tische Anpassung und ist durch die taktile Funktion der Brustflossen und die besondere Struktur und Lage der Augen bedingt. Das Auge von *Platanista* ist durch eine oekologisch bedingte regressive Evolution extrem zurückgebildet und kann praktisch nur axial eintreffende Lichtreize wahrnehmen. Der hochdifferenzierte akustische Apparat (Sonar) steht im Vordergrund der Orientierung. In Seitenlage ist das Schallfeld des Delphins breiter als hoch, was eine günstige Anpassung an die seichten Gewässer des Indus bewirkt. Durch pendelnde Bewegungen des Kopfes in der Horizontalebene während des Schwimmens und Ortens in Seitenlage kann das Tier seinen Sendewinkel noch erheblich vergrössern.

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## Flavonoids of *Parthenocissus* Tissue Culture

In the course of a continuing search for plant tissue cultures which are able to express their biosynthetic potential for flavonoid compounds[1-3], we found that a light-grown callus culture obtained from stem segments of the Virginia creeper, *Parthenocissus tricuspidata* Planch. (Vitaceae) produces 1 anthocyanin and 2 flavonol glucosides. STANKO and BRANDINSKAYA[4] reported the presence of cyanidin, delphinidin and malvidin glucosides in a 'chemical tumor' callus culture obtained from the same species. The investigation of the nature of these flavonoids was undertaken with the view of using this culture system to study the regulation of flavonoid formation in vitro.

*Experimental.* The callus tissue was cultured on HELLER's medium[5] containing glucose (5%), α-naphthalene acetic acid (0.1 mg/l) and solidified with 0.7% agar. The cultures were maintained under continuous illumination (300 fc) and a temperature of 27 ± 1 °C. Standard chromatographic and spectroscopic methods[6,7] were used for the isolation and identification of flavonoids from 4-week old callus tissue.

*Results and discussion.* The spectral characteristics and Rf values of compounds A–C are given in the following Table. Compounds A and B on acid hydrolysis yielded quercetin and glucose which co-chromatographed with authentic samples. The bathochromic shifts exhibited by both compounds in the presence of NaOAC (in the short UV-range) and $AlCl_3$ (in the long UV-range) indicate that the 5- and 7-positions are free. Furthermore, the instability of their $AlCl_3$ complexes in presence of HCl is indicative of glucosylation at the 3-position[7]. On the basis of their Rf values in both organic and aqueous solvents, compounds A and B have been identified as the 3-mono- and 3-diglucosides of quercetin, respectively. Compound C was identified as cyanidin-3, 5-diglucoside by comparison of its chromatographic and spectral characteristics with those of an authentic sample[6]. Its identity was confirmed by acid hydrolysis and characterization of the hydrolytic products.

Whereas these compounds constitute the major flavonoid components of the callus tissue, it is of interest to note that intact leaves of this species contain the same compounds although this has not been previously reported. These flavonoids were only formed when the tissue was cultured on Heller's medium containing 5% glucose. However, when either fructose (5%) or sucrose (2.5%) was used as the carbon source no flavonoid formation was observed[8].

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Chromatographic and spectral characteristics of the major flavonoids of *Parthenocissus* callus tissue

| Spectral values | *γmax* (nm) | | |
|---|---|---|---|
| | A | B | C |
| 80% MeOH | 255, 267[a], 352 | 255, 268[a], 350 | 273, 525 |
| + NaOH | 275, 300, 410 | 278, 310, 414 | |
| + NaOAc | 270, 375 | 272, 372 | |
| + NaOAc + $H_3BO_3$ | 285, 375 | 259, 375 | |
| + $AlCl_3$ | 272, 303[a], 420 | 275, 300[a], 418 | 275, 545 |
| + $AlCl_3$ + HCl | 270, 302[a], 405 | 272, 302[a], 401 | |
| Rf values (×100) | | | |
| BAW[b] | 57 | 48 | 30 |
| 15% HOAc | 30 | 48 | 39 |

[a] shoulder (inflection); [b] *n*-Butanol-acetic acid-water (4:1:2.2, v/v)

Furthermore, this tissue culture loses its potential for flavonoid synthesis when grown as a cell-suspension in the same nutrient medium regardless of the carbon source. Considering its low auxin requirement, this tissue should lend itself favorably to nutritional and hormonal studies of the regulation of flavonoid synthesis in vitro[9].

[9] Acknowledgment. We wish to thank the National Research Council of Canada for financial support of this work.

*Résumé.* Les composés flavonoïdes d'une culture de tissu de vigne vierge cultivée en milieu Héller, contenant 5% de glucose et 0.1 mg/l d'acide naphtyl-acétique, ont été isolés et identifiés comme monoglucoside-3 et diglucoside-3 de quercétine et comme cyanine.

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## 1,2 Benzisothiazol-3-ylacetic Acid as a Growth Promoting Substance for *Helianthus tuberosus* (Jerusalem artichoke) in vitro

Experiments in vivo on a group of plants representative of the most common weeds, and on several plants of agricultural interest, have shown that 1,2 benziso-3-ylacetic acid (BIA), in addition to a strong phytotoxic action, has a remarkable selectivity for Graminaceae[1,2]. Besides this, it was observed that the herbicide action was accompanied with morphological modifications that are very similar to those induced by auxin. Recently it was demonstrated that BIA is characterized by many of the activities possessed by indol-3-acetic acid[3,4]. In fact it was observed that, as occurs for the natural auxins, BIA causes in the third internode of etiolated *Pisum satirum* a strong absorption of water, a considerable cell enlargement (pea test), a greater curvature of split internodes (split test), and moreover a notable production of ethylene.

From this point of view, and for the purpose of identifying more distinctly the biological activity of this new phytoactive molecule, we have subjected BIA to a series of tests for the induction of cellular multiplication in explants of *Helianthus tuberosus* cultivated in vitro.

*Materials and methods.* Explants of dormant tubers of *Helianthus tuberosus* (Jerusalem artichoke) var. OB1 were utilized. Cylindrical explants (3 mm diam., 4 mm height) of the homogeneous medullary parenchyma were placed in vitro in a nutritive medium[5] with glucose 5% and purified agar 1% (Fluka). 1,2 benzisothiazol-3-ylacetic acid (BIA) was used at molar concentrations between $10^{-4}$ and $10^{-7}$ with a control in basal medium alone and basal medium plus indol-3-acetic acid (IAA) at $2\times10^{-6}$ molar concentration. BIA was obtained by synthesis as described[6], melting point: 153–4 °C.

Fifteen replications were utilized for every concentration. The cultures were randomized in a culture room at 24 °C in alternating light (3200 lux). The experiments were repeated twice at different times with similar results. Chlorophyll extraction and determination were made according to Smith and Benitez[7]; the contents were referred to fresh weight, corrected according to the surface.

*Results and discussion.* Observations made during the growth of the explants revealed that, already after 4 days from explantation, the tissues treated with $10^{-5}$ and $10^{-6}$ *M* BIA had visibly grown as much as when IAA was used. No growth was seen to occur at the other concentrations or in basal medium. After 6 days, the explants treated with $10^{-5}$ and $10^{-6}$ *M* BIA or $2\times10^{-6}$ *M* IAA assumed a barrel-shaped form, whereas the tissues treated with $10^{-4}$ *M* and $10^{-7}$ *M* BIA began to grow also and showed a greater growth at the top or bottom of the explant, with the above concentrations respectively. The experiments were stopped after 12 days; fresh and dry weights and chlorophyll content were determined.

BIA has a very strong effect on cellular proliferation (Table), similar to that of IAA. The optimal concentrations $10^{-5}$ and $10^{-6}$ *M* cause an increase in fresh weight of 270 and 290% respectively and in dry weight of 199 and

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Effect of 1,2 benzisothiazol-3-ylacetic acid (BIA) on the growth of dormant tubers explants of *Helianthus tuberosus* in vitro

| | Concentration (*M*) | Fresh wt. (mg) | Fresh wt. of control (%) | Dry wt. (mg) | Dry wt. of control (%) | Dry wt. (%) | Chlorophyll (µg/g fresh wt.) |
|---|---|---|---|---|---|---|---|
| | 0 | 49.1 ± 4.2[a] | 100 | 11.2 ± 2.4[a] | 100 | 23.0 | 7.5 |
| IAA | $2\times10^{-6}$ | 141.6 ± 14.3 | 288 | 18.8 ± 1.8 | 167 | 13.3 | 5.8 |
| BIA | $10^{-4}$ | 88.7 ± 4.8[a] | 180 | 15.2 ± 1.6[a] | 135 | 16.9 | 3.6 |
| BIA | $10^{-5}$ | 135.0 ± 9.2 | 270 | 21.9 ± 3.0[a] | 199 | 16.1 | 4.6 |
| BIA | $10^{-6}$ | 146.5 ± 8.4 | 290 | 18.3 ± 2.7 | 166 | 13.1 | 4.7 |
| BIA | $10^{-7}$ | 87.2 ± 17.2[a] | 177 | 15.9 ± 2.9[a] | 141 | 20.1 | 3.7 |

Average values ± SE were made on about 15 explants, 12 days old. The difference between each average and the control (basal medium alone) is significant at 1%; BIA $10^{-5}$ *M* and $10^{-6}$ *M* averages are significantly different at 1% (Student's *t*-test). [a] The difference of each average with the average of the explants treated with IAA is significant at 1% (Student's *t*-test). Chlorophyll content is corrected according to the surface.