NOTES

PA 789/80, A POLYACETYLENIC ANTIBIOTIC FROM *MARASMIUS ALLIACEUS* (JACQ. *ex* FR.) FR.

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(Received for publication August 25, 1980)

During a systematic screening of basidiomycetes for new antibiotic substances PA 789/80, a polyacetylenic antibiotic with activity against Gram-positive bacteria, yeasts and moulds, was isolated from the culture extract of *Marasmius alliaceus*. The neutral compound showed the same characteristic finger type of ultraviolet spectrum as marasin.[1] It differs from known antibiotic substances found in submerged culture of the genus *Marasmius*[1~8] and closely related genera.[9~12]

Marasmius alliaceus, strain BC 2020, was maintained on BM-medium (glucose, 30 g; Witte peptone, 10 g; KH_2PO_4, 0.5 g; $MgSO_4$, 0.5 g; $Ca(NO_3)_2$, 0.5 g; $FeSO_4$, 10 mg; $MnSO_4$, 6 mg; $ZnCl_2$, 4 mg; $CuSO_4$, 1 mg; inositol, 50 mg; aneurine, 50 μg; biotin, 1 μg; agar, 15 g per liter). Mycelium from ten agar slants was used to inoculate ten 500-ml flasks each containing 80 ml of BM-medium. The seed cultures were incubated on a rotary shaker at 24°C for 8 days. After homogenizing under sterile conditions these cultures were used as inoculum for ten 2-liter flasks each containing 250 ml of the same medium. The fermentation was conducted at 24°C for 12 days with agitation. During the fermentation the antibiotic activity of the culture broth was estimated after extraction with ethyl acetate.

For isolation the culture broth containing the antibiotic substance was filtered through Celite, and the filtrate (3 liters) was extracted twice with 1.5 liters each of ethyl acetate. The organic phase was concentrated by solvent evaporation. The crude concentrate was applied to 2 successive columns of silica gel (Lobar columns C and B, Merck); one column was eluted with chloroform - methanol (9: 1), the other column with diisopropyl ether - methanol (9: 1). The fractions containing the antibiotic were pooled, the solvent evaporated and the residue rechromatographed on silica gel. Elution with chloroform - cyclohexane (97: 3) yielded 5 mg of PA 789/80 as a colourless homogeneous oil. Experiments of crystallization failed.

The polyacetylenic antibiotic is readily soluble in methanol, acetone, ethyl acetate, chloroform and dimethyl sulfoxide, and slightly soluble in carbon tetrachloride, *n*-hexane and water. In solution the neutral compound is rather stable. The chromatographic behaviour of PA 789/80 (silica-gel plates, F-254, 0.25 mm, Merck) is described in Table 1.

The ultraviolet absorption spectrum of the isolated antibiotic indicates the presence of an ene-diyne chromophore with λ max at 225, 237, 248, 262 and 278 nm (Fig. 1).

On treatment with alkali (0.1 N sodium hydroxide) the polyacetylenic substance undergoes changes as evidenced by a quite new type of absorption spectrum (on acidifying, the ultraviolet spectrum does not change). The base-catalysed isomerization caused the disappearance of the typical four-finger spectrum and the appearance of new maxima at 223, 235, 251, 265, 280 and 294 nm. Since the alkali-induced rearrangement is one of the most characteristic properties of allenic compounds[13~16] it is presumed that PA 789/80 is like mycomycin[17], nemotin[13,18], odyssin[19], and marasin[1,14], an allenic diacetylene with the partial structure $-C\equiv C-C\equiv C-\underset{|}{C}=C=\underset{|}{C}-$.

The infrared spectrum of PA 789/80 (Fig. 2) shows absorption maxima at 3540, 3320, 2210, 1955, 1735, 1605, 1450, 1385, 1330, 1045, 950, 875 and 845 cm^{-1}. The neutral polyacetylenic antibiotic has a carbonyl-function (1735 cm^{-1}) which probably does not conjugate with the ene-diyne chromophore. While the presence of acetylenic hydrogen (3320 cm^{-1}) and disubstituted acetylene (2210 cm^{-1}) is evident, that of

Table 1. Chromatographic bebaviour of PA 789/80 on TLC.

Solvent system	Rf value
Ethyl acetate - benzene (3 : 1)	0.44
Diisopropyl ether - methanol (9 : 1)	0.39
Chloroform - methanol (9 : 1)	0.45

the allene (1955 cm^{-1}) and alcoholic hydroxyl (3540 cm^{-1}) is not so well indicated.

Table 2 shows the antimicrobial spectrum of PA 789/80. The polyacetylenic antibiotic is active against Gram-positive bacteria and a variety of moulds and yeasts. Gram-negative bacteria are not affected or affected to a much lesser extent. The antibiotic activity of PA 789/80 was compared to that of two other substances, nystatin (obtained from Lederle) and xeromphalin A. The latter antibiotic was isolated from the agaric *Xeromphalina campanella* (BATSCH *ex* FR.) R. MRE[12].

Fig. 1. U.V. Spectrum of PA 789/80 and its alkali-isomerization product in absolute ethanol.

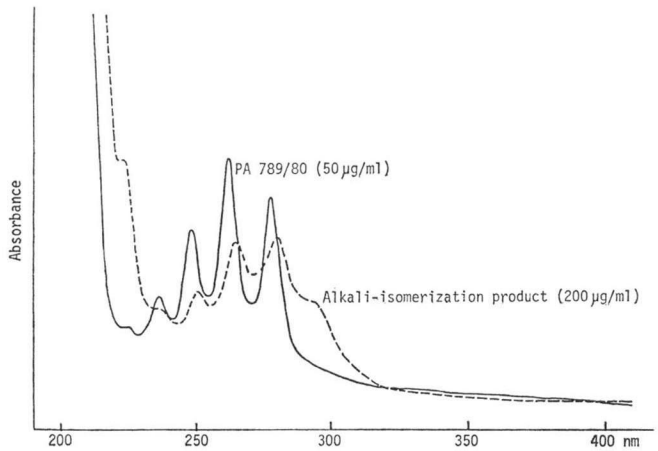

Fig. 2. I.R. Spectrum of PA 789/80 in chloroform.

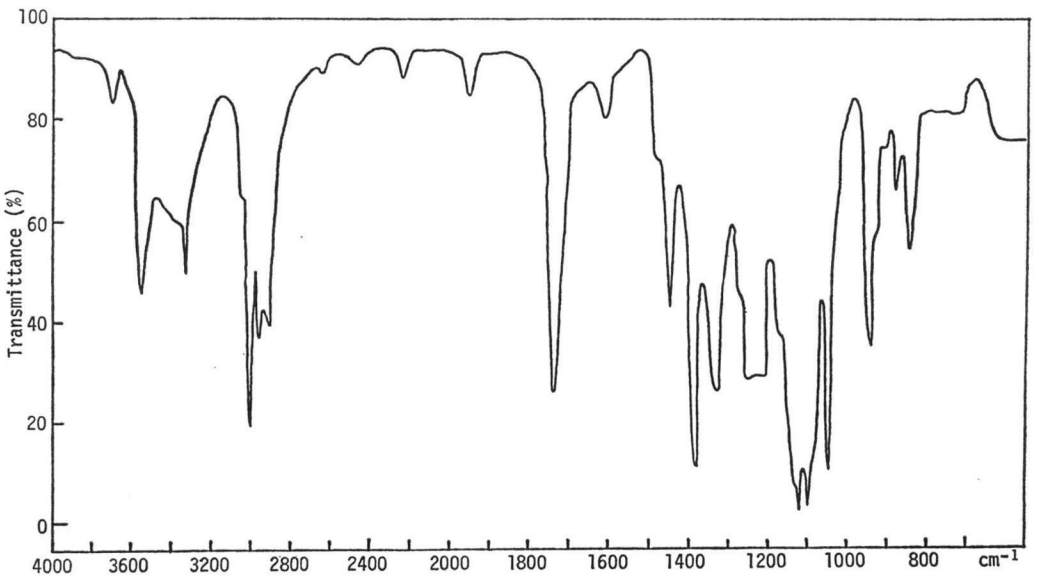

Table 2. Antimicrobial spectrum of PA 789/80 (agar diffusion test).

Test organism	Diameter inhibition zone (mm) with 20 μg antibiotic per agar cup		
	PA 789/80	Xeromphalin A[12]	Nystatin
Bacillus subtilis ATCC 6633	36	26	0
Streptomyces rimosus NRRL 2234	9	25	0
Micrococcus flavus ATCC 10240	32	24	0
Sarcina subflava ATCC 7468	25	26	0
Sarcina lutea 10054	34	27	0
Escherichia coli communis	0	0	0
Pseudomonas aeruginosa 25151	0	0	0
Micrococcus conglomeratus	48	29	0
Staphylococcus aureus SG 511	24	25	0
Sarcina lutea 9341	28	19	0
Candida albicans S-2869	38	16	30
Candida tropicalis S-2173	27	14	25
Aspergillus niger NRRL 67	37	15	27
Neurospora crassa ATCC 9277	0	16	29
Fusarium roseum culmorum	0	13	17
Fusarium oxysporum	0	14	14
Alternaria solani	40	13	30
Cladosporium resinae	44	26	31

Acknowledgements

The authors wish to thank Mrs. K. HÄUSLER for valuable technical assistance.

References

1) BENDZ, G.: Marasin, an antibiotic polyacetylene, isolated from the culture medium of *Marasmius ramealis*. Arkiv Kemi 14: 305~321, 1959
2) BENDZ, G.: 8-Hydroxy-3-methylisocoumarin isolated from the culture medium of *Marasmius ramealis*. Arkiv Kemi 14: 511~518, 1959
3) BENDZ, G.: An antibiotic agent from *Marasmius graminum*. Arkiv Kemi 3: 495~500, 1951
4) BENDZ, G.: A study of the chemistry of some *Marasmius* species. Arkiv Kemi 15: 131~148, 1960
5) KAVANAGH, F.; A. HERVEY & W. J. ROBBINS: Antibiotic substances from basidiomycetes. IV. *Marasmius conigenus*. Proc. Natl. Acad. Sci. U.S. 35: 343~349, 1949
6) HOLROYDE, J. K.; A. F. ORR & V. THALLER: 3,7-Bis(hydroxymethyl)-1-benzoxepin-5(2H)-one, a novel metabolite from cultures of the fungus *Marasmiellus ramealis* (BULL. *ex* FR.) SINGER. J. Chem. Soc., Chem. Comm. 1976: 242~243, 1976
7) KING, T. J.; I.W. FARELL, T. G. HALSALL & V. THALLER: Alliacolide, a new bicyclic sesquiterpene epoxylactone with a novel carbon skeleton from cultures of the fungus *Marasmius alliaceus* (JACQ. *ex* FR.) FR.; X-ray structure. J. Chem. Soc., Chem. Comm. 1977: 727~728, 1977
8) ANKE, T.; J. KUPKA, G. SCHRAMM & W. STEGLICH: Antibiotics from basidiomycetes. X. Scorodonin, a new antibacterial and antifungal metabolite from *Marasmius scorodonius* (FR.) FR. J. Antibiotics 33: 463~467, 1980
9) ANKE, T.; F. OBERWINKLER, W. STEGLICH & G. SCHRAMM: The strobilurins—new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus* (PERS. *ex* FR.) SING. J. Antibiotics 30: 806~810, 1977
10) KUPKA, J.; T. ANKE, F. OBERWINKLER, G. SCHRAMM & W. STEGLICH: Antibiotics from basidiomycetes. VII. Crinipellin, a new antibiotic from the basidiomyceteous fungus *Crinipellis stipitaria* (FR.) PAT. J. Antibiotics 32: 130~135, 1979
11) ANKE, T.; H. J. HECHT, G. SCHRAMM & W. STEGLICH: Antibiotics from basidiomycetes. IV. Oudemansin, an antifungal antibiotic from *Oudemansiella mucida* (SCHRADER *ex* FR.)

HOEHNEL (Agaricales). J. Antibiotics 32: 1112~1117, 1979

12) PALMA, N.: Isolierung und Charakterisierung neuer antibakteriell und antifungal wirksamer Substanzen aus der Pilzart *Xeromphalina campanella*. Dissertation, Innsbruck, 1978

13) BU'LOCK, J. D.; E. R. H. JONES, P. R. LEEMING & J. M. THOMPSON: Chemistry of the higher fungi. VI. Isomerization reactions of naturally occurring allenes. J. Chem. Soc. (London) 1956: 3767~3771, 1956

14) BENDZ, G.: Isomarasin, the alkali-isomerisation product of marasin. Arkiv Kemi 14: 475~481, 1959

15) JOHNSON, A.W.: Natürlich vorkommende Polyacetylen-Verbindungen. Endeavour 24: 126~130, 1956

16) BEW, R. E.; J. R. CHAPMAN, E. R. H. JONES, B. E. LOWE & G. LOWE: Natural acetylenes. XVIII. Some allenic polyacetylenes from basidiomycetes. J. Chem. Soc. (London) 1966: 129~135, 1966

17) CELMER, W.D. & I.A. SOLOMONS: Mycomycin. I. Isolation, crystallization and chemical characterization. J. Amer. Chem. Soc. 74: 2245~2248, 1952

18) BU'LOCK, J.D.; E.R.H. JONES & P.R. LEEMING: Chemistry of the higher fungi. V. The structures of nemotinic acid and nemotin. J. Chem. Soc. (London) 1955: 4270~4275, 1955

19) BU'LOCK, J.D.; E.R.H. JONES & P.R. LEEMING: Chemistry of the higher fungi. VII. Odyssic acid and odyssin. J. Chem. Soc. (London) 1957: 1097~1101, 1957