An Antibiotic Complex from *Alternaria brassicicola*

The genus *Alternaria* has not been extensively surveyed for its ability to produce secondary metabolites. Only 3 antimicrobial compounds from 2 species of *Alternaria* have been described[1-3]. Accordingly, we surveyed 127 *Alternaria* strains representing 30 known species and found that 86 produced antibiotics[4]. From these 86 strains, *Alternaria brassicicola* NRRL 2167 was chosen for further investigation.

Materials and methods. Fermentation procedures have been described[4]. Recovered mycelia were extracted with ether, the supernatant with ethyl acetate; extracts were pooled. Thin-layer chromatography (TLC) was on silica gel N-HR chromatoplates (Brinkmann Instruments[5], Westbury, N.Y.); benzene and acetonitrile (55:45 v/v) was used for the developing reagent. Thin-layer strips were bioautographed against *Bacillus subtilis* NRRL B-765. Column chromatography was on 100-mesh silicic acid (Mallinckrodt Chemical Works, N.Y.) using benzene and gradient addition of acetonitrile for development.

Results and discussion. Bioautography revealed the presence of 5 zones of antibiotic activity. These zones corresponded to areas on the chromatoplates that took up iodine vapor in an iodine chamber.

The first fraction (A) off the chromatographic column was the most active. This fraction was rechromatographed on a silicic acid column. Solvent was removed by flash evaporation and the residue redissolved in ether. This solution was washed with water and then 50% aqueous methanol. The ether solution was dried with anhydrous sodium sulfate and treated with activated carbon to remove a yellow substance. The active material was a white, highly viscous liquid that we were unable to crystallize. However, only a single spot was revealed by TLC on silica gel N-HR with various solvent systems and several detection methods.

This antibiotic had a single sharp maximum in ether at 340 nm. There was no shift of the peak on addition of alcoholic HCl or NaOH. In the IR-spectrum (Figure), a broad peak at 3400 cm^{-1} indicated the presence of an OH group(s) which was further confirmed by nuclear magnetic resonance (NMR) observations in which the broad peaks at 3.1 and 4 ppm disappeared on D_2O addition. A negative $FeCl_3$ test indicates the absence of phenolic hydroxyl. NMR-spectra were too complex for interpretation. Elemental analysis gave C, 55.81%; H, 7,26%; N, 3.21%; oxygen by difference, 33.72% (theoretical: 55.93; 7.27; 3.26; 33.53). Tests for sulfur and halogen were negative. These analyses give a tentative elementary composition of $C_{20}H_{31}O_9$. The antibiotic had an optical rotation in methanol $[\alpha]_D^{25} + 20.1$. It was soluble in ether, chloroform, methanol, acetone, ethyl acetate, and acetonitrile; partially soluble in carbon tetrachloride; and insoluble in water, carbon disulfide, benzene, hexane, and pentane-hexane.

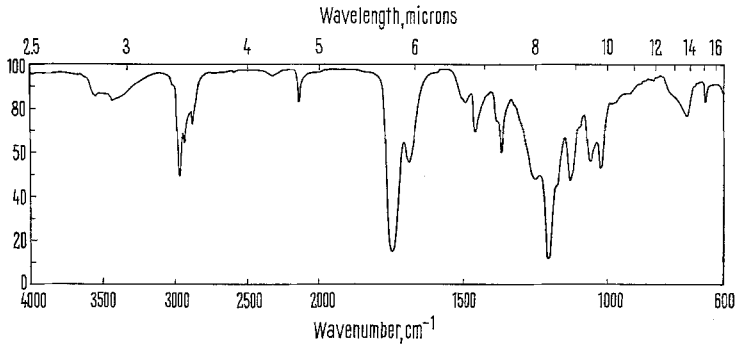
Antibiotic disc assay against *B. subtilis* showed an unusual inhibition pattern after 24 h incubation at 30 °C. There was an inner hazy zone around the disc surrounded by a clear zone of no growth; after 2 additional days of incubation, the inner hazy zone cleared. Addition of an electron acceptor, e.g. methylene blue or triphenyltetra-

In vitro antimicrobial activity of brassicicolin A in agar dilution test

Test microorganism	NRRL No.	Minimal inhibitory concentration (μg/ml)
Bacteria		
Bacillus subtilis	B-765	100
Bordetella bronchiseptica	B-140	12.5
Corynebacterium fascians	B-190	12.5
Proteus vulgaris	B-417	> 1000
Pseudomonas aeruginosa	B-23	> 1000
Staphylococcus aureus	B-313	> 1000
Streptococcus faecalis	B-537	> 1000
Yeasts		
Candida albicans	Y-477	> 1000
Cryptococcus neoformans	Y-5637	12.5
C. neoformans	Y-5638	12.5
Torulopsis glabrata	YB-4025	50
Hansenula jadinii	Y-1542	50
Molds		
Aspergillus fumigatus	1217	> 1000
Colletotrichum circinans	2504	100
Diplodia zeae	2282	10
Mucor ramannianus	1839	1.0
Stemphyllium sarcinaeforme	2188	10
Trichophyton rubrum	A-2846	1.0
T. sulfureum	A-2842	10

[1] H. RAISTRICK, C. E. STICKINGS and R. THOMAS, Biochem. J. *55*, 421 (1953).
[2] P. W. BRIAN, P. J. CURTIS, H. G. HEMMING, C. H. UNWIN and J. M. WRIGHT, Nature *164*, 534 (1949).
[3] J. F. GROVE, J. chem. Soc. 4056 (1952).
[4] L. A. LINDENFELSER and A. CIEGLER, Devs ind. Microbiol. *10*, 284 (1969).

[5] The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.



IR-spectrum of brassicicolin A. The spectrum was recorded in carbon tetrachloride with a Beckman IR-8 infrared spectrophotometer.

zolium, to the medium eliminated the occurrence of the hazy area and also produced larger zones of inhibition.

The in vitro antimicrobial spectrum of the antibiotic (Table) indicates that it has activity primarily against yeasts and some select bacteria and molds.

The trivial name of brassicicolin A is assigned to this antibiotic.

Zusammenfassung. Bei Untersuchungen von *Alternaria brassicicola*, die eine antibiotische Komplexverbindung bildet, wurde eine der aktivsten Verbindungen mit Brassicicolin A bezeichnet, isoliert und gereinigt. Die Substanz konnte jedoch nicht kristallisiert werden. In-vitro-Untersuchungen zeigen primär eine Antiwirksamkeit gegen Hefen.

A. CIEGLER and L. A. LINDENFELSER

Northern Regional Research Laboratory[6], *Peoria (Illinois 61604, USA), 24 February 1969.*

[6] This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

Potassium-Stimulated Respiration of Cerebral Cortex of Rats Poisoned with Phosphamidone

In vitro studies have shown that the rate of oxygen uptake of brain tissue from animals poisoned with organophosphorus anticholinesterases is essentially unchanged even after lethal doses of these compounds[1].

In the present experiments the brain cortical slices taken from rats poisoned with phosphamidone (2-chloro-2-diethyl-carbamyl-1-methyl-vinyl-dimethyl phosphate)[2] were tested in vitro under conditions in which tissue respiration has been stimulated by potassium ions.

Phosphamidone was given s.c. to adult male rats weighing about 200 g. The animals were killed by decapitation 45 min after administration of 15 mg/kg (LD_{50}) and 20–30 min after administration of 30 mg/kg (LD_{90-100}). The brains were quickly removed and 2 brain slices from each hemisphere were used. The oxygen uptake of cerebral cortex was determined at 38 °C by standard Warburg technique, using Krebs-Ringer phosphate solution with glucose as substrate and oxygen as the gas phase. For potassium stimulation 10 m*M* KCl was added.

As can be seen from the Table, the oxygen uptake of cortical slices from phosphamidone-treated rats was lower than that of slices from control animals. The reduction in the tissue respiration was statistically significant ($P < 0.05$) and of the order of 20–35%. The doses inhibitory to the tissue respiration are, however, distinctly higher than those required for inhibition of cholinesterase in cortical slices[3].

The respiratory activity of brain cortex from normal rats is considerably increased by addition of a relatively small concentration of potassium chloride[4]. The addition of the same concentration of potassium chloride to the brain cortex from phosphamidone-treated rats brings about a stronger stimulation of the respiration rate. The increase of respiration augmented as the dose of phosphamidone was increased, amounting to more than 100% at a dose of 30 mg/kg.

It is thus obvious that in phosphamidone-treated rats the respiration of brain cortex is much more sensitive to the stimulant action of potassium chloride than in normal animals. It is interesting, however, that the addition of the same concentration of potassium chloride to the brain cortex taken from animals poisoned with equitoxic doses of paraoxon and TEPP has not such an effect. The true nature of these differences is not quite clear. It is possible, however, that the effect of phosphamidone on the chemically stimulated cellular respiration is due to some of its metabolites, since in concentrations of 10^{-6} to 10^{-2} *M* phosphamidone has no influence on the rate of oxygen uptake in cortical slices stimulated by potassium chloride.

In vitro effect of potassium ions on the respiration of cerebral cortex of rats poisoned with some organophosphates

Organophosphate	(mg/kg)	KCl (m*M*)	QO_2[a]	Change of normal (%)
None	–	–	19.2 ± 1.3 (25)	–
	–	1.0	23.9 ± 1.1 (35)	+ 24[b]
Phosphamidone	15	–	12.5 ± 0.3 (10)	− 34[b]
	15	1.0	28.5 ± 1.5 (8)	+ 48[b]
	30	–	11.4 ± 0.4 (14)	− 40[b]
	30	1.0	37.2 ± 3.7 (6)	+ 93[b]
Paraoxon	0.25	–	18.7 ± 1.7 (8)	+ 2
	0.25	1.0	17.3 ± 1.6 (4)	− 9
	0.5	–	21.5 ± 1.7 (6)	+ 11
	0.5	1.0	20.5 ± 3.4 (4)	+ 6
TEPP	0.5	–	21.8 ± 2.4 (5)	+ 13
	0.5	1.0	17.8 ± 1.7 (4)	− 7
	1.0	–	12.4 ± 1.8 (5)	− 35[b]
	1.0	1.0	17.1 ± 1.3 (4)	− 10

[a] QO_2 = µl O_2/h/mg dry weight of tissue (mean ± S.E.); the figures in parentheses indicate the number of rats. [b] $P < 0.05$.

Résumé. Chez le rat intoxiqué par des doses léthales de phosphamidone, la consommation in vitro d'oxygène des tranches du cortex cérébral stimulées par KCl est plus élevée que celles des témoins ou des rats intoxiqués au paraoxon ou au TEPP.

D. ANDJELKOVIĆ and M. P. MILOŠEVIĆ

Department of Pharmacology, Medical Faculty, Beograd (Yugoslavia), 13 March 1969.

[1] G. PAULET and P. ANDRÉ, J. Physiol. *49*, 335 (1957).
[2] Pure sample of phosphamidone (Dimecron®) was kindly supplied by CIBA A.G., Basel.
[3] D. ANDJELKOVIĆ and M. P. MILOŠEVIĆ, in press.
[4] H. MCILWAIN, *Chemical Exploration of the Brain* (Elsevier Publ. Co., Amsterdam, London, New York 1963), p. 55.