Phytochemistry, Vol. 21, No. 11, pp. 2633–2640, 1982.
Printed in Great Britain.

0031–9422/82/112633–08$03.00/0

LIPID METABOLISM IN AGEING LEAVES OF DARK-GROWN BARLEY SEEDLINGS

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(*Revised received* 17 *March* 1982)

Key Word Index—*Hordeum vulgare*; Gramineae; barley; leaves; senescence; kinetin; glycerolipids; fatty acids; desaturation; [^{14}C]oleate; linoleate; linolenate.

Abstract—The rapid senescence of the etiolated leaves of dark-grown barley seedlings in the dark is accompanied by the loss of those lipids associated with the plastids. The linolenate content of the plastid glycerolipids rapidly decreased whereas it tended to increase in the extraplastidic phospholipids. Kinetin treatment slowed down the loss of the plastid lipids and their constituent fatty acids. The hormone treatment brought about increased linolenate, particularly in phosphatidylcholine and monogalactosyldiacylglycerol. The senescing leaf attempts to adapt to ageing by increased membrane synthesis and/or membrane repair. Kinetin appears to control the sequential desaturation of oleate to linolenate.

INTRODUCTION

The leaves of dark-grown seedlings of barley age relatively quickly when grown in Vermiculite. The seedlings produce maximum chlorophyll in the light after 6–7 days dark-growth, after which their potential for pigment synthesis and plastid development rapidly declines [1, 2]. The senescence phase, which occurs after 7 days growth in the dark, is also marked by the development of hydrolytic enzyme activity [1] and a general decline in amino acid and protein [3].

For a number of years the 'etiolated' leaf has provided experimental material for studies on plastid development [4–7] and chlorophyll synthesis [2, 8–12]. The leaves of dark-grown seedlings have also been used as a source of etio-plastids for porphyrin studies [13–15], and Leech *et al.* [16, 17] have analysed the lipids associated with successive regions of the maize leaf and their plastids along an age gradient from the basal meristem to the leaf tip. Recently, MacKender [18] has reported similar analyses for the leaves of barley and wheat.

The present paper reports the changes in the lipids and their component fatty acids, which are associated with the development and subsequent senescence of the barley leaf growing in the dark. The effect of kinetin treatment on the lipids in the etiolated leaf is also given.

RESULTS

Seedlings of barley were grown in the dark and 4 days after sowing were sprayed with either a solution of kinetin (3 mg/l.) or water. The primary leaves were removed at regular intervals for analysis. The major fatty acids in the polar lipid fraction (Table 1) were palmitate (16:0), oleate (18:1), linoleate (18:2) and linolenate (18:3). The fatty acids in the polar lipid fraction from leaves treated with water reached a maximum between 7 and 9 days and then declined with further age in the dark. Similar trends were observed in the polar-lipid fatty acids in leaves treated with kinetin, however, the 18:3 was always substantially higher (80–90%) than that observed in the water control leaves. The 16:0, 18:1 and 18:2 acids increased only slightly in the leaves treated with kinetin. The results, expressed on a leaf fr. wt, and dry wt basis (not given), also showed the presence of substantially more 18:3 in the polar lipid fraction from the leaves of seedlings treated with kinetin. On a relative basis (Table 1) the polar lipids from the kinetin treated leaves contained over 10% more 18:3 than the water control leaves with a corresponding reduction in the proportion of 18:2.

Similar changes, although not so pronounced, were observed in the relative concentration of 18:3 and 18:2 in the neutral lipids (data not shown).

The etiolated barley leaf contains an age gradient of cells and organelles from the basal meristem to the leaf tip. In order, therefore, to obtain more uniform experimental material, analyses were made using the top 4 cm of the leaf. The fatty acid composition of individual polar lipids in the top region of the leaf is given in Table 2. The major fatty acids declined in nearly all the phospholipids analysed and this was particularly marked in phosphatidylcholine (PC) and monogalactosyldiacylglycerol (MGDG). The decrease in the fatty acids of the major polar lipids was a reflection of both a general loss in the parent lipid (Table 3) and a differential loss in specific component fatty acids (see relative fatty acid content, Table 2). The relative amount of 18:3 increased in most phos-

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Table 1. The effect of kinetin on the fatty acids of the total polar lipid in the leaves of dark-grown seedlings of barley

Leaf age (days)	Acyl fatty acid							
	C16		C18:1		C18:2		C18:3	
	C	K	C	K	C	K	C	K
5	80(24)	—	18(5)	—	100(30)	—	140(41)	—
7	112(23)	146(20)	16(3)	26(4)	131(27)	158(22)	235(47)	382(54)
9	115(24)	131(19)	15(3)	21(3)	139(29)	146(22)	212(44)	376(56)
11	102(22)	118(19)	16(3)	16(3)	144(31)	129(21)	204(44)	345(57)
13	76(23)	108(20)	13(4)	17(3)	108(33)	118(23)	128(40)	281(54)
17	58(23)	70(20)	11(5)	12(3)	80(32)	78(23)	99(40)	187(54)

Seedlings were sprayed daily with water (C) or a solution of kinetin (K). At intervals leaves were taken for analysis. Results are expressed as nmol/leaf. Figures in parentheses are the relative (%) content of each acid in the polar lipid fraction.

Table 2. The fatty acids of the polar lipids in the leaf-tip of dark-grown seedlings of barley

Polar lipid	Leaf age (days)	Acyl fatty acid				
		C16:0	C18:0	C18:1	C18:2	C18:3
PC	6	11.3(32)	0.2(1)	1.8(5)	10.0(29)	11.5(33)
	7	11.3(32)	0.3(1)	1.7(5)	9.3(26)	12.9(36)
	9	8.7(32)	0.2(1)	1.4(5)	6.1(22)	11.0(40)
	11	6.9(33)	0.2(1)	0.6(3)	3.9(19)	9.3(44)
PI	6	3.1(48)	0.06(1)	0.2(2)	1.6(25)	1.6(24)
	7	3.8(46)	0.03(< 1)	0.2(2)	2.2(26)	2.1(25)
	11	1.6(37)	0.03(1)	0.1(2)	1.0(25)	1.5(35)
PE	6	2.7(24)	0.1(1)	0.2(1)	4.8(44)	3.3(30)
	7	3.5(23)	0.2(1)	0.3(2)	6.2(41)	5.1(33)
	11	2.4(27)	0.2(2)	0.2(2)	2.4(26)	3.8(43)
MGDG	6	2.7(7)	Absent	Absent	3.9(11)	29.6(82)
	7	2.9(7)	Absent	Absent	4.9(11)	35.2(82)
	9	2.7(9)	Absent	Absent	4.5(14)	23.5(77)
	11	2.4(11)	Absent	Absent	3.1(14)	16.1(75)
DGDG	6	5.8(19)	0.2(1)	0.5(2)	1.9(6)	21.8(72)
	7	5.6(18)	0.3(1)	0.5(1)	2.2(7)	23.4(73)
	11	2.5(16)	0.1(1)	0.3(2)	1.6(11)	10.5(70)

The top 4 cm of the leaves of dark-grown seedlings were analysed. Results are expressed as nmol/leaf top. Figures in parentheses are the relative (%) amounts of the acid in the parent lipid.

pholipids and decreased in the glycolipids, particularly in MGDG. A corresponding decrease in the relative content of 18:2 occurred in the fatty acids of the phospholipids. In the glycolipids, however, the proportion of 18:2 increased.

In a second series of experiments the leaves of 6 day old dark-grown seedlings were sprayed twice daily with a solution of kinetin and after 48 hr the top 4 cm of the leaves were analysed for the polar lipids (Table 4) and their component fatty acids (Table 5). MGDG was the only major polar lipid to show a substantial decline in the leaf tops. On the other hand, the MGDG level increased appreciably in the hormone treatments. In absolute terms PC contained slightly more 18:3 than any other fatty acid. The glycolipids, MGDG and digalactosyldiacylglycerol (DGDG) were particularly rich in 18:3. The relative content (Table 5) of PC-18:3 and MGDG-18:3 was always higher in the leaf tips from seedlings treated with kinetin. The results expressed as fatty acid per mol

Table 3. The polar lipids in the leaf-tip of dark-grown seedlings of barley

Leaf age (days)	Polar lipid				
	PC	PI	PI	MGDG	DGDG
6	17.4	3.2	5.5	18.1	15.1
7	17.7	4.1	7.6	21.5	15.0
9	13.6	—	—	15.4	—
11	11.9	2.1	4.4	10.8	7.5

The top 4 cm of the leaves of dark-grown seedlings were analysed. The results are expressed as nmol/leaf top.

Table 4. The effect of kinetin on the polar lipids in the leaf-tips of dark-grown seedlings of barley

Polar lipid	Leaf age (days) and treatment		
	6C	8C	8K
PA	6.7	6.3	9.3
PI	4.6	3.3	3.3
PC	20.5	19.0	17.1
DGDG	19.4	21.3	20.0
MGDG	21.0	15.0	24.0

Dark-grown, seedlings (6 days old) were sprayed with water (C) or a solution of kinetin (K) and after 48 hr, the top 4 cm of the leaves were analysed. Results are expressed as nmol/leaf top. Figures in parentheses are the relative (%) amounts of the acid in the parent lipid.

parent lipid (not shown) indicate that treatment with kinetin brings about some enrichment of PC and MGDG with 18:3.

In further experiments the effect of kinetin on lipids and their component fatty acids was followed in ageing excised leaf sections. The 3 cm tips from 7 day old dark-grown seedlings were removed and floated in a solution of kinetin or water in Petri dishes in the dark. At intervals leaf segments were removed for analysis. The total polar lipid (Table 6) of the leaf segments in water diminished by 50% between the 20 and 140 hr treatments, whereas only *ca* 6% reduction was observed in leaf segments treated with kinetin. The fatty acid composition of the total lipid fraction (Table 7) shows that treatment with kinetin slightly increased the relative amount of 18:3. However, the relative 18:3 in the total polar lipid, from leaf segments treated with water decreased by some 12%.

The individual polar lipids decreased in the leaf segments treated with either water or kinetin. Treatment with kinetin, however, diminished the rate of decline in most lipids, particularly MGDG, DGDG and PC. The relative content (Table 8) of phosphatidylinositol (PI), phosphatidylethanolamine (PE) and PC increased quite markedly in leaf segments treated with water and little or no increase occurred in the kinetin treatments. On the other hand, the segments treated with the hormone showed only a slight decrease (compared to the water controls) in the relative amounts of the plastid lipids, MGDG, DGDG, phosphatidylglycerol (PG) and sulpholipid (SL).

The absolute and relative amounts of the major fatty acids in the phospho- and glycolipids are given in Table 9. The 18:3 in the total polar lipid decreased by 66% in the leaf segments in water. This was largely due to a marked reduction in the contribution made by the plastid lipids, whose total 18:3 decreased by nearly 80%. The decrease in the relative content of 18:3 in the plastid lipids indicates a loss of linolenate at a greater rate than the breakdown of the parent lipids. In the phospholipids, PC, PE and PI, the 18:3 was lost only slowly and this resulted in its relative accumulation. This was particularly evident for PC, in which the 18:3 content increased from 36 to 50% between 43 and 140 hr incubation in water. The 18:3 in the total lipid from leaf segments incubated in kinetin only decreased by 22% between the 43 and 140 hr treatments. After 43 hr in kinetin, the 18:3 in the polar lipids was, in most cases, higher than that in the equivalent water control. The relative amount of 18:3 in MGDG and DGDG increased in the kinetin treated segments whereas in the other polar lipids examined it tended to remain similar to the relative quantities found in the lipids from the water control segments.

Linoleic acid (18:2) decreased by 55% in the total polar lipid in leaf segments incubated in water for 140 hr. The relative content of 18:2 in PC and PE declined during the incubation period whereas it increased in the plastid lipids, MGDG, DGDG and PG. In segments incubated in kinetin the absolute loss in 18:2 was smaller than that observed in the water control segments. The total relative content of 16:0, 18:0 and 18:1 changed little in the lipids from leaf segments in either water or kinetin.

The relative distribution of the unsaturated C_{18} acids between the parent polar lipids is given in Table 10. The major contribution to the total 18:3 was from MGDG and DGDG, which contained *ca* 38 and 31% respectively. With prolonged incubation in water the relative contribution of glycolipid 18:3, declined appreciably. In contrast, the contribution to phospholipid 18:3, particularly PC, increased in leaf segments in water. Treatment with kinetin retarded the relative decrease in the glycolipid 18:3 and the relative increase in phospholipid 18:3. The contribution made by the polar lipids to the total 18:2 generally remained similar in all the treatments and incubation times.

The 18:3 content/mol PC increased in leaf segments incubated for long periods in water or kinetin solution (not shown). On the other hand, the specific quantity of 18:3 in MGDG decreased in segments in water and remained high in those in kinetin. The specific quantity of 18:2 in PC and MGDG fell after treatment with kinetin when compared to the equivalent water controls.

Desaturation of [1-^{14}C]*oleate*

Dark-grown seedlings were sprayed daily with a solution of kinetin. At regular intervals 1.5 cm long leaf segments were removed from 1 cm behind the leaf tip and their ability to further desaturate [1-^{14}C]oleate was determined (see Experimental). Little or no incorporation of radioactivity was found in

Table 5. The effect of kinetin on the fatty acid content of the polar lipids in the leaf tip of dark-grown seedlings of barley

Polar lipid	Acyl fatty acid, leaf age (days) and treatment											
	C16:0			C18:1			C18:2			C18:3		
	6C	8C	8K	6C	8C	8K	6C	8C	8K	6C	8C	8K
PA	3.5(26)	3.3(26)	5.3(28)	0.8(6)	0.7(5)	0.9(5)	5.1(39)	4.9(39)	6.8(37)	3.9(29)	3.8(30)	5.5(30)
PI	3.6(39)	3.1(47)	3.2(48)	0.3(3)	0.2(3)	0.2(3)	2.4(26)	1.6(24)	1.6(24)	2.9(32)	1.7(26)	1.7(25)
PC	12.0(29)	11.0(29)	10.8(32)	2.2(5)	1.8(5)	1.5(4)	12.7(31)	11.5(30)	8.8(26)	14.2(35)	13.8(36)	13.0(38)
DGDG	6.7(17)	6.7(16)	6.3(16)	0.7(2)	1.1(2)	0.3(1)	4.3(11)	4.6(11)	4.5(11)	27.2(70)	30.1(71)	28.9(72)
MGDG	1.9(4)	2.2(8)	2.3(5)	0.5(1)	0.4(1)	0.4(1)	4.9(12)	4.3(14)	4.9(10)	34.7(83)	23.2(77)	40.4(84)

Dark-grown, seedlings (6 days old) were sprayed with water (C) or a solution of kinetin (K) and after 48 hr, the top 4 cm of the leaves were analysed. Results are expressed as nmol/leaf top. Figures in parentheses are the relative (%) amounts of the acid in the parent lipid.

Table 6. The total polar and neutral lipid in excised leaf tips incubated in either water or kinetin in the dark

Incubation time (hr)	Polar lipid		Neutral lipid	
	C	K	C	K
20	35	35	6	4
75	24	34	4	3
140	18	33	4	4

The top 3 cm of the leaves of 7 day old seedlings were removed and floated in either water (C) or a solution of kinetin (K) for various times in the dark. The results are expressed as μg/leaf top.

Table 7. The relative fatty acid content (%) of the polar lipid in excised leaf tips, in either water or kinetin, in the dark

Incubation time (hr)	Acyl fatty acid							
	C16:0		C18:1		C18:2		C18:3	
	C	K	C	K	C	K	C	K
20	17	17	3	2	16	16	64	65
75	20	17	3	2	19	14	58	67
140	23	18	3	2	22	13	52	67

The top 3 cm of the leaves of 7 day old seedlings were removed and floated in either water (C) or a solution of kinetin (K) for various times in the dark.

18:3 in leaves treated with water or kinetin. Adequate quantities of [^{14}C]18:2 were, however, produced in both treatments. Maximum incorporation of radioactivity in 18:2 was found in 6–7 day old leaves and this represented *ca* 8 and 12%, of the total radioactivity in the unsaturated acids, in the water and kinetin treated leaves, respectively.

In further experiments 6 day old, dark-grown seedlings, which had been sprayed regularly with water or kinetin, were transferred to the light for 12 hr, after which the leaves were harvested and segments prepared from just behind the leaf tip.

The green leaf segments from both treatments were capable of synthesizing [^{14}C]18:2 and [^{14}C]18:3 from [^{14}C]18:1. In the leaf segments from the seedlings treated with kinetin as much as 23% of the total radioactivity in the unsaturated acids was found in 18:3 and this compared to 10% found in the water treatments. The proportion of radioactivity in 18:2 was similar (*ca* 16%) in leaf segments from both the water and kinetin treated seedlings.

DISCUSSION

The primary leaves of dark-grown seedlings of barley, continue to increase in length, fr. wt and dry wt, until *ca* day 14 of growth in the dark [19]. Linolenic acid (18:3), the major fatty acid of the polar lipid, increased substantially during the early phase in leaf development and was the first fatty acid to decline with further growth in the dark. On the other hand, 18:2 was not lost from the leaf until after 11 days growth. Draper [20] found a relative decrease in 18:3 and an increase in 16:0 in the total esterified fatty acids in ageing green cucumber cotyledons. Similarly Newman *et al.* [21] found a relative decrease in the 18:3 of green barley leaves kept in the dark and in green cocklebur leaf discs senescing in the light. In the present study the percentage of 18:3 decreased with age of the etiolated leaf and this was largely paralelled by a relative increase in 18:2.

In a more detailed study of lipid changes in the top 3–4 cm of the ageing, etiolated leaf, it was found that the major polar lipids were MGDG, DGDG and PC and that most of the 18:3 was present in these lipids. The phospholipids, especially PC, contained the larger portion of 18:2 and 16:0 although DGDG also contained substantial C16:0. During the senescence of the etiolated leaf the absolute amounts of the three major fatty acids, 18:3, 18:2 and 16:0, and the minor fatty acid, C18:1, decreased in all the phospho and glycolipids examined. However, the 18:3 in phospholipids was lost only slowly when compared to the decline in the glycolipid 18:3. In all experiments it was observed that MGDG, DGDG, PG and the SL, decreased with leaf age to a greater extent than the phospholipids, PC, PE and PI. MGDG, PG and SL are concentrated in the plastids [17, 18, 22, 23], whereas the phospholipids, PC, PE and PI are largely in extraplastidic membranes. The pattern of change observed in the glycerolipids in intact and excised leaves of dark-grown seedlings suggest that the extraplastidic membranes are more resistant to

Table 8. The polar lipids in excised leaf tips in either water or kinetin in the dark

Incubation time and treatment	Polar lipid							
	PI	PC	PE	PA	PG	SL	MGDG	DGDG
43 hr water	2.4(4)	15.4(26)	5.7(10)	2.0(3)	2.4(4)	3.2(5)	14.6(25)	13.7(23)
43 hr kinetin	3.1(5)	16.7(27)	6.1(10)	2.2(3)	3.0(5)	3.3(5)	15.4(24)	13.4(21)
140 hr water	1.8(8)	8.9(39)	3.5(15)	0.9(4)	0.8(3)	0.7(3)	3.6(16)	2.7(12)
140 hr kinetin	2.1(4)	12.9(28)	5.2(11)	1.4(3)	1.8(4)	2.1(5)	12.1(26)	8.9(19)

The top 3 cm of the leaves of 7 day old seedlings were removed and floated in either water (C) or a solution of kinetin (K) for various times in the dark. Results are expressed in nmol/leaf top. Figures in parentheses are the relative amounts (%) of each polar lipid.

Table 9. The fatty acid content of the polar lipids in excised leaf tips in water or kinetin in the dark

Polar lipid and incubation time (hr)	Acyl fatty acid and treatment									
	C16:0		C18:0		C18:1		C18:2		C18:3	
	C	K	C	K	C	K	C	K	C	K
PI 43	2.1(44)	2.5(40)	0.03(1)	0.05(1)	0.1(2)	0.1(2)	1.1(22)	1.4(23)	1.5(31)	2.2(34)
140	1.4(40)	1.9(44)	0.02(1)	0.02(1)	0.04(1)	0.04(1)	0.7(21)	0.8(19)	1.3(37)	1.5(35)
PC 43	8.4(27)	9.7(29)	0.2(1)	0.3(1)	1.5(5)	2.1(6)	9.6(31)	9.0(27)	11.0(36)	12.3(37)
140	4.1(23)	6.6(26)	0.1(1)	0.2(1)	0.3(2)	0.6(2)	4.5(25)	5.7(22)	8.7(49)	12.7(49)
PE 43	2.7(24)	2.7(22)	0.1(1)	0.1(1)	0.3(2)	0.3(2)	5.0(44)	5.2(43)	3.3(29)	3.8(32)
140	2.0(28)	2.9(28)	0.1(2)	0.1(1)	0.3(5)	0.4(3)	2.3(32)	3.5(34)	2.3(33)	3.5(34)
PA 43	1.0(24)	1.1(25)	0.1(1)	0.1(2)	0.2(5)	0.2(5)	1.4(35)	1.6(37)	1.4(35)	1.3(31)
140	0.5(28)	0.7(27)	Trace	Trace	0.02(1)	0.04(1)	0.6(34)	0.9(32)	0.6(37)	1.1(40)
PG* 43	1.8(37)	2.1(36)	0.03(1)	0.04(1)	0.05(1)	0.1(2)	0.5(9)	0.7(12)	2.0(43)	2.1(36)
140	0.7(43)	1.2(33)	0.02(1)	0.04(1)	0.07(4)	0.08(2)	0.2(13)	0.5(13)	0.5(32)	1.1(32)
SL 43	1.9(30)	1.8(26)	0.06(1)	0.04(1)	0.06(1)	0.06(1)	0.6(10)	0.8(12)	3.7(58)	4.0(60)
140	0.6(40)	1.4(33)	0.01(1)	0.05(1)	Trace	0.03(1)	0.1(6)	0.6(10)	0.7(53)	2.3(55)
MGDG 43	0.5(2)	0.5(2)	Trace	Trace	Trace	Trace	1.5(5)	1.3(4)	27.2(93)	29.0(94)
140	0.2(3)	0.4(2)	Trace	Trace	Trace	Trace	0.7(9)	1.5(6)	6.2(88)	22.2(92)
DGDG 43	3.1(11)	2.9(11)	0.2(1)	0.1(1)	0.2(1)	0.2(1)	1.4(5)	1.5(5)	22.4(82)	22.1(82)
140	0.6(10)	1.7(10)	0.06(1)	0.1(1)	0.05(1)	0.1(1)	0.5(10)	0.8(5)	4.2(78)	15.0(83)
Total 43	21.4(18)	23.2(19)	0.7(1)	0.7(1)	2.4(2)	3.1(2)	20.9(17)	21.5(17)	72.5(62)	76.8(61)
140	10.0(22)	16.8(18)	0.4(1)	0.5(1)	0.8(2)	1.3(1)	9.6(21)	14.0(15)	24.6(54)	59.5(65)

*PG also contained C16:3 in the following concentrations: 43 hr: C, 0.4(9); 43 hr, K, 0.8(13); 140 hr: C, 0.1(6); 140 hr: K, 0.7(19).

The top 3 cm of the leaves of 7 day old seedlings were removed and floated in either water (C) or a solution of kinetin (K) for various times in the dark. Results are expressed as nmol/leaf top. Figures in parentheses are the relative amounts (%) of the acid in the parent lipid.

Table 10. The relative distribution (%) of the unsaturated C18 acids between the polar lipids

Polar lipid and incubation time (hr)	C18:1		C18:2		C18:3	
	C	K	C	K	C	K
PI 43	5	4	5	7	2	3
140	5	3	8	6	5	3
PC 43	63	68	46	42	15	16
140	37	49	47	40	36	21
PE 43	11	9	24	24	5	5
140	42	28	24	25	9	6
PA 43	7	7	6	7	2	2
140	2	3	6	6	3	2
PG 43	2	4	2	3	3	3
140	8	6	2	4	2	2
SL 43	3	2	3	4	5	5
140	Trace	2	1	3	3	4
MGDG 43	Trace	Trace	7	6	37	37
140	Trace	Trace	7	10	25	37
DGDG 43	9	6	7	7	31	29
140	6	9	5	6	17	25

Data calculated from Table 9.

degradation (or have more efficient repair mechanisms) than the etioplastid. Similar suggestions have been made for the breakdown of the lipids in the photosynthetic apparatus in green tissue [20, 24], and Galliard [25, 26] found a selective degradation of lipids in the non-photosynthetic pulp of pre- and post-climacteric apples.

MGDG was usually the first polar lipid to decrease in quantity during senescence and this was then followed by a decline in DGDG and PG. The ability of the etiolated leaves to produce chlorophyll in the light declines rapidly after 6–7 days dark-growth [1, 2]. The loss in chlorophyll producing potential seems more closely associated with the loss in MGDG than in any of the other lipids. It is noteworthy that Roughan and Boardman [27] suggested that MGDG may play an important role in the formation of granal membranes and hence chlorophyll formation. Ultrastructural studies [28–30] also suggest that the chloroplast is the first organelle to show signs of senescence, and Barton [28] postulated that the primary cause of senescence was the appearance of an enzyme complex that 'decomposed' the chloroplast thylakoids and then moved from the plastids to affect other membranes. Thimann *et al.* [31–33], on the other hand, have produced evidence which may indicate that senescence is initiated in the cytoplasm following the *de novo* synthesis of proteolytic enzymes. It is of inter-

est that Huber and Newman [34] found (in senescing green soybean cotyledons) increased incorporation of [^{14}C]acetate into cytoplasmic lipids as opposed to plastid lipids and they concluded that "senescence commenced with the increased activity of the cytoplasmic lipids while the chloroplast lipids experienced less change". However, from the data of Huber and Newman [34] it should be noted that chlorophyll started to decrease at the same time as the increased incorporation of ^{14}C into PC and before other phospholipids.

Little or no data are available on the fatty acid composition of individual polar lipids during senescence in plants. Interesting changes were observed in the fatty acid composition of these lipids in the apical regions of senescing, intact, etiolated barley leaves. The most marked change occurred in the relative level of 18:3. The 18:3 appreciably decreased in MGDG. In contrast, the precentage of 18:3 in PC, PE and PI dramatically increased with senescence. Similar results were obtained with leaf sections in water in the dark. Although here, the relative increase in 18:3 was particularly marked in PC whereas the major decrease occurred in the plastid lipid, PG. It would appear that there is operating, both inside and outside the plastid, a selective degradation of parent lipid and their constituent fatty acids. The lipids of the thylakoid membranes [22, 35, 36] are richer in 18:3 than the lipids in the plastid envelope. Similarly, Bahl *et al.* [37] found that the lipids of the prolamellar body in wheat etioplasts, were relatively richer in 18:3 than the envelope lipids and most of this acid was present in MGDG. It is possible that in the plastid there is, during senescence, a selective removal of lipids with a higher content of 18:3. The extraplastidic lipids, on the other hand, show a relative increase in 18:3 and only a slight decrease in absolute terms. This suggests the presence, outside the plastid, of phospholipase enzymes with a relatively low deacylating activity towards 18:3, perhaps due to its intramolecular position. However, it should be noted that Galliard and Matthew [38] consider that the enzyme responsible for the complete deacylation of membrane lipids in plants is a non-specific enzyme designated 'lipolytic acyl-hydrolase'.

The cytokinin, kinetin, is known to delay the senescence of various green leaf tissues [39–41] and etiolated leaves [1]. Donaldson *et al.* [42] found that chloroplasts isolated from mature green spinach leaves that had been treated with kinetin contained relatively more total 18:3 than chloroplasts from untreated leaves, and Kull and Buxenstein [43] found that the phytohormone increased total 18:3 in light grown leaves of *Impatiens* and *Populus*. Recently, Le Pabic [44] reported that, in *Spirodele*, BAP treatment increased the 18:2 and 18:3 content of the polar lipids. In the present study kinetin was found to be most effective in retarding the loss of membrane lipids observed during leaf senescence, particularly when leaf sections were floated on a solution of kinetin in the dark. Kinetin treatment retarded or even stopped completely the loss of 18:3 and 16:0 whereas 18:2 still declined at a relatively rapid rate. The hormone also markedly retarded the loss in the plastid lipids, MGDG, DGDG, PG and the SL more than the phospholipids PC, PE and PA. The 18:3 content of MGDG and PC actually increased in barley leaf tissue treated with kinetin, suggesting that the hormone controls the synthesis of lipids and their component fatty acids and/or their degradation. During the senescence of the leaf sections in water there was also a large increase in the relative content of 18:3 in the phospholipids, particularly in PC. This suggests that the increase in C18:3 is somehow 'beneficial' and is a positive process of adaptation to senescence rather than being a mere result of selective removal of phospholipids relatively low in 18:3. During ageing in the leaf perhaps there is an attempt at membrane repair and renewal with a tendency to synthesize specific phospholipids rich in 18:3. On the other hand, it is possible that some 18:3, synthesized outside the plastids is a source of plastidic fatty acid and that the rate of transfer to the plastid becomes limiting. This would result in an accumulation of 18:3 in non-plastidic phospholipids.

Little or no synthesis of 18:3 by the desaturation of [^{14}C]oleate could be demonstrated in leaf sections from dark-grown leaves, a result in agreement with a previous report [45]. However, a light treatment induced the development of an active desaturase system which resulted in the production of relatively large quantities of radioactive 18:2 and 18:3 (see ref. [45]). Kinetin application to the dark-grown leaves prior to the light treatment, however, increased the relative amount of [^{14}C]18:3 synthesized from [^{14}C]18:1. The cytokinins, therefore appear to control, besides the production and/or activation of hydrolytic enzymes, the enzymes involved in the desaturation of oleate and the production of linoleate and linolenate.

EXPERIMENTAL

Barley seeds (*Hordeum vulgare* cv Proctor) were obtained from K. Wilson (South) Ltd., Wellingborough, Northants. [1-^{14}C]oleic acid (58 Ci/mol) was purchased from the Radiochemical Centre, Amersham, U.K.

The seeds were soaked for 18 hr in H_2O and sown in trays of Vermiculite. The seedlings were grown in complete darkness at 20° with regular watering. At intervals leaf material was removed for lipid analysis. In the hormone treatments the seedlings were either sprayed regularly with a soln of kinetin (3 mg/l.) or cut sections floated directly in a kinetin soln of a similar concn.

Lipid extraction and analysis. Leaf tissue was extracted in cold *iso*-PrOH followed by $CHCl_3$–MeOH (2:1) and the extracts bulked. After evaporation by RFE the residue was dissolved in $CHCl_3$–MeOH (2:1) and the H_2O soluble contaminants removed by phase separation against NaCl (0.7%, w/v). The $CHCl_3$ phase was taken to dryness under N_2 and stored in $CHCl_3$ at − 15°. Neutral lipids and polar lipids were fractionated by phase separation against MeOH and petrol [46].

Polar lipids were separated by TLC on Si gel with $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (10:4:2:2:1) or $CHCl_3$–Me_2CO–7 M NH_4OH (60:25:4) and located with I_2 vapour. The gel containing phospholipid was removed for transmethylation in Me_2CO–C_6H_6–H_2SO_4 (20:10:1 by vol.) FAMES were analysed by GC and their concn determined by triangulation and comparison to an int. standard (heptadecanoic acid). Glycerolipid concns were calculated from their molar fatty acid content.

[^{14}C]*Oleate desaturation.* Sections (1.5 cm long) were cut from the leaves in a region 1 cm back from the leaf tip.

Groups of 25 segments were loaded onto pins and placed in a 100 ml conical flask. The 'loaded' pins were covered with 12 ml KPi buffer (0.1 M, pH 7.5) and infiltrated with ammonium [1-^{14}C]oleate (10 μCi) under vacuum. After thorough washing the sections were transferred to fresh buffer and incubated at 25° with constant shaking for 2.5 hr. The tissue was extracted by the method of ref. [47]. The total lipid extract was transmethylated and the FAMES were separated by $AgNO_3$–Si gel TLC (1:9). The plates were developed twice in hexane–Et_2O–HOAc (85:15:1) and the esters located by spraying with dichlorofluorescein (0.05% in EtOH) and viewing under UV light. The methyl esters of mono-, di- and trienoic fatty acids were assayed for radioactivity by liquid scintillation in PPO and POPOP in toluene (4 g PPO + 0.3 g POPOP/l. toluene). All counts were corr. for quenching.

Acknowledgements—S.A.H. is grateful to the Agricultural Research Corporation, The Sudan Government, for financial support.

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