GROWTH INHIBITORS FROM ETIOLATED LEAVES OF BARLEY (*HORDEUM VULGARE* L.)

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Abstract

The unrolling of etiolated barley leaves, *H. vulgare* cv. Pallas, is phytochrome-mediated. After brief illumination with red light unrolling begins after 8 hr. The possibility that illumination results in the fall in content of an endogenous inhibitor led to the work reported.

Inhibitory activity in an "acidic fraction" from etiolated barley leaves, as measured by leaf unrolling and wheat seed germination bioassays, declined in the 6-hr period in the dark following exposure to red light for 10 min.

No abscisic acid (ABA) could be detected, either by paper chromatography and wheat coleoptile bioassay or by gas chromatography, in the inhibitory acidic fractions. The inhibitor is therefore not ABA. The inhibitory region on paper chromatograms did not exhibit a strong phenol-like reaction.

Linoleic and linolenic acids, identified by gas chromatography and mass spectroscopy, were present in a 1 : 1 ratio in acidic fractions and linoleic acid inhibited unrolling. These fatty acids are chromatographically separable from the leaf-unrolling inhibitor(s). As they also increased nearly threefold in the leaves in the 6 hr following exposure to red light, they are not of interest to the present investigation. They possibly originate from etioplasts.

I. INTRODUCTION

During a study of hormonal and phytochrome control of leaf unrolling in barley, *Hordeum vulgare* L. cv. Pallas (Carr *et al.* 1972; Menhenett 1972), it was apparent that extracts of etiolated leaves inhibited growth in selected test systems (i.e. wheat seed germination and barley leaf unrolling itself). Poulson and Beevers (1970) suggested that the lag phase of leaf unrolling following exposure to red light might involve the decline of an endogenous inhibitor, perhaps abscisic acid, in the leaves. An investigation was undertaken to test for the presence of an inhibitor of physiological importance in etiolated barley leaves and to see whether its activity decreased during the lag period of leaf unrolling. The early stages of this study have been briefly reported (Carr *et al.* 1972).

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II. Materials and Methods

(*a*) *Growing and Harvesting Plants and Red Light Treatment*

Seeds of barley cv. Pallas were sown about 1 cm deep in boxes of coarse river-washed sand soaked with distilled water. No further watering was given but the excess was allowed to drain away. The boxes were then placed in a cabinet in a dark room maintained at 25±1°C. After 7 days leaves were harvested by pulling them from the coleoptiles. Leaves were placed in Petri dishes lined with moist filter paper to maintain full turgor.

Where appropriate, leaves were given 10 min exposure to red light while in the dishes. The red source has been described (Carr *et al.* 1972). At the level of the leaves the energy was 6·5 μW cm^{-2}. After the light treatment dishes of leaves were placed in darkness. Other dishes of leaves were kept in the dark throughout.

These operations were all carried out with the aid of a dim green "safelight" described elsewhere (Carr *et al.* 1972). The total energy about 10 cm above bench level was 1·7 μW cm^{-2}.

(*b*) *Extraction and Preliminary Purification of Extracts*

In the first experiments the following extraction procedure was used (method 1). Whole leaves were chopped into half-inch lengths and macerated at high speed in 150 ml of cold 70% ethanol in the 200 ml chamber of a Sorvall Omnimixer in an ice bucket. The extract was filtered and 100 ml ethanol added to the tissue which was left to stand at room temperature (25°C) in the dark for 22–24 hr. It was filtered and washed with ethanol. The filtrates were combined, evaporated to dryness, and stored at −15°C. All operations up to and including the second filtration were carried out in the darkroom using the safelight.

In later experiments, when it seemed possible that abscisic acid (ABA) or auxin-like substances might be of significance, the extraction technique was modified (method 2). The chopped leaves were macerated as before but in 150–200 ml cold 80% methanol. The macerate was then poured into a flask covered with aluminium foil and additional methanol added (about 100 ml per 200 leaves—12·5 g fresh weight). This flask was then kept on ice in the dark for 22–24 hr with occasional shaking. It was then filtered in the darkroom into a receiving flask kept in ice. Washings with 80% methanol were repeated, the extracts combined and evaporated.

For solvent partitioning the extracts were taken up in water, filtered or centrifuged, and the pH adjusted to 3·0 with dilute HCl. The aqueous extract was then extracted three times with double volumes of diethyl ether (with 50 inversions of the separating funnel for each partition). The ether was then evaporated off and the "acidic ether fraction" so obtained stored at −15°C in a flask wrapped completely with aluminium foil.

Paper chromatography of the acidic ether fraction was carried out by streaking or spotting onto Schleicher and Schüll paper (598L). Solvents and methods of elution are mentioned under particular experiments.

(*c*) *Solvent-partitioning Technique to Isolate Abscisic Acid*

This was similar to that described by Milborrow (1967). Leaves (1100, about 78·5 g fresh weight) were extracted as in method 2 above, except that the extract was filtered after 6 hr at 4°C. Following solvent evaporation, the extract was taken up in about 170 ml water and partitioned four times with equal volumes of diethyl ether. The ether solution was then extracted four times with saturated sodium bicarbonate and water alternately. These extracts were combined and after pH adjustment to 7·5 were shaken with ether to remove neutral and weakly acidic compounds. The aqueous phase was then acidified to pH 3·3 and extracted five times with ether. Ether was evaporated off and the residue stored at −15°C in the dark.

(*d*) *Gas Chromatography*

A Varian Aerograph series 1700 instrument was employed. The column was a 6 ft by $\frac{1}{8}$ in. stainless steel tube with 3% SE–30 on Varaport 30 (Varian Aerograph). The column temperature was 165°C. Injection port and detector where at 210°C. The detector was a flame ionization detector. Carrier gas was nitrogen and the flow rate 20 ml/min. Extracts and a sample of (±)-ABA were methylated using diazomethane and the gas chromatograph traces compared.

A Hewlett–Packard 700 chromatograph was used to test for linoleic and linolenic acids. This had a 6 ft by $\frac{1}{8}$ in. glass column, packed with 6% diethylene glycol succinate (DEGS), 80–100 screen, supported on Chromasorb CLP (Johns-Manville). Operating conditions were as follows: injector temperature 200°C, column temperature 170°C, detector temperature 205°C. Carrier gas was nitrogen at 22 lb/in^2 (about 70 ml/min). The hydrogen and air flow rates were 47 and 500 ml/min respectively and the sensitivity and attenuation settings 10 and 4.

(*e*) *Combined Gas Chromatograph–Mass Spectrum*

In one experiment the Varian gas chromatograph was linked to a G.E.C.–A.E.I. MS 962 mass spectrometer. The operating conditions for the mass spectrometer were: ionizing energy 70 eV; 100 μA trap current; 8 kW accelerating voltage; and source temperature 150°C.

(*f*) *Bioassays*

(i) *Wheat Seed Germination Bioassay*

Fifty seeds cv. Pitic were sown in a 7-cm Petri dish on filter paper moistened with 3 ml of solution under test and the dishes kept in the dark room at 25°C. After 24-30 hr the number of seeds germinated, i.e. with radicle protruding, were counted. On some occasions the length of the coleoptile on these seeds was recorded about a day later.

(ii) *Wheat Coleoptile Straight Growth Bioassay*

This was similar to that described by Bentley (1954): 10-mm sections 5 mm from the tip were cut from coeoptiles of 3-day-old seedlings (cv. Pitic) grown in darkness. After 24 hr on test solutions section length was measured.

(iii) *Barley Leaf Unrolling Bioassay*

This has been detailed elsewhere (Carr *et al.* 1972; Menhenett 1972). Briefly, 1-cm sections of 7-day-old etiolated barley seedlings, cv. Pallas, were cut 1 cm from the apex. These segments unroll subsequent to a short period of exposure to red light. Unrolling is due to differential cell expansion in the upper mesophyll tissue and does not involve cell division (Burstrom 1942). The reponse is phytochrome-mediated since far-red light can substantially reduce the red response. A red–far-red–red sequence restores full opening (Menhenett 1972). There were about 20 leaf segments per treatment, 10 in each of two Petri dishes. After 22–26 hr the leaf segments exposed to red light have unrolled. Estimates of leaf width, based on projected leaf width using a photographic enlarger, are made. Extracts were tested for their ability to inhibit this red light response. They were supplied either continuously by floating segments on the solutions or briefly by infiltrating under vacuum for 20–30 min.

III. Results

(*a*) *Demonstration of Inhibitors in Acidic Ether Fractions of Etiolated Whole Leaves of Barley*

Preliminary experiments had indicated that crude extracts of dark-grown barley leaves contained material which inhibited the leaf-unrolling response stimulated by red light.

Whole leaves were exposed to red light for 10 min and extracted immediately or returned to darkness for 6 hr and then extracted. Other batches of leaves were extracted immediately after harvest or after a further 6 hr in darkness. Acidic ether fractions were prepared, taken up in water (pH 6·0), and tested at a range of concentrations on wheat seed germination. The results (Table 1A) show that dark-grown barley leaves may contain inhibitor(s) which decrease in amount following a brief illumination. The time course of the inhibitor decrease in the acidic ether fractions was followed by extracting leaves (by method 1) 1, 4, and 6 hr after exposing them for 10 min to red light. A batch of leaves was also extracted after 6 hr in the dark.

Table 1B shows that extracts prepared 1 hr after red light possess considerable inhibitor, while those extracted 6 hr after contain less. A marked change in inhibitory

TABLE 1

EFFECTS OF ACIDIC ETHER FRACTIONS OF ETIOLATED BARLEY LEAVES ON GERMINATION OF PITIC WHEAT SEEDS

Whole leaves extracted 0 and 6 hr (A) or 1, 4, and 6 hr (B) after light treatments as indicated

Time of extraction (hr)*	Extract concn. (g/ml)†	Light treatment	Germination (%)	Time of extraction (hr)*	Extract concn. (g/ml)†	Light treatment	Germination (%)
A: 0 and 6 hr extracts				B: 1, 4, and 6 hr extracts			
0	0·2	Red light	96	1	0·4	Red light	96
0	0·4		74	4	0·4		92
0	0·6		56	6	0·4		90
0	1·2		20	6	0·4	None	92
6	0·2		90	1	1·2	Red light	90
6	0·4		86	4	1·2		80
6	0·6		100	6	1·2		90
6	1·2		96	6	1·2	None	76
0	0·2	None	92	1	2·4	Red light	48
0	0·4		96	4	2·4		42
0	0·6		86	6	2·4		82
0	1·2		46	6	2·4	None	36
6	0·2		88	—	Distilled water control		90
6	0·4		88				
6	0·6		88				
6	1·2		68				
6	1·6		48				
—	Distilled water control		91				

* Hours after exposure to red light or hours in darkness.

† Expressed as grams of tissue per millilitre of solution, 3 ml per dish.

activity seems to have occurred between 4 and 6 hr after exposure to red light. Germination (after 28 hr) was found to be strongly inhibited by 50 μg ABA per dish (Fig. 1).

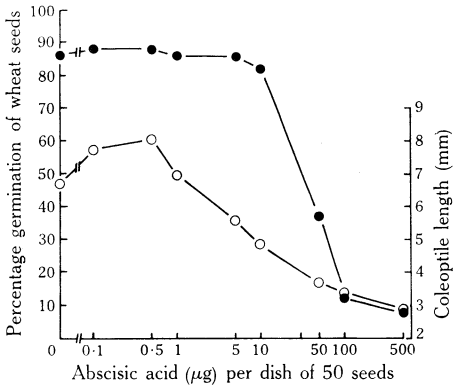

Fig. 1.—Effects of ABA on wheat seed germination recorded after 28 hr.

● Percentage germination.

○ Coleoptile length.

A comparison of Table 1 with Figure 1 shows clearly that to account for the degree of inhibition by the acidic ether fractions unusually large amounts of ABA would have

to be present. For example, the inhibition due to extracts made 4 hr after exposure to red light (Table 1B) would require about 40 μg ABA per 7·2 g fresh weight of leaves.

(*b*) *Attempts to Detect Abscisic Acid in Barley Leaf Extracts*

(i) *Paper Chromatography of an Acidic Ether Fraction*

Dark-grown leaves were harvested, extracted immediately (method 1), an acidic ether fraction prepared, and 0·4 g fresh weight equivalent subjected to paper chromatography. 5 μg ABA was put on as a separate streak and the chromatogram developed in isopropanol–ammonia–water (10 : 1 : 1 by vol.). After development ABA was seen under ultraviolet light as a dark area at R_F 0·68–0·76. The chromatogram zones were assayed using wheat coleoptiles. Inhibition was strong at R_F 0·6–0·8 in the the ABA chromatogram but there was no corresponding inhibition in the leaf extract (Table 2). At R_F 0·8–0·9 indole-containing compounds appeared which showed a small promotion of coleoptile growth (Table 2).

TABLE 2

EFFECTS OF ELUATES FROM PAPER CHROMATOGRAMS ON WHEAT COLEOPTILE STRAIGHT-GROWTH TEST

Acidic ether fractions either chromatographed directly or subjected to solvent-partitioning schedule as described in Section II(*c*) prior to chromatography of final fraction. Standard errors of assay given in parenthesis

R_F	Coleoptile length (mm)		Coleoptile length (mm)	
	Acidic ether fraction chromatographed directly	ABA	Solvent partitioning of acidic ether fraction	ABA
0–0·1	16·48 (0·43)	16·25 (0·35)	18·98 (0·45)	
0·1–0·2	16·16 (0·41)	15·43 (0·39)	17·75 (0·41)	
0·2–0·3	16·20 (0·37)	16·46 (0·34)	17·18 (0·62)	
0·3–0·4	15·58 (0·32)	15·77 (0·29)	17·11 (0·35)	
0·4–0·5	15·18 (0·38)	15·82 (0·49)	17·57 (0·31)	
0·5–0·6	17·01 (0·42)	16·20 (0·30)	17·13 (0·70)	
0·6–0·7	15·63 (0·35)	12·65 (0·12)	18·05 (0·48)	13·15 (0·23)
0·7–0·8	15·94 (0·23)	12·60 (0·17)	18·48 (0·23)	12·38 (0·13)
0·8–0·9	17·25 (0·40)	15·61 (0·25)	18·25 (0·26)	
0·9–1·0	16·52 (0·28)	16·49 (0·34)	17·40 (0·30)	
Control	16·05 (0·32)	16·05 (0·32)	17·42 (0·24)	

A further aliquot of this acidic ether fraction was subjected to the solvent-partitioning technique to isolate ABA [Section II(*c*)]. An amount of the final ether fraction equivalent to 4·15 g fresh weight of tissue was chromatographed and assayed but again with a negative result as above (Table 2).

(ii) *Gas Chromatography of Leaf Extracts*

Leaves (1100) were extracted immediately after harvest as described in Section II(*c*). An aliquot of the final ether fraction, equivalent to 63 g tissue fresh weight, was methylated, taken up in ether, and aliquots injected into the Varian gas chromatograph. A sample of methyl abscisate (MeABA) was chromatographed under identical conditions in the same series of runs. MeABA had a retention time of 19 min on the

3% SE–30 column and no corresponding peak was present in the extract chromatogram (Fig. 2). The retention time of the peak to the right of MeABA was a little over 20 min. The leaf extract was again treated with diazomethane and rechromatographed but Figure 2 remained typical of the results. No MeABA could be detected.

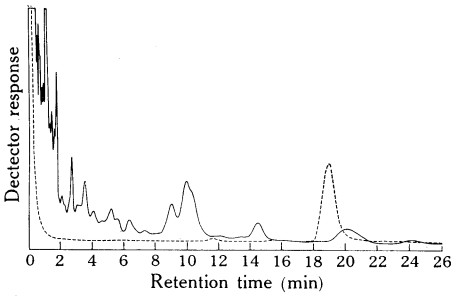

Fig. 2.—Gas chromatograms of methyl abscisate (- - - -) and methylated leaf extract (——).

An unmethylated portion of this final ether fraction was tested, soon after its preparation, on leaf unrolling stimulated by red light. No significant reduction of leaf unrolling was observed after 25 hr as the following tabulation indicates:

Extract concn.*	0†	0	0·05	0·1	0·4	0·6	1·2
Mean leaf width (mm)	2·16	3·40	3·49	3·27	2·99	3·29	3·09

* Expressed as grams of tissue per millilitre, 6 ml per treatment. † Dark control.

It seemed possible that the inhibitor of leaf unrolling remained in the first ether fraction during the solvent-partitioning procedures. A new extract was made using method 2 and an acidic ether fraction prepared and tested, by vacuum infiltration at one concentration, on barley leaf unrolling. The response to red light was strongly inhibited, mean leaf widths being 3·67 and 2·70 mm respectively for extract concentrations equivalent to 0 and 0·3 g tissue per millilitre (6 ml used per treatment). For the dark controls mean leaf width was 1·93 mm. Acidic ether fractions prepared similarly also inhibited wheat seed germination.

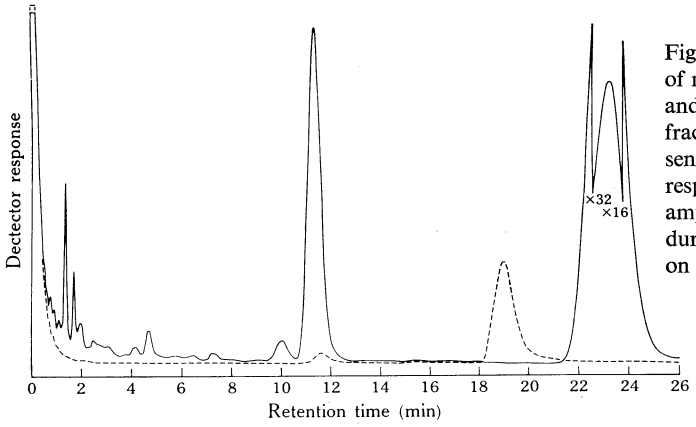

Fig. 3.—Gas chromatograms of methyl abscisate (- - - -) and methylated acidic ether fraction (——). Initial sensitivity setting (detector response) was 16×10^{-11} amp. Changes in sensitivity during the run are indicated on the figure.

Another portion (3·81 g fresh weight) of the extract used in the preceding experiment was methylated and subjected to gas–liquid chromatography. No sub-

tance in the acidic ether fraction had a retention time corresponding to that of MeABA (Fig. 3). There were two intense peaks in the extract chromatogram of which the first, with a retention time of approximately 11½ min, was possibly dibutyl phthalate, an ether impurity. The second, the most intense peak, had a retention time of approximately 23¼ min. Its presence was confirmed in other similarly prepared methylated acidic ether fractions. During one such run a proportion of the peak was fed into the mass spectrometer. A comparison with mass spectra of fatty acids provided strong evidence that it was a mixture of the methyl esters of linoleic and linolenic acids (Hallgren *et al.* 1959). Separation of the two methyl esters would not have been obtained on the 3% SE–30 column used.

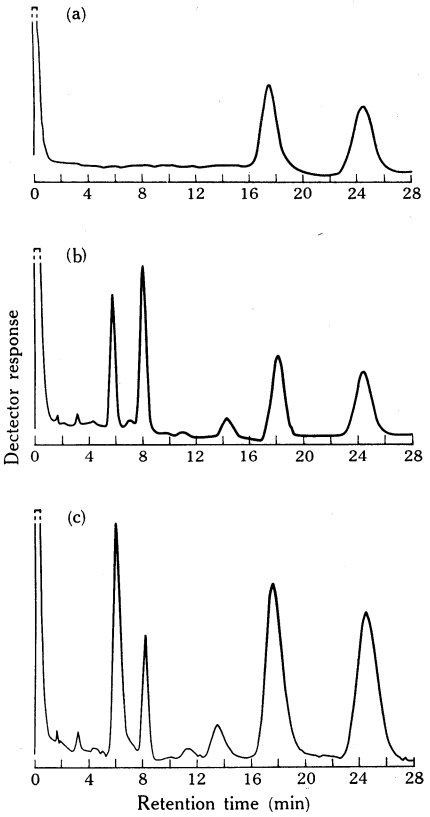

Fig. 4.—(*a*) Gas chromatogram of methylated linoleic and linolenic acids. (*b*) Gas chromatogram of methylated acidic ether fraction prepared from whole leaves extracted immediately after red light (equivalent to 0·52 g fresh weight of leaves). (*c*) Gas chromatogram of methylated acidic ether fraction prepared from whole leaves extracted 6 hr after red light (equivalent to 0·50 g fresh weight of leaves).

It was of interest to determine if these constituents of acidic ether fractions affected leaf unrolling. Rather surprisingly linoleic acid in water solution strongly inhibited red light-stimulated barley leaf unrolling, as indicated in the following tabulation:

Linoleic acid concn. (mg/l):	0*	0	1	5	10	20	40	100
Mean leaf width (mm):	1·85	3·19	2·99	3·04	2·68	2·16	2·17	2·12

* Dark control.

A concentration of 20 mg/l decreased the level of unrolling almost down to the level of the dark control. As a result of this experiment it was decided to separate the two

unsaturated fatty acids in acidic ether fractions and to find out if the amount in the leaves might decrease during the lag phase of unrolling. Acidic ether fractions were prepared from leaves extracted (method 2) immediately after, and 6 hr after, a 10-min red light treatment. These fractions were methylated with diazomethane, as were samples of linoleic and linolenic acids, taken up in ether, and tested in the Hewlett–Packard gas chromatograph. The solutions were kept in an ice-box during the experiments. Results of typical runs are presented in Figure 4. Figure 4(*a*) shows the trace of methylated linoleic and linolenic acids combined. The average retention times indicate the presence of peaks corresponding very closely to the methyl esters of linoleic and linolenic acids. These data, taken together with the evidence from mass spectrometry, confirm the identification. However, the amounts actually increase from the 0- to the 6-hr extracts. Measurement of peak areas (height × width at half height) showed that both increased 2·8 times but a 1 : 1 ratio of methyl linoleate to methyl linolenate was maintained. The identity of the other peaks on the extract chromatograms is unknown. These unsaturated fatty acids do not constitute the postulated inhibitor whose concentration falls during the lag phase of unrolling. Nevertheless, it seemed possible that linoleic could be responsible for part or all of the observed inhibition of unrolling by the acidic ether fraction. Using a calibration curve and the data of Figures 4(*b*) and 4(*c*) amounts of the methyl esters in the 0- and 6-hr extracts were calculated. These results, considered together with those in the proceding tabulation, revealed that sufficient amounts of the fatty acids to cause part or all of the observed inhibition of unrolling were indeed present in both the 0- and 6-hr acidic ether fractions. However, neither acid inhibited wheat seed germination, even at very high concentrations, as the following tabulation shows (germination for controls = 76%):

Acid	Concn. (mg/l)	Germination (%)	Acid	Concn. (mg/l)	Germination (%)
Linoleic	100	82	Linolenic	100	76
	500	88		500	82
	1000	84		1000	76

This suggests that the differential inhibitory effects of 0- and 6-hr acidic fractions on seed germination were caused by other substances in the extracts.

(*c*) *Evidence from Paper Chromatography of an Inhibitor of Leaf Unrolling*

It was obviously desirable to separate the linoleic and linolenic acids present in acidic ether fractions from any possible leaf unrolling inhibitor. Chromatography of the fatty acids on Schleicher and Schüll 598L paper using n-butanol–acetic acid–water (12 : 3 : 5 by vol.) and examination under ultraviolet light revealed that both ran close to the front at R_F 0·89–0·99.

Two batches of whole leaves were harvested, extracted (method 2) 0 and 6 hr after exposure to red light for 10 min, and acidic ether fractions prepared. An amount equivalent to 9·8 g fresh weight of leaves from each treatment was chromatographed on paper (acid washed for 2 days with 5% acetic acid) using the above solvent. The solvent front was allowed to advance 22½ cm. Coloured material migrated to R_F 0·8–1·0. The chromatograms were not examined under ultraviolet light but cut into

0·1 R_F zones and eluted with 30 ml ether overnight at 4°C in the dark. The ether was than decanted and elution repeated with a further 30 ml. After evaporation of the ether the eluates were taken up in 6·5 ml 0·01M phospate buffer (pH 5·5) and tested, by vacuum infiltration, for their ability to inhibit red light-stimulated unrolling (Fig. 5).

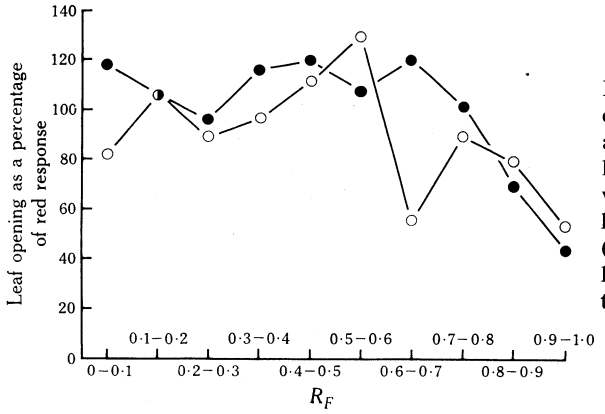

Fig. 5.—Differential effect of eluates from chromatograms of acidic ether fractions on barley leaf unrolling. These fractions were prepared from whole leaves harvested immediately (○) and 6 hr (●) after the leaves were exposed for 10 min to red light.

Only one zone, at R_F 0·6–0·7, exhibited a striking differential inhibitory effect. At this region the eluate from the 0-hr chromatogram inhibited the response to red light by about 50%, while the corresponding region from the 6-hr extract chromatogram gave, perhaps, a small increase in response. Both extracts produced a small inhibitory effect at R_F 0·8–0·9 and a larger inhibitory response at 0·9–1·0. The latter response was quite possibly due to the linoleic and linolenic acids present. The R_F value of ABA is 0·79–0·98 in this chromatographic system.

TABLE 3

EXAMINATION WITH ULTRAVIOLET LIGHT OF A CHROMATOGRAM OF A 0-HR ACIDIC ETHER FRACTION TESTED FOR PHENOL-LIKE COMPOUNDS

R_F	Phenol test reaction	R_F	Appearance under ultraviolet light
0–0·1	None		Normal
0·1–0·2	None		Normal
0·2–0·3	None	0·23–0·25	Very dark region
0·3–0·4	None	0·34–0·38	Blue fluorescence
0·4–0·48	None	0·43–0·46	Very dark region
0·48–0·59	Green-blue colour associated with yellow fluorescence	0·46–0·52	Yellow fluorescence
0·59–0·72	Weak green-blue coloration	0·60–0·71	Weak whitish fluorescence
0·72–0·86	Very strong prussian blue		Normal
0·86–1·0	Strong prussian blue		Normal

Examination of a chromatogram prepared with a 0-hr acidic ether fraction indicated several ultraviolet-absorbing and fluorescent zones. A strip of this chromatogram was tested for phenol and tannin-like compounds using the ferric chloride–potassium ferricyanide dip technique (Smith 1969). Results are summarized in

Table 3. It is apparent that the area from which the inhibitor was eluted in the previous experiment does not exhibit a strong phenol reaction. Areas corresponding to the regions of strong absorbance and fluorescence did not appear to affect leaf unrolling. It is possible that the inhibitor at R_F 0·6–0·7 is not a phenolic compound.

IV. Discussion

The experiments described have yielded evidence to support the hypothesis, put forward by Poulson and Beevers (1970), that the lag period prior to the rapid phase of leaf unrolling might involve a decrease in level of inhibitory compounds. They suggested ABA as a possible candidate but no ABA could be detected, either by paper chromatography and wheat coleoptile bioassay or by gas chromatography, in extracts from barley leaves. ABA can therefore be eliminated as the inhibitor in the acidic ether fractions. In pea seedlings grown in darkness at high humidity Burden *et al.* (1971) have shown that little ABA is present and the amount is not significantly altered when the seedlings are exposed to red light treatment.

Although auxin has been shown to inhibit unrolling in etiolated grass leaves (Burström 1942; Kang 1971; Menhenett 1972), generous estimates of the possible auxin levels in the "0 hr" acidic ether fraction make it highly unlikely that there was sufficient to cause the observed inhibition of leaf unrolling (Menhenett 1972).

Xanthoxin, a neutral growth inhibitor recently isolated from seedlings of wheat and dwarf bean, has been shown to possess activity comparable with ABA in several tests. Is it possible that the inhibitor in the acidic ether fractions was xanthoxin? It seems quite probable that if present in the original extracts some might have partitioned from an aqueous layer at pH 3 into diethyl ether. However, at concentrations inhibitory to leaf unrolling, crude or chromatographed acidic ether fractions (0 hr) did not inhibit growth in the wheat coleoptile section test, in which xanthoxin showed strong inhibitory action (Taylor and Burden 1970). Preliminary experiments with a crude xanthoxin preparation showed little effect on leaf unrolling. Moreover, red light has been shown to increase, rather than decrease, xanthoxin levels in etiolated pea and wheat seedlings (Burden *et al.* 1971). Whether the inhibitor shown to prevent wheat seed germination corresponded to that which blocked leaf unrolling is unknown. For both responses the inhibitory effect decreased in extracts made 0 and 6 hr after exposure to red light.

It is possible that the inhibition is due to the presence in these extracts of an unidentified inhibitor which may have a regulatory role in leaf unrolling since it was shown to decline in the 6-hr period following irradiation with red light. This is the time interval during which the unrolling response escapes from phytochrome control (Carr *et al.* 1972).

It is interesting that linoleic and linolenic acids were present in equal quantities in the acidic ether fractions. This was shown to be the case for these fatty acids in the etioplasts of *Phaseolus vulgaris*, whereas in the chloroplasts linolenic acid predominates (Wallace and Newman 1965). This predominance of linolenic over linoleic (unsaturated over saturated) was also found in the chloroplasts of light-grown wheat plants. In dark-grown plants approximately equal amounts were present (Wolf *et al.* 1966). In the experiments here a short burst of red light led to increases over 6 hr in both linoleic and linolenic acids in the ratio 1 : 1. It seems probable that the major source of these fatty acids in the acidic ether fractions was the etioplasts. If the increase

produced by red light can be shown to be prevented by immediate far-red light these fatty acid changes could form part of the phytochrome-mediated elimination of the lag phase in chlorophyll formation (e.g. Augustinussen and Madsen 1965).

It is of interest to consider why linoleic acid should inhibit unrolling stimulated by red light. There is considerable evidence that exogenously supplied fatty acids, such as oleic and linoleic, lead to uncoupling of oxidative phosphorylation in mitochondria of animal cells (Van den Bergh 1966: Van den Bergh *et al.* 1969). This phenomenon has also been demonstrated in mitochondria from plant cells (Lotlikar and Remmert 1968). It is possible therefore that linoleic acid prevented the unrolling of barley leaves by reducing the available supply of energy.

V. Acknowledgments

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