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GROWTH INHIBITING ACTIVITY OF SOME STEROID GLYCOALKALOIDS ON HIGHER PLANTS

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The relations between the fungicidal activity and the molecular structure of some steriod glycoalkaloids have recently been established (Ferenczy and Kevei, 1967a,b; Kevei, 1968). The antifungal activity of these compounds is strictly proportional to their ability of complex formation with ergosterol. Their primary acting site is the ergosterol-containing cell membrane system. The release of compounds of low molecular weight (e. g. amino acids) is very characteristic.

In the present study we intended to obtain data on the effects of some steroid glyalkaloids of plant origin on higher plants.

Materials and Methods

The compounds used were chromatographically pure and crystalline tomatin, solaradixin, solamargin, solasonin, and β-solamargin. All compounds were isolated from plants by various methods. (Kevei, 1968.) Melting points: tomatin (I) 272—274°C (decomp.), solaradixin (II) 264°C (decomp.), solamargin (III) 301—303°C (decomp.), solasonin (IV) 292—294°C (decomp.) and β-solamargin (V) 225—226°C.

The concentrations generally applied were 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7} M. Each compound at each concentration was used at two pH-values (5 and 7) using M/300 phosphate buffer.

Preliminary tests were made with four plant species: *Cucumis sativus* "Kínai Kígyó", *Papaver somniferum* "Fertődi kék", *Sinapis alba* and *Solanum laciniatum*. As the four species gave similar responses, detailed work was carried out with *Cucumis sativus*. The seeds were of the 1967 crop.

Seed germination test. — Two-layer filter papers were placed into 9 cm Petri-dishes and wetted with 6 ml of distilled water (control) or with the test solutions at pH 5 and 7. Four replicates, each with 50 seeds were prepared, incubated in the dark at 25°C for 36 hours, then examined for the percentage of germination. At four-hour intervals the results were checked again.

Growth tests. — After germination on filter papers wetted with distilled water, the seedlings having uniform radicles of 15 mm length were selected and transferred in to the test solutions.

Pieces of bobbin net were stretched on 50 ml beakers filled with the solutions. The seedlings (10 in each beaker) were placed on the net with their radicles dipping into the solution. The controls were buffers at pH 5 and 7. The beakers were placed inside basins covered with glass plates to maintain a humid atmosphere.

After 72 hours of dark incubation at 25°C the seedlings were removed, measured, and the differences between the initial and final lengths of roots and hypocotyls were calculated.

Glycoside absorption, translocation and amino acid release. — To study the absorption and translocation solamargin has been chosen owing to its pronounced biological activity and sensitivity to Hansen and Dam reagent (Hansen and Dam, 1957) modified by Vahouny et al (Vahouny, Borja and Weersing, 1963). These experiments were carried out under aseptic conditions to avoid the interference of micro-organisms. The wetted filter papers in Petri-dishes, the glassware and the solutions were sterilized by autoclaving and the seeds were disinfected by 0,5 % bromine water. After germination, the seedlings were cultivated in buffer solution (pH 7) at 25°C for three days, then 40 uniform plants (the total weight of roots corresponded to 55 $\pm$3 mg dry weight) were transferred to 50 ml solamargin solution (10^{-4} M) at the same pH value. Samples were taken from the solution in every second hour, of incubation and analysed for solamargin and amino acids. Also, plant samples were taken in the same periods. The plants were extracted with methanol for 30 minutes under reflux, and the methanolic solutions were concentrated and applied to chromatoplates in various amounts. The quantity of solomargin in the solutions, roots and hypocotyls was estimated by using thin-layer chromatography as previously described (Ferenczy and Kevei, 1967a). Silica gel G (Merck, nach Stahl), activated at 110° C for one hour, was used as adsorbent and methanol-benzene--ammonia (25 %) (10:20:1) as solvent system. Modified Hansen — Dam reagent was applied (100 mg $FeCl_3$ dissolved in 100 ml glacial acetic acid + 100 ml conc. H_2SO_4) as colour reagent.

In some experiments with β-solamargin, the treatment of roots and the quantitative thin-layer chromatography was carried out as in case of solamargin.

The total quantity of amino acids was determined as previously described (Ferenczy and Kevei, 1967a) using 0,1 ml of each solution, applying on chromatographic paper, drying and developing with ninhydrin. The spots were eluted by 50 % methanol and the colour intensity was measured fotometrically at 520 nm. The extinction values were referred to a standard calibration curve made by alanin. The determination of individual amino acids from both solutions and root extracts was carried out with paper chromatography (Kevei, 1968). Butanol — glacial acetic acid-water (2:2:1) was used as solvent system and Schleicher — Schüll 2043b as paper. For the sake of comparison, a mixture of 20 amino acids was run simultaneously.

When translocation of solamargin from leaves to other organs was studied, 0,1 ml solamargin solution (2×10^{-3}M, pH 7 containing 0,1 % Tween 80 and 0,1 % polyethylenglycol) was applied to the upper surface (17,8$\pm$1,3 cm^2) of one leaf per plant and dispersed. Before treatment, *Cucumis plants* were grown in water culture for two weeks, after treatment for 24 hours (one group in light at 6000 lux, and another in the dark) at 25°C, then cut, the identical parts collected and extracted. The extracts were concentrated and analysed as mentioned before.

Statistics. The standard errors were calculated in the usual manner (Cavalli-Sforza, 1965.) Every experiment was carried out in four replicates.

Results and discussion

Seed germination test. The steroid glycoalkaloids used did not inhibit the germination of *Cucumis* seeds even at the highest concentration used (10^{-4} M). There was only a slight retardation (up to 19 %) in germination rate in case of tomatin, solasonin, and solamargin. The germination rate lagged behind that of the control only in the first 36 hours, but four hours later it reached the control value (98 %). The radicles treated with the above mentioned compounds and with β-solamargin were deformed, shorter and thicker than those of the controls.

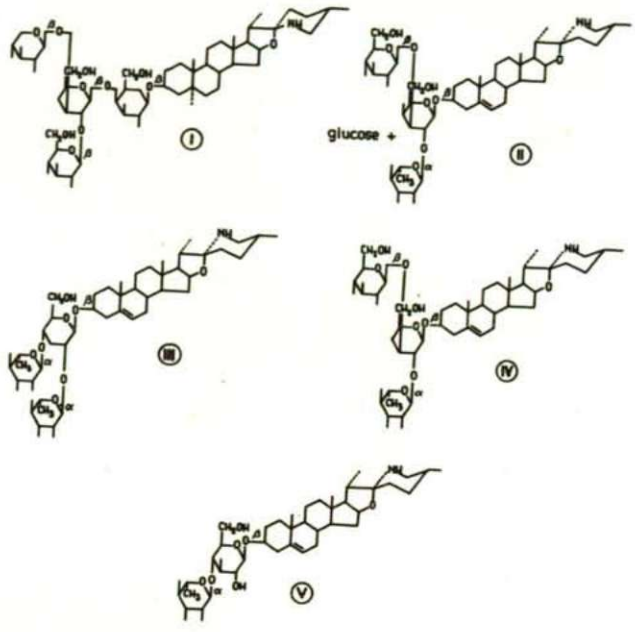

Fig. 1. The comopounds tested. I:tomatin, II:solaradixin, III:solamargin, IV: solasonin, V:β-solamargin.

This morphological aberration was characteristic (Fig. 2).

In an earlier work (K e v e i, 1968) it was also found that filamentous fungi growing in culture media containing the same compounds showed similar features (short, thick and disterted hyphae).

G r o w t h t e s t. — The applied compounds except solaradixin exert a strong inhibitory effect on roots (Fig. 3 and 4). As a consequence, the hypocotyls were also inhibited (Fig. 5 and 6). The inhibition was proportional to the concentrations and was more pronounced at pH 7. In some cases lower concentrations caused slight promotions of the root and hypocotyl growth.

Beside the retardation of growth of the main roots, there occurred also characteristic deformations (Fig. 7).

Comparing these results with those of the earlier antifungal tests (F e r e n c z y and K e v e i, 1967a,b; K e v e i, 1968), it can be established that there is a close similarity in the sequence of activity of the compounds examined. Both on higher plants and on fungi the sequence of activity was as follows: tomatin $\rangle$ solamargin $\rangle$ solasonin $\rangle$ β-solamargin $\rangle$ solaradixin. The activity of β-solamargin is higher in *Cucumis* growth test than in the microbiological tests. The more pronounced activity is not attributable to the alteration of β-solamargin molecule in higher plants as was thought. The quantitative thin-layer

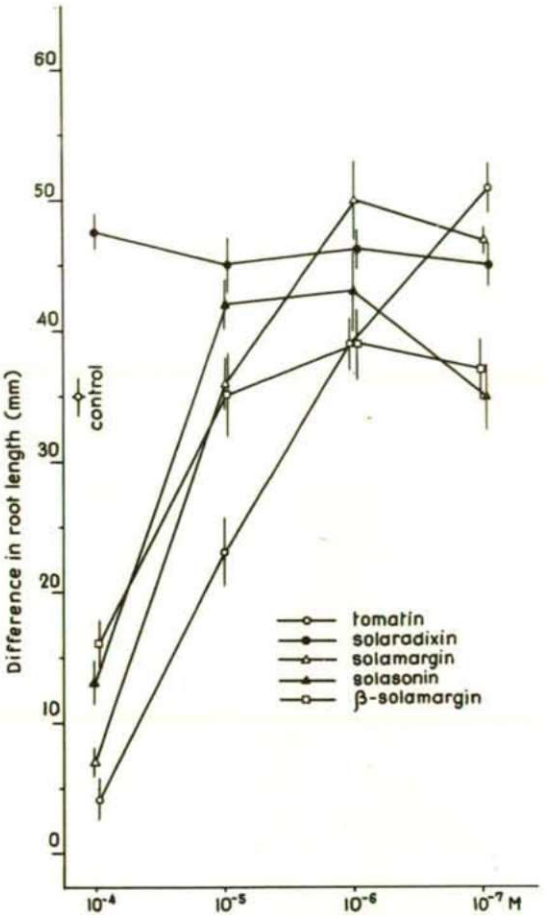

Fig. 3. Growth inhibiting effects on roots of *Cucumis* seedlings at pH 5. Vertical lines represent the standard errors.

chromatography for β-solamargin and for hypothetical derivatives indicated a more intensive absorption of the compound into the roots than into fungi, but no change in the molecular composition. Solaradixin proved to be inactive both in case of higher plants and of microorganisms.

As reported previously, the activity of these compounds is strictly related to the sugar components of the molecules. E.g. the great difference in activity between tomatin and solaradixin (both having four sugar molecules) cannot be attributed to the difference of stereo-position of ring-F or to the absence or presence of double bond in ring-B but to the difference in composition and sequence of the sugar molecules.

Glycoside absorption, translocation and amino acid release. Examining both the solutions and the roots for the amount of solamargin, it was foud that 40 $\pm$ 5% of the quantity of solamargin was absorbed by the roots from the solution. It was also proved that there was no decomposition or other change of the solamargin was absodbed by the rots from the solution. It was also proved that there was no decomposition or other change of the solamargin molecule in plants or in the solution.

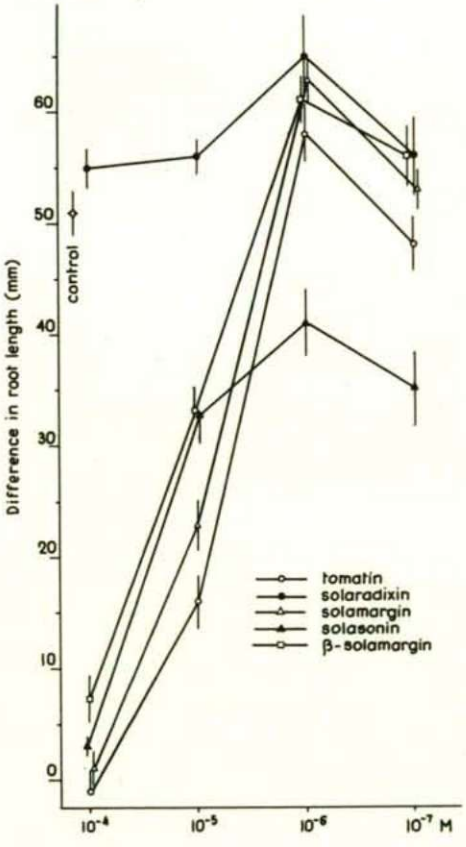

Fig. 4. Growth inhibiting effects on roots of *Cucumis* seedlings at pH 7. Vertical lines represent the standard errors.

The results obtained by chromatography of plant extracts show no translocation of the compound from roots to hypocotyls or from treated leaves to other leaves, stems or roots.

The data of the release of amino acids from roots are shown in Fig. 8. The typical effect of solamargin is the release of amino acids from the roots into the external solution in the first two hours. The subsequent increase in the amount of released amino acids is slight.

It was also found that the release of amino acids was not selective. All kinds of free amino acids to be present in the pool of plant cells were released and their proportion in the external solution was the same as in the cells. The proportoin of amino acid did not show any variation with time.

Concerning the release of amino acids, very similar data were obtained earlier with certain microorganisms too (Ferenczy and

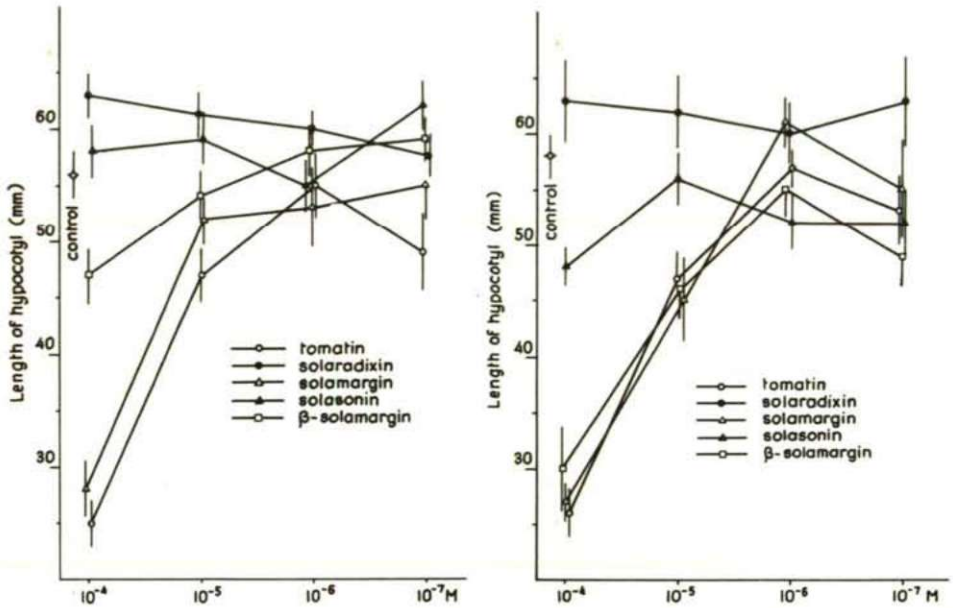

Fig. 5. Growth inhibiting effects on hypocotyls of *Cucumis* seedlings at pH 5. Vertical lines represent the standard errors.

Fig. 6. Growth inhibiting effects on hypocotyls of *Cucumis* seedling at pH 7. Vertical lines represent the standard errors.

Kevei, 1967a,b; Kevei, 1968).

In experiments with various yeast and filamentous fungi it was established that the mode of action of these compounds is the destruction of the ergosterol-containing cell membrane. From the data reported here it may be concluded with fair probability, that the growth inhibiting activity of the steroid glycoalkaloids on roots of higher plants is attributable to the disorganization of the β-sitosterol-containing membrane system.

This research work was carried out during a scholarship granted by the Hungarian Government to one of us (M. R. Rezk) for which he offers his sincere gratitude.

Fig. 2. Distorting effect of tomatin (10^{-4} M); upper row:treated seeds. Lower row:controls.

Fig. 7. Growth inhibiting effect of solamargin at pH 7. Concentrations from left to right:10^{-4},10^{-5},10^{-6},10^{-7} M and control.

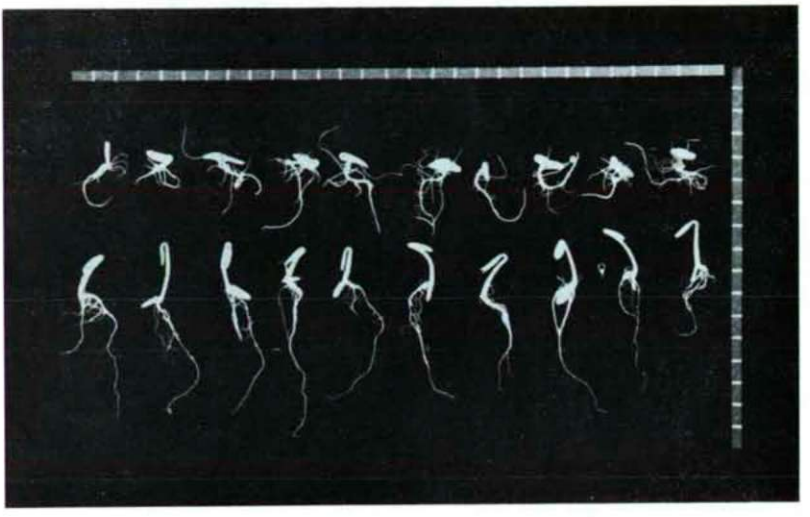

Fig. 2

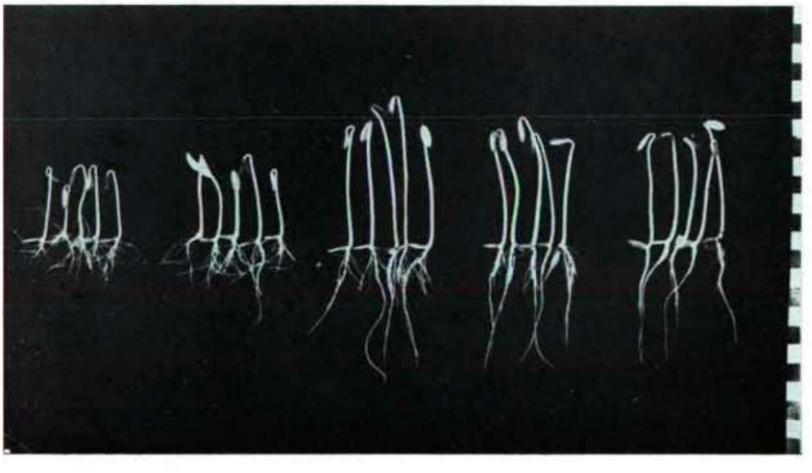

Fig. 7

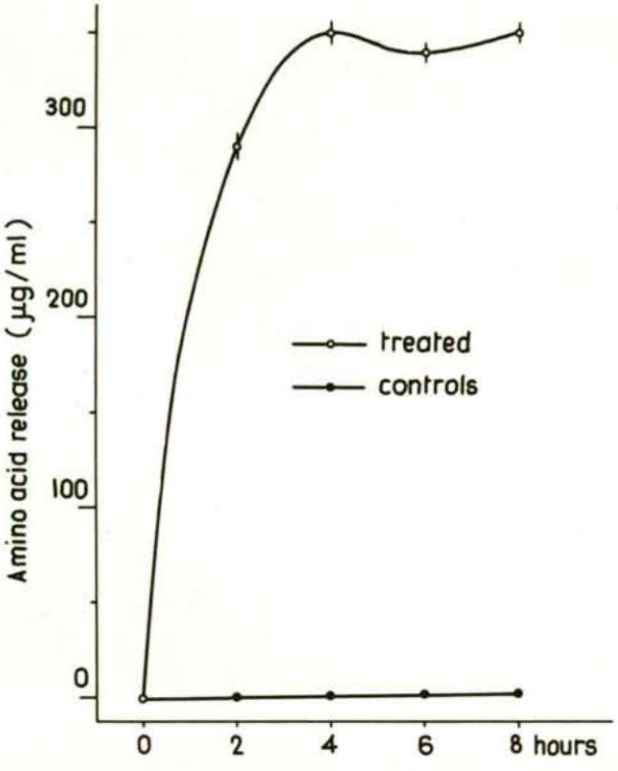

Fig. 8. Effect of solamargin on release of amino acids from roots of *Cucumis* seedlings. Vertical lines represent the standard errors.

Abstract

Five steroid glycoalkaloids (tomatin, solaradixin, solamargin, solasonin, and β-solamargin) isolated from plants were examined for growth inhibiting activity. It was found that these compounds had no inhibiting effect on germnaiton, but four of them caused characteristic root growth inhibition and distortion. There are close relations between the chemical structure and the biological activity. The sequence of activity is: tomatin ⟩ solamargin ⟩ solosonin ⟩ β-solamargin ⟩ solaradixin (inactive).

There was no sign of translocation of solamargin from the roots to the hypocotyls and from the leaves to other organs. This compound caused a quick and non-selective release of amino acids from the root cells. The mode of inhibitory action is probably the disorganization of the sterol-containing cellular membrane system.

These data are in good agreement with the results of earlier work on fungi.

References

Cavalli-Sforza, L. (1965): Grundbegriffe der Biometrie. — Jena.

Ferenczy, L. and Kevei, F. (1967a): Studies on antifungal steroid glycosides. I. Data on the mode of action of some antifungal steroid glycoalkaloids. Symposium on Mechanisms of Action on Fungicides and Antibiotics. — Berlin, pp. 59—68.

Ferenczy, L. and Kevei, F. (1967b): Mechanism of action of antifungal steroid glycoalkaloids. — Acta Microbiol. Acad. Sci. Hung. *14*, 127.

Hansen, P. W. and Dam, H. (1957): Paper chromatography and colorimetric determination of free and esterified cholesterol in very small amounts of blood. — Acta Chem. Scand. *11*, 1658—1662.

Kevei, F. (1968): Hatásmódvizsgálatok szelektív antifungális aktivitású szteroid glikozidokkal (Studies on the mode of action steroid glycosides of selective antifungal activity). — Doctoral Dissertation, A. József University, Szeged, Hungary.

Vahouny, G. V., Borja, C. R. and Weersing, S. (1963): Radioactive and analytical determination of free and esterified cholesterol following micro thin-layer silisic acid chromatography. — Anal. Biochem. *6*, 555—559.

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