Quantitative Changes of the Lipid and Fatty Acid Composition of Leaves of *Aleurites montana* as a Consequence of Growth under 700 ppm CO_2 in the Atmosphere

P. He*, A. Radunz, K. P. Bader and G. H. Schmid

Lehrstuhl Zellphysiologie, Fakultät für Biologie, Universität Bielefeld, Postfach 100131, D-33501 Bielefeld, Bundesrepublik Deutschland

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Dedicated to Professor Wolfgang Kowallik on the occasion of his 65th birthday

Aleurites montana, Leaf Lipids, Glycolipids, Phospholipids, Chlorophyll

Leaf lipids of *Aleurites* plants that were cultivated for 5 months in air containing 700 ppm CO_2, were compared to those of control plants cultivated at 350 ppm CO_2. The content of ether soluble lipids referred to dry matter is the same in CO_2- and control plants. The comparison of lipids analyzed as the pigments chlorophyll and carotenoids, phospholipids and glycolipids shows that the ratio of phospholipids and glycolipids is slightly shifted in favor of phospholipids in CO_2-plants. Thus, within the group of phospholipids, phosphatidylglycerol and phosphatidylinositol occur in higher concentrations in CO_2-plants.

Although the differences in the lipid content appear moderate in CO_2- and control plants, it is the saturation degree of fatty acids that differs substantially. The fatty acids of CO_2-plants contain according to the higher phospholipid content approx. 5% more saturated fatty acids. Stearic acid is three-fold increased. Whereas in the phospholipid fraction saturated fatty acids comprise one half of all fatty acids, the unsaturated fatty acids make up for 80 to 90% in the glycolipid fraction. In CO_2-plants not only in the phospholipid fraction but also in the glycolipid fraction saturated fatty acids occur in a higher portion. This means that not only in the cell membrane of CO_2-plants but also in the thylakoid membrane the fluidity is decreased. Also in the wax-fraction long-chained carbonic acids with 20–26 carbon atoms occur. As the portion of these carbonic acids is twice as high in CO_2-plants, it is concluded that a stronger formation of the wax layers exists in CO_2-plants.

By means of Western blotting and by the use of lipid and carotenoid antisera the binding of lipids onto proteins of photosystem II and photosystem I was analyzed. It is seen that besides the major amount of lipids which build up the thylakoid membrane, some lipids are also bound to membrane peptides. Whereas monogalactolipid is bound to the LHCP-complex peptides, to the OEC_1-peptide and the 43 and 47 kDa chlorophyll binding peptides, the anionic lipids sulfoquinovosyldiglyceride and phosphatidylglycerol and digalactolipid are bound to the core peptides of PS II and PS I. β-carotene and the xanthophylls were found to be bound to the core peptides and β-carotene and violaxanthin were also bound to the light-harvesting pigment complex.

Introduction

In previous publications we have reported on the influence of an increased CO_2-content of the atmosphere (700 ppm) and on the influence of a SO_2-content of 0.3 ppm in air on growth of *Aleurites montana* (He *et al.,* 1996). We were able to show that under the influence of 700 ppm CO_2 30–40% more biomass is formed. In dependence on the increased CO_2-content differences are seen in the protein, chlorophyll and sugar content of leaves. Moreover, the content of the CO_2-fixing enzyme ribulose 1,5-bisphosphate carboxylase (Rubisco) and that of the energy conserving enzyme of the CF_1-complex of the ATPase are

Abbreviations: PS I, photosystem I; PS II, photosystem II; LHCP, light harvesting pigment protein complex; MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; OEC_1, oxygen evolution complex peptide (33 kDa); PAGE, polyacrylamide gel electrophoresis; Tris-HCl, tris[hydroxymethyl]amino-methane; SDS, sodium dodecylsulfate, EDTA, ethylendiamine tetraacetic acid; DTT, dithiothreitol; BPB, bromophenol blue (3,3′,5,5′-Tetrabromphenolsulfone phthalein); Tricine, (N-tris[Hydroxymethyl]methylglycine).

* *Present address:* Central South Forestry University, Zhuzhou/Hunan 41206, People's Republic of China.

Reprint requests to Prof. Dr. Georg H. Schmid.
Fax: 0521-1066410.

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changed. Also the peptide composition of PS II shows that molecular modifications have occurred. In contrast to tobacco, photorespiration is completely suppressed by 700 ppm CO_2 (He *et al.*, unpubl.).

In the present publication we report on changes of the lipid pattern of leaves of *Aleurites* plants and on the increase of the saturation degree of fatty acids in dependence on the CO_2-content. In context with fatty acids analyses it is of great interest, whether the C_{18} trienoic fatty acid occurring in the triglycerides of *Aleurites* seeds by 60–80% having Δ^9 *cis*, Δ^{11} *trans*, Δ^{13} *trans* configuration also occurs in leaf lipids (Fang *et al.*, 1981, 1985; Gunstone *et al.*, 1994). The plant belongs to the *Euphorbiaceae* and is cultivated as a crop plant in China yielding annually 4×10^5 tons of oil. Due to its increased content of trienoic-fatty acids, this oil is a highly appreciated raw material in the lacquer industry (Fang *et al.*, 1981, 1985).

Materials and Methods

Cultivation of plants

Aleurites montana plants were cultivated in an automatic fully climatized growth chamber with a light/dark cycle of 14/10 hours and a day temperature of 26 °C, a night temperature of 22 °C and 60% relative humidity. *CO_2-plants* were transferred to glass compartments in the same growth chamber 3 months after germination where they were exposed to air containing 700 ppm CO_2. 5 months later the leaves were harvested. Leaves were lyophylized after fixation in liquid nitrogen.

Preparation of ether soluble lipids and quantitative determination of lipids

The lyophylized leave material was ground in a coffee mill and the powder extracted on a glass funnel in sequence with methanol, acetone and diethylether. From this crude extract the lipids were taken up in diethylether. Thereafter oligosaccharides and proteins still present were separated from the lipids by Sephadex G25 purification according to Wuthier (1966). For the quantitative determination of glycolipids and phospholipids the lipid mixture was first separated by thin layer chromatography on silica gel G layers in the solvent system: chloroform, methanol, acetic acid, water (85/5/10/3.5, v/v). Glycolipids were quantitatively determined via the determination of galactose with anthrone-sulfuric acid and the phospholipids via determination of phosphorus according to Fiske-Subbarow. Composition of the anthrone reagent was: 0.2 g anthrone were dissolved in 100 ml concentrated H_2SO_4 p.a and diluted with 50 ml bidest. H_2O. The galactolipids were heated with this reagent in a test tube for 15 minutes at 85 °C and the content measured photometrically as absorbance at 625 nm. The determination of the sulfolipid was carried out with the same reagent by heating the lipid for 20 min at 100 °C and photometric determination at 595 nm (Radunz, 1969). The carotenoids were also determined photometrically after the separation by thin layer chromatography on silica gel in the mobile phase system: benzene (100–140 °C), isopropanol, water 100/12/0.25 (v/v) following the method of Davies (1965) and Hager and Meyer-Bertenrath (1966).

Lipid analysis by column chromatography

In order to separate the lipids into their main components a chromatographic analysis on a silica gel column was carried out. The unpolar lipids such as hydrocarbon-components, waxes, tocopherols, plastoquinones, chlorophylls and carotenoids were eluted with chloroform. The elution with acetone yielded glycolipids, steroyl glycosides and the remaining carotenoids (violaxanthin and neoxanthin). Phospholipids were eluted with methanol.

Analysis of fatty acids

The lipids were first saponified by heating with 0.5% methanol-NaOH. From this methanol-NaOH solution the hydrocarbon-components and carotenoids were taken up with petrol ether (40–60 °C). After acidification with HCl, free fatty acids were partitioned in petrol ether/diethyl ether (1:1, v/v). Thereafter the fatty acids were transformed into their methylesters by 1-hour heating in 5% methanolic HCl and taken up in petrol ether (Radunz, 1966b). The gas chromatograph from Hewlett Packard, Typ 5890, Serie II using a 10 m ethylene-glycol-succinated capillary column. The column temperature was 190 °C. The carrier

gas was nitrogen. For comparison purpose and for the characterization of the fatty acids standard fatty acids were used.

Isolation of chloroplasts, determination of chlorophyll and protein

Chloroplasts were isolated following the methods described by Oku and Tomita (1976) and He (1987) by fractioning centrifugation using Tricine buffer, pH 7.4 (buffer composition: 50 mM Tricine, 0.4 M sucrose, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM EDTA and 0.6% polyethyleneglycol 4000). For purification of chloroplasts the thus obtained chloroplast sediment was centrifuged according to Kreutz and Menke (1960) on a sucrose gradient. Chlorophylls were determined in methanol/water (90/10) according to Schmid (1971). Protein determinations were carried out according to Smith *et al.* (1985) and according to Lowry *et al.* (1951).

Detection of binding of carotenoids and lipids onto peptides

SDS PAGE and Western blotting

Polypeptides of chloroplasts were modified in the SDS polyacrylamide gel electrophoresis according to the methods of Weber and Osborn (1969) and Laemmli (1970). A 1.5 mm gel with a 10–20% gradient separation gel and a 3% collection gel was used. Prior to electrophoresis, samples of chloroplasts (10 μg chlorophyll) were solubilized with 10 mM DTT, 2% SDS, 10% glycerol, 0.01% BPB and 10 mM Tris-HCl buffer, pH 8.4, at 50 °C for 30 min. Electrophoresis was carried out at a constant current of 25 mA for 4 h at room temperature. Following electrophoresis the gel was stained with Coomassie Brilliant Blue.

Western blotting was performed as described by Renart *et al.* (1979). The separated polypeptides were transferred by pressure from SDS gels to nitrocellulose membranes (Protan BA85, Schleicher & Schuell) for 20 h at room temperature. The membranes were blocked with 2.5% fish gelatine. The dilution of the first antibody (lipid and carotenoid antibody) depends on the antiserum used. The second antibody, peroxidase-conjugated pig immunglobulins (swine anti-rabbit immunoglobulins, Code No. P0217, DAKO, Hamburg) was diluted 500-fold. Specifically bound antibodies were stained by the reaction of peroxidase with H_2O_2 and 4-chloro-1-naphthol (Sigma).

Antisera

The used antisera to lipids and to the carotenoids were obtained by immunization of rabbits and are characterized in earlier publications (Radunz, 1971, 1972, 1976, 1984; Radunz and Berzborn, 1970; Radunz and Bader, 1982; Radunz and Schmid, 1973, 1975; Lehmann-Kirk *et al.*, 1979a, b; Radunz *et al.*, 1984a, b; Schmid *et al.*, 1993).

Results and Discussion

Influence of 700 ppm CO_2 on the lipid composition

Aleurites plants that have been cultivated for 5 months under a CO_2-content of 700 ppm in the atmosphere have, in comparison to control plants, built up 30–40% more biomass (He *et al.*, 1996). The extraction of leaf lipids with methanol, acetone and diethyl ether has led to the result, that *Aleurites* leaves, despite their crude appearence, have a relatively high lipid content. The lipid content of the control plants is 11.9% and that of *CO_2-plants* practically the same, namely 12.1% of the dry weight. This value corresponds to the lipid concentration in leaves of other higher plants (Radunz, 1966a, 1968; Bednarz *et al.*, 1988). The composition of the lipids of CO_2- and control plants is given in Table I. As expected at least with the glycolipids, phospholipids and carotenoids no qualitative differences are seen. However, small differences in the quantitative composition become apparent. CO_2-plants contain 14% more chlorophyll, 8% more phospholipids and 6.5% more carotenoids, and glycolipids are reduced by 8%. Whereas in control plants the ratio of phospholipids to glycolipids is 1/3.5, this ratio is shifted in CO_2-plants to 1/3, that is in favor of the phospholipids. Phospholipids essentially build the membranes of mitochondria and cell membranes such as tonoplasts, plasmalemma and the membrane of oligosomes (Frentzen and Heinz, 1983), i.e. peroxysomes, glyoxysomes and oleosomes which are cell organells with different contents but surrounded by a bimolecular phospholipid membrane. In the chloroplast membranes phospholipids occur only with 4–6%. However, glycolipids participate in the formation of the thylakoid mem-

Table I. Composition of the purified ether soluble leaf lipids of *Aleurites montana* grown for 5 months under 700 ppm CO_2 in air.

	Chlorophyll %		Glycolipids %		Phospholipids %						Carotenoids %					Chlorophyll	Carotenoid to	Phospholipids to
	a	b	MGDG	DGDG	SL	CA	PE	PG	PC	Pi	β-Ca	Lu	Vio	Neo	Zea	*a/b*	chlorophyll	glycolipids
Control plants	15.2	4.7	32.5	22.9	3.3	0.8	3.6	2.9	8.5	1.2	0.91	2.1	0.45	0.7	0.24	1/3.23	1/4.5	1/3.5
CO_2-plants	16.1	6.6	30.1	21.5	2.7	0.7	2.9	3.4	8.8	2.5	0.92	2.1	0.27	0.57	0.84	1/2.44	1/4.8	1/3.0

Lipid values are given in per cent of total lipids which were put 100%. Not included in this calculation are plastoquinones, tocopherols and waxes. Values are averages of 6 to 8 individual determinations. The deviation of the values given is between 2 to 3 percent.
MGDG, Monogalactosyldiglyceride; DGDG, Digalactosyldyglyceride; SL, Sulfoquinovosyldiglyceride; CA, Cardiolipin; PE, Phosphatidylethanolamine; PG, Phosphatidylglycerol; PC, Phosphatidylcholine; Pi, Phosphatidylinositol; β-Ca, β-Carotene; Lu, Lutein; Vio, Violaxanthin; Neo, Neoxanthin; Zea, Zeaxanthin.

brane where they make up for 40–50% of the total membrane lipids (Quinn and Williams, 1983). The ratio of carotenoids to chlorophylls has shifted slightly from 1:4.5 in control plants to 1:4.9 in CO_2-plants. The chlorophyll$_{a/b}$ ratio is in CO_2-plants, due to the high chlorophyll-*b* content, smaller than in control plants. Whereas the chlorophyll-*a* content has increased in CO_2-plants by 9% the chlorophyll-*b* increase represents 20%. From the higher chlorophyll content as well as from the reduced chlorophyll$_{a/b}$ ratio it is concluded that the light harvesting complex is stronger developed in CO_2-plants than in control plants. Also the higher carotenoid content speaks in favor of this interpretation. Peptide analyses by SDS-polyacrylamide gel electrophoresis confirm this interpretation (Fig. 1).

Concerning the phospholipids it is seen that phosphatidylinositol which essentially contributes to the structure of plasma membranes occurs in twice the amount in CO_2-plants. Phosphatidylglycerol, which contributes to the structure of the

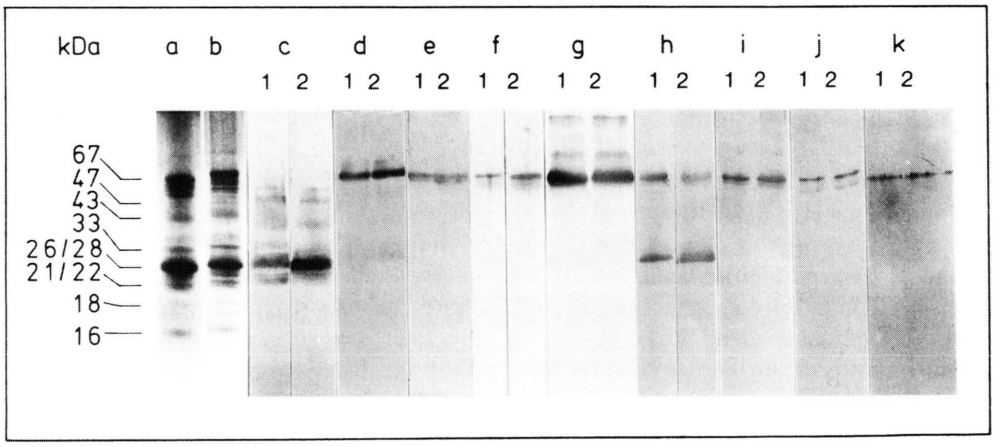

Fig. 1. Detection of lipid and carotenoid binding onto peptides of photosystem I and photosystem II in plants of *Aleurites montana*, cultivated for 5 months under a CO_2-content of 700 ppm in air, by means of the Western blot procedure.
a. SDS-PAGE of chloroplasts from CO_2-plants; b. SDS-PAGE of chloroplasts from control plants; c_1.-k_1. nitrocellulose membranes with chloroplast preparations of CO_2-plants after treatment with lipid and carotenoid antisera; c_2.-k_2. nitrocellulose membranes with chloroplast preparations of control plants after treatment with lipid- und carotenoid antisera; c. monogalactolipid; d. digalactolipid; e. sulfolipid; f. phosphatidylglycerol; g. β-carotene; h. violaxanthin; i. neoxanthin; j. zeaxanthin; k. lutein.
The antiserum dilution was 1:100 to 1:500 (v/v). The concentrations of the chloroplast preparations of CO_2- and control plants were equivalent to 10 μg of chlorophyll. As the CO_2-plants contain more chlorophyll, this corresponds to a protein concentration of 200 μg for CO_2-plants and 280 μg for control plants.

light harvesting complex and which also occurs on the core peptides of photosystem I and II (Kruse *et al.*, 1993; Makewicz *et al.*, 1995, 1996) is present in CO_2-plants in 15% higher concentration. In contrast phosphatidylethanolamine and cardiolipin which contribute to the structure of the inner mitochondrial membrane are reduced in CO_2-plants. Phosphatidylserine occurs only in traces and was quantitatively not determined owing to its low content.

The two galactolipids and the second anionic lipid sulfoquinovosyldiglyceride, which are structural elements of the thylakoid membrane occur in higher concentrations in control plants. The reduction of these glycolipids in the CO_2-plants hints at a modification of the chloroplast structure. The elucidation of this interrelationship remains to be proven. Studies by electron microscopy by Cave *et al.* (1981) with *Trifolium* chloroplasts have shown that cultivation under increased CO_2 lead to starch accumulation which in turn leads to a structural alteration of the thylakoid membrane.

With monospecific antisera to lipids and carotenoids the binding of these lipid components onto peptides of the photosystem II and photosystem I complex was determined using the Western blot procedure (Fig. 1). Western blot analyses of the peptides of photosystem II and of photosystem I with other higher plants such as *Nicotiana tabacum* or with the cyanobacterium *Oscillatoria chalybea* (Kruse *et al.*, 1993; Makwicz *et al.*, 1994; Voß *et al.*, 1992) have shown that lipids and carotenoids are tightly bound to the D1-core peptide (which under the SDS gel electrophoretic conditions used occurs as a dimer) and to the core peptides of PS I (CP1) but also to the peptides of the light-harvesting complex (LHCP). With this immunological method it was shown that the antisera to digalactolipid and the anionic lipids sulfoquinovosyldiglyceride and phosphatidylglycerol react with the core peptides of PS II and PS I (polypeptide band with the molecular weight of 66 kDa) and the antisera to monogalactolipid react with the LHCP-peptides, the OEC_1-peptide and with the chlorophyll binding peptide with the molecular mass of 43 and 47 kDa. From the carotenoids β-carotene and xanthophylls occurring in higher plants, lutein, zeaxanthin and neoxanthin are bound to the core peptides (66 kDa peptide). β-carotene and violaxanthin antisera label also LHCP-complex peptides. Certainly, only a few molecules of these carotenoids are present on the core peptides and should have a protective function. The bound lipid molecules probably serve for an anchoring of the respective proteins in the lipid membrane.

Influence of the increased CO_2-content in air on the fatty acid composition

To analyze this influence of the increased CO_2-content on the fatty acid composition not only leaf lipids from leaves of *Aleurites montana* plants that have been grown in the growth chamber under the respective conditions were studied but also from *Aleurites* plants that have been grown in a greenhouse and in the open field. We included into these analyses also the fatty acids from leaf lipids of an additional *Cypressacea* namely of *Euphorbia cyparissias* which had been grown in the field.

Fatty acids of leaves of *Aleurites montana* qualitatively do not differ from the fatty acid composition of other higher plants (Table II), (Radunz, 1966b, 1968; Quinn and Williams, 1983; Bednarz *et al.*, 1988). Linolenic acid is with 60–80% the main component of fatty acids. Among saturated fatty acids palmitic acid with 9–20% appears as the main component. The C_{16} monoenoic acid as well as stearic and oleic acid occur only in low concentrations. Only linoleic acid occurs with 5–15%. The comparison of fatty acids in plants cultivated under different CO_2-conditions shows that only the concentrations of stearic, oleic and linoleic acid show some differences. Whereas in CO_2-plants an increase of the stearic and a decrease of linoleic acid is observed, in control plants oleic and linoleic acids are higher with the stearic acid portion being lower. The saturation degree of total fatty acids is considerably higher in CO_2-plants. This increase is apparently due to the increase in phospholipids in comparison to that of glycolipids in CO_2-plants as phospholipids in comparison to glycolipids always bear a higher portion of saturated fatty acids. With the higher saturation degree of fatty acids the fluidity of the membrane is lowered. The comparison of *Aleurites* plants grown in a greenhouse and in the open field, clearly show that the modification of the fatty acid composition in dependence on different light intensities, on temperature differences and different humidity conditions are larger than those induced by the

Table II. Comparison of the fatty acid composition of the leaf lipids of *Aleurites montana* plants grown in the open field, in the green-house and in a growth chamber under an increased CO_2-content in air. The values are compared to an additional *Euphorbiacea, Euphorbia cyparissias* which has been grown in the field. Fatty acids are given in % of total fatty acids.

Plants	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{16:1\ trans}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20-26}
Aleurites montana Control plants from growth chamber	0.1	0.8	9.4	0.4	1.4	1.4	3.3	7.3	75.9	–
Aleurites montana *CO_2-plants* from growth chamber	0.5	0.2	10.9	0.3	1.5	4.6	1.7	5.3	75.0	–
Aleurites montana green-house plants	0.2	2.7	16.4	0.3	1.0	2.6	3.0	14.9	58.9	–
Aleurites montana Field plants	0.3	1.2	19.6	0.4	1.7	3.0	2.6	10.8	60.4	–
Euphorbia cyparissias Field plants	0.5	3.3	7.9	0.4	2.4	0.1	0.5	5.0	79.9	–

Lipids	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{16:1\ trans}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	C_{20-26}
Control plants										
Unpolar lipids	1.5	8.8	23.3	2.5	–	7.0	6.0	12.9	24.4	13.6
Glycolipids	0.1	0.1	7.7	0.4	–	3.2	1.9	3.2	83.4	–
Phospholipids	0.2	0.9	26.6	1.1	6.5	17.1	8.7	13.4	25.5	–
CO_2-plants										
Unpolar lipids	2.3	11.5	22.4	1.8	–	6.5	2.8	7.2	22.8	22.6
Glycolipids	0.1	0.3	9.4	0.5	–	7.7	1.8	3.4	76.5	0.3*
Phospholipids	0.2	1.2	29.6	1.2	6.4	19.1	9.1	15.4	17.3	0.5*

Phospholipids are composed of: cardiolipin, phosphatidylethanol amine, phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol. Glycolipids are composed of monogalactosyldiglyceride, digalactosyldiglyceride, Sulfoquinovosyldiglyceride and sterylglycoside. The fraction of unpolar lipids is composed of waxes, carotenoids, tocopherols and plastoquinones. C_{20-26} means carbonic acids with 20, 21...26 carbon atoms without identification of saturated or unsaturated fatty acids. * Given is the portion of $C_{20:0}$. Values are averages of 3 individual determinations.

increased CO_2-content, when plants are grown in a growth chamber. Thus, the saturation degree in *greenhouse plants* and *open-field plants* increases stronger, due to the increase of palmitic acid and the decrease of linolenic acid, than in dependence on the CO_2-content. On the other hand it should be also noted that due to the formation of a thicker wax layer on the leaf surface of *open-field* and *greenhouse plants* a stronger increase of saturated and monoenoic acids has occurred than is the case with the *growth-chamber cultivated plants.* An exception is the fatty acid composition of *Euphorbia cyparissias.* Here the unsaturated fatty acids make up for 88% and saturated fatty acids for 12% of total fatty acids. Also the ratio of fatty acids with 16 and 18 carbon atoms is practically the same in CO_2-plants and control plants. Differences in the modification of chain lengths occur also if fatty acids of *Aleurites* plants are studied that have been cultivated under different out-door conditions. Thus, the C_{16}-fatty acids increase from 12% in *growth chamber plants* to 18% in *greenhouse-plants* and 22% in *open-field plant.* The C_{18}-fatty acids decrease correspondingly.

Since, as shown above, the ratio of phospholipids to glycolipids is changed as a consequence of the increased CO_2-content in air, also the fatty acids of the glycolipid and phospholipid fraction obtained by column chromatography as well as those of the unpolar lipids were determined. From the comparison of the fatty acid composition of these three lipid fractions from CO_2- and control plants it is unequivocally seen (Table II) that in glycolipids the portion of unsaturated fatty acids

is very high wheras it is very low in phospholipids. Whereas the unsaturated fatty acids in glycolipids from control plants make up for 89% and from CO_2-plants for 82%, the unsaturated fatty acids in phospholipids from control plants make up for 55% and in phospholipids from CO_2-plants for 49% of total fatty acids. This shows that not only in phospholipids but also in glycolipids of CO_2-plants the saturated character of fatty acids has increased strongly.

This means that not only in cell membranes but also in the thylakoid membrane fluidity is lowered. As a characteristic difference it is seen that hexadecenoic acid with *trans*-configuration occurs only in phospholipids. In the unpolar lipids, which comprise waxes, carotenoids, tocopherols and plastoquinones the portion of unsaturated fatty acids is unusually high. It makes up in the unpolar lipid fractions of control plants still for 46% and in CO_2-plants for 35% of total fatty acids. As expected here long-chain carbonic acids with 20 to 26 carbon atoms occur, and also carbonic acids with uneven numbers of carbon atoms such as 21, 23...25 are found. These long-chain fatty acids occur as ester components of waxes. As the content of these acids is twice as high in CO_2-plants in comparison to control plants we conclude that the protecting waxes in the leaf surfaces of CO_2-plants are stronger developed.

The C_{18} trienoic fatty acid with conjugated double bounds and Δ^9 *cis*, Δ^{11} *trans*, Δ^{13} *trans* configuration, occurring in the tryglycerides of *Aleurites* seeds, was not found in the leaf lipids.

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