ISOLATION OF A NEW ANTIBIOTIC FROM A SPECIES OF *PSEUDOMONAS*

Sir:

During a programme for screening antibiotic substances produced by bacteria we isolated an antibiotic from the culture broth of a species of *Pseudomonas*. Physicochemical properties of the compound, called antibiotic B371, suggest it to be indoleacryloisonitrile (I), hitherto undescribed.

The bacterium, NCIB 11237, was isolated from muddy water-weed found near Harefield, Middlesex, England and classed as an unidentified species of *Pseudomonas*.

The organism was cultured for 18 hours at 28°C in nutrient broth (bacteriological peptone, 1%; beef extract, 1%; NaCl, 0.5%). Inoculum (0.7 %) was transferred to 5-litre vessels and the organism grown in medium (4 litres) containing milled soya flour (A. E. Staley Mfg. Co.,Decatur, Illinois, U.S.A.) 2.9%, glycerol 1% and K_2HPO_4 0.1% and the fermentation liquor was stirred at 250 rev./minute for 72 hours at 22°C.

Antibiotic production was assayed by agar diffusion (cup-plate method) with *Staphylococcus aureus* Oxford H strain VI as test organism.

Culture broth was extracted with diethylether and the extract purified by column chromatography on dry silica (Woelm, activity III; ICN Pharmaceuticals GmbH & Co., 3440 Eschwege, West Germany) with toluene-diethylamine (99: 1) as eluant. Fractions active against *Staphylococcus aureus* were combined, taken to dryness under reduced pressure, the residue dissolved in methanol and further purified by column chromatography on Sephadex LH20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) packed in methanol and with methanol as eluant. Active fractions were combined and evaporated under reduced pressure to a colourless solution containing about 2 mg antibiotic B371/ml methanol. Antibiotic B371, unstable in broth, was stabilized by extraction into organic solvent but tended to form inactive red solids when evaporated to dryness.

Physicochemical properties of the methanolic solution of antibiotic B371 were: Mass spectrum; found, *m/e* 168.0683 (M^+); calcd. for $C_{11}H_8N_2$, *m/e* 168.0688. UV_{max}^{MeOH}, nm; 275(sh), 313, (Fig. 1); addition of HCl or $NaBH_4$ resulted in an irreversible shift, associated with loss of bioactivity, to give UV max, nm; 273, 279, 288, (Fig. 1). IR; $CHBr_3$, cm^{-1}; 2138(s). NMR; 100 MHz, CD_3OD, TMS as internal standard; no signals were detected outside δ 5.0~8.5 ppm, (Fig. 2). Positive colour reactions were given with VAN URK, PROCHAZKA and SALKOWSKI reagents.

The antibacterial spectrum obtained for a methanol solution of antibiotic B371 is given in Table 1.

Antibiotic B371 gave positive colour reactions with three reagents used for the detection of indoles and the UV spectrum obtained on addition to the antibiotic of either acid or $NaBH_4$ resembled that of the 3-substituted indole chromophore of tryptophane[1]. Strong absorption of antibiotic B371 at 2138 cm^{-1} (IR) suggested N≡C stretching vibration. A singlet (δ 8.05) in the nmr spectrum of the antibiotic was assigned as the proton on the 2-position of a 3-substituted indole and the four multiplets (δ 7.1~7.7) as the adjacent protons on the unsubstituted homocyclic ring. The remaining two signals (δ 6.85, m, and δ 5.77, d, J9 Hz) were assigned as *cis*-CH=CH with the aid of spin decoupling. When δ 6.85, m, was irradiated, δ 5.77, d, collapsed to a slightly broadened singlet while the remaining signals in the spectrum remained unaltered. When δ 5.77, d, was irradiated, δ 6.85, m, was simplified as illustrated (Fig. 2). The nature of the further coupling to δ 6.85, m, remains unexplained, but MATTESON & BAILEY[2] have shown that α,β-unsaturated isonitriles show ^{14}N–H coupling. This may be the source of the additional coupling that

Fig. 1. Ultraviolet absorption spectra of antibiotic B371.

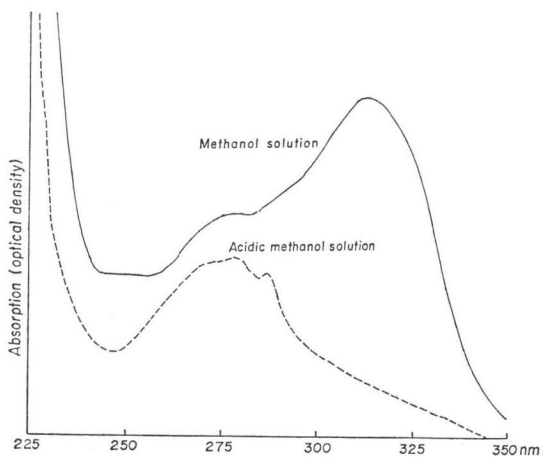
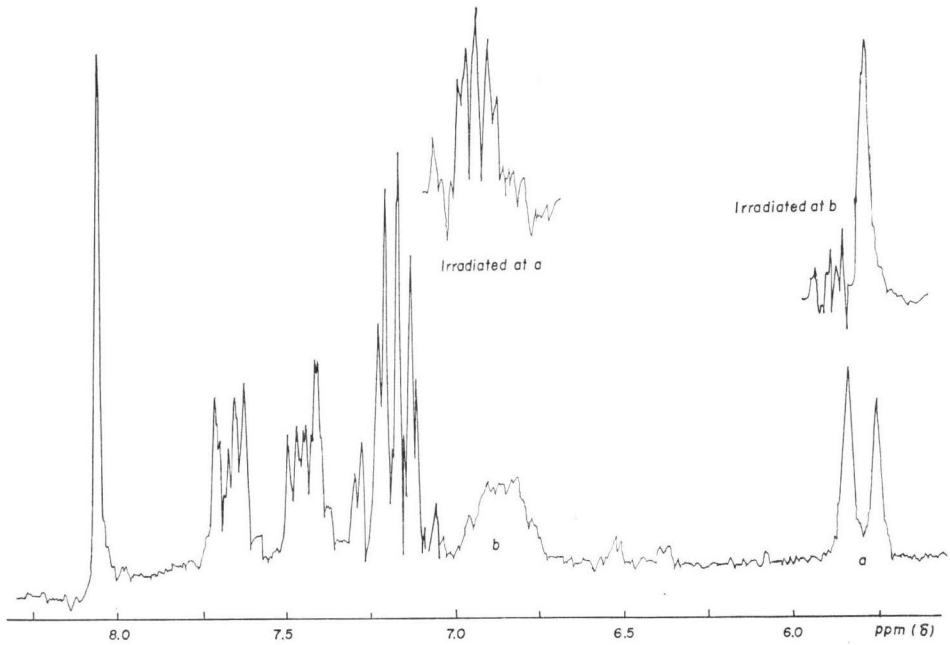
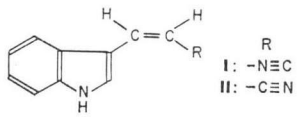


Fig. 2. Nuclear magnetic resonance spectrum of antibiotic B371.



cannot be explained by H–H splitting.

These properties suggest that antibiotic B371 has structure **I** or **II**.



However, from the values quoted by BELLAMY[3] for C≡N frequencies of unsaturated nitriles and isonitriles and from the value, 2230 cm^{-1}, obtained by SUVOROV *et al.*[4] for the known *trans* isomer of **II**, antibiotic B371 is thought to have the structure, **I**.

Acknowledgement

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Table 1. Antibacterial spectrum of B371.

Test organisms*	Lowest concentration at which a zone of inhibition was detected (μg/ml)
Staphylococcus aureus Oxford H strain VI	8
Escherichia coli NCIB 9482	62
Escherichia coli Glaxo C1343	16
Proteus mirabilis Glaxo C1299	62
Shigella sonnei Glaxo C1894	62
Pseudomonas pyocyanea NCTC 8203	>500
Klebsiella aerogenes NCIB 418	250
Flavobacterium sp. Glaxo C1980	2
Saccharomyces carlsbergensis NCYC 530	125

* Agar diffusion cup-plate method (nutrient agar 37°C 18 hours)

References

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