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THE EFFECT OF ARCTIINE ON GERMINATION, ON ROOT TISSUES AND ON NUCLEIC ACIDS

By

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This paper consists of three parts. A: Further data are furnished on the relation between the annual rhythm of germination and the arctiine content; the arctiine content of the various organs is examined and a relation between the arctiine content and the *Compositae* flower-type is established. B: In the second part the effect of arctiine on root tissues is demonstrated by histological examinations. No substantial effect due to arctiine can be observed in the tissues of the stem. C: The interaction of arctiin with nucleic acids is demonstrated by circular dichroism. In the presence of arctiine the conformation (into a double helix?) of the nucleic acids changes markedly.

In a previous paper (SZABÓ, GARAY 1970) it was pointed out that arctiine inhibits the germination of various plants. The question, however, whether this effect manifests itself at a histological level, or, more exactly, what tissue element of the stem and the root are sensitive to arctiine, was not investigated. No mention was made about the effect of arctiine on macromolecules, although there are some data on the interaction between nucleic acids and phenolics. According to D'AMATO and HOFFMAN—OSTENHOF (1956), ortho- and para-phenols exert a mutagene effect in plants. CHAJLAHJAN (1961) remarks that in the effect of gibberellins on nucleic acids cinnamic acid plays a roll. Growth stimulation induced by cumarin appears also on the RNA level (KNYPL 1966). It is not known to the authors whether the interaction between plant phenolics and nucleic acids has been examined *in vitro*. The question, however, is justified, since according to the present theory the substances regulating the growth and organization probably act on the level of the nucleic acids.

Material and methods

Arctiin and arctigenin has been determined as described previously (SZABÓ, GARAY 1970). The seeds were germinated in Petri dishes on wet filter paper, and with 5 mg/ml arctiine solution, respectively. Germination took place in an incubator at 4000 lux, 14000—16000 erg/sq. cm, at 24°C. Three to seven days old seedlings were fixed and preserved in 50 per cent alcohol. The pieces of root and hypocotyl were embedded in celloidine. Cutting was carried out with a sliding microtome. After removing the celloidine, the slides were treated with a solution of 5—10 per cent sodium-hypochlorite and 2 per cent acetic acid. Then they were stained with Erlich-type acidic haemotoxyline, while those from which celloidine had not been removed were stained with gentiana violet. Evaluation was based on about 750 slides.

Four nucleic acids of different origin (yeast, y-RNA, Merck; t-RNA, Calbiochem; and highly polimerized p-RNA, Calbiochem;) were used for the investigation of interaction between arctiine and nucleic acids. The investigated DNA was prepared from blood of chicken embryo.

Unfortunately, there was no opportunity for studying the interaction between plant nucleic acids and arctiine. The nucleic acids were dissolved in ion-exchanged water (pH 6—6.5) containing 0.01 M NaCl, while the arctiine in ion-exchanged water. Measurements were carried out at room temperature, with Jasco-type ORD/UV-5 spectrophotometer equipped with CD attachment.

To determine whether arctiine acts through nucleic acids, the following experiments were carried out. The absorption and CD spectra of four different nucleic acids and of arctiine were taken. Then arctiine was mixed with the individual nucleic acids. After half an hour the absorption and CD spectra were again plotted, to check whether some kind of interaction between arctiine and nucleic acids had happened? In the absence of interaction we would obviously get spectra composed of the 2 individual spectra by simple addition i.e. (NS) + (A) = = (NS + A). If arctiine and nucleic acid enter into any kind of interaction, the CD spectra — which are extremely sensitive to various conformations — should differ from the sum of spectra taken separately.

Results and discussion

(*a*) *New data on the effect of arctiine on germination and its occurence in the various organs*

It has already been suggested in the previous paper that there is a connection between the arctiine content and the annual rhythm of germination in light and in darkness. The investigations had been prolonged for another year and so it was observed that there is a parallelism in the change of the arctigenine content of the ethanol-soluble fraction and the annual rhythm of germination. The higher percentage of germination observed in autumn and in spring is associated with the rise in the arctigenine content of the ethanol-insoluble fraction. However, the effect is not entirely definite. The ethanol-insoluble fraction gradually decreases with the annual changes of germination. The lowest values were recorded during the intensive spring germination (Fig. 1).

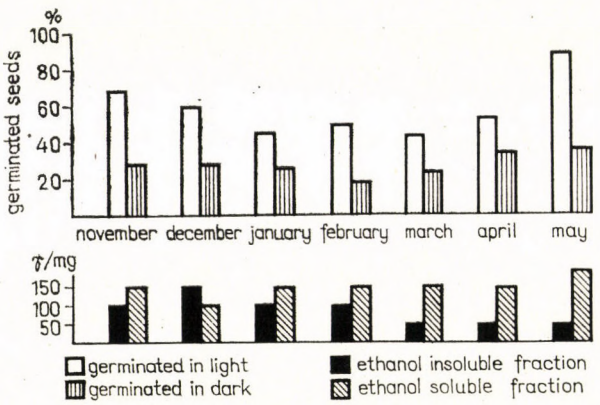

Fig. 1. Annual rhythm of germination of *Arctium lappa* as a function of arctigenine content of the seeds

The arctiine content of the organs of *Arctium lappa* was examined. As seen in Table I the occurence of arctiine is restricted to the inflorescence and the seed. It is interesting from chemotaxonomical point of view that there is connection between the *Compositae inflorescens*-type and the occurence of arctiine. No arctiine could be demonstrated in plants whose inflorescence axis is elongated (Table II) similarly as in the case of *Matricaria*-type inflorescence.

Table 1

Arctigenine content of Arctium lappa

Organs		Ethanol soluble	Ethanol insoluble
Leaf		—	—
Stem		—	—
Root		—	—
Seed		***	*
Full inflorescence	young	*	—
	old	***	—

Table 2

Inflorescence type and arctigenine content within the Compositae

Type of inflorescence	Species	Ethanol soluble	Ethanol insoluble
	*Arctium lappa*	***	**
	Arctium tomentosum	***	**
	Senecio cruentus	***	—
	Helianthus annuus	***	*
	Lactuca sativa	—	**
	Onopordum acanthium	***	—
	Tagetes patulus	*	**
	Zinnia elegans	***	—
	Carthamus tinctorius	—	—
	Bidens tripartitus	—	—
	Callistephus chinensis	—	—

(b) The effect of arctiine on the morphology and tissue structure of seedlings

The 5 mg/ml arctiine solution had an effect on all of the examined species. The size of the treated seedlings in comparison with the control seedlings became twice to three times smaller (Plate I). A similar inhibition manifested itself also in *Arctium lappa* seedlings despite the high endogene arctiine content of its seed.

Though the longitudinal growth of root was inhibited, arctiin had no effect on the shape and thickness of the root. Form remained also unchanged, while fewer and shorter radicals grew. The inhibiting effect of arctiine was manifest also in the epiblema formation. It can be stated — although no statistical examinations were carried out — that in the unit area of absorbing zone the number of root hairs is smaller and the hairs are shorter.

In 3—7 day-old arctiine-treated seedlings the shortening of the hypocotyl could still be observed.

For the examinations of the stem only hypocotyl pieces of a few mm length were at our disposal. No essential deviation could be observed in their tissue structure. Minimal deviations — apparent in the cell dimensions of phloem parenchyma, in the thickness of phloem, and in the change in shape of the starchy capsule cells — were found only in *Phaseolus*.

Concerning the roots arctiine caused changes only in the primary phloem and in the morphology of the root hairs. The endodermis often became polycellular and disarranged (Pictures 1 and 2, Plate II). The epiblasts of phloem parenchyma in *Cucumis* rapidly aged. Suberose-walled many-layered protective tissue appeared often on the whole surface, sometimes even on the top, in a few days. The intensive suberification is observable also on the young radicles. In certain areas this reaches such an extent that a destroying effect of the arctiine can be claimed (Pictures 3—5 in Plate II). This is confirmed also by the fact that no epiblema can be seen on the *Cucumis* roots.

Arctiine disturbed the normal activity of trichoblasts in the *Phaseolus*, *Arctium* and *Lactuca* species. It was frequently observed that on the same rhizodermal cell several root hairs were induced (Picture 3, Plate III).

The root hairs are extremely varying (Pictures 2—4 and 5, Plate III; Pictures 1—6, Plate IV). There are spirally twisted hairs among the one-celled undifferentiated hairs with smaller or larger side tubers, having longer or shorter side branches as well as hairs with different longitudinal axis, and even branching-off hairs. In the case of *Arctium lappa* also multicellular, branching-off root hairs could be observed (Picture 2, Plate IV).

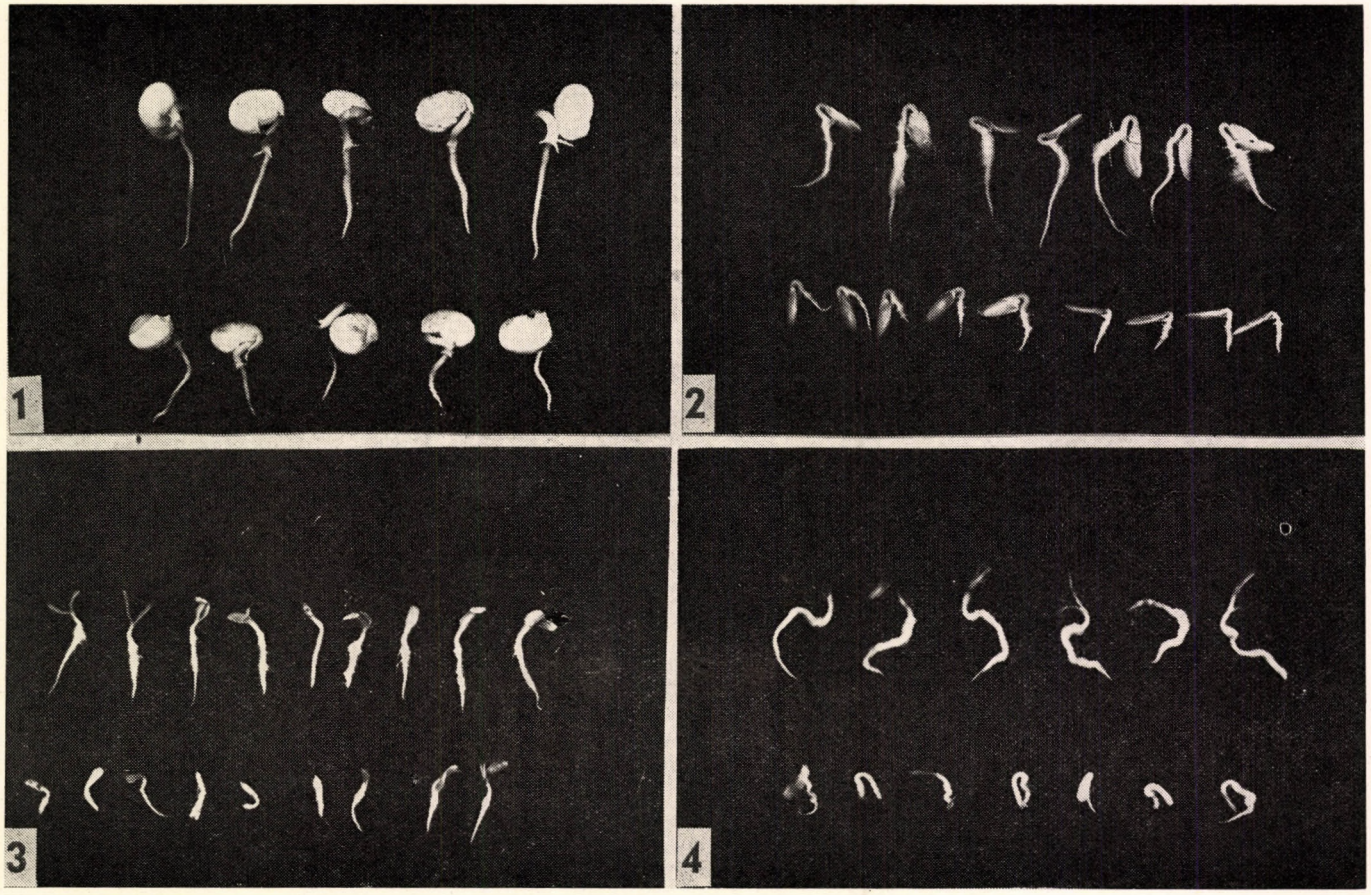

Plate I. Picture 1: 5 day control (above), and arctiine-treated (below) *Phaseolus vulgaris* seedlings. Picture 2: 5 day control (above), and arctiine-treated (below) *Cucumis sativus* seedlings. Picture 3: 3 day control (above), and arctiine-treated (below) *Lactuca* seedlings. Picture 4: 7 day control (above), and arctiine-treated (below) *Arctium lappa* seedlings

(c) *The interaction of arctiine and nucleic acids*

The results are shown in Figures 2—5. The corresponding absorption and CD spectra are given together. It is conspicuous that the absorption curves give no information about the interaction between nucleic acids and arctiine, i.e. the sums of the arctiine and the nucleic acids spectra taken separately do

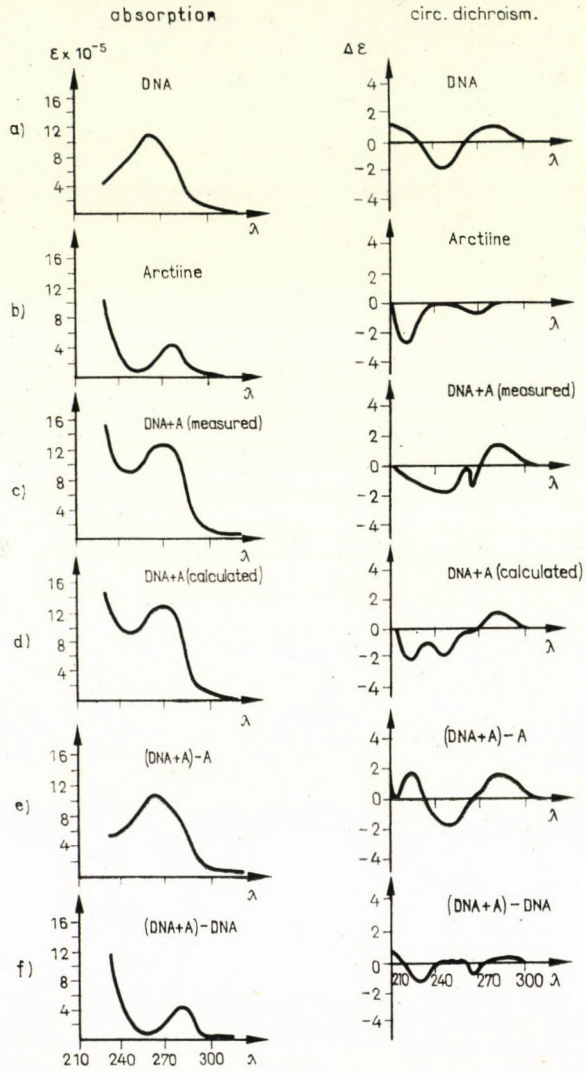

Fig. 2. Interaction between DNA and arctiine. (a) Absorption and circular dichroism of DNA. (b) Absorption and circular dichroism of arctiine. (c) Absorption and circular dichroism of DNA and arctiine together. (d) Calculated absorption and circular dichroism of DNA and arctiine. (e) (DNA + A)—A difference spectrum. (f) (DNA + A)—DNA difference spectrum

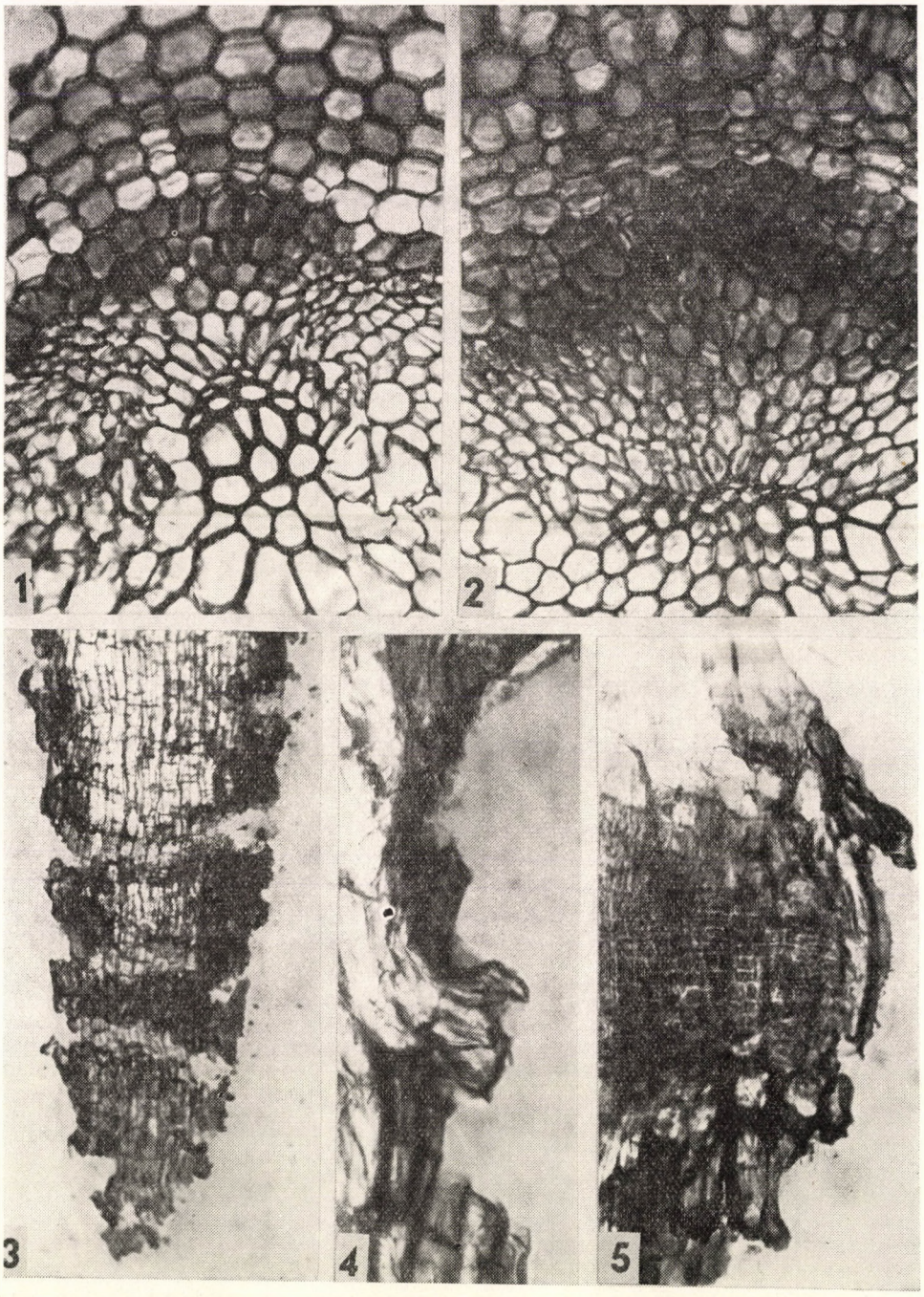

Plate II. Picture 1: Control root cross-section in *Phaseolus vulgaris* (×200). Picture 2: Root cross-section of *Phaseolus vulgaris* treated with arctiine (×200). Picture 3: Suberification and destruction in the main root of *Cucumis savitus* treated with arctiine (×80). Picture 4: Suberification of root-phloem parenchyma in *Cucumis sativus* treated with arctiine (×500). Picture 5: Suberification of radicle in *Cucumis sativus* treated with arctiine (×500)

not differ from the spectrum showing the absorption by the two compounds mixed. On the other hand, from the CD curves it is clear that arctiine and the nucleic acids have entered into some kind of interaction; the so-called sum-curves essentially differ from the spectra taken after half an hour's interaction.

It is primarily the CD band in the higher wavelength, which becomes more expressed; the plus values considerably increase. The negative CD band

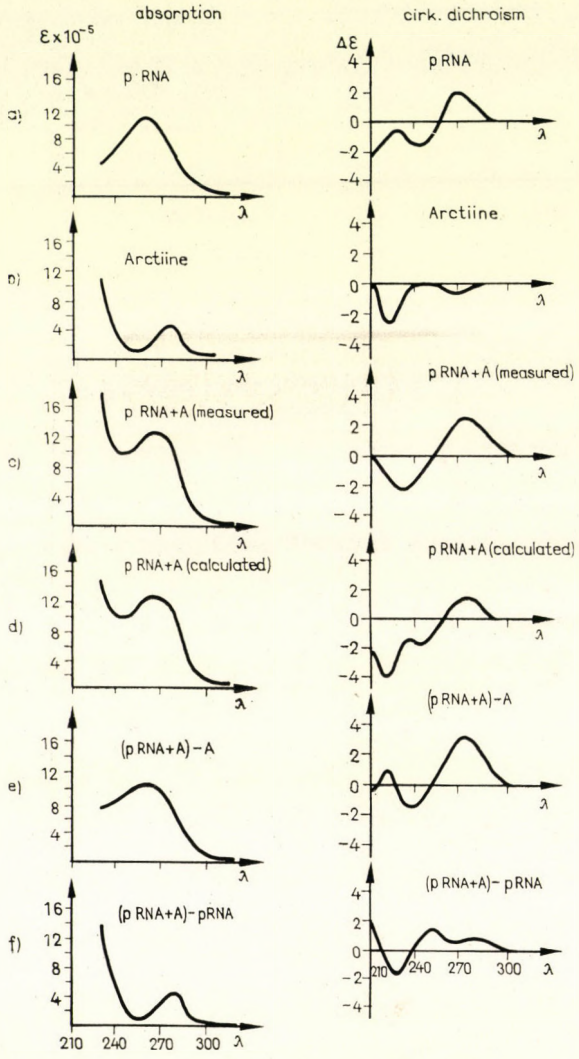

Fig. 3. Interaction between polymerized RNA and arctiine. (a) Absorption and circular dichroism of pRNA. (b) Absorption and circular dichroism of arctiine. (c) Absorption and circular dichroism of pRNA and arctiine together. (d) Calculated absorption and circular dichroism of pRNA and arctiine. (e) (pRNA+)A − A difference spectrum. (f) (pRNA+A) − pRNA difference spectrum

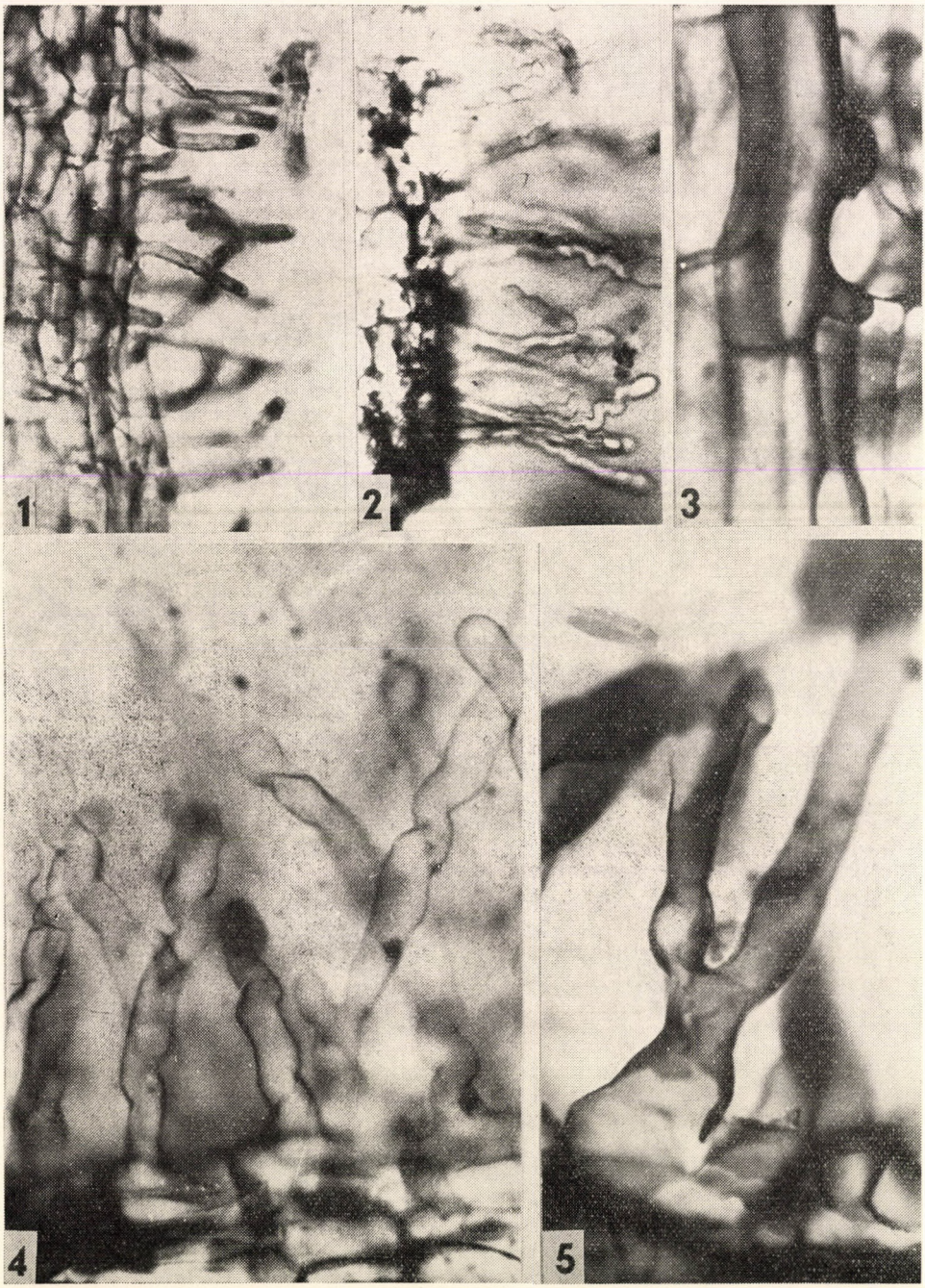

Plate III. Picture 1: Control epiblema in *Lactuca sativa* (×200). Picture 2: *Arctium lappa* epiblema treated with arctiine (×200) Picture 3: Growing rhizodermis cell in *Phaseolus vulgaris* treated with arctiine (×500). Picture 4: Root-hairs in *Phaseolus vulgaris* treated with arctiine (×500) Bifurcating. Picture 5: root hair in *Phaseolus vulgaris* treated with arctiine (×500)

in the low wavelength region does not change so definitely, but the interaction is clear on the basis of this band as well. If the differences between the so-called sum-spectra are considered (Figs 3 and 5) it will be seen that arctiine has entered most strongly into interaction with the yeast RNA and with the highly polymerized RNA. The spectrum taken from DNA and tRNA together with arctiine suggests essentially smaller changes in conformation (Figs 2 and 4).

No closer aspect of the interaction between the phenolic germination inhibitor and nucleic acids is known. Unfortunately, the CD maxima of arc-

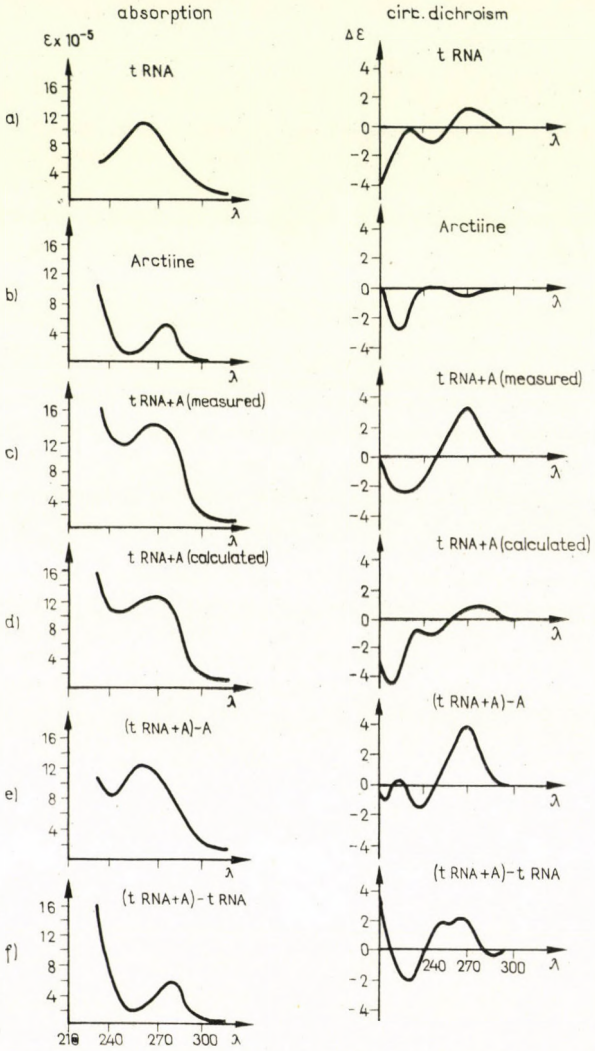

Fig. 4. Interaction between tRNA and arctiine. (a) Absorption and circular dichroism of tRNA. (b) Absorption and circular dichroism of arctiine. (c) Absorption and circular dichroism of tRNA and arctiine together. (d) Calculated absorption and circular dichroism of tRNA and arctiine. (e) (tRNA+A)—A difference spectrum. (f) (tRNA+A)—tRNA difference spectrum

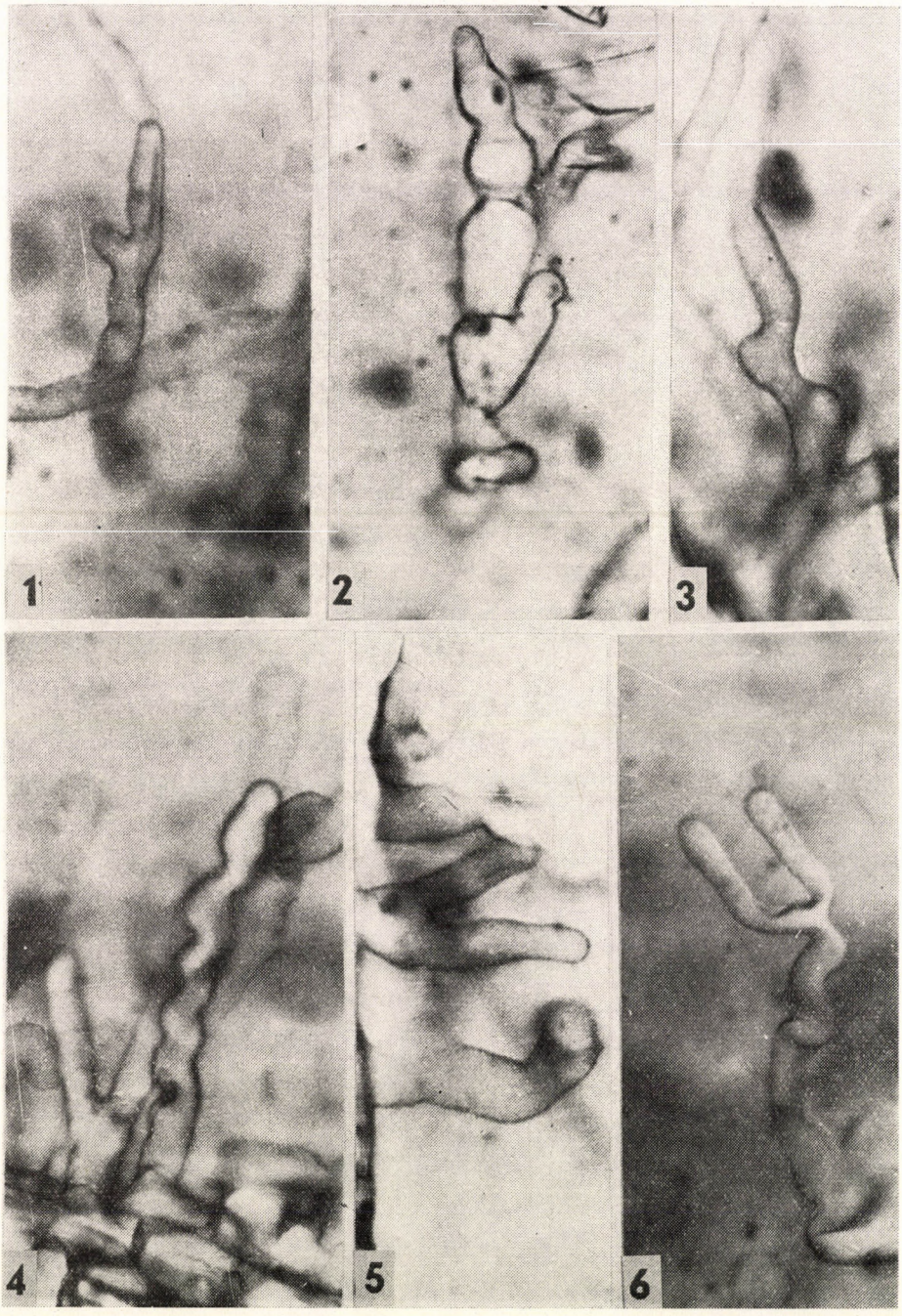

Plate IV. Picture 1: Bifurcating root-hair in *Arctium lappa* treated with arctiine (×500). Picture 2: Multi-celled, bifurcating root-hair in *Arctium lappa* treated with arctiine (×500). Picture 3: Root-hair in side-tuberred *Lactuca sativa* treated with arctiine (×500). Picture 4: Spiral and bifurcating root-hairs in *Arctium lappa* treated with arctiine (×500). Picture 5: Bifurcating and irregularly growing root-hairs in *Lactuca sativa* treated with arctiine (×500). Picture 6: Branching-off root-hairs in *Lactuca sativa* treated with arctiine (×500)

tiine and of nucleic acids are too near to each other in the system, hence it is very difficult to determine what the difference between the "joint"-spectra and the sum spectra can be attributed to. Several works are known in the literature about investigations of the interaction between nucleic acids and dyes. The absorption maximum of dyes are very far from the absorption band of DNA, so it was easy to tell to what extent the dye and the CD curve of the nucleic acid, respectively changed.

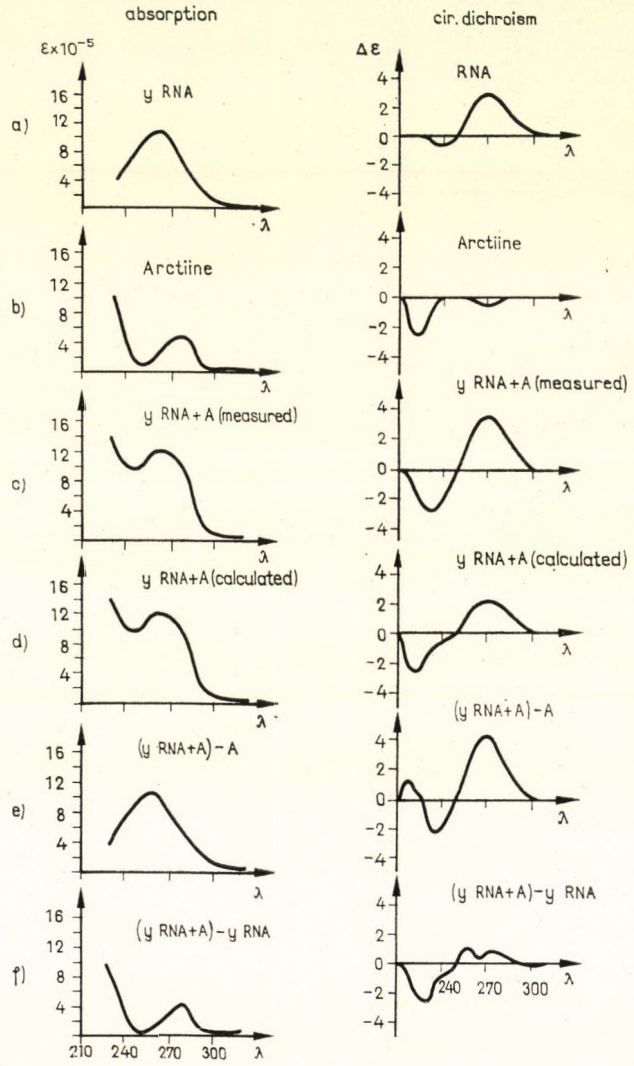

Fig. 5. Interaction between yeast RNA and arctiine· (a) Absorption and circular dichroism of yRNA. (b) Absorption and circular dichroism of arctiine. (c) Absorption and circular dichroism of yRNA and arctiine together. (d) Calculated absorption and circular dichroism by yRNA and arctiine. (e) (yRNA+A)−A difference spectrum. (f) (yRNA+A)−yRNA difference spectrum

Two kinds of interaction can in essence be distinguished: (1) Bindings occur between the anion binding sites and the anions so that the dye molecule coils itself as it were around the nucleic acid helix. (2) The dye molecule penetrates in between the bases and thus a so-called dispersion or exciton interaction may evolve. In the present case — as has been said above — it is extremely difficult to say anything definite, as the CD bands of arctiine and of the nucleic acids are very near to each other. Even so, the problem was approached in the following way.

(a) The hypothesis was that the CD spectrum of arctiine does not change substantially in the presence of nucleic acid. The spectrum of arctiine was sub-

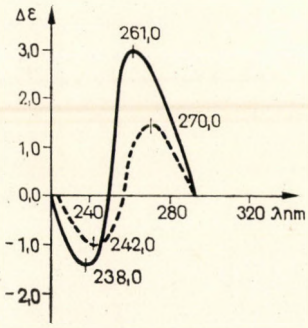

Fig. 6. Circular dichroism of RNA at 40°C and at 20°C

stracted from the "joint" spectrum. The difference spectra so obtained show the conformation change of the nucleic acid (see "e" in Figs 2—5). It must be emphasized here that the difference spectra obtained in this way are not irreal, but characteristic of nucleic acids having a higher helix content. In order to prove this, experiments were carried out. It is well known that the structure of the nucleic acid is very sensitive to temperature. At a high temperature helix conformation decreases. According to Fig. 6, the CD spectrum shows a higher maximum due to a higher conformation at 20°C than at 40°C. The spectrum of yeast RNA + arctiine is closer to the type measured at 20°C than to the spectrum observed at 40°C. In the presence of arctiine the CD spectrum changes as if the temperature had been reduced. This is valid for all nucleic acids.

(b) On the other hand, if the conformation of the nucleic acids is not supposed to change in the presence of arctiine then one has to substract from the "joint" spectrum ("f" in Figs 2—5). However, in this way a plotting will be obtained which is difficult to treat theoretically, at least according to the present state of knowledge. For, if the arctiine had coiled up round the nucleic acid helix, then both CD bands should have changed at the same rate in the

positive direction, similar to the observations of BLOUT and co-workers (1965) concerning the interaction between helix molecules and dyes. According to their experiments, dyes coiled up round the left handed helix produce a negative CD.

Shortages in data preclude a more detailed speculation. It can be inferred only that arctiine enters into interaction with nucleic acids resulting in an conformational change of the nucleic acids. The nature of the interaction is unknown. According to our knowledge this is the first case that an *in vitro* interaction between nucleic acids and growth regulating phenolic has been proved. It should be noted here that the *in vitro* interaction between gibberellic acid and nucleic acids was already demonstrated, but of a quite different type from the one observed here (KESSLER 1969).

Summary

The physiological effect of a phenol-type substance, arctiine and arctigenine, was investigated on different levels of organization.

(1) During the germination of *Arctium lappa*, the arctigenine content gradually decreases. The germination shows a endogene rhythm which is followed by the quantitative change of the arctigenine in alcohol insoluble fraction. Arctigenine is detectable in the generative organs of *Arctium lappa*. Arctigenine is not specific for one species, it can be found in the seeds of other plants within the family *Compositae* as well. According to experiments, its occurrence depends on the systematic place of the particular plant.

(2) Treatment with arctiine markedly inhibits the growth of seedlings, although it has no uniform effect on the various species. It does not influence the anatomy of the stem. Concerning the root, arctiine above all disturbs the growth of trichoblasts, it induces abnormal growth. By its effect, short bifurcating, twisted root hairs occur frequently. It has a strong destructive influence on the root of certain plants, resulting in an early suberification of the primary phloem. Presumably this causes serious disturbances in nutrient absorption.

(3) The interaction between arctiine and the nucleic acids of different origin shows that arctiine changes the structure and increases the double helix (?) conformation of the nucleic acids. The nature of the interaction is not known and it cannot be brought into relation with the germination regulating effect of arctiine or arctigenine.

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