Phytochemistry 1974 Vol 13 pp 39 to 44 Pergamon Press Printed in England

EFFECT OF CYTOKININS ON THE LIPID FATTY ACIDS OF LEAVES

ULRICH KULL and ROLAND BUXENSTEIN
Biologisches Institut der Universitat Stuttgart, Stuttgart, Germany F R

(*Received* 19 *March* 1973 *Accepted* 29 *June* 1973)

Key Word Index—*Coleus blumei* Labiatae *Impatiens sultani* Balsaminaceae, *Populus balsamifera* Salicaceae, kinetin, zeatin, fatty acids linolenic acid

Abstract—Kinetin and zeatin were administered to leaves of intact plants of *Coleus*, *Impatiens* and *Populus* in long-term experiments (4 weeks) and the variations of the fatty acid content of saponifiable lipids determined In the same species there are no significant differences between the effects of the two cytokinins In *Impatiens* and *Populus* the content of linolenic acid increases The behaviour of palmitic and linoleic acids is variable *Coleus* plants were treated with zeatin at 15 and 25° At the higher temperature the hormone causes a distinct rise of palmitic and a diminution of linolenic acid

INTRODUCTION

IT HAS been known for several years that in ageing leaves the synthesis of lipids is promoted by kinetin [1] Although fatty acid synthesis is slightly inhibited in spinach chloroplasts incubated with kinetin,[2] kinetin-treated spinach leaves contained more fatty acids than controls, which may be attributed mainly to an increase in the linolenate component of the plastids [2] Plastids are known to be an important site of action of cytokinins,[3–5] but they are not the only targets of these hormones in plant cells, for example in germinating wheat grains the catabolism of a part of the triglyceride reserves is induced by cytokinins [6] Earlier, in 5 out of 7 species investigated we found an increased amount of total lipids in leaves after the application of kinetin,[7] but in several of these experiments the content of saponifiable lipids was lowered Continuing these investigations, the present study was designed to determine the long-term influence of kinetin and of the naturally occurring cytokinin zeatin on the fatty acid composition of saponifiable lipids of leaves

RESULTS

Leaves of intact plants of *Coleus*, *Impatiens* and *Populus* were treated with cytokinins (see Experimental) An influence of temperature on the effects of cytokinins is known from several reports [8,9] One set of experiments with zeatin was therefore made with *Coleus*

[1] LETHAM D S (1967) *Annu Rev Plant Physiol* **18**, 349
[2] DONALDSON R LOESCHER W and NEWMAN D W (1969) *Am J Botany* **56** 1167
[3] SRIVASTAVA, B I S (1963) *Arch Biochem Biophys* **103**, 200
[4] DENNIS, D T, STUBBS, M and COULTATE T P (1967) *Can J Botany* **45**, 1019
[5] RICHMOND A E SACHS B and OSBORNE D J (1971) *Physiol Plant* **24**, 176
[6] TAVENER, R J A and LAIDMAN, D L (1972) *Phytochemistry* **11**, 981
[7] KULL U (1972) *Botan Studien (Jena)* **19**, 1
[8] ADAMSON D (1962) *Can J Botany* **40** 719
[9] FEDERBUSH J (1970) *Z Pflanzenphysiol* **62**, 70

at a higher temperature than usual (25) This series is designated as 'zeatin-warm" (Table 2) The fatty acid patterns are presented in Tables 1-4 as percentages of the total fatty acids, and for *Coleus* in Tables 1 and 2 also as the percentage of dry weight The fatty acid profiles are in good agreement with those found in leaves of several higher plant species [10] One notable deviation is the relatively low content of linolenic acid in *Populus* leaves

TABLE 1 FATTY ACIDS OF SAPONIFIABLE LIPIDS FROM LEAVES OF *Coleus blumei* (in % of total fatty acids and in % of dry wt) TREATED WITH KINETIN (harvested in September)

Fatty acid	% of total fatty acids				10^{-2} % of dry wt			
	Control	100 ppm	200 ppm	P*	Control	100 ppm	200 ppm	P*
10 0 + 10 1	0 2	0 4	0 8	0 4	0 3	0 4	1 0	0 4
12 0	0 4	0 6	1 3	1 9	0 5	0 6	1 6	2 0
12 1	0 6	0 5	0 5	0 3	0 8	0 6	0 6	0 3
14 0	1 5	1 2	1 1	0 4	2 0	1 4	1 4	0 4
14 1	0 5	0 2	0 2	0 1	0 6	0 2	0 2	0 1
16 0	31 9	31 9	28 0	28 6	41 0	38 3	33 9	29 8
16 1	3 2	3 1	2 4	1 1	4 2	3 7	3 0	1 1
16 3	0 5	0 4	0 5	0 5	0 6	0 5	0 6	0 6
18 0	6 1	6 2	5 9	6 2	7 8	7 4	7 0	6 5
18 1	3 9	3 8	5 2	6 0	5 0	4 6	6 3	6 3
18 2	18 8	19 9	19 8	18 7	24 1	23 9	24 0	19 5
18 3	31 8	31 2	34 5	34 8	40 8	37 2	41 8	36 3
Longer chain	0 6	0 8	0 8	0 9	0 8	0 8	0 9	0 9

* 10 ppm solution (see Experimental)

A decrease in the content of the short-chain fatty acids (to C_{14}) was observed in several cases Only in one series (*Impatiens*—zeatin) did we find a rise, dependent on an increase of myristoleic acid (14 1) The behaviour of palmitic acid (16 0) is variable In *Impatiens* the content decreases, in *Coleus*, only in the series "zeatin-warm" is a significant increase to be seen In most cases no changes were found in the amounts of unsaturated C_{16}-acids The alterations of stearic (18 0) and oleic (18 1) acids are variable and quantitatively not very important The linoleic acid (18 2) content decreases in *Impatiens* in leaves of *Populus* and in two out of three series of *Coleus* the content rises The amounts of linolenic acid (18 3) increase remarkably in *Impatiens* A small rise is found in *Populus* and kinetin-treated *Coleus* leaves In the zeatin experiments with the latter species decreasing amounts were found, especially in the "zeatin-warm" series The content of margaric acid in *Populus* (see Ref 11 on the occurrence of margaric acid in *P balsamifera*) decreases in leaves

In *Coleus* leaves treated with kinetin and in the "zeatin-warm" series the total content of fatty acids decreases but in "zeatin-cold" the total content rises Therefore in the latter series the absolute content of all the important fatty acids increases (This is true for linolenic acid too, with the exception of the "P" series) For the other experiments with *Coleus* the contrary is found

[10] NICHOLS B W and JAMES A T (1968) *Progress in Phytochemistry* (REINHOLD L and LIWSCHITZ Y, eds), Vol 1 p 1 Academic Press New York

[11] KULL U and JERMIAS K (1972) *Z Pflanzenphysiol* **68**, 55

The proportion of multiple unsaturated C_{18}-acids to palmitic acid ((18 2 + 18 3)/(16 0)) is increased by kinetin in *Coleus* and *Impatiens* and by zeatin in the latter species In the zeatin experiments with *Coleus*, on the contrary, it decreases

TABLE 2 FATTY ACIDS OF SAPONIFIABLE LIPIDS FROM LEAVES OF *Coleus blumei* (in % of total fatty acids and in % of dry wt), TREATED WITH ZEATIN AT 15° (harvested in October) AND AT 25° (harvested in March)

	% of total fatty acids							
	15° (October)				25° (March)			
Fatty acid	Control	10 ppm	100 ppm	P*	Control	10 ppm	100 ppm	P*
10 0 + 10 1	0 2	0 3	0 2	0 1	2 7	2 6	2 4	0 7
12 0	0 5	0 5	0 7	0 5				
12 1	0 6	0 5	0 4	0 1	2 1	1 1	1 3	0 7
14 0	0 4	0 4	0 4	0 9	0 6	0 4	0 5	0 4
14 1					0 4	0 2	0 2	0 2
16 0	28 5	27 2	28 1	27 5	15 7	20 1	22 1	22 5
16 1	1 2	0 8	1 1	2 4	1 6	3 3	3 0	1 8
16 3	0 9	0 7	0 9	0 3	1 4	1 0	1 2	1 5
18 0	6 0	6 4	7 2	7 7	4 7	3 3	4 1	4 2
18 1	4 5	4 6	5 6	7 7	3 4	3 0	2 6	3 4
18 2	21 8	23 1	20 5	20 9	17 5	17 7	18 8	21 5
18 3	34 8	34 7	33 6	30 9	48 6	44 1	41 2	41 7
Longer chain	0 6	0 8	1 1	1 0	1 3	3 2	2 6	1 4
	10^{-2} % of dry wt							
10 0 + 10 1	0 2	0 3	0 2	0 1	7 6	4 6	3 6	1 6
12 0	0 4	0 4	0 7	0 4				
12 1	0 5	0 4	0 4	0 1	5 9	1 9	1 9	1 8
14 0	0 3	0 3	0 4	0 4	1 6	0 6	0 7	1 0
14 1					1 1	0 3	0 4	0 4
16 0	22 6	22 5	27 1	23 1	44 0	36 0	33 0	56 0
16 1	1 0	0 7	1 1	2 0	4 5	5 8	4 5	4 5
16 3	0 7	0 6	0 9	0 3	4 1	1 6	1 8	3 6
18 0	4 7	5 3	6 9	6 4	13 0	5 9	6 0	10 5
18 1	3 5	3 8	5 4	6 5	9 0	5 3	3 8	8 4
18 2	17 3	19 2	19 8	17 5	50 0	31 0	48 0	50 3
18 3	27 5	28 8	32 1	25 9	138 0	79 0	61 0	104 0
Longer chain	0 4	0 6	1 1	0 9	3 8	3 0	3 8	3 5

* See Experimental

DISCUSSION

There are no significant differences between the effects of kinetin and zeatin The decrease in the short-chain fatty acid content may be due to a stimulation of anabolic metabolism[7] and may therefore be associated with an enhanced synthesis of longer chain acids However, this stimulation is in general not related to the increasing amounts of total fatty acids In comparing the "cold" and "warm" series of the *Coleus* zeatin experiments the relatively large differences in the controls (especially for palmitic and linolenic acids) cannot be explained Perhaps there is an influence caused by the different seasons, in which the experiments were made The patterns of the controls are contrary to what would be expected, for it is well known that increasing temperature causes a decrease of multiple unsaturated fatty acids and an increase of the saturated

ones[12–16] Within the "warm" series the effect of zeatin is analogous to that of increasing the temperature At the concentration applied, it seems possible that higher temperature will give rise to inhibitory effects of zeatin on synthesis of multiple unsaturated fatty acids

TABLE 3 FATTY ACIDS OF SAPONIFIABLE LIPIDS FROM LEAVES OF *Impatiens sultani* (in % of total fatty acids) TREATED WITH KINETIN (harvested in September) AND ZEATIN (harvested in March)

Fatty acid	Control	Kinetin 100 ppm	Kinetin 200 ppm	Zeatin Control	Zeatin 100 ppm
10 0 + 10 1	1 2	1 5	1 2	1 2	1 0
12 0 + 12 1	2 8	2 6	2 5	2 4	2 5
14 0	1 0	0 7	0 5	0 8	0 9
14 1	3 4	2 9	2 7	0 8	1 5
16 0	30 2	26 5	25 2	34 2	29 4
16 1	3 0	5 2	5 7	3 0	2 6
16 3				1 1	1 1
18 0	7 5	6 2	6 2	2 7	3 0
18 1	7 3	7 0	7 1	4 1	4 3
18 2	10 5	8 8	7 6	8 6	7 0
18 3	33 0	38 5	41 1	41 0	46 5
Not identified	0 1	0 1	0 2	0 1	0 2

Donaldson *et al* [2] after incubating leaves for 28 hr with kinetin, found no enhancement of linolenate synthesis but the degradation was not as rapid as in control tissues In our experiments perhaps by reason of their long duration this effect is not so obvious Moreover it is likely that a significant accumulation of linolenate in leaves requires the availability of either UDPglucose or UDPgalactose[17] An effect of cytokinins on

TABLE 4 FATTY ACIDS OF SAPONIFIABLE LIPIDS FROM LEAVES OF *Populus balsamifera* (in % of total fatty acids) TREATED WITH KINETIN (harvested in September)

Fatty acid	Control	100 ppm	200 ppm
10 0 + 10 1	1 9	2 1	1 6
12 0 + 12 1			
14 0	1 4	1 1	0 5
14 1	5 2	5 2	2 4
16 0	22 8	22 9	25 7
16 1	1 3	1 7	1 2
16 3 (?)	0 8	0 9	0 8
17 0 (?)	22 4	23 0	16 0
18 0	3 4	4 0	2 2
18 1	3 0	3 6	5 5
18 2	9 7	8 9	12 0
18 3	25 8	25 1	27 9
Not identified	2 3	1 5	1 2

[12] HOLTON, R W, BLECKER, H H and ONORI M (1964) *Phytochemistry* **3**, 595
[13] GERLOFF E D RICHARDSON T and STAHMANN, M A (1966) *Plant Physiol* **41**, 1280
[14] RINNE R W (1969) *Plant Physiol* **44** 89
[15] BERINGER H (1971) *Z Pflanzenernahr Bodenkde* **128** 115
[16] SCHUSTER W (1971) *Fette Seifen- Anstrichmittel* **73**, 305
[17] ONGUN A and MUDD J B (1968) *J Biol Chem* **243**, 1558

the availability of these sugar-nucleotides is very probable, because from several reports, it is known that they affect carbohydrate metabolism (e g ,[18,19] and further lit cited in Ref 7) In our long-term experiments there are also considerable changes in the contents of starch and sugars in leaves For experiments with kinetin this was described earlier [7]

The increase in linolenic acid content, which we found in several experiments with *Impatiens* and *Populus*, is in agreement with the data of Donaldson *et al* [2] It is in accord with the well known effects of cytokinins on plastids, especially those of retardation of senescence Provided that cytokinins inhibit some kinases [18] glucose-6-phosphate-isomerase and glucose-6-phosphate-dehydrogenase,[19] a restriction of the glucose metabolism is to be expected Therefore more UDPglucose can be isomerized to UDPgalactose, the formation of which is prerequisite for an intensified synthesis of galactolipids, which contain the greatest proportion of linolenic acid

Cytokinins are known to have an influence on the properties of cellular membranes, e g permeability and ion-transport [5,20,21] It would be of interest to know whether one of the causes of this might be an alteration of the fatty acid composition of membrane lipids There is some evidence in this direction [22] The great importance of the fatty acid composition for the properties of membranes was considered recently [23,24] An approach to this problem can be made by short-term experiments and fractionation of the lipids

In some preliminary experiments carried out with the same material which gave the results described in this paper, an increase in the content of glycolipids and of some of the phospholipids and a decrease of the fraction of neutral lipids under the influence of zeatin was shown This agrees with earlier results showing a rise in the content of lipid-bound sugars in leaves after application of kinetin [7]

EXPERIMENTAL

Plant material *Coleus blumei* Benth , and *Impatiens sultani* Hook were propagated vegetatively from the clone used previously [7 25] On application of cytokinins these plants were 5–7 cm high The investigations with *Populus* were made with homogeneous cuttings of *Populus* × *balsamifera* L CV Oxford *ca* 30 cm All plants were grown in flower pots under natural light in a greenhouse of the Botanical Garden The temp during the period of application was 15–18° One set of experiments with *Coleus* ("zeatin-warm") was made at a higher temp (*ca* 25°) in a hothouse

Application of cytokinins The application of kinetin and zeatin took place 4 × in weekly intervals One week after administration of the last dose the plants were harvested and the material lyophilized The harvesting times are shown on the tables In the series named "P" 10 ppm solutions of the cytokinin were administered by pencil to all green leaves of the plants In the other series only the young, not yet fully expanded leaves near the apex were treated with the solutions by a micropipette Each set included 7–10 plants of almost identical height and shape

Extraction of lipids and preparation for GLC Lyophilized leaves were extracted with $CHCl_3$–MeOH (2 1) and the extract concentrated under red pres [15 26] Saponification and esterification were accomplished as described by Kull and Jeremias [11]

GLC of fatty acid methyl esters A Varian Aerograph 1840-4 gas chromatograph, equipped with a hydrogen FID was used with a steel column (6 m × 3 mm), 10% EGSS-X on 60-80 Chrom WAW-DMCS The column was maintained at 180° injector at 200° detector at 270° N_2 flow 30 ml/min The identity of the compounds

[18] Tuli, V Dilley D R and Wittwer S H (1964) *Science* **146**, 1477
[19] Simpkins, I and Street H E (1970) *J Exp Botany* **21**, 170
[20] Ilan, I Gilad T and Reinhold L (1971) *Physiol Plant* **24**, 337
[21] Ilan, I (1971) *Physiol Plant* **25**, 230
[22] Schaffeler G W and Sharpe Jr F T (1971) *Physiol Plant* **25**, 456
[23] Traub H (1971) *Naturwissenchaften* **58**, 277
[24] Kreutz W (1972) *Angew Chem* **84**, 597
[25] Kull U (1971) *Ber Dtsch Bot Ges* **84**, 299
[26] Wintler E (1963) *Z Lebensmittel-Unters* **123**, 205

was established by comparison of the retention times with those of the methyl esters of fatty acids and by co-injection with these standards [11]

Acknowledgements –Thanks are due to Dipl Chem E Jenkins and to Mr P Cleary for their corrections of the English text and to the Deutsche Forschungsgemeinschaft for financial support to the senior author