days before the carrageenin injection. Exposure to cold surrounding temperature (as in the first experiment) 3 days later elicited the usual cutaneous and renal lesions but, in addition, the tumor transplants underwent severe hemorrhagic necrosis (Fig. 2). Controls receiving the same carrageenin treatment without exposure to cold exhibited no hemorrhagic lesions in the tumor transplants.

This model may be of use in the study of the so-called diseases *a frigore*; it also furnishes us with a new technique for the production of hemorrhagic necrosis in tumors.

HANS SELYE

Institut de Médecine et de Chirurgie Expérimentales, Université de Montréal, Montreal, Quebec

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3. Work supported by the National Cancer Institute of Canada and the Medical Research Council of Canada.

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Phytotoxin Isolated from Liquid Cultures of Ceratocystis ulmi

Abstract. *Phytotoxic material has been isolated from liquid cultures of* Ceratocystis ulmi. *One component of the material has been obtained in pure form and has proved to be a rather thermostable glycoprotein. This compound induced disease symptoms, similar to those produced by the fungus itself, in elm sprouts and trees.*

Phytotoxic material present in the filtrate of a nutrient medium in which *Ceratocystis ulmi* (Buisman) C. Moreau had been grown was discovered by Zentmyer (*1*) and the production of toxic substances has been confirmed (*2–5*). To Zentmyer and Horsfall (*6*) this thermostable toxic material appeared to consist of more than one component, one of which Dimond (*2*) characterized as a polysaccharide. Feldman *et al.* (*3*) and Lafayette and Howard (*7*), however, considered this component as rather unimportant in relation to the pathogenicity of the fungus. Beckman (*5*) showed a pectinase and cellulase to be present in the culture filtrate.

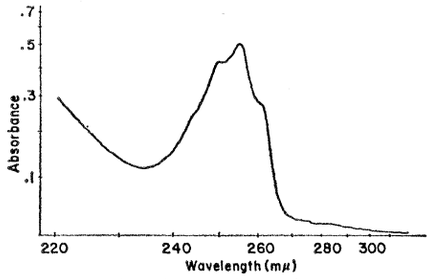

Fig. 1. Ultraviolet absorption spectrum of compound A.

In our experiments *Ceratocystis ulmi* was cultured at 24°C in a nutrient medium of the following composition (per liter of tap water): glucose, 20 g; L-asparagine-monohydrate, 2 g; KH_2PO_4, 1.5 g; $MgSO_4 \cdot 7H_2O$, 1 g; $ZnSO_4 \cdot 7H_2O$, 20 mg; $FeCl_3$, 10 mg; pyridoxin, 1 mg; and thiamin, 1 mg. After 2 to 3 weeks the mycelium was separated from the shake culture by centrifugation and filtration.

The culture filtrate, all fractions, and all substances derived from it during the process of isolation and purification were tested for their toxicity to elm sprouts. Sprouts were raised from root callus according to the method of Tchernoff (*8*); these had the advantage of being genetically homogeneous. All sprouts originated from the susceptible elm *Ulmus hollandica* Mill, clone *belgica*. Each sprout was placed with its base in an aqueous solution of an isolated fraction or substance in concentrations that were present in the original filtrate. The control consisted of similar shoots placed in water, in nutrient medium, and in culture filtrate. If the isolated fractions were toxic, the sprouts showed some of the typical symptoms of elm disease, that is, young leaves wilted and died, older leaves curled upward or developed necrotic spots between the veins. Saplings of the *belgica* clone, aged 1 to 2 years, were used for testing the toxicity of the substances isolated from the culture filtrate. Solutions were taken up by wounds cut into the stems (*9*). For control, spore suspensions, culture filtrate, or water was administered to the trees.

Concentration of the culture filtrate 15 times (in a vacuum) and addition of an equal volume of ethanol to the concentrate caused the precipitation of a polysaccharide. After filtration of the solution and evaporation of the ethanol, the concentrate (*p*H 3.5 to 4) was dialyzed against running tap water until the *p*H of the solution became 5; above *p*H 6.5 loss of phytotoxicity might occur (*3, 10*). Therefore, after 2 days the process was continued with 0.05*M* pyridine–acetic acid buffer (*p*H 4.8). The buffer was changed once a day for 1 week. Concentration and subsequent freeze-drying of the remaining colorless and viscous solution yielded 50 to 150 mg of a white powder (per liter of culture filtrate) which was highly soluble in water and rather thermostable with regard to toxicity. Ultracentrifugation of this substance in 0.1*M* NaCl revealed inhomogeneity. A main compound (A) comprising approximately 95 percent of the total amount, with a molecular weight of about 25,000, and a polydisperse minor constituent (B), with a molecular weight of about 10^6, were present. So far it has not been possible to separate the components from each other by gel filtration on Sephadex G-100 or on Biogel P-100. However, through preparative ultracentrifugation of crude toxin (A + B) isolated from 10 liters of culture filtrate we obtained 300 mg of the pure compound A.

The compound B has not yet been isolated in pure form. Its phytotoxic properties are therefore still unknown. The toxin (A + B) induced in the shoots phytotoxic phenomena similar to those induced by the culture filtrate of *Ceratocystis ulmi*. It appeared to be rather thermostable with regard to toxicity. This material did not possess the activities of pectinase and cellulase as reported by Beckman (*5*). To each tree of a group of 25, a dose of 25 to 200 mg of crude toxin (A + B), dissolved in 5 ml of water, was administered. The quantity used depended on the size of the treated elm. The discoloration of the leaves, the vascular browning, and the plugging of the vascular system with gums and tyloses were similar to those induced by the culture filtrate and also similar to the initial symptoms shown by susceptible trees after inoculation with a spore suspension. Compound A, when applied to shoots, appeared to be as effective as the crude toxin, even in concentrations as small as 10^{-5} mole/liter.

Compound A proved to be a glycoprotein. Figure 1 shows the typical ultraviolet absorption spectrum of this substance. The protein moiety probably consists of one peptide chain, as shown by oxidation of the glycoprotein with sodium periodate and isolation of the product by paper electrophoresis. Total

hydrolysis of the oxidized part yielded several amino acids. Hydrolysis of the carbohydrate and subsequent paper chromatography showed mannose and galactose.

C. A. SALEMINK
H. REBEL
Laboratory of Organic Chemistry, State University, Utrecht, Netherlands
L. C. P. KERLING
V. TCHERNOFF
Phytopathological Laboratory, Willie Commelin Scholten, Baarn, Netherlands

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Oxidation of Dimethyl Sulfoxide to Dimethyl Sulfone in the Rabbit

Abstract. *A white, crystalline compound was obtained from a butanol extract of the urine of rabbits injected subcutaneously with dimethyl sulfoxide. The melting point and infrared spectrum of the compound were identical with those of authentic dimethyl sulfone.*

During studies involving the subcutaneous injection of estrone benzoate into rabbits, injection was carried out daily in 1.5 ml of steroid-suspending vehicle (*1*) and 0.5 ml of dimethyl sulfoxide. The dimethyl sulfoxide aided in the dispersion of the solute, and it was hoped that it would increase its absorption. Urine from twenty daily collections was extracted with ethyl acetate, adjusted to *p*H 2.0, and extracted with butanol (*2*). The butanol extract was subjected to countercurrent distribution in a mixture of ethyl acetate, butanol, and water (3 : 1 : 4), and a fraction with the partition coefficient of the estrogen conjugate under study (*2*) was removed. After three distributions in the same system, the dried fraction contained, in addition to the steroid conjugate, a crop of fine needle-like crystals.

After being thoroughly washed with ethanol, the material was twice recrystallized from ethanol and dried in a vacuum. The crystals melted at 108.5° to 110°C and gave the infrared spectrum (KBr) shown in Fig. 1, curve *A*. This suggested that the compound was dimethyl sulfone. A sample of authentic dimethyl sulfone was prepared by oxidation of dimethyl sulfoxide with hydrogen peroxide in a mixture of ethanol and acetic acid. This material melted at 109.5° to 110.5°C, and the infrared spectrum in KBr is shown in Fig. 1, curve *B*. When this sample of dimethyl sulfone was mixed with the material isolated from rabbit urine, the mixture melted at 109.5° to 110°C.

The crystals of dimethyl sulfone were not observed in the urine of rabbits when dimethyl sulfoxide was not included in the injection medium (*2*), but were found in urine from two rabbits injected with dimethyl sulfoxide in the absence of estrone benzoate and steroid suspending vehicle; this leaves no doubt that the dimethyl sulfone arose from the administered dimethyl sulfoxide. The urine was collected in metabolism cages which, while retaining feces on a screen, do permit some leaching of fecal material by the urine. The possibility that part of the dimethyl sulfone may have been excreted in the feces cannot therefore be excluded.

The infrared spectrum of the dimethyl sulfoxide used indicated that the maximum amount of dimethyl sulfone which might have been present in the starting material was 1 percent. The recovered sulfone accounted for 2.5 percent of the injected sulfoxide, even though the conditions of our experiments were designed for the isolation of a steroid conjugate (*2*) and were certainly not optimum for the recovery of dimethyl sulfone.

Boursnell *et al.* (*3*) suggested that β:β'-dichlorodiethyl sulfoxide might be oxidized to the corresponding sulfone by the rabbit, while Snow (*4*) found

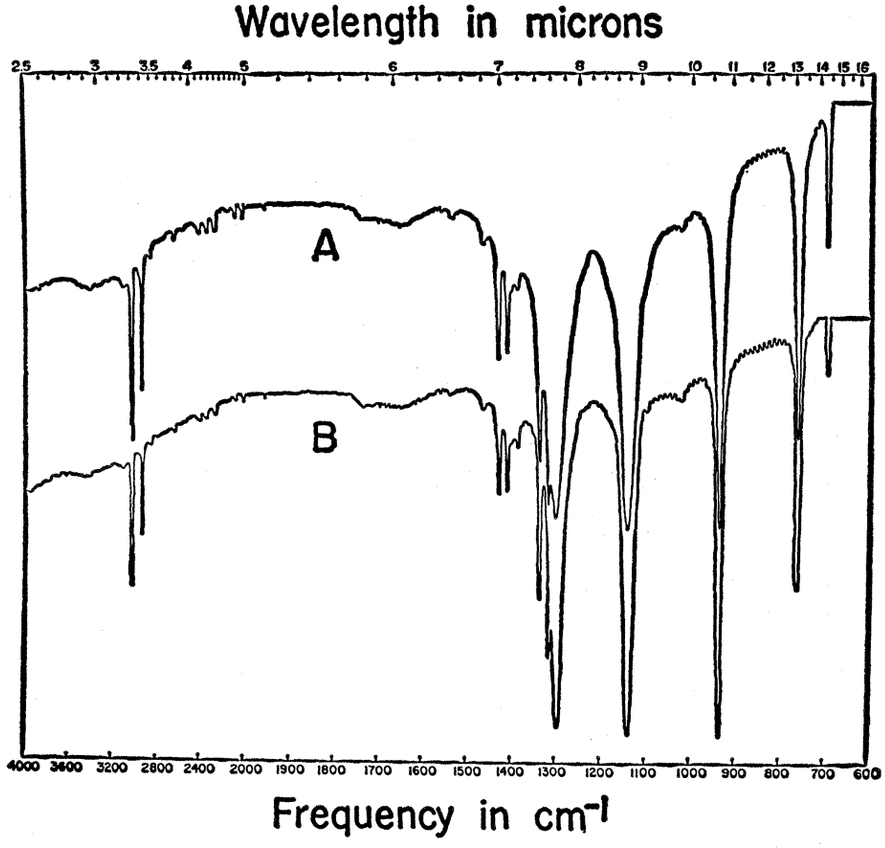

Fig. 1. Infrared spectrum in KBr of dimethyl sulfone isolated from rabbit urine after administration of dimethyl sulfoxide (curve *A*) and of authentic dimethyl sulfone (curve *B*).