

BRIEF COMMUNICATION

On the Auxin Activity of Glucobrassicin in Biological Tests

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It was reported in a previous paper (KUTÁČEK 1964) that high doses (10^{-2} M and 10^{-3} M) of the indole thioglycoside glucobrassicin(S- β -D-1-(glucopyranosyl)-3-indoleacetothiohydroxymyl-O-sulphate) occurring in the families *Brassicaceae* (KUTÁČEK, OPLIŠTILOVÁ 1964), *Resedaceae*, *Capparidaceae* and *Tovariaceae* (SCHRAUDOLF 1965) have an inhibitory effect on the growth of wheat and clover. On diluting glucobrassicin (GLUBR) considerably, the inhibition gradually passes over into growth stimulation.

In the present communication the effect of GLUBR on segments of wheat coleoptiles is described, together with its effect on the curvature of pea epicotyl, in comparison with other auxins, indoleacetic acid (IAA) and indoleacetonitrile (IAN). Also the interreaction of GLUBR and kinetin was verified.

GLUBR was isolated according to GMELIN and VIRTANEN (1961). In the tests we always used freshly prepared aqueous solutions of its twice recrystallized tetramethylammonium (TMA) salt. The preparation as well as the incubation solutions were tested for the absence of IAN by thin-layer chromatography on silica gel G (KUTÁČEK, PROCHÁZKA 1964).

The tests on segments of wheat coleoptile (*Triticum aestivum*, cv. Kaštická osinatka) was carried out in the modification by NITSCH and NITSCH (1956), the test for curvature of pea epicotyl (*Pisum sativum*) was performed as described by LARSEN (1955).

The growth activity of increasing concentrations of GLUBR (TMA salt) in the tests on segments of wheat coleoptile is shown in Fig. 1. For the sake of comparison, we also measured the activity of IAA and IAN. The optimum concentration of GLUBR, bringing about maximum increase of growth of coleoptile segments, corresponded to 10^{-3} M GLUBR (i.e. 521 μ g GLUBR/ml); the optimum concentrations of IAN and IAA were $5 \cdot 10^{-5}$ M and 10^{-5} M,

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respectively. Maximum stimulation of growth attained with GLUBR TMA salt amounted to 80% maximum stimulation by IAA and to 62.5% maximum stimulation by IAN. In no experiment could we demonstrate by thin-layer chromatography the presence of IAN released from GLUBR.

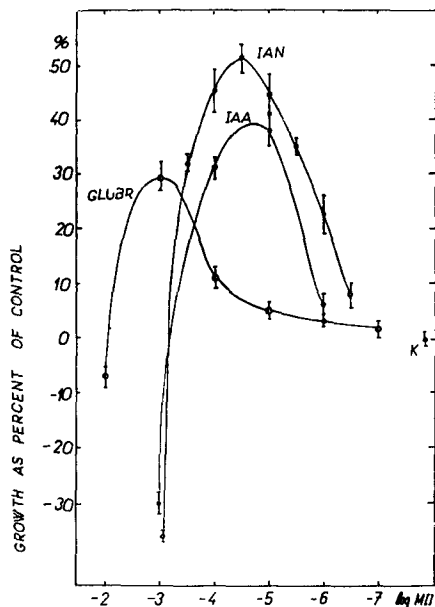


Fig. 1. Growth curves of glucobrassicin (GLUBR) as compared with indoleacetic acid (IAA) and indoleacetonitrile (IAN) determined by the wheat coleoptile test. The individual experimental points show the probable error of the mean of the results. K = control.

GLUBR (100 μ g) stimulated the growth of wheat coleoptiles even after rechromatography (butanol- $\text{CH}_3\text{COOH-H}_2\text{O}$ -4 : 1 : 2) and immediate elution of the corresponding spot on the paper chromatogram.

Increasing doses of kinetin will gradually remove the stimulatory effect of GLUBR on the longitudinal growth of wheat coleoptiles (Fig. 2).

In the test for pea curvature GLUBR (TMA salt) was ineffective, similarly to IAN whereas IAA was active (Fig. 3). The concentration in this test was 10^{-4} M for GLUBR and IAN and 10^{-5} M for IAA.

The above results demonstrate rather convincingly that GLUBR exhibits growth activity in the classical auxin test — applied to segments of wheat coleoptile. The auxin character of GLUBR is also indicated by the antagonism with kinetin in the wheat-coleoptile section test. A similar antagonism was observed with kinetin and IAA where kinetin reduced the effect of IAA on the longitudinal growth of apical segments of sunflower (de Ropp 1956).

Two hypotheses can be put forward concerning the mechanism of action of GLUBR on the growth of wheat coleoptiles: Either GLUBR is active per se, or else it acts via IAN which can be liberated from the GLUBR complex by metabolic transformations in the coleoptile tissue. Results obtained with

high doses of GLUBR on the growth of experimental plants of wheat and clover where the morphological changes observed after treatment with GLUBR suggested the effect of high doses of IAN (KUTÁČEK 1964). Steric reasons do not seem to support the hypothesis of growth activity of GLUBR per se,

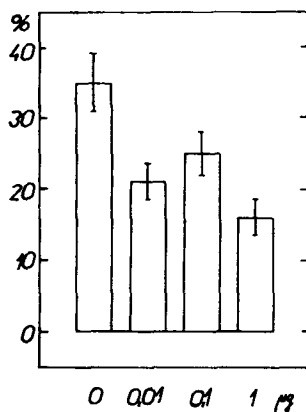


Fig. 2. The reduction of the stimulation effect of 10^{-3} M glucobrassicin on the longitudinal growth of segments of wheat coleoptiles under the influence of kinetin. The probable error of the mean of experimental results is shown for each point. Ordinate: growth as percent of the control.

since the distance of the positive charge on the indole nucleus from the negative charge at the sulphate group will be relatively greater than in the common growth substance (see e.g. THIMANN 1963). The negative result obtained in testing GLUBR (TMA salt) on pea curvature does not contribute much to the distinction between the two hypotheses. GLUBR can be either ineffective itself or else the IAN liberated from it can show no effect. The pea test is negative with respect to IAN (BENTLEY, BICKLE 1952, THIMANN 1953, WIGHTMAN 1962). A positive result would support the hypothesis on the growth effect of GLUBR per se, but no such result was obtained.

The results prove that the growth activity of GLUBR must be taken into account when studying auxins from plants of the *Brassicaceae*, *Tovariaceae*,

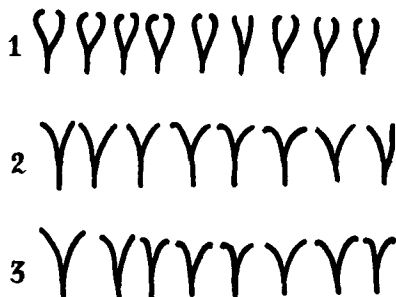


Fig. 3. Qualitative test of efficiency of glucobrassicin (3) on pea epicotyls, as compared with indoleacetic acid (1) and indoleacetoneitrile (2).

Capparidaceae and *Resedaceae* families by the classical auxin tests. The reason of this activity, i.e. whether GLUBR is efficient per se, or only after its decomposition into IAN is so far not definitely clear. Neither is the physiological function of GLUBR in plants containing GLUBR, owing to the fact that the growth tests were performed on plants, in which no GLUBR is present.

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M. KUTÁČEK, R. BULGAČOV, K. OPLIŠTILOVÁ, Ústav experimentální botaniky ČSAV, Praha: **O auxinové aktivitě glukobrasiceinu v biologických testech.** — Biol. Plant. **8** : 252—255, 1966.

Bylo shledáno, že glukobrasicein působí na dlouhivý růst úseků koleoptile pšenice. Stimulační efekt glukobrasiceinu může být snížen přidávkem kinetinu. Auxinovou aktivitu glukobrasiceinu nutno vzít v úvahu při vyhodnocování růstových látek v rostlinách čeledi *Brassicaceae*, *Tovariaceae*, *Capparidaceae* a *Resedaceae*.

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В работе приведены количественные данные о установленном стимуляционном действии тетраметиламмонийной соли глюкобрасисина при испытании на секциях коллеоптилей пшеницы и о снижении стимуляции при добавлении кинетина. Дискутируется возможный механизм действия.