NOTES

NORPLICACETIN, A NEW ANTIBIOTIC FROM *STREPTOMYCES PLICATUS*

J. R. EVANS and GLENIS WEARE

Biochemistry Department, Glaxo Research Ltd.
Sefton Park, Stoke Poges,
Buckinghamshire, England

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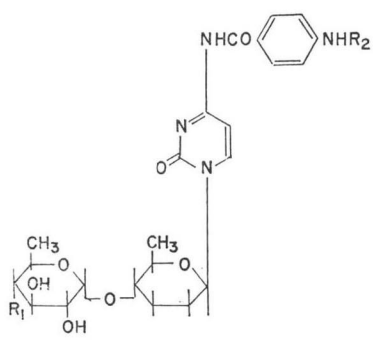
HASKELL *et al*[1] isolated amicetin (**I**), bamicetin (**II**) and plicacetin (**III**) from the fermentation broth of *Streptomyces plicatus*. We have isolated a fourth antibiotic, the chemical and physical properties of which suggest it to be norplicacetin (**IV**), from cultures of a streptomycete isolated from a soil sample from Ghana.

This organism produced fine aerial hyphae carrying more than 10 grey, smooth walled spores in unbranched chains ending in a loop or open spiral. Colonies had yellow vegetative growth and produced no melanoid or soluble pigment. Growth took place on L-arabinose, D-fructose, D-galactose, D-glucose, L-rhamnose, D-mannitol, D-xylose and *i*-inositol but not on raffinose and sucrose. Growth was inhibited by streptomycin sulphate. These characteristics and its ability to produce amicetin, bamicetin and plicacetin indicate the organism to be closely related to or identical with *Streptomyces plicatus*[2]. The isolate has been deposited with the National Collection of Industrial Bacteria, Aberdeen, Scotland and designated as NCIB 11305.

Antibiotic production during fermentation was assayed by agar diffusion (cup-plate) with *Staphylococcus aureus* Oxford H strain VI as test organism. To detect and quantify the nature and amount of the various antibiotics produced by this streptomycete, samples of culture fluids and extracts were applied to thin-layer silica gel sheets (EK6061; Eastman-Kodak Co. Ltd., Rochester, N.Y., U.S.A.); these were developed with ethylacetate - methanol (3:1 by volume) and dried sheets were subjected to bioautography with *Staphylococcus aureus*.

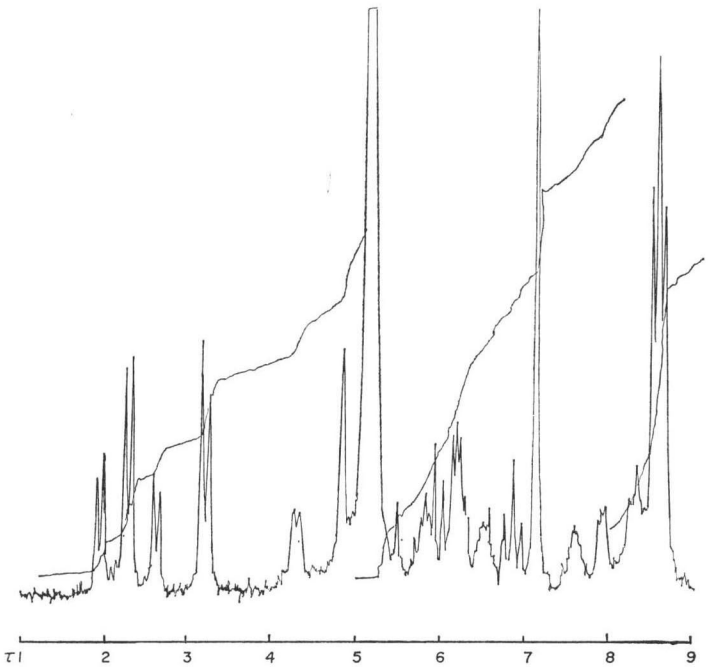
The organism was grown in 250-ml conical flasks in a medium (60 ml/flask) containing sucrose, 3%; milled soya bean (J. Bibby & Sons Ltd., Liverpool, Lancashire, England), 2%; beet molasses (United Molasses Co. Ltd., London, England), 2%; corn steep liquor (Garton & Sons Ltd., London, England), 0.6%; NaCl, 0.5%; $CaCO_3$, 0.4%; $(NH_4)_2SO_4$, 0.2%. The inoculated medium was shaken (220 rev/minute on a rotary shaker with a 2-inch throw) for 48 hours at 28°C. A portion (80 ml) of the shake flask fermentation was transferred to 4 liters of medium (glycerol, 2%; milled soya bean meal, 1.5%, casein digest (Bengers Ltd., Holmes Chapel, Cheshire, England), 0.1%; $NaNO_3$, 0.3%) in a 5-litre fermenter. The mixture was stirred (350 rev/minute) and aerated (1.5 litres air/minute) for 118 hours at 28°C.

Fermented broth was centrifuged and the supernatant extracted at pH 8 with *n*-butanol. The extract was evaporated under reduced pressure and the residue was applied to the top of a column of dry silica (Woelm, activity III, 80 × 2.5 cm; ICN Pharmaceuticals GmbH & Co., 3440 Eschwege, West Germany). Impurities were eluted with ethylacetate and antibiotics with ethyl acetate - methanol (4:1 by volume). Fractions active against *Staphylococcus aureus* were



	R_1	R_2
I :	$-N(CH_3)_2$	$-CO-\overset{\overset{CH_3}{\vert}}{\underset{\underset{NH_2}{\vert}}{C}}-CH_2OH$
II :	$-NHCH_3$	$-CO-\overset{\overset{CH_3}{\vert}}{\underset{\underset{NH_2}{\vert}}{C}}-CH_2OH$
III :	$-N(CH_3)_2$	H
IV :	$-NHCH_3$	H

Fig. 1. Nuclear magnetic resonance spectrum of norplicacetin



combined and evaporated. The residue was dissolved in methanol and further purified by chromatography on a column of Sephadex LH20 (80×4.5 cm; Pharmacia Fine Chemicals AB, Uppsala, Sweden) packed in methanol and with methanol as eluant. Combined active fractions contained 4 components (A, B, C, D) of Rf 0.65, 0.53, 0.42, 0.2 respectively.

The combined fractions were applied to a column of dry silica (120×2.5 cm) and eluted with a stepwise gradient of ethyl acetate-methanol (9 : 1~4 : 1 by volume). Components eluted in the order A, B, C, D and appropriate fractions were combined. Each pool was evaporated, applied to a column of Sephadex LH20 (100× 4.5 cm) and eluted with methanol. The main component from each pool was collected and evaporated to a residue. Components eluted in the order D, B, A, C.

Compound B was identified as amicetin[8] and D as bamicetin[1] by UV absorption spectroscopy and by a TLC comparison with authentic samples of amicetin and bamicetin (International Centre of Information on Antibiotics, Liege, Belgium).

Compound A was recrystallized from methanol as white crystals (m.p. 169~174°C, $[\alpha]_D^{23}+$

Table 1. Antimicrobial spectrum of norplicacetin

Organism	MIC* (μg/ml)
Staphylococcus aureus Glaxo 663	31
Staphylococcus aureus Glaxo 853E	31
Streptococcus pyogenes Glaxo 618	4
Streptococcus pneumoniae	4
Streptococcus pneumoniae	8
Haemophilus influenzae Glaxo 1184E	16
Escherichia coli Glaxo 573	>500
Escherichia coli 1661	>500
Klebsiella aerogenes Glaxo 1082E	>500
Pseudomonas aeruginosa Glaxo 1371E	>500
Mycobacterium smegmatis ATCC 607	62
Mycobacterium smegmatis NCTC 8158	62
Mycobacterium bovis BCG 1077	0.5
Mycobacterium bovis BCG 1173	0.5
Candida albicans Glaxo C316	>500

* Serial dilution method in tubes (nutrient medium, 37°C). The results obtained with *Mycobacterium smegmatis* were recorded after 2 days incubation of the cultures, those obtained with *Mycobacterium* BCG after 14 days and those obtained with the other organisms after 18 hours.

177° (*c* 1.3, methanol)) and identified as plicacetin[1] by UV absorption spectroscopy, nmr spectroscopy and by mass spectrometry (field desorption; M^+, *m/e* 517; $(M+1)^+$, *m/e* 518. $C_{25}H_{35}N_5O_7$ requires MW 517).

Compound C was isolated as white needles m.p. 168~171°C; $[\alpha]_D^{23}+125°$ (*c* 1, methanol). The UV spectrum λ_{max}^{MeOH} ($E_{1cm}^{1\%}$), 253 (230), 325 (500) nm was identical with that of plicacetin. The mass spectrum (field desorption; M^+, *m/e* 503, $(M+1)^+$, *m/e* 504) suggested the molecular formula $C_{24}H_{33}N_5O_7$. The nmr spectrum (Fig. 1; 100 MHz, D_2O containing 10% CH_3COOD, TMS as internal standard) was similar to that of plicacetin with the difference that the singlet at τ 7.16 (N–CH_3 protons) integrated for 3 protons whereas that for plicacetin (τ 6.92) integrated for 6 protons. These results suggest that compound C is norplicacetin (**IV**), an undescribed member of the amicetin group of antibiotics.

The antimicrobial effect of norplicacetin against several microorganisms is shown in Table 1. In these tests norplicacetin resembles plicacetin with moderate activity against Gram-positive bacteria and mycobacteria.

Acknowledgement

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References

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