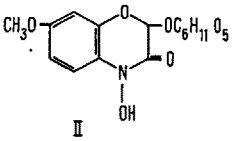
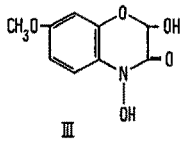
Bei der Durchsicht der Arbeiten über die Isolierung von Inhaltsstoffen des Mais lenkte sich unsere Aufmerksamkeit auf eine kürzlich von VIRTANEN et al.[7–13] bearbeitete Substanzgruppe. Ausgehend von der fungiziden Aktivität des Benzoxazolons[7] sowie des 6-Methoxybenzoxazolons[8], isoliert aus Roggen bzw. Mais und Weizen, fanden sie, dass diese Körper in Wirklichkeit Artefakte sind. Die in den Pflanzen vorkommenden Prekursoren sind Glucoside, denen die Konstitution (II) zukommt. Durch Enzymeinwirkung bildet sich beim Zerstören des pflanzlichen Gewebes das entsprechende Aglucon (III), das unter Hitzeeinfluss in das Benzoxazolon (IV) übergeht[9–12]:

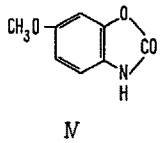


II



III

2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-on



IV

6-Methoxybenzoxazolon

Die oben aufgezählten Charakteristika der von uns aus Mais isolierten, offensichtlich mit Simazin (I) interferierenden Substanz $C_9H_9NO_5$ lassen auf Identität mit (III) schliessen.

Die Fähigkeit dieses 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-ons (III), mit Simazin (I) reagieren zu können, wurde in folgendem Versuch bestätigt: 2,5 mg der Verbindung III wurden in 10 ml Phosphatpuffer pH 3,6 (0,15 m) gelöst und mit 0,1 mg Simazin, das seinerseits in 25 ml Wasser gelöst war, versetzt. Nach viertägigem Aufbewahren des Reaktionsgemisches im Dunkeln bei Raumtemperatur wurde das unveränderte Simazin mit Chloroform extrahiert, mit Schwefelsäure zu Hydroxy-Simazin hydrolysiert und die Extinktion des Kations dieser Substanz bei 240 mμ gemessen[6]. Es konnten nur noch 15% des eingesetzten Simazins aufgefunden werden. Demgegenüber war im entsprechenden Kontrollversuch ohne Zugabe von (III) noch sämtliches Simazin unverändert vorhanden.

Gleiche Umsetzungsraten wurden auch erhalten, wenn an Stelle von 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-on das 2,4-Dihydroxy-1,4-benzoxazin-3-on oder die beiden entsprechenden Glucoside eingesetzt wurden[14]. Verbindungen vom Typ der Benzoxazolone (IV), wie sie durch Erhitzen der 2,4-Dihydroxy-benzoxazinone entstehen, vermögen mit Simazin nicht zu reagieren; die eingangs erwähnte Hitzeempfindlichkeit der Abbauaktivität des Maispreßsaftes findet damit ihre Erklärung.

VIRTANEN fand in den Pflanzen Glucosidgehalte von Promille-Grössenordnung[13]; demgegenüber liegen die in den Pflanzen gefundenen Simazinmengen in der Grössenordnung eines hundertstel Promilles, so dass das in den Abbauversuchen eingesetzte Verhältnis ungefähr dem natürlichen entsprechen dürfte.

Die genauen Zusammenhänge der Interferenz von Simazin und ähnlichen Verbindungen mit Substanzen vom Typ II und III bedürfen noch weiterer Abklärung. Widerspruchsvoll erscheint zunächst die Tatsache, dass Substanzen vom Typ II und III auch aus Pflanzen wie Weizen und Roggen isoliert wurden, die gegen Simazin relativ empfindlich sind. Abgesehen davon, dass ihr Gehalt unter den einzelnen Pflanzenspecies und sogar Sorten variieren kann und sich auch mit dem Entwicklungsstadium der Pflanzen ändert[13], zeigen zum Beispiel neuere Untersuchungen, dass der Mais seine aussergewöhnliche Resistenzfähigkeit gegenüber Simazin nicht nur der Fähigkeit, diesen Wirkstoff abzubauen, verdankt. Diese ist auch andern Pflanzen eigen[4,15,16]. Als weiterer wichtiger Faktor wurde aber gefunden, dass der Mais in manchen Fällen relativ weniger Simazin aufnimmt als andere Pflanzen[5,15]; so dürfte ein günstiges Verhältnis der Menge des vorhandenen abbauenden Systems zur aufgenommenen Menge des abzubauenden Substrates das Auftreten phytotoxischer Konzentrationen verhindern. Ferner scheint es, dass noch weitere, anders geartete physiologische Systeme existieren, die eine Resistenz gegenüber Triazinherbiziden bedingen können. Untersuchungen in diesen Richtungen sind im Gange.

Summary. 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (III), isolated from corn sap, detoxifies the herbicide Simazine, 2-chloro-4,6-bis-ethylamino-s-triazine, *in vitro*. 2,4-Dihydroxy-1,4-benzoxazin-3-one and the corresponding natural precursors of it and of (III), glucosides, behave in the same manner. It is very likely, therefore, that these glucosides and aglucones play a role in the resistance phenomena of some plant species against Simazine and related phytotoxic compounds. 6-Methoxybenzoxazolone and benzoxazolone, derived from the abovementioned benzoxazinones by heating, do not interfere with Simazine.

W. ROTH und E. KNÜSLI

Wissenschaftliche Laboratorien der J. R. Geigy A.G., Basel, 14. April 1961.

[7] A. I. VIRTANEN und P. K. HIETALA, Acta chem. scand. *9*, 1543 (1955).
[8] A. I. VIRTANEN, P. K. HIETALA und O. WAHLROOS, Suomen Kemist. *B 29*, 143 (1956).
[9] O. WAHLROOS und A. I. VIRTANEN, Acta chem. scand. *13*, 1906 (1959).
[10] A. I. VIRTANEN und P. K. HIETALA, Acta chem. scand. *14*, 499 (1960).
[11] P. K. HIETALA und A. I. VIRTANEN, Acta chem. scand. *14*, 502 (1960).
[12] E. HONKANEN und A. I. VIRTANEN, Acta chem. scand. *14*, 504 (1960).
[13] A. I. VIRTANEN, Angew. Chemie *70*, 544 (1958).
[14] Wir sind Herrn Prof. A. I. VIRTANEN für die freundliche Überlassung von Mustern dieser Substanzen zu grossem Dank verpflichtet.
[15] D. E. DAVIS, H. H. FUNDERBURK, jr., und N. G. SANSING, Weeds *7*, 300 (1959).
[16] T. J. SHEETS, Weeds *9*, 1 (1961).

Electronmicrographs on the Plastids in the Root of *Azolla imbricata*

Plastid structures of higher plants have been previously investigated in detail with the light microscope as well as with the electron microscope. As to the chloroplasts of higher plants, it is generally recognized that they consist of the grana, which contain the pigment, and of a colourless stroma[1]. However, in the chloroplasts of lower plants, e.g. some algae (*Euglena, Chlamydomonas, Chlorella, Spirogyra, Nitella,* and *Fucus*)[2,3], the defined grana structures are not observed, but the lamellae are fairly well ordered[3].

[1] E. STEINMANN and F. S. SJÖSTRAND, Exp. Cell Res. *8*, 15 (1955).
[2] D. VON WETTSTEIN, Hereditas *43*, 303 (1957).
[3] A. J. HODGE, J. D. MCLEAN, and F. V. MERCER, J. biophysic. biochem. Cytol. *1*, 605 (1955).

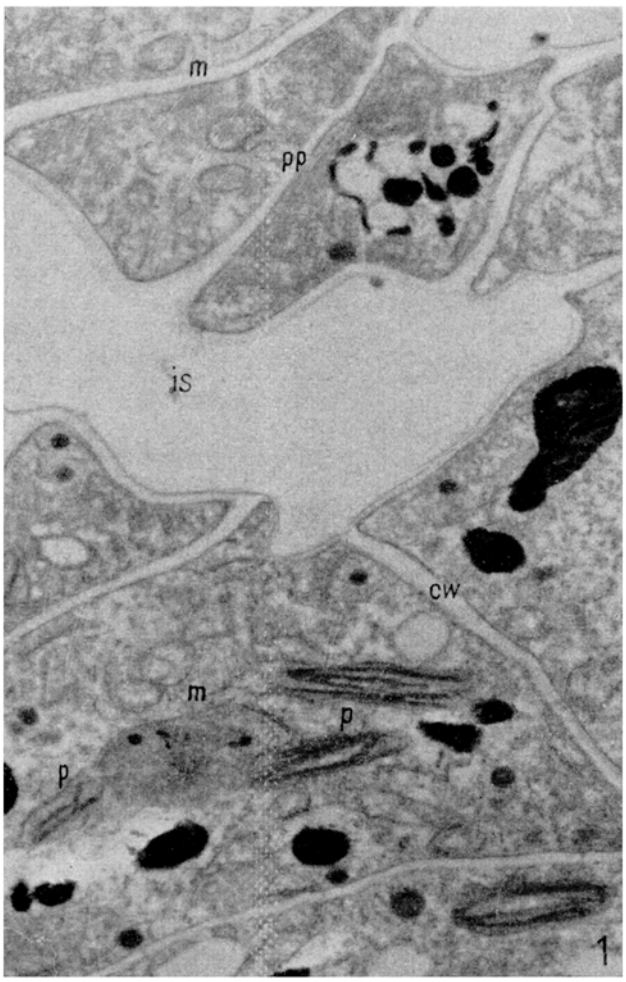


Fig. 1. Central area of the root cells. × 12 000.

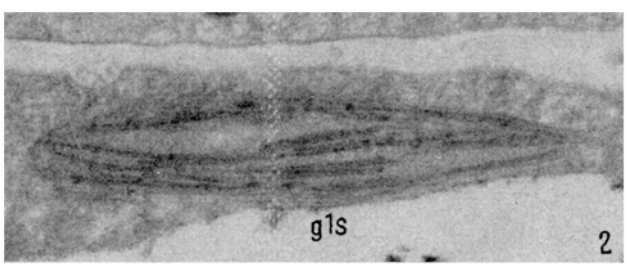


Fig. 2. A plastid which has lamellae and grana-*like* structures but no starch grain. × 28000.

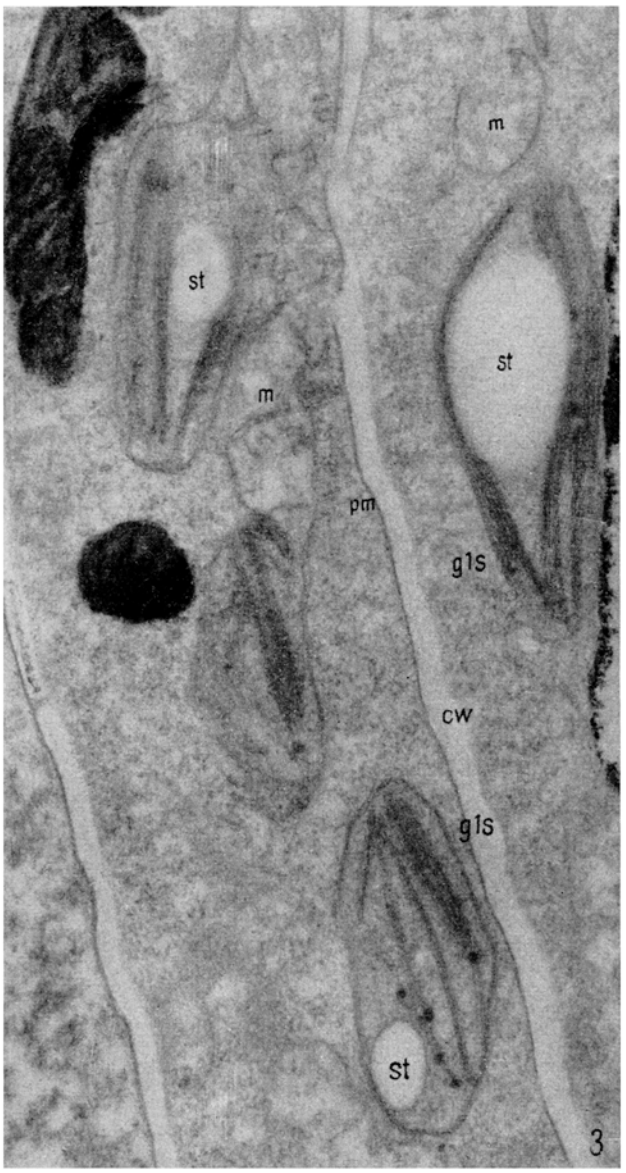


Fig. 3. Three plastids which contain starch grains and one which does not. × 35000.

p = plastid, pp = proplastid, gls = grana-*like* structure, st = starch grain, m = mitochondrion, pm = plasma membrane, cw = cell wall, is = intercellular space.

In the present investigation, with the electron microscope the author studied the structure of plastids in the root cells of an aquatic fern species, *Azolla imbricata* Nakai; and it was revealed that the plastids in the root of this fern are of a chloroplast nature: the internal structure, especially, exhibits the grana-*like* structure which is assumed to be an intermediate structure between those of higher and lower plants.

The roots were fixed by 1% osmic tetraoxide solution buffered to pH 7.4 with veronal-acetate, embedded by methacryl resin (butyl-methacrylate 8: methyl-methacrylate 2), sectioned with glass-knives by a JUM-type microtome, and photographed with a JEM-5G type electron microscope.

Almost all plastids have a structure of a chloroplast nature, i.e. a lamellar and grana-*like* structure (Figures 1–3). In Figure 2 a typical one is presented: its boundary membrane is of double structure; several parallel lamellae of double structure are observed as the internal structures; some piles of the grana-*like* structure are located here and there on the lamellae; in the stroma are observed some spherical bodies of high electron density having the appearance of carotenoid-*like* pigments. These structural features of chloroplast (lamellae and grana-*like*), on the basis of the observations made in this investigation, are therefore rightly considered to be an intermediate structure between those of higher and lower plants.

As to the question of the origin and development of the plastid structure (lamellae and grana), the following contradictory observations have been previously reported: (1) on the one hand, the grana are differentiated after formation of the lamellae (WETTSTEIN[2], HODGE[4]), and (2) on the other, precisely the opposite opinion (MÜHLETHALER et al.[5]). In the case of the chloroplasts found in the roots of this fern, the grana-*like* structures seem to be differentiated after formation of the lamellar structures.

Figure 1 shows the central portion of one root near the tip where the cells are in the more undifferentiated state. Plastids in various developmental stages can be observed in each cell. The young undifferentiated plastids of simpler structure consist of only 2 or 3 lamellae (Figure 1). They would be regarded as the typical proplastids by MÜHLETHALER et al.[5] and BOPP-HASSENKAMP[6], although there are some opposing arguments by several investigators as to the proper definition of proplastid.

Some, but not all, of the plastids contain starch grains (Figures 1 and 3). The one shown in Figure 3 is filled with large grains. Such plastids may be called amyloplasts? They also clearly possess the lamellar and grana-*like* structure. Therefore, they have the appearance of both amyloplasts and chloroplasts. Herein they are called chloroplasts tentatively: only the plastids, in which starch grains are included and other structural elements cannot be positively recognized, are called amyloplasts.

It should be noted further that the plastids of the roots of this fern are all of a chloroplast nature and some of them seem to have the functions both of photosynthesis (proper only to chloroplasts) and of starch reservoirs (proper only to amyloplasts). The roots of higher plants normally lack the function of photosynthesis; i.e., they have not chloroplasts: the plastids of their roots include proplastids, leucoplasts and amyloplasts.

However, since the fern studied here always floats on the water, it seems very likely that the roots suspended as they are in the water are accessible to light and would possess the function of photosynthesis. The light microscope reveals furthermore that almost all plastids are yellowish-green, like the ordinary chloroplasts, and that they react positively to I_2-KI as well as to Lillie's polysaccharide staining. It may be supposed, therefore, that there might be some relationship between the grana-*like* structure and the starch-containing power of the chloroplasts and the above-mentioned habitat.

Zusammenfassung. Alle Plastiden in den Wurzelzellen von *Azolla imbricata* erweisen sich elektronenmikroskopisch als von Chloroplastiden-Natur. Sie zeigen granenartige Strukturen und enthalten zum Teil Stärkekörner. In einigen Zellen der Wurzelmitte werden auch Proplastiden festgestellt.

S. KAWAMATU

Biological Institute, Aichi Gakugei University, Okazaki (Japan), February 15, 1961.

[4] A. J. HODGE, J. biophysic. biochem. Cytol. *2*, 597 (1956).
[5] K. MÜHLETHALER and A. FREY-WYSSLING, J. biophysic. biochem. Cytol. *6*, 507 (1959).
[6] G. BOPP-HASSENKAMP, Z. Naturforsch. *15b*, 91 (1960).

The Action of a Phenothiazine Derivative on Enzymes of the Pentose Phosphate Pathway[1]

Phenothiazine derivatives have been implicated as inhibitors of many enzyme systems[2-4]. It has also been observed that they are inhibitors of human erythrocyte metabolism in the presence of methylene blue[5], presumably by inhibiting an enzyme of the pentose phosphate pathway. The present report extends the observations on the pentose pathway by studying the effect of phenothiazine derivatives on glucose-6-phosphate dehydrogenase (G-6-PD) and 6-phosphogluconate dehydrogenase (6-PGD) in human erythrocytes.

Blood from healthy donors was collected in tubes containing heparin. The cells were washed three times with 0.14 *M* NaCl and packed by centrifugation after each washing. They were subsequently taken up in distilled water and hemolyzed by alternate freezing and thawing. This preparation was used as a source of enzymes and was prepared fresh daily. All operations were carried out at 4°. Oxidation of glucose-6-phosphate (G-6-P) and 6-phosphogluconate (6-PG) was followed by conventional Warburg techniques[6].

Observations showed that the phenothiazine derivative (thioperazine) exerted an inhibitory effect when either G-6-P or 6-PG was used as substrate (Table I). Preincubation of the phenothiazine with the enzyme at 27° for 30 min prior to the addition of G-6-P or triphosphopyridine nucleotide (TPN) increased the inhibition. TPN, on the other hand, when preincubated with the phenothiazine partially protected the enzyme system against inhibition. In those cases where 6-PG was used as substrate, the results obtained on preincubation were equivocal and are still under investigation.

The TPN effect was further studied by varying the TPN concentration in the system. It can be noted from Table II that an increase in the TPN concentration had a significant effect on the inhibition produced by the phenothiazine. For example, a system containing 0.46 μmoles of TPN in the presence of a constant amount of phenothiazine resulted in an inhibition of 49% of the G-6-PD activity, whereas 1.84 μmoles of TPN decreased the inhibition to 19%.

Tab. I. Effect of thioperazine[a] on G-6-PD activity

Conditions	% Inhibition (range)
No preincubation	31–33
Preincubation 30 min with inhibitor	49–59
Preincubation 30 min with inhibitor plus TPN	28–38

Assay System: 0.10 ml hemolyzate; 0.50 ml glycylglycine buffer, pH 7.4; 0.31 μmoles methylene blue; 40 μmoles $MgCl_2$; 0.92 μmoles TPN; 15 μmoles G-6-P; 1.5 μmoles phenothiazine. Total vol. 3.0 ml.

[a] This particular phenothiazine used because of its solubility at pH 7.4.

[1] Supported by the Nebraska Fund for Psychiatric Research.
[2] J. BERNSOHN, I. NAMAJUSKA, and L. S. G. COCHRANE, Arch. Biochem. Biophys. *62*, 274 (1956).
[3] A. S. MORACZEWSKI and K. P. DUBOIS, Arch. int. Pharmacodyn. *120*, 201 (1959).
[4] E. W. HELPER, M. J. CARVER, H. P. JACOBI, and J. A. SMITH, Arch. Biochem. Biophys. *76*, 354 (1958).
[5] M. J. CARVER and N. ROESKY, Exper. *15*, 138 (1959).
[6] F. M. HUENNEKENS, L. LIEU, H. A. P. MYERS, and B. W. GABRIO, J. biol. Chem. *227*, 253 (1957).