ISOLATION AND STRUCTURE OF OXIRAPENTYN

Sir:

A new antibiotic, oxirapentyn was isolated from the culture broth of fungus identified as *Beauveria felina* SANK 13682. It was characterized to have a new skeleton with two isoprene units and an activity against Gram-positive bacteria. In this paper we report the isolation, biological activities and structure of oxirapentyn.

Oxirapentyn was produced in a shaken culture of *B. felina* SANK 13682 for 3 to 5 days at 26°C in a medium containing 2% sucrose, 2.5% mashed potatoes, 1% Casamino Acids, 0.5% KH_2PO_4 and 0.25% $MgSO_4 \cdot 7H_2O$. The culture filtrate (8.7 liters) was extracted with ethyl acetate (9 liters) and the solvent layer was concentrated *in vacuo* to syrup. This syrup was dissolved in 10 ml of benzene - ethyl acetate (9: 1) and further purified by silica gel column chromatography. The active fractions were concentrated to yield the crude oxirapentyn. The pure oxirapentyn (1.3 g) was obtained by recrystallization from the solvent mixture of hexane - ethyl acetate as colorless needles.

Oxirapentyn (**1**): soluble in methanol, acetone, ethyl acetate, and chloroform and insoluble in water; mp 114~115°C; *Anal.* calcd. for $C_{18}H_{20}O_6$: C 65.05, H 6.07, found: C 65.42, H 6.05; MS *m/z* 332 (M^+); UV λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 225 (480); $[\alpha]_D^{20}$ −111.7° (*c* 1, $CHCl_3$); IR ν_{max}^{KBr} 2230 (triple bond), 1740 (acetyl), 1710 (ketone) and 1608 cm^{-1} (double bond). The ^1H and ^{13}C NMR spectra of **1** are shown in Figs. 1 and 2. The complete structure of **1** was determined by J_{CC} coupling and the long-range J_{CH} resolved 2-D method in the NMR spectrum, the result of which will be discussed elsewhere. However, the structure of **1** with its relative configuration was elucidated by means of X-ray analysis as follows: The crystals are trigonal, space group $P3_121$ (or $P3_221$) with a=11.078 (2), c=25.170 (4) Å, Z=6. Intensity data were measured on a Rigaku AFC-5 apparatus equipped with a rotating-anode X-ray generator (graphite-monochromated Cu-Kα radiation). The structure was solved by MULTAN[1] and refined by block-diagonal least-

Fig. 1. ^1H NMR spectrum of oxirapentyn (90 MHz, $CDCl_3$).

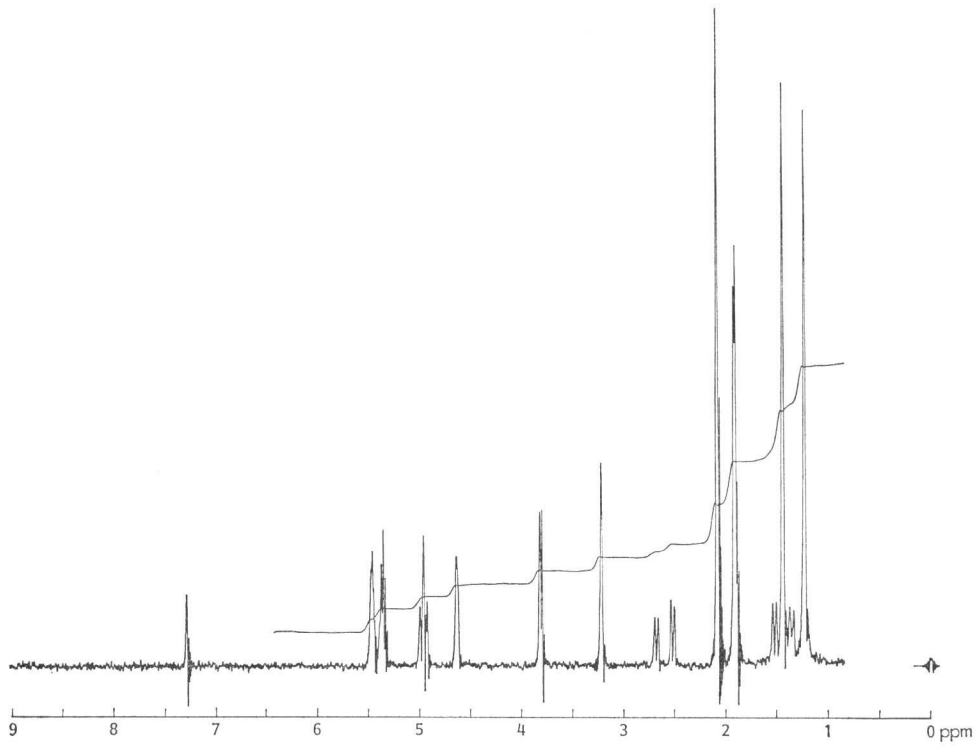


Fig. 2. ^{13}C NMR spectrum of oxirapentyn ($CDCl_3$).

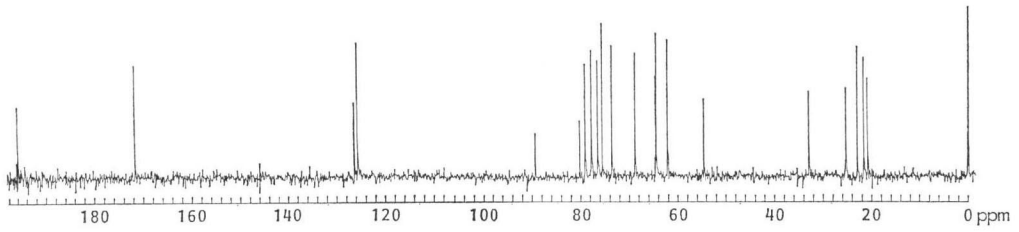
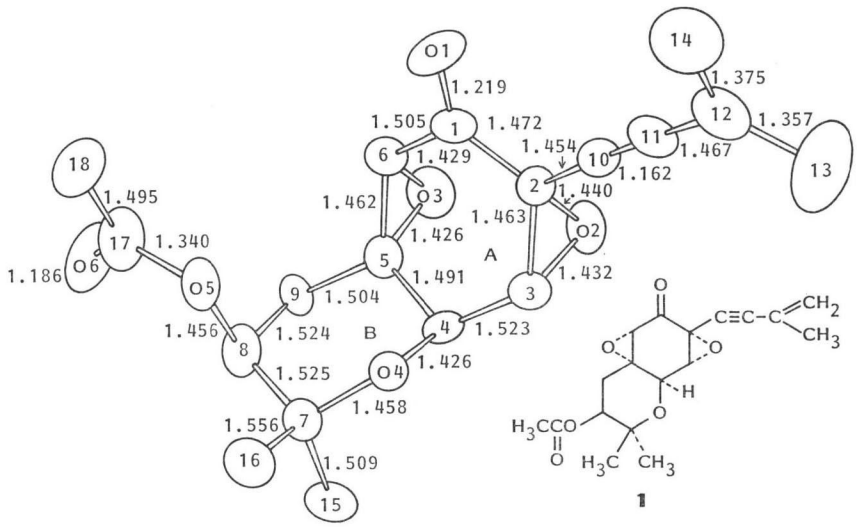


Fig. 3.



squares methods. Hydrogen atoms were located from a difference Fourier synthesis. The final least-squares refinement with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms lowered the R value of 6.7% for 1,020 observed reflections ($Fo \geq 1.5\ \sigma\ Fo$). The final atomic parameters have been deposited with the Crystallographic Data Center. The molecular skeleton thus obtained is shown in Fig. 3 drawn by ORTEP[2]. The two six-membered A and B rings are *cis*-fused, and the A ring takes a flattened boat form with C(1) as a top and C(4), an end atom, while the B ring takes a chair form with O(4) as a stem and C(9), a stern atom. The two epoxy groups attached to the A ring are *syn* with respect to each other. The *O*-acetyl group attached to the B ring is in an β-axial position. The relatively high temperature parameters (C(13)=8.8 and C(14)=11.6 Å^2) of the isopropenyl carbon atoms may reflect a large thermal motion and/or the disordering of atoms. Thus, the bond

Table 1. Antimicrobial spectrum of oxirapentyn.

Test organism	Medium*	MIC (μg/ml)
Staphylococcus aureus FDA 209P JC-1	1	25
Streptococcus faecalis S-299	1	800
Bacillus subtilis PCI 219	1	25
Micrococcus luteus PCI 1001	1	25
Mycobacterium smegmatis ATCC 607	1	100
Escherichia coli NIHJ JC-2	1	>800
Klebsiella pneumoniae PCI 602	1	800
Proteus vulgaris OX19	1	>800
Pseudomonas aeruginosa NCTC 10490	1	>800
Bacteroides fragilis SANK 71176	2	800
Eubacterium aerofaciens SANK 72276	2	>800
Propionibacterium acnes SANK 71976	2	400
S. faecalis S-299	2	>800

* 1. Mueller Hinton agar.
2. GAM agar, under anaerobic condition.

order of the C (12)-C (13) and C (12)-C (14) bonds could not be given. The absolute configuration of **1** is still ambiguous and under investigation by X-ray analysis and CD spectrum.

The antimicrobial activity of **1** determined by the agar dilution method is shown in Table 1. The LD_{50} of **1** to mice injected intraperitoneally was 6.25 mg/kg.

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