Inhibitors of Chlorophyll Biosynthesis from Bulbs of *Gladiolus* spp.

Joachim Wübert, Ulrike Oster and Wolfhart Rüdiger

Botanisches Institut der Universität München, Menzinger Str. 67, D-80638 München

Dedicated to Professor Werner Wehrmeyer on the occasion of his 65th birthday

Z. Naturforsch. **51c,** 548–557 (1996); received April 19/May 2, 1996

Gladiolus spec., *Lepidium sativum* L., 3-Hydroxybenzoic Acid Methylester, 5-Hydroxymethylfurfural, Gas Chromatography/mass Spectrometry

Compounds extracted with methanol from the bulbs of *Gladiolus* spp. were tested for inhibition of Chl accumulation in greening cress (*Lepidium sativum* L.) seedlings. For the biotest, cress seeds were imbibed with water for 8 h and then incubated in the dark with the test solution for 40 h. Chlorophyll accumulation was determined at the end of the subsequent irradiation period of 8 h. Compounds active in this biotest were purified by several chromatographic steps and identified by thin-layer chromatography with staining reactions and by gas chromatography/mass spectrometry. The following compounds were identified and their concentration for 50% inhibition of Chl accumulation determined: sucrose (75 mM) plus glucose (30 mM), 3-hydroxybenzoic acid methylester (0.49 mM) and 5-hydroxymethylfurfural (6.7 mM). The inhibitory action was compared with several substituted derivatives of the natural compounds.

Introduction

Chlorophyll (Chl) biosynthesis has frequently been investigated in angiosperm seedlings that become green only in the light. Dark-grown seedlings accumulate several Chl precursors, among which protochlorophyllide is the major one (reviews: Rüdiger and Schoch, 1988; Jordan, 1991; Rüdiger, 1993; Senge, 1993). Irradiation of dark-grown seedlings leads to phototransformation of protochlorophyllide to chlorophyllide, and subsequent esterification and incorporation of the newly-formed Chl into Chl-binding proteins. The latter step results in mutual stabilization of pigment and apoprotein. Chl that is not protein-bound is a target for rapid degradation (Hörtensteiner *et al.,* 1995; Matile and Kräutler, 1995; Gossauer and Engel, 1996) and apoproteins that are not complexed with pigments do not accumulate but are likewise degraded (Bennett, 1981; Eichacker *et al.,* 1990; Mullet *et al.,* 1990; Paulsen *et al.,* 1993). According to the present knowledge, plastid-encoded apoproteins are produced in excess so that any newly-formed Chl is immediately incorporated and stabilized (Kim *et al.,* 1994). Accumulation of Chl during light-dependent greening can therefore be taken as a measure for Chl biosynthesis under these conditions.

Several inhibitors have been used to investigate the details and significance of various steps in Chl biosynthesis. For example, gabaculin inhibits formation of 5-aminolevulinate (ALA) in the C-5 pathway. This inhibitor has been used to demonstrate formation via the C-5 pathway not only of Chl (Flint, 1984) but also of phytochromobilin (Gardner and Gorden, 1985; Elich and Lagarias, 1987) and cytochromes (Anderson and Gray, 1991) in angiosperms. Glutamate-1-semialdehyde, the direct precursor of ALA, accumulated and was isolated after gabaculin treatment (Kannangara and Schouboe, 1985). Diphenylether herbicides, e.g. acifluorfen, inhibit protoporphyrinogen oxidase (Matringe *et al.,* 1992). Accumulation of protoporphyrinogen, distribution of it in all cell compartments and autoxidation to protoporphyrin lead to subsequent photooxidation, especially of membrane lipids, as a result of the particular herbicide action (Sherman *et al.,* 1991). Metal chelators (2,2'-bipyridyl, 1,10-phenanthroline, 8-hydroxyquinoline) can cause accumulation of protochlorophyllide (Kittsteiner *et al.,* 1991) and other Chl precursors (Ryberg and Sundqvist, 1976). Application of metal chelators together with ALA results in strong photodynamic damage

Reprint requests to Prof. Dr. W. Rüdiger.
Telefax: 089-17861-185.

0939–5075/96/0700–0548 $ 06.00

to the plants (Rebeiz *et al.*, 1984, 1988). Magnesium protoporphyrin monomethylester accumulates also after application of thujaplicin (Oster *et al.*, 1996). The finding of this inhibitor of Chl biosynthesis in non-green parts of *Thuja occidentalis* (Oster *et al.* 1996) prompted us to search for other inhibitors of Chl biosynthesis in natural sources. We describe here the inhibitory activity of substances that occur in bulbs of *Gladiolus* spp. and the elucidation of their structures. The structures were also used as key structures for detection of additional inhibitors with similar chemical structures.

Material and Methods

Extraction of plant material and purification of inhibitory compounds

Bulbs (500 g) of *Gladiolus* spec. (Garten-Kölle, München/Germany) were minced with 1 L methanol in a Waring blendor. After addition of 4 L methanol to the homogenate, the mixture was stirred for 24 h at room temperature and then filtered through a folded filter (Schleicher and Schüll, No. $595^1/_2$). The clear filtrate (5 L) was evaporated to dryness in a rotary evaporator. The remaining residue was dissolved in 50 ml water and applied to a column (4.5 x 40 cm) of Sephadex LH 20 (Pharmacia, Uppsala, Sweden) equilibrated with water [and degassed]. Elution with water (360 ml) yielded the first fraction („water fraction") that inhibited Chl biosynthesis (see below for the biotest). The water fraction was analyzed by thin-layer chromatography on silica gel KG 60 (Merck). The solvent system toluene/acetic acid/methanol (3:2:5, v:v:v) resolved glucose (R_F = 0.59) and sucrose (R_F = 0.52) as the main compounds of this fraction. The staining reagent (aniline/diphenylamine/phosphoric acid, Stahl (1967)) did not reveal any other compounds comparable in abundance to glucose and sucrose. The column was washed with additional 800 ml water. No bioactivity was found in this eluate. The subsequent washing with methanol (1 L) resulted in elution of additional inhibitory activity. The eluate was evaporated to dryness („methanol fraction").

Silicia gel KG 60, particle size 0.015–0.04 mm (Merck, Darmstadt/Germany) was equilibrated with $CHCl_3$ and used to pack a column (3.2 x 2.5 cm) . The dried „methanol fraction" was dissolved in 10 ml $CHCl_3$: methanol (4:1) and applied to the column. Elution was performed with a step gradient consisting of several 300 ml volumes of $CHCl_3$ containing 0, 25, 50 and 75% methanol, respectively. The last elution step was performed with 300 ml methanol. Bioactivity was found in fractions II (25% methanol eluate) and IV (75% methanol eluate) (see Table I).

Purification of fraction II and isolation of 3-hydroxybenzoic acid methyl ester

Fraction II was concentrated in vacuo to a small volume (ca. 3 ml) and applied to a silica gel column (2 x 12 cm silica gel KG 60, particle size 0.015–0.04 mm, Merck) equilibrated with toluene/methanol (4:1). Fractions of 4 ml each were collected during elution with a step gradient of toluene/methanol 4:1 (30 ml), 3:2 (40 ml) and 2:3 (30 ml). Fractions 14–21 that contained bioactivity were combined (= fraction II.2) and evaporated. The residue was dissolved in 2 ml toluene/methanol (1:1) and applied to another silica gel column (2 x 17 cm) equilibrated with toluene. The column was developed with a step gradient consisting, in sequence, of toluene (25 ml), the mixture of toluene with 5% (100 ml), 20% (69 ml) and 50% methanol (60 ml) and methanol (100 ml). Fractions of 6 ml each were collected and tested for bioactivity. Bioactivity was detectable in fractions 29–44. The residue of these combined fractions (= fraction G) was applied to a third silica gel column. Elution with toluene (35 ml) and toluene mixed with 2% (90 ml), 4% (60 ml), 8% (90 ml), 20% (100 ml) and 50% methanol (35 ml) yielded bioactivity in fractions (6 ml each) 36–45 (= fraction G2). The active fractions were combined and evaporated. The residue was further investigated after microdistillation at reduced pressure (0.05 mbar, bath temperature 150°C). Votatile compounds were collected in a trap cooled with liquid nitrogen. Bioactivity was completely recovered in the trap. This fraction was further investigated by gas chromatography/mass spectrometry (see Figs. 2 and 3) and found to contain 3-hydroxybenzoic acid methyl ester as the major compound.

Purification of fraction IV and isolation of 5-hydroxymethylfurfural

Fraction IV was evaporated to dryness. The residue was dissolved in water/trifluoro acetic acid

(1:1) and heated in a closed tube to 120°C for 1 h. The hydrolysate was evaporated. The residue was dissolved in a small volume of acetone and applied to a silica gel column (2 x 10 cm) equilibrated with toluene/acetic acid/methanol (75:20:5). Elution of the column started with the same mixture (60 ml) and was continued with toluene/acetic acid/methanol (50:20:30). Fractions (3 ml each) were collected and tested for bioactivity. Fractions 18–22 that contained most of the bioactivity were combined and evaporated. The residue was subjected to microdistillation. The volatile compounds were then investigated by gas chromatography/mass spectrometry with the instrument 4500 (Finnigan-MAT, Bremen) (see Figs. 4 and 5). The main compound was identified as 5-hydroxymethylfurfural.

Biotest

Fifteen cress seeds (*Lepidium sativum* cv. Armada, J. Wagner Samenzucht, Heidelberg/Germany) were placed on filter paper (4.5 cm diam.) in a Petri dish and incubated with 1.5 ml deionized water in the dark at 25°C. The water was removed with a pipette after 8 h and replaced by 1.5 ml of a test solution. Incubation was continued in the dark for 40 h. Several experiments were performed with longer periods (up to 120 h) of incubation in the dark. Incubation with inhibitors was always performed during the last 40 h before the start of the irradiation period.The Petri dishes were then irradiated with white light (5 W · m^{-2}, fluorescent tubes LI8W/25, Osram) for 8 h. The plantlets were harvested by cutting at 1 cm under the hypocotyl hook and placed into 3 ml N,N-dimethylformamide such that the tissue was entirely covered by the solvent. The samples were incubated overnight at 4°C, and their Chl *a* and *b* content were determined by measuring absorbance and calculating according to the formulae (Moran, 1982):

$$\text{Chl } a \text{ [nmol/ml]} = 14.15 \cdot (E_{662}\text{-}E_{750}) - 3.34 \cdot (E_{644}\text{-}E_{750}) - 0.05 \cdot (E_{624}\text{-}E_{750})$$

$$\text{Chl } b \text{ [nmol/ml]} = 25.83 \cdot (E_{644}\text{-}E_{750}) - 6.00 \cdot (E_{662}\text{-}E_{750}) - 1.10 \cdot (E_{624}\text{-}E_{750})$$

Results and Discussion

For detection of inhibitors of Chl accumulation, a biotest with cress seedlings (*Lepidium sativum* L.) was developed with the following considerations: the solution to be tested should not be added to dry seeds because possible inhibition of water uptake was not the matter of investigation. After imbibition, early steps of Chl biosynthesis up to the step of Pchlide formation and other germination-related metabolic events occur in the swollen seeds in the dark. Light is then needed for the Pchlide-Chlide transformation. Thus, Chl accumulation occurs only in the light. Preliminary experiments had shown that the first phase of weight increase (corresponding to water uptake) was completed within 8 h. The water was removed from the Petri dishes at this time and replaced with the test solution. After periods of dark incubation (40 h), and irradiation (8 h), the Chl content at the end of the irradiation period was determined. The value of the water control was taken as the basis (= 100%) for calculation of the percentage of inhibition. Two effects were observed in these experiments: (1) Light induced greening was gradually reduced as incubation time in the dark was increased. This indicated a gradual decrease of metabolic activity including steps of Chl biosynthesis. (2) The percentage of inhibition of Chl accumulation was likewise reduced if the incubation with inhibitors started later. This suggested that part of the observed inhibition could not be attributed to inhibition of enzymatic steps of Chl biosynthesis but rather to inhibition of formation of enzymes of Chl biosynthesis. It has been shown before that the capacity of the tetrapyrrole pathway changes dramatically with the age of young etiolated cress seedlings, indicating a strong turnover of the relevant enzymes (Oster *et al.*, 1991). In the present investigation, the described inhibitory activity on Chl accumulation may be due to factors affecting both formation of enzymes and the steps of Chl biosynthesis.

For quantitation of the inhibitory activity, dilution series of extract and fractions were applied to the biotest. Because the concentration of soluble compounds present in 5 L methanol extract of 500 g bulb is not known, it is assumed to be 1.0 for use as a reference point. Since methanol had inhibitory activity, an aliquot (40 ml) of the crude extract was evaporated to remove methanol completely. The residue was dissolved in the same volume (40 ml) water to regain the relative concentration 1.0. The solvent was completely removed in the same man-

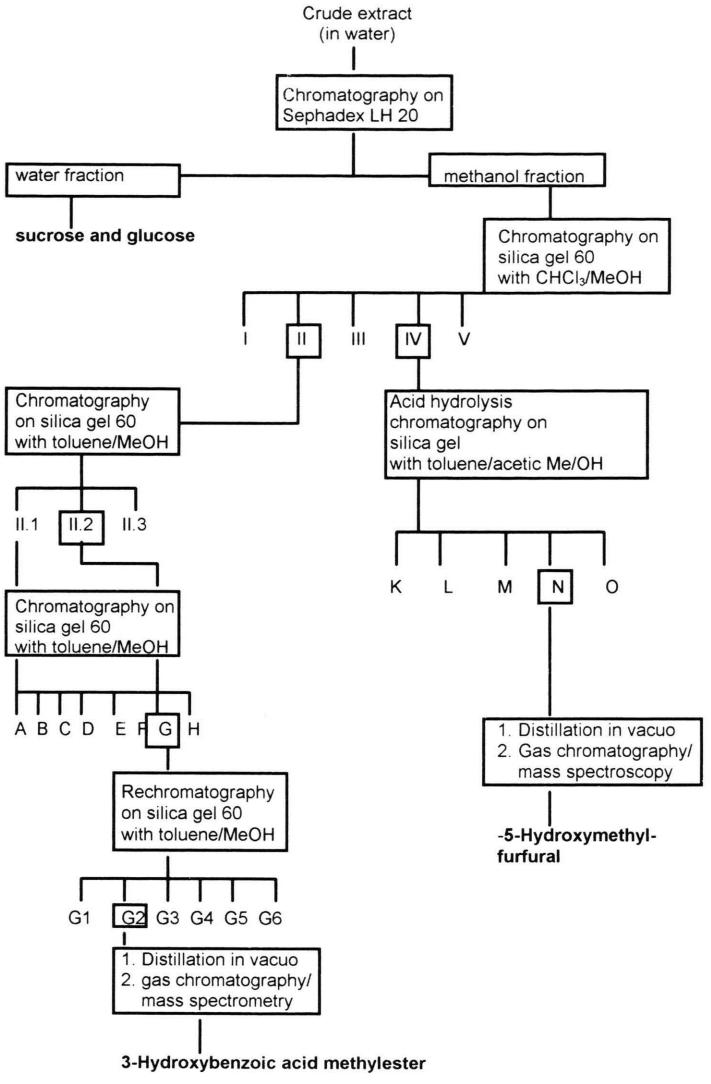

Fig. 1. Scheme for isolation of inhibitors of Chl biosynthesis from bulbs of *Gladiolus* spp.

ner also for the biotest of all fractions obtained later (see below). Chl accumulation was inhibited up to 50% after about 10 fold dilution (= relative concentration 0.09±0.01). The same extent of inhibition was found whether incubation started 8, 24 or 32 h after imbibition. No inhibition was found with the same concentration when the incubation started 48 h after imbibition. Apparently, the inhibition concerned early steps of Chl synthesis.

Isolation of the bioactive compounds started with chromatography on Sephadex LH 20 (Fig. 1). Elution with water yielded a single peak of bioactivity („water fraction"). Analysis of this fraction revealed the presence of sucrose and glucose. From dilution series on TLC and comparison with authentic samples, the concentration in the original extract was calculated to be 100 mM sucrose and 40 mM glucose. The biotest with the authentic sugars results in 50% inhibition of Chl biosynthesis with either 84±30 mM sucrose or 110±5 mM glucose; application of a 5:2 mixture of sucrose/glucose resulted in 50% inhibition at 75±16 mM sucrose plus 30±6 mM glucose. We conclude that a considerable part of inhibition can be attributed to the sugar content of the bulbs. When glucose was supplied to mature leaves of spinach and to-

bacco, the amount of Chl decreased within several days together with enzymes of photosynthesis (Krapp *et al.*, 1991; Polle and Eiblmeier, 1995). Decrease in Chl content due to glucose was also found in cell cultures of *Chenopodium rubrum* (Schäfer *et al.*, 1992). The authors considered glucose as one of the possible effectors for the „sink" regulation of photosynthesis. The mechanism of Chl loss was not investigated but it might have been caused by a change in Chl turnover. A possible mechanism can be suggested based on results from an investigation of phycobilin biosynthesis in *Cyanidium caldarium*. In this case, light induces ALA synthesis and glucose represses the inducing effect on ALA synthesis (Rhie and Beale 1994). Further investigations are needed to check whether glucose (and other sugars) can modulate the formation or rather the activity of enzymes of Chl biosynthesis and degradation.

The methanol fraction of the LH 20 chromatography that contained much inhibitory activity was rechromatographed on silica gel. Elution with $CHCl_3$ that contained increasing concentration of methanol yielded inhibitory activity in fractions II and IV but not in fraction I, III and V (Table I). Chromatography of fraction II on silica gel with toluene containing 20–60% methanol resulted in fractions II.1, II.2 and II.3. In the biotest with fraction II.1, the cotyledons of the treated seedlings did not pretrude through the seed coat; consequently, no greening could be detected in the light. A clear inhibition of Chl accumulation was observed with fraction II.2 but not with fraction II.3.

The inhibition in fraction II.2 was larger than that in the parent fraction II. This could be due to an antagonism between compounds present in the same fraction. Only after separation from such an antagonist, the true effect of an inhibitor can be measured. In agreement with this assumption, Chl accumulation was stimulated by fraction A obtained by subfractionation of fraction II.1 (see Table II) and by fraction M obtained by subfractionation IV (see Table III) whereas other subfractions showed more or less inhibitory activity.

Table I. Chromatography of the methanol fraction of LH20 on silica gel 60. Aliquots of each fraction were adjusted to the relative concentration 20 and used for the standard test of Chl accumulation. The% Chl accumulation values are based on that of the water control = 100%.

Fraction Number	Eluted with %methanol in $CHCl_3$	Chl accumulation [%]
I	0	98
II	25	35
III	50	96
IV	75	36
V	100	94

Table II. Rechromatography of fraction II (see Table I) on silica gel 60 with toluene containing increasing amounts of methanol. Aliquots of each fraction were adjusted to the relative concentration 20 and used for the standard test of Chl accumulation. The % Chl accumulation values are based on that of the water control = 100%.

Fraction number	Eluted with %methanol in toluene	Chl accumulation [%]
II. 1	20	not detectable
II. 2	40	8
II. 3	60	74
II. 1 A	0	110
B	0–5	50
C	5–20	75
D	20–100	67
II. 2 E	0–20	92
F	20	96
G	50	9
H	100	6
G 1	0–2	95
G 2	4	31
G 3	8	50
G 4	8–20	50
G 5	20	79
G 6	20–50	53

Further fractionation of fraction II.1 with a flat methanol gradient in toluene yielded fractions A-D (Table II). Thin-layer chromatography revealed several additional compounds in each of the fractions (not shown). Neither the original germination inhibition of fraction II.1 nor a strong inhibition of Chl accumulation was observed in any of these fractions. The corresponding subfractionation of fraction II.2 yielded 2 fractions (G and H) with inhibitory activitiy (Table II). Fraction H contained polar compounds that could not be purified further. Fraction G was further separated into 6 fractions. Of these, only fraction G2 contained volatile inhibitory activity that was recovered by microdistillation. The minor inhibitory activity found in fractions G3 – G6 was completely destroyed under microdistillation conditions. No traces of

Table III. Chromatography of fraction IV on silica gel 60. The biotest for Chl accumulation was performed using the relative concentration of 30.

Fraction	Eluted with	% Chl accumulated
K	toluene/acetic/methanol	56
L	75:20:5 (v:v:v)	43
M	toluene/acetic acid/methanol	104
N	50:20:30 (v:v:v)	27
O	acetic acid/methanol 20:80 (v:v)	3

any inhibitory activity were detectable in the distillate or in the residue.

Fraction G2 showed one main peak with a retention time of 24.6 min in gas chromatography (Fig. 2). The mass spectrum (Fig. 3) of this peak resembled that of hydroxybenzoic acid methylesters with the mass peak at *m/z* 152. The base peak in the mass spectrum of the main compound of fraction G2 was at m/z 121. The base peak is also at *m/z* 121 in 3-hydroxybenzoic acid methylester and 4-hydroxybenzoic acid methylester but at *m/z*

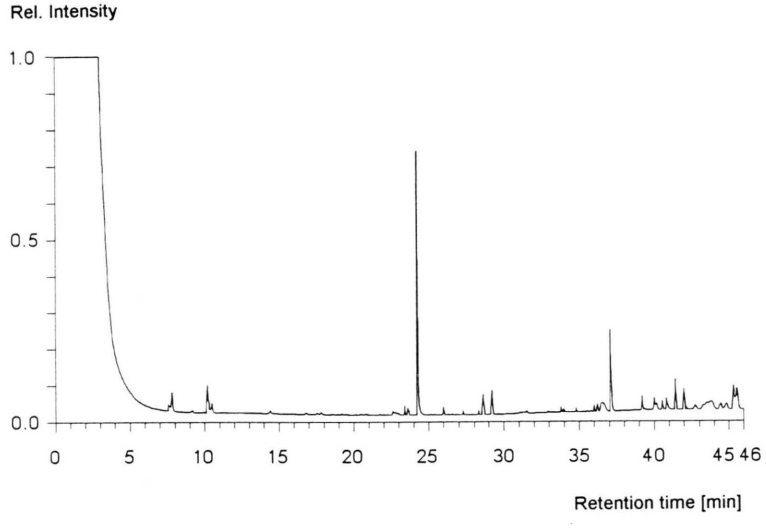

Fig. 2. Gas chromatogram of fraction G2 after vacuum distillation. The column was a quartz column (DB5, length 25 m). The temperature started at 50°C (1 min), was then increased (5°C x min^{-1}) to 250°C and remained at this temperature for 5 min. The injection was splitless, the injector was heated to 250°C.; and detection was by FID.

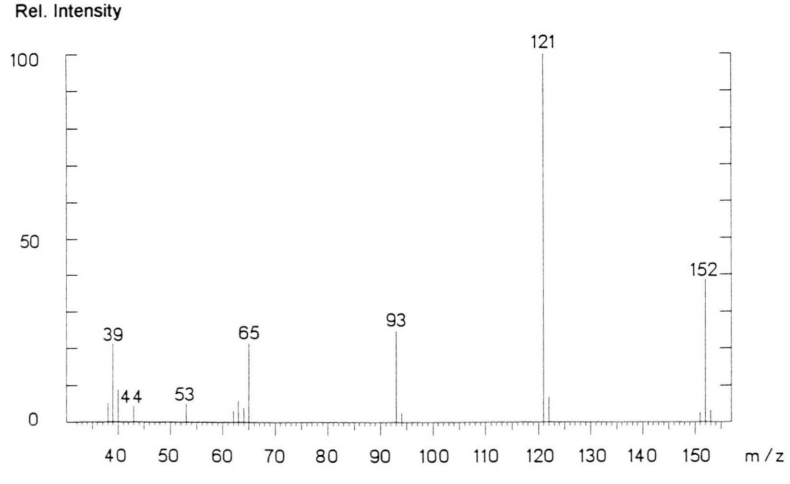

Fig. 3. Mass spectrum of the main peak of Fig. 2. The ionisation energy was 70 eV, and the ionisation temperature at 120°C. The spectrum is identical with that of 3-hydroxybenzoic acid methylester (NBS spectrum Nr. 7561).

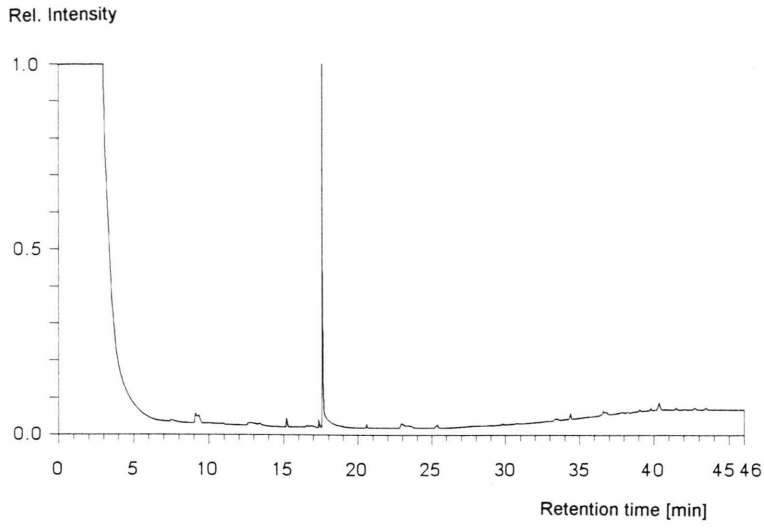

Fig. 4. Gas chromatography of fraction N after vacuum distillation. Conditions are as in Fig. 2.

120 in 2-hydroxybenzoic acid methylester. Otherwise, the fragmentation of the isomeric compounds is the same with fragments at *m/z* 93, 65, 53, 44 and 39. This fragmentation pattern was also found for the compound present in fraction G2 (Fig. 2). To identify the compound from *Gladiolus* spp. and to distinguish between the isomeric structures, we prepared all isomeric hydroxybenzoic acid methylesters (from the corresponding acids with diazomethane). Of these, only the retention time of 3-hydroxybenzoic acid methylester was 24.6min. The 2-hydroxy-compound has a retention time of 17.7 min, and the 4-hydroxy-compound 25.3 min. Thus the inhibitor of fraction G2 was identified as 3-hydroxybenzoic acid methylester. Hydroxybenzoic acids are widely distributed in plants. The best known examples are salicylic acid (2-hydroxybenzoic acid) and its derivatives because of their bacteriostatic and analgetic effects. Some hydroxybenzoic acids have been described as germination inhibitors (Karl and Rüdiger 1982, Rasmussen and Einhellig, 1979; Ray *et al.*, 1980: Lohaus *et al.*, 1983). Inhibition of Chl accumulation is added here as another effect of this class of compounds.

As a precondition for application of GC/MS, fraction IV was at first investigated for volatile inhibitors. After microdistillation, no bioactivity was found either in the distillate or in the residue. The same result was obtained after reaction of fraction IV with diazomethane. These data suggest that the active compounds were destroyed or inactivated by the heat. Either they did not react with diazomethane, or methylation did not sufficiently increase the volatility. The fraction IV was therefore treated with trifluoroacetic acid to hydrolyze any glycosidic linkages. Subsequent chromatography on silica gel yielded 5 fractions, K-O (Table III). The complete inhibition of Chl accumulation by fraction O turned out to be an artefact of residual trifluoroacetic acid. No inhibitory activity was found in fraction M and only minute inhibitory activity was detected in fractions K and L. The bioactivity of fraction N was completely recovered in the volatile fraction from a microdistillation. The gas chromatogram (Fig. 4) showed one major peak and its mass spectrum (Fig. 5) was identical with that of 5-hydroxymethylfurfural (NBS spectrum No. 3454). The identity was confirmed by comparison of the retention time and mass spectrum with that of the authentic compound.

Using greening cress seedlings as a biotest system, natural compounds containing a five-membered ring (5-hydroxymethylfurfural, this paper), a six-membered ring (3-hydroxybenzoic acid methyl ester, this paper), and a seven-membered ring (thujaplicin, Oster *et al.* (1996)) were identified as inhibitors of chlorophyll biosynthesis. Each compound had different substituents. To learn more about the role of substitutents, we tested the authentic natural compounds and synthetic com-

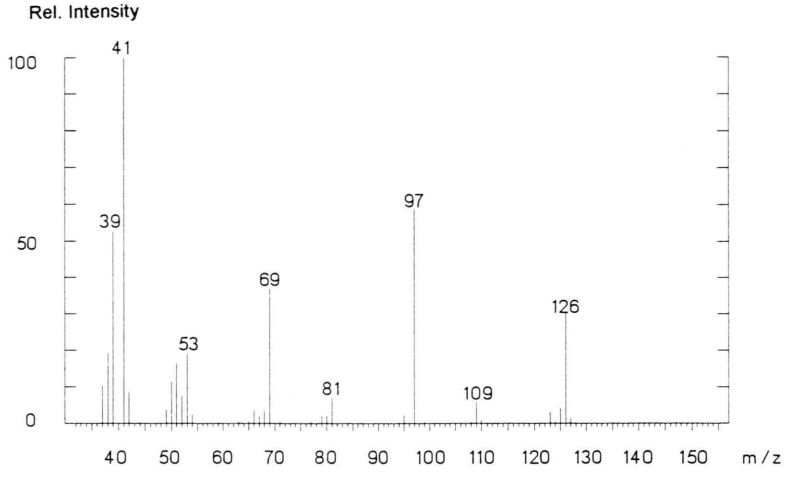

Fig. 5. Mass spectrum of the main peak of Fig. 4. Conditions are as in Fig. 3. The spectrum was identical with that of 5-hydroxymethylfurfural (NBS spectrum Nr. 3454).

pounds with identical ring systems but different substituents for inhibition of Chl accumulation (Table IV). The concentration required for 50% inhibition (CBS-50) was <10 mM for all tested compounds. In the series of substituted benzoic acids, the methyl esters were more active than the free acids. This could be due to differences in the uptake efficiency which usually increases with decreasing polarity of the compounds. Only the free 2-hydroxybenzoic acid is nearly as active as the methylester and much more active than the isomeric free acids. This could be due to the metal chelating property of 2-hydroxybenzoic acid since it is known that metal chelators inhibit greening under the experimental conditions used here (Kittsteiner *et al.*, 1991). The tested furane derivatives were generally less effective than the benzoic acid derivatives. The unsubstituted aldehyde furfural was more effective than its hydroxymethyl derivative. In the series of furane carboxylic acids, the electron-withdrawing methyl group has no effect but the bromo- and nitro-groups increase the inhibitory effect by an order of magnitude. In the series of tropone derivatives, ß-thujaplicin (4-isopropyltropolone), which has recently been described as a new inhibitor of Chl biosynthesis (Oster *et al.*, 1996), is by far the most effective compound. In this case, the isopropyl group increases the inhibitory activity by about an order of magnitude. In summary, natural compounds with entirely different chemical structures are able to inhibit Chl accumulation in greening seedlings. The inhibitory action can be modified by exchange of substituents so that the action can be optimized. It will be interesting to find out whether the mechanism of inhibition is similar or different for these different compounds.

Table IV. Inhibition of Chl biosynthesis in cress seedlings by compounds from *Gladiolus* spp. and analogues. The CBS-50 values are the concentration of compounds necessary for 50% inhibition. The values are means and standard deviation of 6 parallel samples.

Compound	CBS-50 [mM]
2-Hydroxybenzoic acid	0.26 ± 0.02
3-Hydroxybenzoic acid	1.6 ± 0.2
4-Hydroxybenzoic acid	2.2 ± 0.7
2-Hydroxybenzoic acid methylester	0.18 ± 0.03
3-Hydroxybenzoic acid methylester	0.49 ± 0.09
4-Hydroxybenzoic acid methylester	0.34 ± 0.1
Furfural	1.0 ± 0.3
2-Hydroxymethylfurane	4.1 ± 0.7
5-Hydroxymethylfurfural	6.7 ± 1.9
2-Furane-carboxylic acid	5.1 ± 2.6
5-Methyl-2-furane carboxylic acid	4.8 ± 3.2
5-Bromo-2-furane-carboxylic acid	0.4 ± 0.05
5-Nitro-2-furane-carboxylic acid	0.3 ± 0.07
Tropon	1.4 ± 0.5
Tropolon	0.3 ± 0.14
Tropolon methylester	0.68 ± 0.1
β-Thujaplicin	0.03 ± 0.01

Acknowledgements

We thank Dr. A. Esen for carefully reading the manuscript. Thanks are due to the BASF AG, Ludwigshafen, for the gift of several furane derivatives. The work was supported by the Deutsche Forschungsgemeinschaft, Bonn.

Anderson, C.M. and Gray, J.C. (1991), Effect of gabaculine on the synthesis of heme and cytochrome *f* in etiolated wheat seedlings. Plant Physiol. **96,** 584–587.

Bennett, J. (1981), Biosynthesis of the light-harvesting Chl *a/b* protein. Polypeptide turnover in darkness. Eur.J.Biochem. **118,** 61–70.

Eichacker, L., Soll, J., Lauterbach, P., Rüdiger, W., Klein, R.R. and Mullet, J.E. (1990), *In vitro* synthesis of Chl *a* in the dark triggers accumulation of Chl *a* apoproteins in barley etioplasts. J.Biol.Chem. **265,** 13566–13571.

Elich, T.D. and Lagarias, J.C. (1987), Phytochrome Chromophore Biosynthesis. Both 5-aminolevulinic acid and biliverdin overcome inhibition by gabaculine in etiolated *Avena sativa* L. seedlings. Plant Physiol. **84,** 304–310.

Flint, D.H. (1984), Gabaculin inhibits δ-ALA synthesis in chloroplasts. Plant Physiol. **75,** suppl. 170.

Gardner, G. and Gorton H.L. (1985), Inhibition of phytochrome synthesis by gabaculine. Plant Physiol. **77,** 540–543.

Gossauer, A. and Engel, N. (1996), Chl catabolism – structures, mechanisms, conversions. J.Photochem.-Photobiol. B: Biology **32,** 141–151.

Hörtensteiner, S., Vicentini, F. and Matile, P. (1995), Chl breakdown in senescent cotyledons of rape, *Brassica napus* L.: Enzymatic cleavage of phaeophorbide *a in vitro*. New Phytol. **129,** 237–246.

Jordan, P.M. (Ed.) (1991), Biosynthesis of Tetrapyrrols, Elsevier Science Publs. B.V., Amsterdam, pp. 1-66.

Kannangara, C.G. and Schouboe, A. (1985), Biosynthesis of δ-aminolaevulinate in greening barley leaves. VII. Glutamate-1-seminaldehyde accumulation in gabaculine treated leaves. Carlsberg Res.Commun. **50,** 179–191.

Karl, R. and Rüdiger, W. (1982), Natürliche Hemmstoffe von Keimung und Wachstum, I Ausarbeitung eines quantitativen Biotests und Anwendung auf Extrakte aus Spelzen von *Avena sativa* L. Z.Naturforsch. **37c,** 793–801.

Kim, J., Eichacker, L.A., Rüdiger, W. and Mullet, J.E. (1994), Chl regulates accumulation of the plastid-encoded Chl proteins P700 and D1 by increasing apoprotein stability. Plant Physiol. **104,** 907–916.

Kittsteiner, U., Mostowska, A. and Rüdiger, W. (1991), The greening process in cress seedlings. I. Pigment accumulation and ultrastructure after application of 5-aminolevulinate and complexing agents. Physiol.Plant. **81,** 139–147.

Krapp, A., Quick, W.P. and Stitt, M. (1991), Ribulose-1,5-bisphosphate carboxylase-oxygenase, other Calvin-cycle enzymes, and Chl decrease when glucose is supplied to mature spinach leaves via the transpiration stream. Planta **186,** 58–69.

Lohaus, E., Blos, I. and Rüdiger, W. (1983), Carboxylic acids in wheat, rhy and barley. Z.Naturforsch. **38c,** 524–530.

Matile, P. and Kräutler, B. (1995), Wie und warum bauen Pflanzen das Chl ab? Chemie in unserer Zeit **29,** 298–306.

Matringe, M., Mornet, R. and Scalla, R. (1992), Characterization of [^{3}H]acifluorfen binding to purified pea etioplasts, and evidence that protoporphyrinogen oxidase specifically binds acifluorfen. Eur.J.Biochem. **209,** 861–868.

Moran, R. (1982) Formulae for determination of chlorophyllous pigments extracted with N,N-dimethylformamide. Plant Physiol. **69,** 1376–1381.

Mullet, J.E., Klein, P.G. and Klein, R.R. (1990), Chl regulates accumulation of the plastid-encoded Chl apoproteins CP43 and D1 by increasing apoprotein stability. Proc.Natl.Acad.Sci.USA **87,** 4038–4042.

Oster, U., Blos, I. and Rüdiger W. (1991), The greening process in cress seedlings. III. Age-dependent changes in the capacity of the tetrapyrrole pathway. Z.Naturforsch. **46c,** 1052–1058.

Oster, U., Brunner, H. and Rüdiger, W. (1996), The greening process in cress seedlings. V. Possible interference of Chl precursors with light-regulated expression of Lhc genes. J.Photochem.Photobiol., in press.

Paulsen, H., Finkenzeller, B. and Kühlein N. (1993), Pigments induce folding of light-harvesting Chl *a/b*-binding protein. Eur.J.Biochem. **215,** 809–816.

Polle, A. and Eiblmeier, M. (1995) Carbohydrate accumulation affects the redox state of ascorbate in detached tobacco leaves. Bot.Acta **108,** 432–438.

Rasmussen, J.A. and Einhellig, F.A. (1979), Inhibitory effects of combinations of three phenolic acids on grain sorghum germination. Plant Science Letters **14,** 69–74.

Ray, S.D., Guruprasad, K.N. and Laloraya, M.M. (1980), Antagonistic action of phenolic compounds on abscisic acid – induced inhibition of hypocotyl growth . J.of Experimental Botany **31,** 1651–1656.

Rebeiz, C.A., Montazer-Zouhoor, A., Hopen H.J. and Wu, S.M. (1984), Photodynamic herbicides: I. Concept and phenomenology. Enzym.Microb.Technol. **6,** 390–401.

Rebeiz, C.A., Montazer-Zouhoor, A., Mayasich, J.M., Tripathy, B.C., Wu, S.M. and Rebeiz, C.C. (1988), Photodynamic herbicides. Recent developments and molecular basis of selectivity. Crit.Rev.Plant.Sci. **6,** 385–436.

Rhie, G. and Beale, S.I. (1994), Regulation of heme oxygenase activity in *Cyanidium caldarium* by light, glucose, and phycobilin precursors. J.Biol.Chem. **269,** 9620–9626.

Rüdiger, W. (1993), Biosynthese von Tetrapyrrolen bei Pflanzen. Naturwissenschaften **80,** 353–360.

Rüdiger, W. and Schoch, S. (1988), Chlorophylls. In: Plant Pigments (Goodwin T.W., Ed.), Academic Press, London.

Ryberg, M. and Sundqvist, C. (1976), The influence of 8-hydroxyquinoline on the accumulation of porphyrins in dark-grown wheat leaves treated with δ-aminolevulinic acid. Physiol.Plant. **36,** 356–361.

Schäfer, C., Simper, H. and Hofmann B. (1992), Glucose feeding results in coordinated changes of Chl content, ribulose-1,5-bisphosphate carboxylase-oxygenase acitivity and photosynthetic potential in photoautotrophic suspension cultured cells of *Chenopidium rubrum*. Plant Cell Environment **15,** 343–350.

Senge, M.O. (1993), Yearly review: Recent advances in the biosynthesis and chemistry of the chlorophylls. Photochem.Photobiol. **57,** 189–206.

Sherman, T.D., Becerril, J.M., Matsumoto, H., Duke, M.V., Jacobs, J.M., Jacobs, N.J. and Duke, S.O. (1991), Physiological basis for differential sensitivities of plant species to protoporphyrinogen oxidase-inhibiting herbicides. Plant Physiol. **97,** 280–287.

Stahl, E. (1967), Dünnschichtchromatographie. Springer Verlag, Berlin, Heidelberg, New York, 2. Auflage.