ACTA CHEMICA SCANDINAVICA 8 (1954) 1332—1334

Stemphylone, a Root-Killing Substance from *Stemphylium radicinum*

O. ROSENLUND HANSEN

A/S Ferrosan, Copenhagen, Denmark

In testing some plant pathogenic fungi for antibiotic and germ-inhibiting effects substrates from *Stemphylium radicinum* were found to inhibit the germination of *i.a.* seeds of *Lepidium sativum*.

The active substance can be extracted with chloroform and recrystallized from ethanol or acetone. The substance is slightly soluble in water, but soluble in many organic solvents, concentrated sulphuric acid, and hydrochloric acid. It is optically active.

With dinitrophenylhydrazine an orange dinitrophenylhydrazone is obtained. As reactions for aldehydes are negative, the substance is consequently supposed to be a ketone. It has been called stemphylone.

The formula $C_{12}H_{12}O_5$ is proposed as composition of the substance.

A solution of stemphylone in concentrated H_2SO_4 has an intensive blue fluorescence in ultraviolet light.

The maxima of the UV absorption spectrum are reached at 340 mμ and 271 mμ.

Neergaard [1] has found production of crystals in some *Stemphylium* cultures on agar substrates. These crystals have been proved to consist of stemphylone.

Stemphylone is slightly inhibitory to *Escherichia coli* and *Streptococcus aureus*.

By the technique used an effect has been observed at concentrations down to 5×10^{-5} on germinating seeds of *Lepidium sativum*.

At concentrations permitting germination but inhibiting complete development, no visible changes are seen on the stem and the seed-leaves, whereas the root has changed somewhat. It is much shorter than normal, and is brown to brownish black coloured while the root-hairs are missing.

EXPERIMENTAL

All melting points are uncorrected micro melting points. The microanalyses are made by Mr. P. Hansen.

Production of stemphylone. A 2 % solution of malt extract (A/S Alfred Benzon) was poured into glass-bottles in layers of 3 cm, sterilized, and inocula-

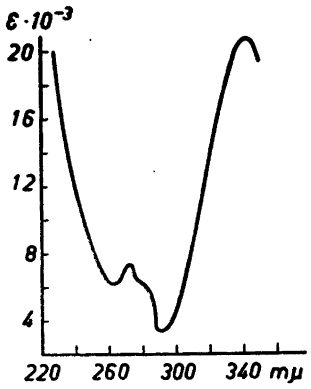

Fig. 1. UV spectrum o stemphylone in ethanol..

ted with spores from *Stemphylium radicinum* (M., Dr. & E.) Neerg. 1939 „Carrot IV" [2], which had previously been cultivated on slices of raw carrot. The bottles were left at a temperature of 22°, and the production of stemphylone was followed by a fluorescence reaction. One drop of the substrate was added to 5 ml of concentrated H_2SO_4, and the fluorescence compared with a standard solution.

After four weeks the mycelium was filtered off, and the filtrate extracted with 1/5 vol. of chloroform. The chloroform layer was dried with Na_2SO_4, added charcoal, and filtered. After evaporation the residue was a brownish crystal mass.

Yield 1 g per litre of substrate. Recrystallized from ethanol and then from acetone. Almost colourless needles. M.p. 220° with destruction. (Found: C 61.3; H 5.3. Calc. for $C_{12}H_{12}O_5$ (236.2): C 61.3; H 5.1.)

Optical activity; With a solution of 126 mg of stemphylone in 10 ml of a mixture of ethanol and chloroform (1 vol. + 2 vol.) $[\alpha]_D^{22} = -125°$ was found.

Fluorescence. A solution of 0.06 mg of stemphylone in 100 ml of concentrated H_2SO_4, and a solution of 0.05 mg of quinine in 100 ml of dilute H_2SO_4 had equal intensity of fluorescence when compared in a simple photoelectric fluorophotometer.

UV spectrum. The absorption spectrum was measured in a solution of ethanol in a Beckmann model DU quartz spectrophotometer (Fig. 1).

Dinitrophenylhydrazone. After recrystallization from pyridine orange needles were obtained. M.p. 245° (dest.). An alcoholic solution is coloured scarlet by NaOH. (Bamberger's test for nitrophenylhydrazones). The sample used for analysis was dried at 165° for 1 h. (Found: C 52.2; H 3.9; N 13.4. Calc. for $C_{18}H_{16}O_8N_4$ (416.3); C 52.0; H 3.9; N 13.5.)

Isolation of crystals from an old agar culture; A few milligrams of crystals were collected with a needle and recrystallized from ethanol. M.p. 220°. The isolated compound was in all respects identical with stemphylone.

Determination of the effect of stemphylone on germinating seeds of Lepidium sativum. Filter paper 9 cm in diameter was placed on the bottom of a Petri dish, and 3 ml of an aqueous solution of stemphylone were poured

on it. About 50 seeds were placed on the moist filter paper and left in the thermostat at a temperature of 22°. At concentrations down to 10^{-3} there was no germination. Growth-inhibition could be observed at concentrations between 10^{-3} and 5×10^{-5}.

SUMMARY

A ketonic compound, stemphylone, was isolated from substrates of *Stemphylium radicinum*. Production, purification, and analysis of the compound are described.

The most interesting property of stemphylone is the toxic effect on germinating seeds, where especially the root development is inhibited.

My thanks are due to P. Neergaard, Dr. agron., for having drawn my attention to the production of crystals in the agar cultures of *Stemphylium* species, and for the fungus cultures used.

REFERENCES

1. Neergaard, P. *Danish Species of Alternaria and Stemphylium.* Copenhagen (1945), p. 348.
2. *Ibid.* p. 335.

Received May 13, 1954.