Le dosage in vitro de la Δ^4 réductase par la méthode que nous avons décrite précédemment[12] montre que l'activité spécifique de cette enzyme est 12 fois plus importante dans les cellules cultivées 85 h dans le milieu A par rapport aux cellules provenant du milieu B.

D'autre part, quand des cellules provenant d'une culture sur le milieu B, en l'absence de stéroïde, sont récoltées après 40 h, puis remises en suspension, après lavage, dans un tampon phosphate 0,18 *M* de pH 7,0 en présence d'une source de carbone et d'énergie à 20 g/l, on observe les faits suivants. Si on incube ces cellules pendant 48 h à 27°, en aérobiose, en l'absence de sources d'azote et en présence de (4-^{14}C) progestérone (I), les pourcentages de réduction de I menant à II, en fonction de la nature de la source de carbone, sont respectivement de 39% avec le glucose, 36% avec le fructose, 11% avec l'acétate de sodium, 4% avec l'hexanoate de sodium et 3% avec l'oléate de sodium; en l'absence de source de carbone le pourcentage de réduction est de 6%. Le métabolisme des sources carbonées glucidiques dans un milieu déficient en sources d'azote stimule donc la réduction de la Δ^4 des stéroïdes.

Ces résultats sont à rapprocher de quelques observations d'auteurs[4,18,19] qui avaient constaté des résultats variables dans certaines réactions d'oxydation et de réduction des stéroïdes, en fonction de la composition du milieu de culture. L'hydrogénation des stéroïdes par les *Nocardia* est sans doute liée à la capacité qu'ont ces microorganismes d'effectuer la biosynthèse d'acides gras à longues chaînes hydrogénées[20]. Il est probable que le catabolisme des stéroïdes, oxydatif quand l'organisme utilise le stéroïde, comme source de carbone et d'énergie, soit dévié dans le sens réducteur quand l'organisme cessant sa croissance à cause de la déficience en sources d'azote, synthétise alors des composés de réserve hydrogénés, notamment des lipides, à partir de l'excès de source glucidique. Dans ces conditions les stéroïdes hydrogénés seraient incorporés dans les lipides cellulaires[21].

Summary. Nocardia corallina hydrogenates progesterone to 5α-pregnane-3,20-dione in growth medium containing excess glucose. The Δ^4 steroïd reductase appears in such conditions, in the acellular extracts. Likewise in non-proliferating cells incubated in nitrogen-deficient medium containing different carbon sources, glucidic sources stimulate hydrogenation of the Δ^4. The significance of oxydations and reductions of steroïds among Actinomycetales are discussed in relation to the metabolic characteristics of these organisms.

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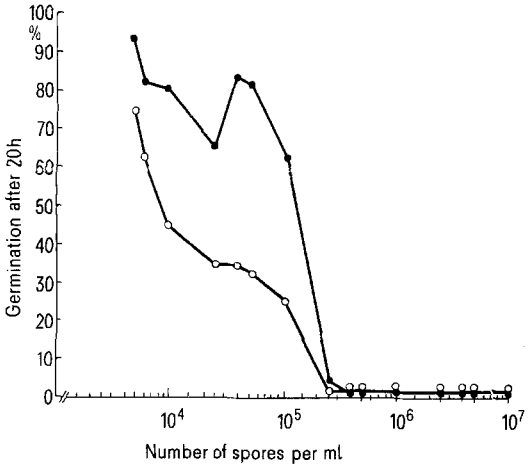
Elemental Sulfur Responsible for Self-Inhibition of Spores of *Phomopsis viticola*

Alpha spores of *Phomopsis viticola*, causal agent of dead-arm disease of grapevine, do not germinate, with or without an external nutrient source, if their concentration is greater than 5×10^5 spores/ml (Figure). This phenomenon of self-inhibition has been studied in other fungi and discussed by several authors[1–3]. The self-inhibitor of *P. viticola* was extracted from the cirrhus with $CHCl_3$-MeOH (1:1) at room temperature under continuous stirring for 2 h. Spores were removed by centrifugation and the supernatant evaporated to dryness and resuspended in petroleum ether (PE). The soluble phase in PE was then fractionated using a column of Kieselgel H in PE with a mobile phase consisting of a mixture of 200 ml of PE and 4 ml of diethyl ether (DE). The fraction PE-DE collected, was evaporated to dryness and resuspended in $CHCl_3$. The self-inhibitor was then purified by TLC on Kieselgel HF (250 μ) using cyclohexane-$CHCl_3$ (99:1). Only the spot at Rf = 0.85, after elution in $CHCl_3$, was inhibitory to spore germination in tests using 5 mm diameter assay disks on 2% malt agar in petri plates. Mass spectrum analysis of this spot, using a Hewlett-Packard GC-MS, indicated sulfur in form of S_8: m/e 255.8 (theoretically 255.776) (100%) with a relative intensity of 37% for the peak P + 2 (theoretically 35%). The mass spectrum of the compound also gives peaks corresponding to S_2: *m/e* 64 (69.1%), S_3: *m/e* 96 (27.7%), S_4: *m/e* 128 (59.7%), S_5: *m/e* 160 (40.6%) and S_6: *m/e* 192 (24.2%). The final extract from a blank extraction, made by the same procedure, was not inhibitory to germination and no spot appeared to Rf = 0.85. As a result of this, comparisons were made between standard elemental sulfur, and self-inhibitor extracted from the cirrhus of *P. viticola*. In each case spore germination was inhibited, and chromatographic, and chemical tests gave similar results.



Effect of the number of spores of *Phomopsis viticola* on germination. Incubating washed spores in: ○, no nutrient (water); ●, nutrient consisting of 0.5 mg/ml of freeze-dried water-soluble matrix of *P. viticola*.

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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.