Tetrahedron Letters No.27, pp. 3175-3178, 1968. Pergamon Press. Printed in Great Britain.

A NEW ANTIBIOTIC PRODUCED BY A STRAIN OF ASPERGILLUS FLAVIPES

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(Received in UK 1 April 1968; accepted for publication 11 April 1968)

During the researches on thermophylic fungi our attention was drawn to a strain belonging to Aspergillus flavipes series (1) which demonstrated a strong activity against gram-positive and gram-negative bacteria.

The strain F-2091/7 of our collection was grown in submerged culture in Erlenmeyer flasks of 500 ml capacity containing 100 ml of the following nutrient medium, a modification of that reported by Stoll and coworkers (2): Sucrose g.100; $Ca(NO_3)_2$ g.1; KH_2PO_4 g. 0.250; $MgSO_4 \cdot 7H_2O$ g. 0.250; KCl g. 0.125; $FeSO_4 \cdot 7H_2O$ g. 0.033; $ZnSO_4 \cdot 7H_2O$ g. 0.027; L-asparagine g.10; L-cysteine hydrochloride g. 0.01; yeast extract g. 0.1; stock solution ($MnSO_4 \cdot 7H_2O$ g. 2; KJ g. 0.5; $NiCl_2 \cdot 6H_2O$ g. 0.05; $CoCl_2 \cdot 6H_2O$ g. 0.05; $CuSO_4 \cdot 5H_2O$ g. 0.05; H_3BO_3 g. 0.05; H_2SO_4 ml. 1; distilled water to 1000 ml) 3 drops; distilled water to 1000 ml; pH 5; sterilization: 30 min. at 100°C.

The antibiotic production began at 24°C after 3 days culture, reaching the highest activity after 7 days. At this time the maximum concentration of the antibiotic was about 300 μg/ml (the activity was measured in agar plate using a strain of B. subtilis as test organism).

The antibiotic was extracted from the fermentation broth, acidified to pH 3,5, with the same amount of chloroform, and purified by chromatography on acid alumina (C. Erba), using $CHCl_3$:Et_2O=4:1 as eluent. Repeated crystallizations of the active fraction from benzene gave the new antibiotic (I) as optically active ($[\alpha]_D^{21°} = -71.8°$; c=1% in 95% ethanol) white needles, m.p. 130-131°C.

Elemental analysis gave results consistent with a crude formula $C_{12}H_{15}NO_4$, confirmed by mass spectrometry (found M^+=237.100). The new

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compound is neutral and has a characteristic u.v. spectrum (λ_{max} 330 mμ in neutral ethanol, $\varepsilon = 5,4.10^3$). Its i.r. spectrum (KBr) shows NH and/or OH bands at 3200-3000 cm^{-1} as well as saturated (1725 cm^{-1}) and unsaturated (1650 and 1615 cm^{-1}) carbonyl. The presence of a $CH_3-\underset{CH_3}{CH}-CH_2-\underset{}{C}=$ group is easily inferred from its NMR spectrum*: two doublets ($J = 6.5$), each corresponding to three protons, at 0.88 and 0.91 δ, a one-proton multiplet at 2,22 δ, a two-proton multiplet (showing the 8-peak pattern characteristic for the AB part of an ABX system) centred at 2.89 δ. A three-proton sharp singlet at 2.02 δ is attributable to a $CH_3-\underset{}{C}=$. Another singlet at 5.60 δ is easily interpreted as due to a $\begin{matrix}C\\C\end{matrix}\!\!>C=\underline{C}H-CO-$, while a broad singlet at 5.25 δ (9.30 δ in $CDCl_3$), easily exchangeable with D_2O, is due to an imidic hydrogen. This explains the evolution of ammonia when (I) is treated with NaOH. A singlet (one-proton) appearing at 4.32 δ, which does not exchange with D_2O, will be accounted for later.

The NMR data and the successive loss of fragments of 85 and 43 m.u. in the mass spectrum are consistent with the presence in (I) of $CH_3-\underset{CH_3}{CH}-CH_2CO-$ and CH_3-CO-substituents.

Catalytic hydrogenation of (I) (PtO_2 in AcOH) gives, after absorption of three moles of hydrogen, a tetrahydrocompound (II) (white prisms from AcOEt, m.p. 189-190°) analysing for $C_{12}H_{19}NO_3$ (found $M^+ = 225.136$), which on treatment with NaOH does not give NH_3; its i.r. spectrum does no longer show the 1725 cm^{-1} band. (II) gives, by boiling with Ac_2O, a diacetate (III), m.p. 175° (white needles from 90% ethanol; $[\alpha]_D^{21°}$ + 6.1°; c = 1% in 95% ethanol). Some interesting features appear in the NMR spectra of (II) and (III): both show the presence of the CH_3-CO- singlet as well as the pattern of the $CH_2-CH\begin{matrix}CH_3\\CH_3\end{matrix}$ group, referred to above, displaced to lower δ values and transformed in a more complex multiplet, thus indicating some additional coupling. Their comparison shows that acetylation is accompanied by the disappearance of an amidic proton (1H, extremely broad signal at 6-7 δ in II) and by a shift of a one-proton multiplet from 4.35 (in II) to 5.45 δ (in III); hence (III) is a N,O-diacetate.

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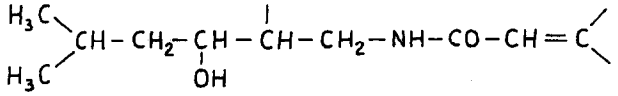
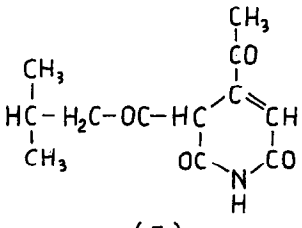
* NMR spectra were recorded on a Varian HA-100 apparatus, solvent C_5D_5N, internal standard TMS; chemical shifts are given in ppm, coupling constants in cps.

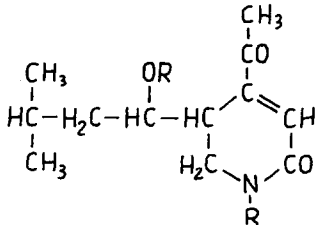
NMDR shows that the proton on the acetylable function arosen by reduction of a saturated keto group in (I) couples with a proton which is, in turn, also coupled to a new 8-peak pattern (two protons) centred at about 3.2 δ in (II) and at about 2.85 δ in (III), lacking in (I). Remembering that (II) has an oxygen less than (I) we deduce that catalytic hydrogenation also involves the reduction of a -CO-NH- to a $-CH_2-NH-$ group. All the evidence reported so far demonstrates, in our opinion, that (II) contains the sequence:



Taking into account that in (II) the CH_3-CO- group is yet present, as stated above on NMR basis and as confirmed both by the easy loss of a 43 m.u. from the M^+ ion and by u.v. spectrum, which cannot be accounted for only on the basis of an unsaturated amide group, we can deduce structure (II) for the tetrahydroderivative.



(I)



R=H (II) $COCH_3$ (III)

Consequently, the new antibiotic must have structure (I), in which the absolute stereochemistry of the asymmetric carbon remains to be elucidated.

From the allowed formula, we can classify (I) as belonging, at least formally, to the cycloheximide family, of which several members are known(3), although this is the first time that a member of this family is found in fungi.

At the contrary of cycloheximide, this new antibiotic, as shown in Table No. 1, is active against gram-positive and gram-negative bacteria, and inactive against fungi.

T A B L E I

Tested microrganism	Minimal inhibiting concentration (µg / ml)
B. subtilis	25
St. aureus Oxford	25
St. aureus 108 (penicillin resistant)	25
E. coli	25
Mycobacterium phlei	>100
Nocardia sp.	<100
Streptomyces sp.	<100
Saccharomyces cerevisiae	>1000
Candida albicans	>1000
Penicillium sp.	>1000
Claviceps cynodontis	< 100

Studies on possible therapeutic applications are in program.

References.

(1) K.B. Raper and D.I. Fennel, The genus Aspergillus, The Williams and Wilkins Co., Baltimore (1965).

(2) A. Stoll, A. Brack, A. Hofmann and H. Kobel, U.S.Pat. 2.809.920 (1957).

(3) S.A. Waksman and A.H. Lechevalier, The Actinomycetes, vol.III, p.50, The Williams and Wilkins Co., Baltimore (1962).