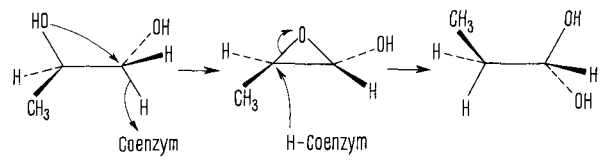
sprechenden optisch aktiven Lactaldehyde mit H_2 ^{18}O und anschliessende Reduktion mittels $LiAlH_4$ gewonnen. Die Stellung des schweren Isotops am C-1 folgt unmittelbar aus der hohen optischen Reinheit (> 95%) der zwei Diole, welche die Möglichkeit einer mit dem Austausch parallel laufenden Tautomerisierung der Edukte ausschliesst, und wurde im übrigen durch massenspektrometrische Messungen an den entsprechenden Bisphenylurethanen bestätigt. Analoge Behandlung von Acetoxyaceton gab eine racemische Probe von 2-^{18}O-Propan-1,2-diol, **3**, in welchem die Lage des schweren Isotops ebenfalls auf massenspektrometrischem Wege bestätigt werden konnte.

Die drei ^{18}O-indizierten Substrate **1**, **2** und **3** wurden unter identischen Bedingungen mit einem zellfreien Extrakt aus *A. aerogenes* in Gegenwart des α-(Dimethylbenzimidazolyl)-Co-5′-deoxyadenosyl-cobamids inkubiert und der jeweils gebildete Propionaldehyd zwecks Vermeidung eines Austausches mit den Sauerstoffatomen des Wassers in situ mittels Hefe-Alkoholdehydrogenase und NADH zu Propanol reduziert. Nach Bildung der Phenylurethane wurde der ^{18}O-Gehalt der drei Proben massenspektrometrisch bestimmt (vgl. Tabelle).

Die erhaltenen Resultate zeigen, dass je nach der (*S*)- oder (*R*)-Konfiguration des Substrates das Sauerstoffatom des Produktes aus der primären bzw. sekundären OH-Gruppe stammt.

In der wohlbegründeten Annahme, dass beide Enantiomeren des Propan-1,2-diols nach einem prinzipiell gleichen Mechanismus umgesetzt werden, scheinen folgende Schlussfolgerungen unumgänglich: (i) im Laufe *beider* Umsetzungen wandert die sekundäre OH-Gruppe stereospezifisch von C-2 nach C-1 unter Ausbildung des Propan-1,1-diols **4**; (ii) die Dehydratation des letzteren zu Propionaldehyd ist enzymatisch kontrolliert, wobei das Enzym zwischen den sterisch nichtidentischen OH-Gruppen von **4** zu unterscheiden vermag.

Für die Propandioldehydrase-Reaktion ist es nunmehr verlockend, ein Reaktionsschema zu postulieren (vgl. **a**→**b**→**c**) in welchem die Halbacetalform des Lactaldehyds als oxydierte Zwischenstufe vorkommt[8].



Summary. Investigation of the propanediol dehydrase reaction with ^{18}O-labelled substrates indicates that the conversion of propane-1,2-diol to propionaldehyde involves transfer of the oxygen atom from C-2 to C-1. The dehydration of the so formed propane-1,1-diol is sterically controlled by the enzyme.

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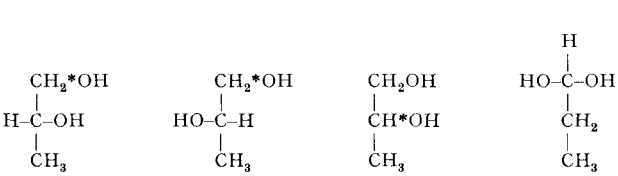
J. Seibl und D. Arigoni

Organisch-chemisches Laboratorium, Eidg. Technische Hochschule, Zürich (Schweiz), 25. Mai 1966.

Substrat	^{18}O-Überschuss (in %)		% Retention
	Edukt[a]	Produkt	
(*R*)-1-^{18}O-Propan-1,2-diol, **1**	12	1	8
(*S*)-1-^{18}O-Propan-1,2-diol, **2**	9	8	88
(*RS*)-2-^{18}O-Propan-1,2-diol, **3**	12,8	5,5	43

[a] Gemessen am Fragment m/e = 195, entstanden durch die Abspaltung von Phenylisocyanat aus dem Molekül-Ion. Fehlergrenze: ± 0,5%.



1 **2** **3** **4**

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X-Ray Microanalyser Study on the Localization of Minerals in Native Plant Tissue Sections

Histo- and cytochemical methods are important aids to the investigation of the mineral metabolism in plants. On the whole, the common colour tests do not give a good localization[1]. This disadvantage has been overcome by the spodogramme technique[2], but except for Si, the identification of the minerals in the ash is difficult[3]. Recently a very sensitive method, microautoradiography, has been developed. This technique has been used extensively on plant material by Lüttge and Weigl[4] and by Branton and Jacobson[5]. But its value is diminished by the fact that a high resolution is only reached with isotopes of low radiant energy.

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The X-ray microanalyser is a sensitive instrument and its resolution is very high owing to the use of an electron beam of 1 μ diameter. All elements that are heavier than Na can be determined qualitatively and also quantitatively in relative units, if identical preparation procedures are followed.

No work with the X-ray microanalyser on plant tissues has so far been reported in literature, but several investigations on zoological and medical materials have been carried out[6].

Our experiments have shown that the X-ray microanalyser is a powerful tool for the localization of minerals in plant tissues and cells. In this paper the distribution of the nutrient elements K and P in leaves of *Zea Mays* is described.

Methods. The experiments were carried out with *Zea Mays* (Ohio M 34). The cultivation of the plants in nutrient solution under greenhouse conditions is described elsewhere[7]. Tissue samples (third leaf, middle of the blade) were excised from plants 5 weeks old.

Preparation. K and P partly occur in a water-soluble state in plants. The localization of these diffusible substances is guaranteed by the use of the cryostat technique described by LÄUCHLI[8,9]. The tissue samples were embedded in fresh brain, frozen with CO_2, and sectioned in a Dittes-Duspiva cryostat at -15 °C, 20 μ thick. The sections were affixed to polished aluminium platelets and coated with a layer of carbon 200 Å thick. Aluminium backings were preferred to glass slides for their better discharge of heat and current during analysis.

An X-ray microanalyser of Japan Electron Optics was used. Principles and applications of the instrument are given by BIRKS[10].

Analysis conditions. X-ray microanalyser type JEOL JXA-3A; high voltage, 25 kV; beam diameter, 1 μ; beam current, 50 μA; sample current, 0.3 μA; spectrometer 1, quartz crystal for potassium (K_α-radiation, 67° 50′); spectrometer 2, KAP crystal for phosphorus (K_α-radiation, 26° 38′); vacuum, 10^{-5} mm Hg (column and spectrometers); gas flow proportional counters (90% Ar, 10% methane).

Results and discussion. The elements K, Ca, Sr (if present in the nutrient solution), Fe, Si, P, and S are detectable in the leaf sections, mainly in the vascular bundles of the midrib.

Figure 1 shows a cross section through the midrib. Vascular bundles are arranged at the lower side with parenchyma cells containing chloroplasts between them. The rest consists of colourless parenchyma and some sclerenchyma.

Examination of the bordered area with the X-ray microanalyser gives the following results: In Figure 2a the electron linear scanning line is visible between A and B. The line starts in the brain, passes the sclerenchyma and the vascular bundle, and ends in the colourless parenchyma. K- and P-concentrations along the electron linear scanning line are represented in Figure 2b. Both elements are mainly localized in the sclerenchyma and the vascular bundle. The principal sites of deposition are in the cell walls and they are partly limited to narrow areas of one to a few μ.

The sites of accumulation are not always the same for K and P. K is chiefly localized in the sclerenchyma and the cell walls of the bundle-sheath. Moreover, the K-peak between 6 and 7 must be assigned to the lumen of a vessel; this is the direct proof of a high concentration of K in the transpiration stream. No deposition of K is detectable in the parenchyma.

The main sites of concentration of P lie in the sclerenchyma and in the walls of bundle-sheath cells and vessels. Smaller quantities are found in the phloem and the protoxylem, the deposition of P in the parenchyma is only low. The P-peak in the lumen of the bundle-sheath cell (3) seems to demonstrate a fairly high concentration in the nucleus.

The distribution of K proves that a transverse transport from the vessels takes place with a favoured deposi-

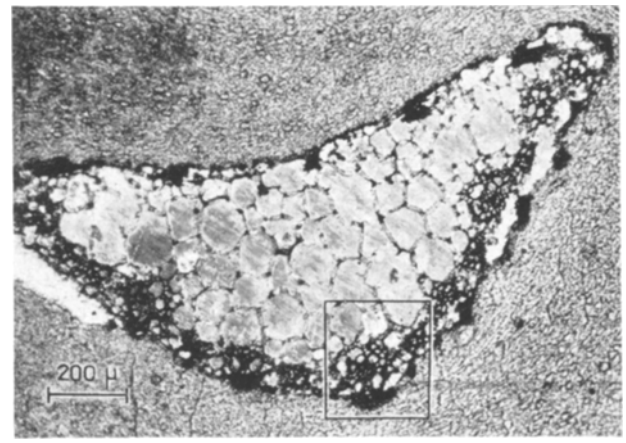

Fig. 1. Cross section through the midrib of a maize leaf affixed to a polished aluminium platelet and coated with carbon. The bordered area was examined with the X-ray microanalyser. × 50.

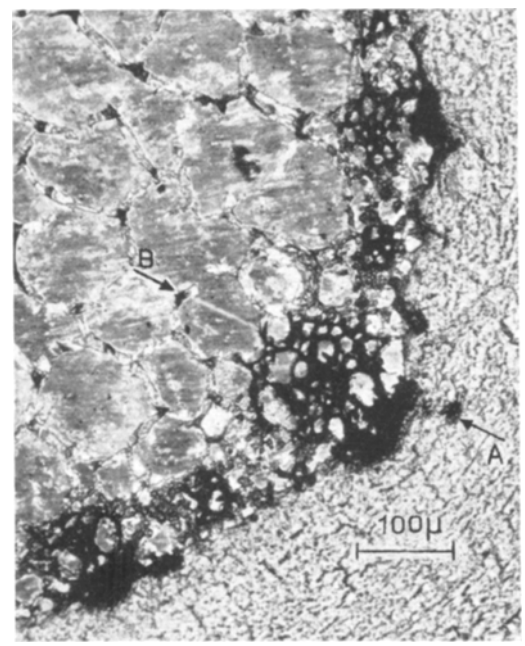

Fig. 2a. Electron linear scanning line between A and B after analysing the bordered area in Figure 1. × 125.

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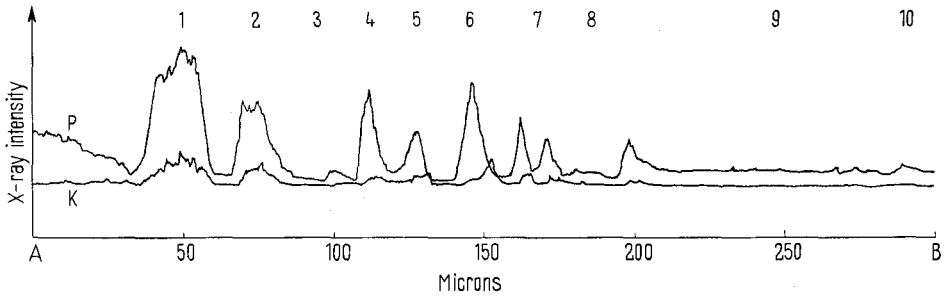

Fig. 2b. K- and P-concentration along the electron linear scanning line. 1 = Sclerenchyma; 2 = Outer cell wall of bundle-sheath; 3 = Lumen of a bundle-sheath cell; 4 = Inner cell wall of bundle-sheath; 5 = Phloem; 6 = Vessel wall; 7 = Protoxylem; 8 = Bundle-sheath cell; 9 = Lumen of a parenchyma cell; 10 = Parenchyma cell wall.

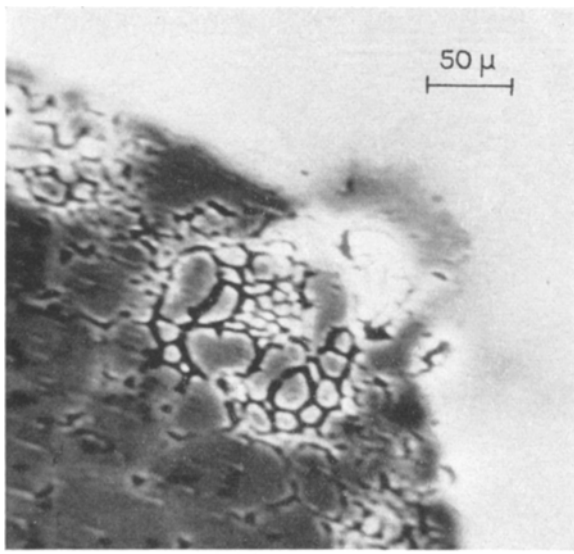

Fig. 3. Absorbed electron image of a part of the midrib showing a dark phase, presumably caused by Fe deposition, mainly in parts of the vascular bundle. × 225.

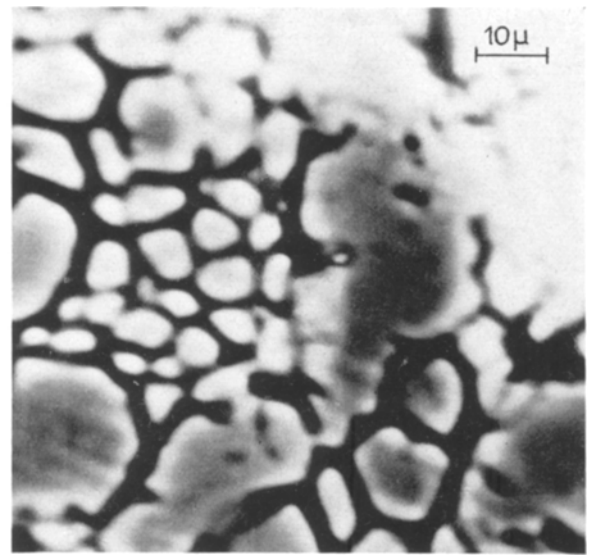

Fig. 4. Absorbed electron image of a vascular bundle showing the dark phase localized to the cell walls. × 900.

tion in cell walls. An earlier study[11] has shown that K exists in maize leaves only in a water-soluble form. Hence the K-peaks localized to the cell walls must originate from a precipitation of soluble potassium salts. FREY-WYSSLING[12] supposes that K may be found in cell walls as carbonate and sulphate.

Remarkable are the high depositions of P in cell walls which point to precipitations of calcium phosphate. Preliminary experiments showed a rather low content of Ca; furthermore, one has to consider the content of Ca in the middle lamellae. Since a precipitation of potassium phosphate is improbable from the curves in Figure 2b, P must be bound also organically in cell walls.

Electron images of the same preparations show different phases. There are principally three phases in the absorbed electron images (Figures 3 and 4). The bright phase may originate from organic materials, the grey one from the aluminium backings, while the dark phase presumably shows the distribution of a heavy element, namely Fe. In Figure 3 the dark phase appears foremost in the vascular bundles and in the parenchyma containing chloroplasts; this phase does not exist in the sclerenchyma. In the absorbed electron image of the vascular bundle (Figure 4) the dark phase is localized to the cell walls. Further experiments to determine its nature are in progress.

From this investigation it follows that, combined with a cryostat technique, the X-ray microanalyser is a suitable means of study; owing to its high resolution it allows demonstration of very small mineral depositions in plant tissues. The localization of soluble minerals is a proof that diffusions in the tissue are prevented by our preparation method.

A report on the distribution of several elements (mainly K, Ca, Sr, P) occurring in different tissues of higher plants is in preparation[13].

Zusammenfassung. Mit Hilfe der elektronischen Röntgen-Mikrosonde konnten die Elemente K, Ca, Sr, Fe, Si, P und S in Kryostatschnitten von nativem Pflanzengewebe lokalisiert nachgewiesen werden. Versuche über die Verteilung von K und P in Maisblättern zeigen eine bevorzugte Anhäufung in den Leitbündeln und im Sklerenchym der Mittelrippe. Elektronenbilder lassen eine dunkle Phase, vermutlich die Verteilung von Fe anzeigend, hauptsächlich in den Zellwänden der Leitbündel erkennen.

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[13] The first author wishes to thank Prof. E. WENK for permission to work in his institute and Prof. M. GEIGER-HUBER for his interest in this investigation.