PAPERCHROMATOGRAPHIC EXAMINATION OF GERMINATION- AND GROWTH-INHIBITING SUBSTANCES FOUND IN THE DRY FRUITS OF THE GLEDITSIA

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Introduction

In recent years numerous papers have been published concerning the germination- and growth-inhibiting substances of the fleshy fruits (EVENARI, 1949; EPERJESSY and NAGY, 1956; FERENCZY, 1957 a, b; VARGA and FERENCZY, 1957; VARGA, 1957 a, b) whereas the examination of the inhibiting substances of the dry fruits has been somewhat neglected. Investigations pertinent to this problem have been carried out so far, among others, by EVENARI, 1949; ELLIOT and LEOPOLD, 1953; KÖVES, 1957; reliable and systematic examinations, relating to inhibitory substances of the dry fruits, however, are still missing. To gather informations I have investigated the germination- and growth-inhibiting properties of extracts of the Gleditsia legume and the quality and quantity of the inhibitor-substances found in those.

Material and method

10 g of the crushed dry legumes of the *Gleditsia triacanthos* L. were measured and extracted in hot water for quarter of an hour. Then the hot, aqueous extract was placed in Petri-dishes containing filter paper, wherein 50—50 seeds respectively of each of two monocotyledonous (*Secale cereale* L. »Kisvárdai P«, *Hordeum vulgare* L. »Hatvani 308«) and of two dicotyledonous plants (*Amaranthus albus* L., *Papaver somniferum* L. »Fertődi kék«) were germinated. The control seeds were germinated on filter paper wetted with distilled water. Seeds were as germinated considered when the radicula broke the seed-coat.

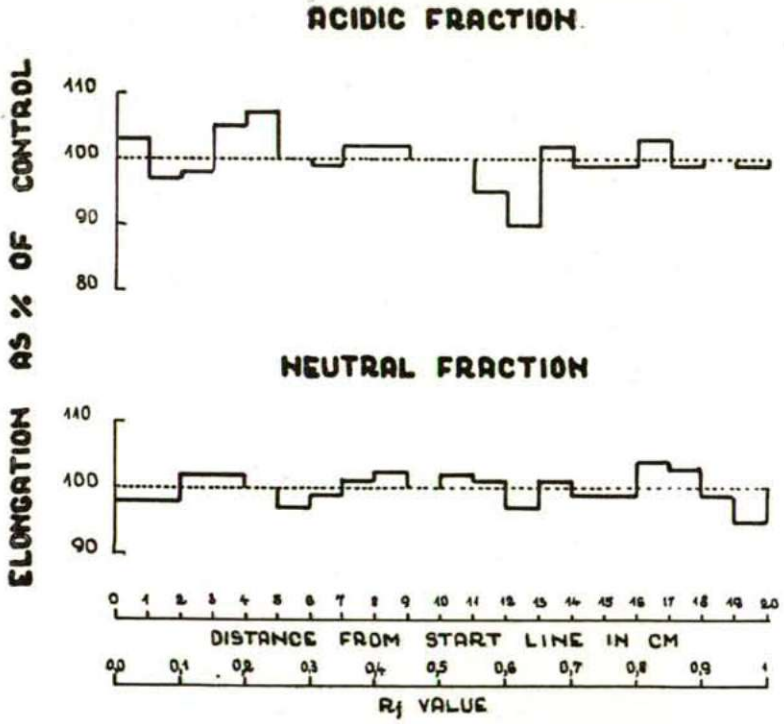
The ethereal extract has been made also from 10 g smashed and cut legumes with peroxide free ether. The extract was partitioned on LARSEN's principle (1949), with BONDE's method (1953) between pH 4,5 and pH 9,5. For acidification, instead of tartaric acid, 0,5 n HCl, suggested by LARSEN (1955), was used; namely, in his opinion tartaric acid may also cause inhibition in the coleoptile sections. The acidic and neutral fraction obtained by partition was paperchromatographically fractionated and examined partly by *Avena* coleoptile-section test (with BENTLEY's, 1950, and BENTLEY and HOUSLEY's method, 1954), partly by *Papaver* germination test (FERENCZY's method, 1957).

For paperchromatography Sch. et Sch. 2043b paper and isopropylalcohol- ammonia-water (10 : 1 : 1) solvent and for the biological examination *Avena* sativa L. »Fleischmann« coleoptile sections (GRACZA, 1957), and seedlings of *Papaver somniferum* L. »Fertődi kék« respectively were used.

As to other details of the biological test I would refer to my earlier paper (*Gracza*, 1957).

Experimental results

The hot-water extract inhibited the seeds of all the four experimental plants in 100 % as compared to the controls. The pH of the extract examined with universal indicator paper was found acidic (pH 6). Two inhibition spots could be noted (Fig. 1) on the chromatogram. The Rf value of the first spot was 0,1, and that of the second one 0,55—0,65. On the chromatogram of the neutral fraction a single spot appeared up to Rf 0,95—1,0. The inhibition is expressed in percent with reference to the elongation of the control coleoptile



sections. The percentile inhibitory effects were converted into salicylic acid mg by using a standard curve (VARGA, 1957) obtained on the basis of the inhibitory effects of known amounts of salicylic acid and were referred to 1 g of the fresh weight.

The Rf 0,1 spot of the acidic fraction corresponds to the inhibitory effect of 1 mg/g salicylic acid; the Rf 0,55—0,65 to that of 8 mg/g salicylic acid and the inhibitory effect of the Rf 0,95—1,0 spot of the neutral fraction corresponds to that of 1 mg/g salicylic acid.

Discussion

Concerning the character of the germination- and growth-inhibiting substances the followings may be stated. They are water and ether soluble. They are thermostabile as they were not inactivated following hot water extraction (100 C°). They are of acidic character because on the one hand the extract, testing with universal indicator paper showed a pH value 6, and on the other the real inhibition spots appeared on the chromatogram of the acidic fraction. The following data furnish informations on the quality of the spots. The spot of 0,1 Rf value due to its position may, in all probability, be a mixture of open carbonchain substances (tannic acids) (VARGA, 1957).

The spot of 0,55—0,65 Rf value may be indentified with the β-inhibitor described by BENNET-CLARC, KEFFORD (1953) due to its position and on the basis of its effect on the *Avena* section test as well as on that of the *Papaver germination*. It has been demonstrated that this β-inhibitor is a mixture of substances composed of aromatic acids (e. g. presumably cinnamomic acid and benzoic acid) (VARGA, 1957).

Im my opinion the inhibitory spot of 0,95—1,0 Rf value of the neutral fraction may be identified with the ω-inhibitor described by FERENCZY (1957) composed, according to him, of, in all probability, of the volatile oils running in the frontline. On the basis of the above results it seems desirable to decide with comprehensive and systematic investigations and examinations whether all the dry fruits contain inhibiting-substances.

Summary

1. The dry legume fruit of the *Gleditsia triacanthos* L. contains germination- and growth-inhibiting substances.

2. The hot-water extract of the fruits inhibits the germination of the seeds of mono- and dicotyledonous plants used for test.

3. Having examined the ethereal extract with *Avena* coleoptile cylinder and *Papaver* germination test following paperchromatographic fractionation it could be stated that the acidic fraction contains two inhibiting substance or mixture of substances. Presumably the inhibiting concentration of the tannic acids is responsible for the inhibitory effect of Rf 0,1 spot, the second, i. e. the Rf 0,55—0,65 spot is identical with the β-inhibitor described by BENNET-CLARK, KEFFORD (1955).

4. Extensive and systematic examinations of the inhibiting substances of dry fruits seem to be necessary.

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