The existence of free sulfur in certain bacteria has been known for some time. Sulfur was found to accumulate on the exterior of the cells of *Thiobacillus thioparus*[4]. This phenomenon has never been reported in fungi, however. It has been shown that molecular sulfur furnished to certain fungi causes the production of hydrogen sulfide, indicating that these microorganisms are capable of metabolizing free sulfur. In this case, the sulfur interfers as a hydrogen acceptor in hydrogenation and dehydrogenation reactions[5]. If elemental sulfur is added to a suspension of spores of *P. viticola*, H_2S is produced after 30 min. These spores, without an addition of external sulfur, produce hydrogen sulfide detected, after 2 days, by filter paper impregnated with lead acetate according to the method of FELLERS[6], confirming the presence of free sulfur in the cirrhus of *P. viticola*. Work is continuing to understand better this self-inhibitor phenomenon. It is important to known how the free sulfur accumulates, its origin from an organic or inorganic source, and whether it accumulates in the interior of the spores or externally in the matrix. With this knowledge, it may be possible to control the self-inhibitory mechanism and thus the plant disease, either by blocking the production of inhibitor and causing the spores to germinate prematurely in the cirrhus, or by augmenting the inhibition so that the spores will not germinate even in favorable periods when the cirrhus is diluted by rain.

Résumé. Les spores alpha de *Phomopsis viticola*, agent de l'excoriose de la vigne, ne germent plus si leur concentration est supérieure à 5×10^5 spores/ml. Il s'agit d'un phénomène d'auto-inhibition. Des extractions, purifications et analyses du spectre de masse ont montré que l'auto-inhibiteur était du soufre moléculaire (S_8). Les recherches ne permettent pas de savoir si ce soufre libre est présent dans les spores ou dans la gelée sporifère.

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Thiabenzazonium, a New 1,5-Benzodiazepine Derivative with Antimicrobial Activity

Investigating 1,5-benzodiazepine derivatives, interesting antimicrobial activities for 2-dialkylaminoalkylthio-4-aryl-3H-1,5-benzodiazepines and for 2-trialkylammonium-alkylthio-4-aryl-3H-1,5-benzodiazepine iodides[1,2] were found. The substance with the highest activity is the 2-methyldiethylammoniumethylthio-4,*p*-phenylthiophenyl-3H-1,5-benzodiazepine iodide, i.e. thiabenzazonium (M. W. 601.62), obtained by iodomethylation of 2-diethylaminoethylthio-4,*p*-phenylthiophenyl-3H-1,5-benzodiazepine, which was prepared by S-alkylation of 4, *p*-phenylthiophenyl-1,3-dihydro-2H-1,5-benzodiazepin-2-thione with 1-chloro-2-diethylaminoethane.

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Table I. Antimicrobial activity in vitro (minimal inhibitory concentrations)

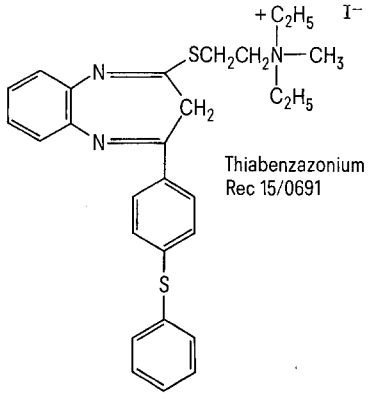
Strains	Minimal inhibitory concentrations (μg/ml)		
	Thiabenzazonium	Chloramphenicol	Bacitracin
Stp. pyogenes humanus A 821	0.25	2	0.25
Stp. pyogenes humanus Ac/203	0.5	1.25	0.5
Stp. pyogenes humanus 823	1	1.25	1
Stp. pyogenes humanus 827	0.5	2.5	0.5
Stp. faecalis ATCC 10541	4	5	8
Stp. β haemolyticus [a]	1	—	0.25
Stp. β haemolyticus [a]	4	—	1
Stpt β haemolyticus [a]	1	—	0.25
Stp. β haemolyticus [a]	1	—	2
St. aureus SG 511	0.5	4	>32
St. aureus ATCC 6538 P	1.25	2.5	—
Bacillus subtilis	1	1	—
Clostridium novii ISM	16	32	—
Escherichia coli 100	4	1	—
Pseudomonas aeruginosa	32	32	—
Proteus vulgaris 0	>64	8	—
Salmonella tiphymurium	32	2	—
Candida albicans	32	64	—
Tricophyton mentagrophytes 2538	>64	>64	—

[a] From pharingeal swabs.

Table II. LD_{50} and p 0.05 fiducial limits of thiabenzazonium and of its cleavage products

Substance	Animal species	Route of administration	LD_{50} (mg/kg)
Thiabenzazonium	Mouse	os	9000 (6570–12300)
Thiabenzazonium	Moose	i.p.	42 (34–52)
Thiabenzazonium	Rat	os	> 10.000
Thiabenzazonium	Rat	i.p.	35 (28–43)
CA	Mouse	i.p.	> 3000
CB	Mouse	os	880 (704–1100)
CB	Mouse	i.p.	37 (34–40)

CA, 4,p-phenylthiophenyl-1,3-dihydro-2H-1,5-benzodiazepin-2-one. CB, 2-methyldiethylammoniumethylthiole iodide.



Thiabenzazonium
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In acid medium thiabenzazonium cleaves to 4,*p*-phenylthiophenyl-1,3-dihydro-2H-1,5-benzodiazepin-2-one and 2-methyldiethylammoniumethylthiole salt, which have no antimicrobial activity.

Antimicrobial activity. The in vitro minimal inhibitory concentrations (MIC) are given in Table I, in comparison with chloramphenicol and bacitracin.

The bactericidal activity against a number of Gram positive strains (2 *Stp. pyogenes humanus* gr. A, 2 *Stp. equisimilis* gr. C, 2 Stp. species gr. G, 3 *Stp. mitis viridans*, 2 *Diplococcus pneumoniae*, 1 *St. aureus* and 2 *Corynebacterium diphtheriae*) after 5, 15, and 30 min of contact at 2 concentrations of the substance (5 µg/ml and 10 µg/ml) was studied and thiabenzazonium was found active against *Streptococcus*, *Diplococcus* and *Corynebacterium*.

The phenol coefficient, determined according to the Rideal-Walker method, showed a bactericidal activity of thiabenzazonium about 250 times higher than that of phenol. In vitro *St. aureus* and *Stp. pyogenes* did not develop resistance against thiabenzazonium during 10 successive transfers.

Virucidal activity was tested against Influenza APR-8 virus and Influenza A2 Ann. (Arbor 60) virus. The infectivity was then evaluated on embryonated Leghorn eggs as previously described[3]. After 1 h of contact at 37 °C, thiabenzazonium inactivated APR-8 virus at a concentration of 0.125 µ*M*/ml and the A2 virus at a concentration of 0.0156 µ*M*/ml.

Toxicity. The toxicity of thiabenzazonium after a single administration is uneventful and the animals die 10–20 h after oral or i.p. treatment. The LD_{50}, given in Table II, show that there is a striking difference between the oral and the i.p. toxicity of thiabenzazonium, probably due either to a poor intestinal absorption of the drug, or to a splitting into less toxic substances. A 6 months chronic toxicity study in rats with 4, 40 and 400 mg/kg/die orally and in dogs with 4, 30 and 200 mg/kg/die orally was also practically uneventful. Also fetal toxicity studies in rats and rabbits yielded similar uneventful results.

Conclusion. Thiabenzazonium is a substance with potent antimicrobial properties, especially on some Gram positive bacteria which are agents of oropharyngeal infections. The drug shows also virucidal activities on influenza-virus strains. These antibacterial and antiviral properties, combined with a very low oral toxicity, allow one to classify thiabenzazonium as an antimicrobial drug, potentially indicated for local treatment and for prophylaxis of oropharingeal infections.

Riassunto. Si descrivono la sintesi, le caratteristiche fisico-chimiche e le proprietà antimicrobiche del thiabenzazonio, un nuovo derivato ammonico quaternario della 1,5-benzodiazepina. Il thiabenzazonio è dotato di una spiccata attività batteriostatica e battericida su alcune specie microbiche Gram-positive, che spesso sono responsabili di infenzioni del cavo orofaringeo.

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Paracrystallization of Actomyosin

The interaction of actin and myosin lies at the basis of muscular contraction. For this reason actomyosin, as gels or threads, has been used as a useful contractile model[1,2]. There would be merit in refining such a model by assembling actomyosin into a thick and thin filament order similar to that of the muscle sarcomere. This paper reports the formation of such aggregates having a degree of order not previously achieved[3–5].

Natural actomyosin of high purity and retaining calcium sensitivity was prepared from leg muscle of the hen[6]. It was dissolved to 0.4–0.6 mg/ml in a relaxing medium of high ionic strength (6 m*M* ATP, 6 m*M* $MgCl_2$, 2 m*M* EGTA, 0.01 *M* imidazole, 0.5 *M* KCl, pH 7.0), and then dialyzed for 24 h at 2 °C against a similar medium (ionic strength 0.15) in which KCl was reduced to 0.05 *M*, allowing thick myosin filaments to

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