
Irumamycin[1~3], an antifungal antibiotic produced by *Streptomyces subflavus* subsp. *irumaensis*, is a 20-membered macrolide antibiotic involving 3′-*O*-carbamoyl-2-deoxy-β-D-rhamnoside moiety. During the course of our studies with the large-scale production of irumamycin (**2**), a new antibiotic designated as 3′-*O*-decarbamoylirumamycin (**1**) was isolated from the EtOAc extract of the broth filtrate. In this paper, we wish to describe the isolation, structure elucidation and biological activity of **1**.

Fermentations were carried out under the conditions reported earlier[1]. The whole broth (100 liters) was centrifuged and the filtrate extracted twice with EtOAc. After evaporation of the extract, the residue was treated with *n*-hexane to afford a crude powder (25 g). Application of this powder on a column of the high-porous synthetic resin Diaion HP-20 gave an active fraction containing irumamycin and its new minor component. The powder (420 mg) obtained by evaporation of the active fraction was chromatographed on silica gel using benzene - acetone mixtures (10: 1, 6: 1, 3: 1). The resulting powder was further purified by silica gel preparative thin-layer chromatography with a benzene - acetone mixture (3: 1) to afford a new antibiotic **1** (40 mg) as a white amorphous powder.

The antibiotic **1**, mp 78~80°C, $[\alpha]_D^{25}$ +13.5° (*c* 1, $CHCl_3$), *Anal* found: C 66.57, H 8.77, O 24.86%, is structurally similar to **2** from its IR spectrum (ν_{max}^{KBr} 3450, 2860, 1687, 1350, 1200, 1020, 944 cm^{-1}, Fig. 1). The ^{13}C NMR spectrum (100 MHz in $CDCl_3$, Fig. 2) of **1** showed the presence of nine methyls, eight methylenes, fourteen methins including nine carbons bonded to oxygen, and an anomeric carbon (δ 98.7), a hemiketal carbon (δ 94.2), six olefinic carbons (δ 117.0, 129.7, 133.6, 134.3, 134.9, 135.4), an ester carbonyl (δ 173.7) and a ketone carbonyl (δ 217.5). The chemical shifts for each carbon signal arising from the aglycone part involving an alkyl side chain in **1** were coincident with those of **2**. However, the signal of a carbamoyl carbon attached at 3′-position of the sugar moiety in **2** lacks in the ^{13}C NMR spectrum of **1**. This spectral evidence indicates that **1** possesses the same 20-membered lactone structure involving a C_{11} alkyl side chain as in **2**, but differs in the structure of the sugar moiety glycosidated at 13-position of the aglycone. The existence of 2′-deoxy-β-D-rhamnose in **1** was supported



Decarbamoylirumamycin (**1**) R=H
Irumamycin (**2**) R=$CONH_2$

Fig. 1. IR spectrum of **1** (KBr).

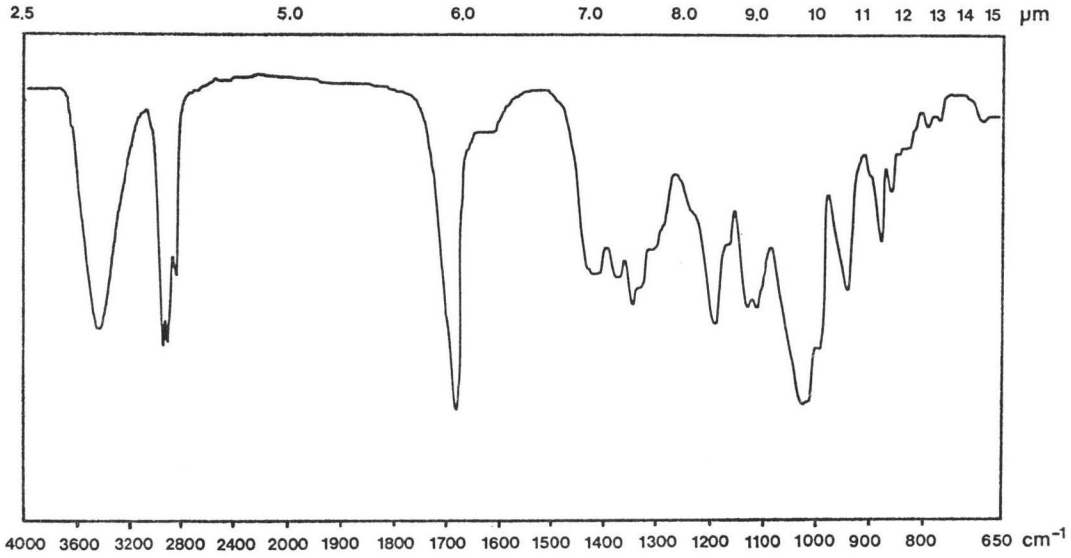


Fig. 2. ^{13}C NMR spectrum of **1** (100 MHz, $CDCl_3$).

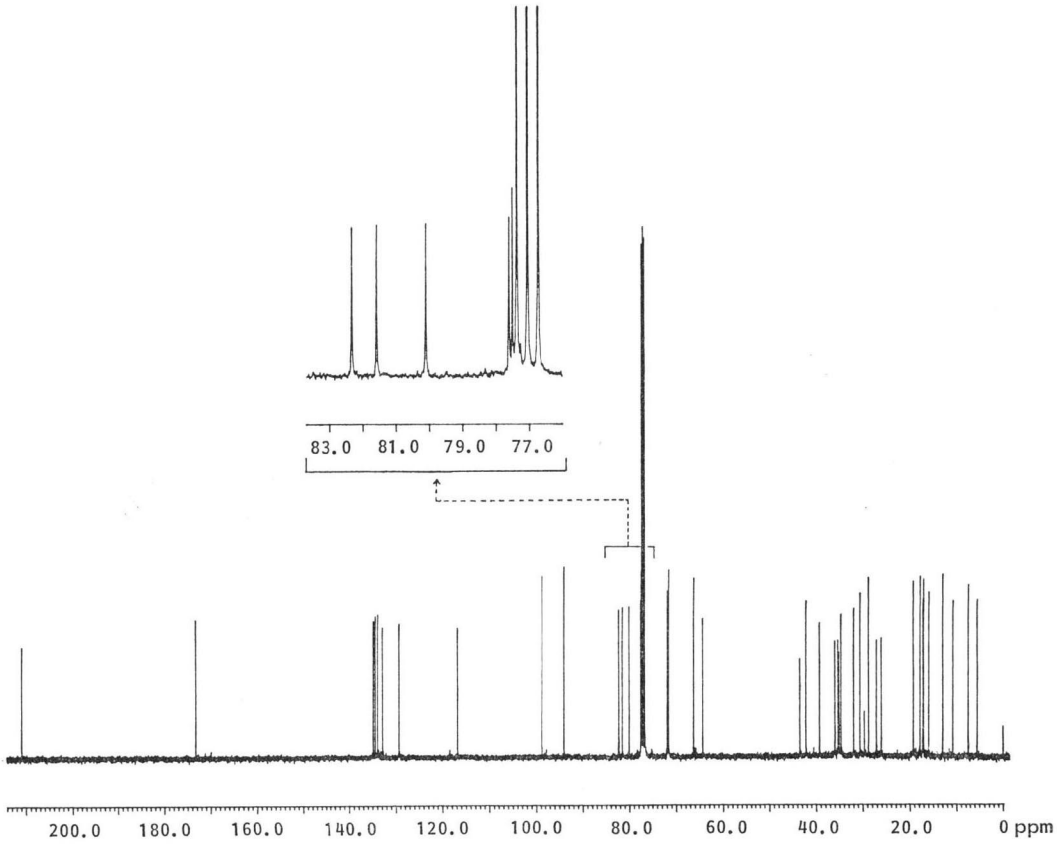


Fig. 3. ^1H NMR spectrum of **1** (400 MHz, $CDCl_3$).

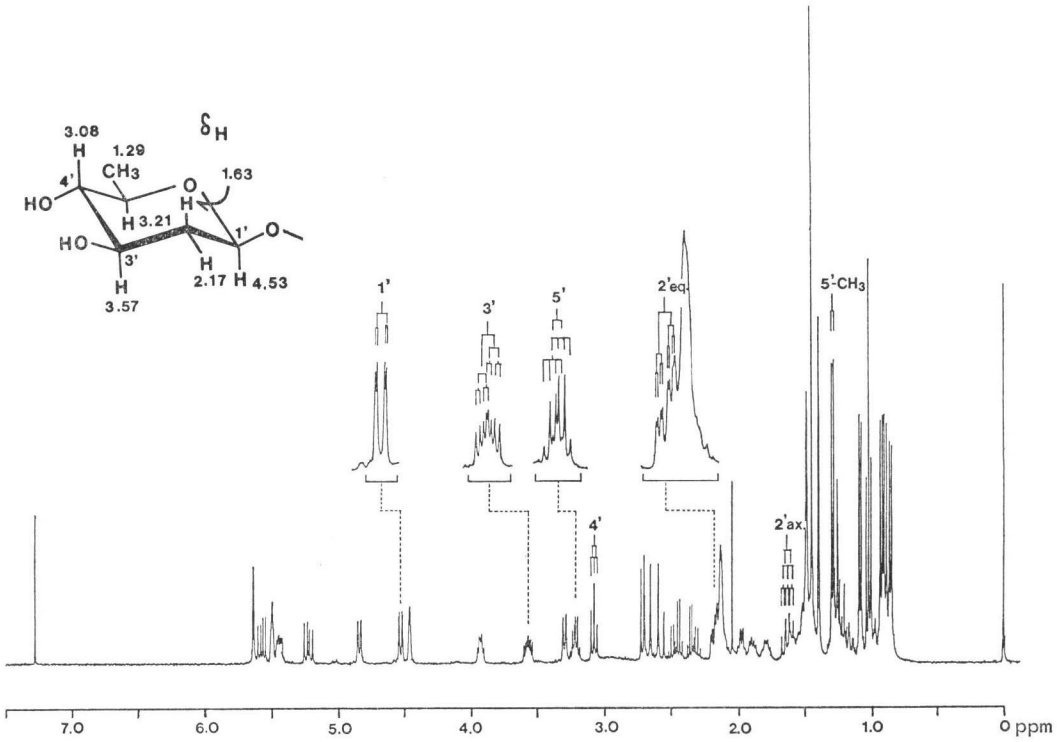


Table 1. Antifungal spectra of 3′-*O*-decarbamoylirumamycin (**1**) and irumamycin (**2**).

Microorganism*	MIC (μg/ml)	
	1	**2**
Alternaria kikuchiana KF-185	100	50
Botrytis cinerea KF-184	50	12.5
Piricularia oryzae KF-180	0.4	<0.1
Pellicularia sasakii KF-219	100	25
Sclerotinia cinerea KF-181	0.4	<0.1
Aspergillus niger KF-102	25	25
Candida albicans KF-1	>100	>100

* Organisms were grown on potato - glucose agar (pH 6), 27°C, 3 days.

from the following proton spin decoupling experiments [in the 400 MHz ^1H NMR spectrum] (Fig. 3) in addition to the appearance of an anomeric carbon (δ 98.7, J_{CH}=166.8 Hz) and an anomeric proton at δ 4.53 ($J_{1',2'eq}$=9.7 Hz, $J_{1',2'ax}$=1.6 Hz). The proton signal (δ 4.53, ddd) at 3′-position observed in **2** disappeared in **1**. In its place, the corresponding proton (δ 3.57, ddd) at the base of the hydroxyl group observed in **1** couples with the proton (δ 3.08, dd) at the base of the hydroxyl at 4′-position and the methylene proton (2′ax: δ 1.63, ddd, 2′eq: δ 2.17, ddd). On the other hand, the proton (δ 3.21, q) at 5′-position couples with the methyl proton (δ 1.29, d) and the proton (δ 3.08) at the base of the hydroxyl. The combined evidence of the ^1H and ^{13}C NMR spectra supports the identity of 3′-*O*-decarbamoylirumamycin for **1**.

3′-*O*-Decarbamoylirumamycin inhibits the growth of the phytopathogenic fungi *Piricularia oryzae*, and *Sclerotinia cinerea*, but exhibits no activity against bacteria (Table 1). The acute toxicity of 3′-*O*-decarbamoylirumamycin is low: LD_{50} 300 mg/kg (ip) or more on mice. Venturicidins A and B[4,5] and X-14952B[6] have been reported as a family of macrolides possessing a 20-membered ring lactone. The lack of a carbamoyl group at 3′-position on rhamnose moiety in **1** results in a small decrease of antifungal activity, contrasting with venturicidin B (3′-*O*-decarbamoylventuricidin A), which shows stronger antifungal activity than venturicidin A[5].

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