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Epoxidation – a Consequence of Cell Damage

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Summary. Products obtained after plant cell injury were studied by dividing white cabbage leaves (*Brassica oleracea*) in two parts. One part was heated to denaturate enzymes, homogenized, and stirred for six hours. After chromatographic separation and appropriate derivatization, the fractions were analyzed for low molecular weight compounds by GCMS. The other part was homogenized without cooking, but treated and analyzed in exactly the same way as the non-cooked sample. Comparison of the thus obtained products revealed that – besided already well known lipid peroxidation processes, *e.g.* generation of stress hormones as well as liberation and oxidation of phenolic compounds – a main but less known way of oxidative destruction was observed: epoxidation. The reaction not only involves unsaturated fatty acids, but also sterols and terpenes. This seems to be a typical response of plant cells to injury.

Keywords. *Brassica oleracea*; Cell injury; Lipid peroxidation; Epoxidation.

Epoxidierung – Eine Folge von Zellverletzung

Zusammenfassung. Zur Untersuchung der Prozesse, die nach Zellverletzung ablaufen, wurde ein Weißkohlkopf geteilt. Die eine Hälfte wurde gekocht, um Enzyme zu zerstören, und dann homogenisiert. Das Homogenisat wurde bei Raumtemperatur sechs Stunden an der Luft gerührt. Nach chromatographischer Trennung wurden die einzelnen Fraktionen nach entsprechender Derivatisierung mittels GC-MS auf niedermolekulare Inhaltsstoffe untersucht. Die andere Hälfte des Weißkrautes wurde ungekocht in gleicher Weise homogenisiert, aufgearbeitet und analysiert. Der Vergleich der so erhaltenen Produkte ergab, daß neben bereits bekannten Lipidperoxidationsprozessen wie z.B. der Bildung von Streßhormonen und der Freisetzung sowie Oxidation von Phenolen ein weniger bekannter Weg oxidativer Umwandlung beobachtet wurde: Epoxidierung. Sie betrifft nicht nur ungesättigte Fettsäuren, sondern auch Sterole und Terpene. Diese Reaktion scheint eine typische Antwort von Pflanzen auf Zellverletzung zu sein.

Introduction

It is well known that damage of plant cells causes a change in the composition of low molecular weight compounds: membrane phospholipids and galactosides are cleaved immediately by action of esterases [1]. The thus produced unsaturated acids with a *cis*-1, 4-pentadienyl system – mainly linoleic acid – are converted to hydroperoxides in a lipoxygenase catalyzed reaction [1]. Fatty acid hydroperoxides are the starting compounds for a great number of secondary degradation products, *e.g.* aldehydes [2] and epoxyhydroxy acids [3–5]. In addition, cell injury initiates

the production of stress hormones, *e.g.* jasmonic acid [6–9], ethylene [10], and abscisic acid [11].

It is also well known that injury of plant cells induces the cleavage of phenolic glucosides, *e.g.* causes liberation of *p*-hydroxy cinnamic acid [12], and stimulates their *de novo* synthesis [13, 14]. Further oxidation reactions, initiated by peroxidases, produce lignin and *o*-chinoidic compounds [15].

In some instances, epoxidation products of unsaturated fatty acids had been found after injury of plants [16–20]. Recently, we have observed that epoxidation always accompanies processes in which lipid peroxidation is involved [21, 22]. Active species are the peroxyl radicals generated as intermediates in peroxidation processes. In this communication, we report on the epoxidation of sterols. To the best of our knowledge, these processes have not been observed so far after plant injury.

Results and Discussion

White cabbage leaves were divided in two parts. One part was heated to denaturate enzymes, followed by homogenization and extraction with organic solvents (no change in the content of compounds was observed if the action of enzymes was restricted by immediate addition of methanol, another method to avoid enzymatic action [23, 24]). The other part of the cabbage leaves was homogenized without heating, keeping the enzymes active. This homogenisate was stirred in aqueous solution for 24 h on air. Both samples were further treated in exactly the same way. Extraction of lipids was achieved according to *Bligh* and *Dyer* [25]. The lipid fraction was transesterified to obtain methyl esters of good volatility for subsequent separation by gas chromatography (GC). This method offered the advantage to convert epoxides to the corresponding 1-hydroxy-2-methoxy derivatives which allowed easy detection by mass spectrometry after trimethylsilylation [26]. In contrast, epoxides usually don't show very informative mass spectra [27]. The methyl esters were separated by column chromatography (CC) and thin layer chromatography (TLC). After trimethylsilylation, the samples were analyzed by GC-MS [23]. Since it turned out that an unambiguous localization of original hydroxyl groups was not possible in all cases, the fractions were alternatively hydrogenated to remove double bonds [28, 29]. The obtained trimethylsilylated saturated hydroxy fatty acid methyl esters allowed the localization of oxygen functions.

The qualitative results of this investigation are compiled in Table 1. The results agree with previous findings [16–18]. 1,2-Substituted dihydroxy acids are derived from epoxides (*e.g.* 9,10-dihydroxy stearic acid is a hydrolysis product of the epoxide of oleic acid), whereas 12,13- and 9,10-dihydroxy acid 18:1 are derived from the 9,10- and the 12,13-epoxide of linoleic acid. Similarly, three 1,2-dihydroxy substituted 18:2 acids derived from linolenic acid were found. A 9,10-dihydroxy derivative of linolenic acid was also detected. Such a compound has been described previously [30], generated by non-enzymatic oxidation of linolenic acid with Fe^{2+}/ascorbate. It was previously unknown as a lipid peroxidation product of plants, as well as two other dihydroxy products identified containing the hydroxy function in 1,5- and 1,6-position of the C18 fatty acid chain, respectively.

Table 1. Dioxygenated fatty acid methyl esters obtained from wounded cabbage leaves (identified as trimethylsilyl-ethers); *not described previously as lipid peroxidation product of plants; DiOH= dihydroxy

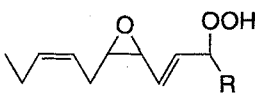
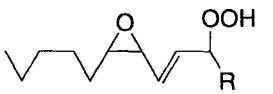
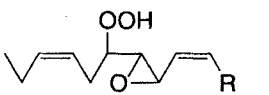
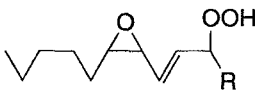
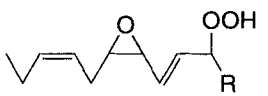
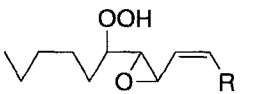
R_i(DB-1)	Dioxygenated fatty acid	Precursor
2460	11,12-DiOH-18:0	oleic acid epoxide
2479	9,10-DiOH-18:0	oleic acid epoxide
2444	12,13-DiOH-18:1	linoleic acid epoxide
2455	9,10-DiOH-18:1	linoleic acid epoxide
2435	9,10-DiOH-18:2	linolenic acid epoxide
2438	12,13-DiOH-18:2	linolenic acid epoxide
2514	15,16-DiOH-18:2	linolenic acid epoxide
2431	9,16-DiOH-18:0* (after hydrogenation with Pd/C)	
2487	9,13-DiOH-18:0* (after hydrogenation with Pd/C)	
2466	9,14-DiOH-18:0* (after hydrogenation with Pd/C)	

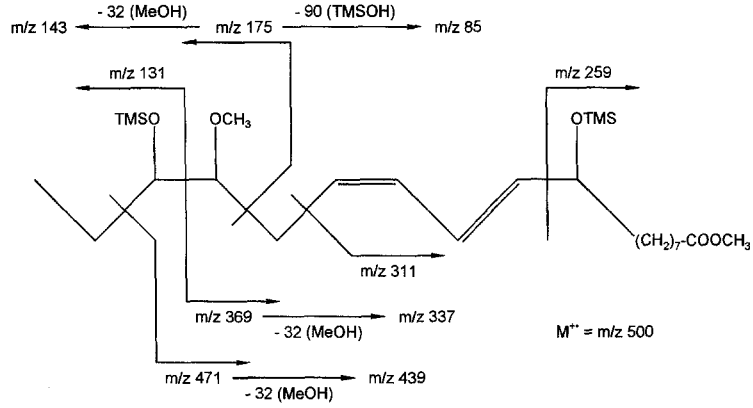
An investigation of the fraction containing methoxydihydroxy acids (Table 2) indicated that the primarily produced hydroperoxides may suffer a further epoxidation. GC measurements demonstrated that the 12,13-epoxide is the main epoxy derivative of linoleic and linolenic acid oxidized at carbon atom 9. Most of these products have been described earlier in soybean, pea, and cereals [16, 19, 20, 31]. The occurrence of two previously unknown epoxides (9-hydroxy-15, 16-epoxy-10, 12-octadecadienoic acid and 16-hydroxy-14, 15-epoxy-9, 12-octadecadienoic acid) is in agreement with the prediction that any double bond is attacked by epoxidation.

The structures of the new compounds were derived unambiguously from their mass spectra. Main fragments of 1,2-substituted hydroxymethoxy derivatives of saturated acids stem from a cleavage of the C–C bond between the two carbons carrying the functional groups (Scheme 1). Thus, 9,16-trimethylsilyloxy-15-methoxy stearic acid methyl ester suffers fragmentation to an ion of $m/z = 131$. The alternative cleavage product of $m/z = 369$ cannot be detected because of its quick decomposition by loss of methanol leading to a fragment of mass 337. In addition to these fragments, other characteristic ions are recognized, although in lower yield, result from alternative α-cleavages (*e.g.* ions of $m/z = 175$ and 259, Scheme 1). Trihydroxy fatty acids, already detected in rice plant, taro tuber and, soybean [32–34], have also been identified.

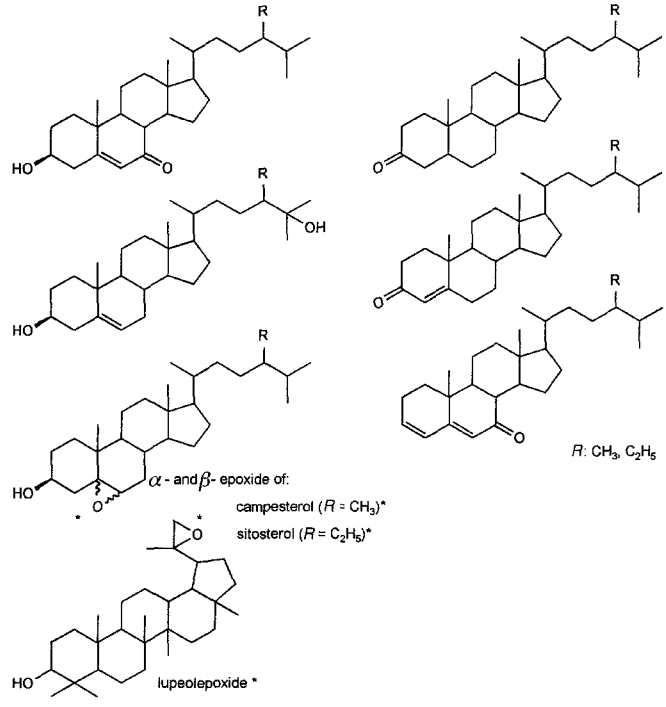
Oxidized sterols and pentacyclic triterpenes were obtained after TLC of methanol extracts and detected by GC-MS after trimethylsilylation. In addition to 7-hydroxy and 7-oxo derivatives of sitosterol and campesterol, their dehydration products (probably artefacts generated by elimination of water) and derivatives hydroxylated in position 25 were recognized epoxides as well as (Scheme 2). These epoxides could not be detected even in traces in not injured plants.

Table 2. Dihydroxy methoxy fatty acid methyl esters obtained from wounded cabbage leaves (identified as trimethylsilyl ethers); $R = (CH_2)_7$-COOH; *previously unknown; DiOH=dihydroxy

R_i(DB-1)	Identified structure	Precursor	R_i(DB-1)	Identified structure	Precursor
2579	14-Methoxy-15,16-DiOH-*c*9,*c*12-18:2*		2550	13-Methoxy-9,12-DiOH-*t*10,*c*15-18:2	
2568	15-Methoxy-9,16-DiOH-*t*10,*c*12-18:2*		2552	12-Methoxy-9,12-DiOH-*t*10,18:2	
2478	11-Methoxy-12,13-DiOH-*c*9,*c*15-18:2*		2550	13-Methoxy-9,12-DiOH-*t*10-18:2	
2550	12-Methoxy-9,13-DiOH-*c*10,*c*15-18:2*		2595	11-Methoxy-12,13-DiOH-*t*9-18:2	



Scheme 1. Fragmentation pattern for main mass spectrometric ions of 9,16-trimethylsiloxy-15-methoxy-10-octadecadienoic acid methyl ester



Scheme 2. Oxidized sterols and triterpenes in leaves of white cabbage; *only found in injured cabbage leaves

The same TLC fraction contained lupeolepoxide, also absent in not injured leaves (Scheme 2). Lupeol was found to be a main constituent of the non polar fraction accompanying the epoxidized sterols.

In vivo, the yield of products in plant tissue initiated by cell injury (*e.g.* immediately after attack of an insect or a fungus) is usually rather low. This fact makes it difficult to carry out kinetic measurements. Therefore, we tried to amplify the reactions occurring in plants after injury. As already outlined [23], we used for

this purpose homogenization, a process in which a great number of cells is injured within a short time. For comparison, we destroyed the enzymes prior to homogenization in another sample by cooking or cut off their action by homogenization in an organic solvent.

Changes in the composition of different plant constituents with low molecular weight were observed as an answer of plant cells to mechanic injury or attack of fungi as well as insects [35, 36]. Most of the previous investigations have been restricted to a single group of compounds, the so-called phytoalexines [37, 38]. The production of these components is described to be a rather long lasting process which reaches its maximum after several hours.

In contrast, an almost instant enzymatic attack to unsaturated fatty acids was reported [1]. These are converted to monohydroperoxides during this reaction. Decomposition of fatty acid hydroperoxides generates simple aldehydes, *e.g.* hexanal (derived from linoleic acid) [2], but also induces further reactions, *e.g.* production of jasmonic acid [6]. Synthesis of the latter compound requires a multi step reaction which needs considerable time to reach its climax production. Interestingly, we detected jasmonic acid in the plant even after boiling, indicating that low levels of jasmonic acid are obviously always present.

Lipid peroxidation of unsaturated fatty acids is observed in many diseased states of mammalians [39] and was studied therefore in detail. It has been shown that oxidation does not stop at the step of a monohydroperoxide, but that also dihydroperoxides are produced, detected either directly after HPLC/negative ion mass spectrometry [40] or indirectly after reduction and derivatization followed by a GC-MS electron impact analysis [41]. Recently it was found that such a twofold lipid peroxidation does not only transform linolenic acid to its 9,16-dihydroperoxy derivative [40], but also 9,13- and 9,14-derivatives are produced [41]. We were now able to detect these compounds in the injured leaves of cabbage, demonstrating that oxidation processes similar to those in mammalian tissue occur also in plants.

Generation of epoxides of unsaturated fatty acids is an already known process in plants after injury [2]. The detection of previously unknown 16-hydroperoxy-14, 15-epoxy-octadecadienoic acid demonstrates unambiguously that at least this epoxide was produced after hydroperoxidation at carbon atom 16 of linolenic acid; direct epoxidation of double bonds in linolenic acid produces exclusively a 9-, 10-, a 12-, 13-, or a 15-, 16-epoxide. Generation of a 14,15 epoxide requires the presence of a double bond in position 14 which is present only in a 16-hydroperoxide. This finding is in agreement with our recent observation that epoxidation processes are obviously slower than the generation of hydroperoxides [42].

Epoxidation is not restricted to unsaturated fatty acids as proven by the occurrence of sterol and terpene epoxides after homogenization of plant tissue in an oxygen atmosphere without previous destruction of enzyme activity. Remarkably, we were not able to detect these epoxides, even in traces, in samples with inactive enzymes. In contrast, other oxidation products derived from fatty acids or phenolic compounds were present in injured leaves as well as in not injured ones, even though in much lower yields in the latter. This seems to indicate that hydroperoxides of fatty acids are produced in normal plant metabolism, but epoxides of sterols and triterpenes are obviously generated only after injury. Their production seems to be connected with cell death: the occurrence of lupeolepoxide was

reported earlier in several dried plants extracts [43–45], and drying is connected with cell damage. Probably fresh plant tissue contains lupeol but not its epoxide.

Epoxidation products of sterols have been reported in investigations of nutrition compounds. Sitosterol and campesterol epoxides were detected in plant oils [46], dried leaves [47], and especially in fats [48, 49]. During storage, hydroperoxides are generated, and these have been shown to epoxidize double bonds [50, 51]. In agreement with this deduction, epoxidation of cholesterol could be demonstrated to occur *via* lipid peroxidation [51–53]. Results from our laboratory [21, 22] and other [16] suggest that peroxyl radicals are able to epoxidize unsaturated compounds. These peroxyl radicals are formed as intermediates by attack of lipoxygenases on unsaturated fatty acids, but also in the course of a radical induced lipid peroxidation reaction [54]. In addition, it cannot be excluded that the observed epoxidation reactions of sterols and terpenes might be due to the action of epoxidases. Such enzymes were found to cause the epoxidation of arachidonic acid in mammalian tissue [55, 56].

In addition to sterol epoxides, 7-oxo and 7-hydroxy sterols were detected. We suspect that these compounds are generated in a different way. They are also produced in absence of a lipid medium which seems to be necessary for the formation of sterol epoxides [57, 58], thus indicating that they are products of a non-enzymatic induced lipid peroxidation.

In conclusion, cell damage obviously activates a great number of enzymatic processes which do not only involve phenolic compounds or selected phytohormones (*e.g.* jasmonate) but also unsaturated fatty acids, sterols, and terpenes [59].

Experimental

Chemicals

N-methyl-N-trimethylsilyltrifluoroacetamide (*MSTFA*) was obtained from Macherey & Nagel (Düren, Germany). All other chemicals were purchased from Fluka (Neu Ulm, Germany). Solvents, obtained from Merck (Darmstadt, Germany), were distilled before use.

Plant material

White cabbage was obtained from local stores. Outer leaves were withdrawn, and the residue was divided in two samples: on the one hand, white cabbage leaves were cooked in H_2O for 15 min and homogenized with solvents; on the other hand, leaves of the same plant were homogenized with H_2O and stirred for 24 h at room temperature. Both samples were subjected to analogous extraction procedures.

Extraction of fatty acids

Lipid extraction followed the method of *Bligh* and *Dyer* [25]. Lipids were transesterified in *THF* with 1*M* $NaOCH_3$ (in methanol) at room temperature. The fatty acid methyl esters were subjected to CC on silica gel 60 (Merck), collecting three fractions (A: cyclohexane (*CH*): ehtylacetate (EtOAc) 98:2 (v/v), not oxidized fatty acid methyl esters. B: *CH*:EtOAc 1:1 (v/v), C: EtOAc:methanol (MeOH) 95:5 (v/v), both oxidized fatty acid methyl esters. Further separation was achieved by preparative TLC using a solvent system of *CH*:EtOAc 8:2 (v/v) (monohydroxy fatty acid methyl esters,

$R_f = 0.5–0.6$; dihydroxy fatty acid methyl esters, $R_f = 0.2–0.25$; methoxydihydroxy fatty acid methyl esters, $R_f = 0.1–0.15$; trihydroxy fatty acid methyl esters, $R_f = 0.0–0.1$). Trihydroxy fatty acid methyl esters were enriched by a second TLC in a solvent system containing CH:EtOAc3:7 (v/v) ($R_f = 0.5–0.3$).

TLC

Preparative TLC was performed on home-made plates (20 × 20 cm) coated with silica gel 60 PF_{254} (thickness 0.75 mm) (Merck). TLC zones were eluted with EtOAc.

Hydrogenation with Pd/H_2 on activated charcoal (5% Pd)

The methyl esters were hydrogenated for 2 h with palladium on activated charcoal (5% Pd) in MeOH at room temperature and a H_2 pressure of 1.5 bar.

Trimethylsilylation

Approximately 0.1–0.5 mg of the dried sample was dissolved in 10 µl EtOAc (purified and dried), and 20 µl *MSTFA* were added. The mixture was kept in darkness at room temperature for 12 h. 1 µl of the solution was subjected to GC and GC-MS.

Extraction of oxidized sterols

MeOH extracts of wounded and intact cabbage leaves were successively extracted with *CH* and EtOAc as described above. The sterols were obtained from EtOAc extracts by subsequent TLC separations (A: *CH*:EtOAc 8:2 (v/v), $R_f = 0.6–0.8$, not oxidized sterols; B: *CH*:EtOAc 1:1 (v/v), $R_f = 0.4–0.6$, oxidized sterols). Identification was achieved by GC-MS.

Gas chromatography

GC analyses were carried out with an United Technologies Packard Model 438S chromatograph equipped with a flame ionisation detector using a DB-1 fused silica capillary column (length: 30m; i.d.: 0.32 mm; film thickness: 0.1 µm; J&W Scientific, Mainz-Kastel, Germany). Temperature program: 80°C isotherm for three minutes, from 80°*C* to 280°C at 3°C/minute, 280°C isotherm for 15 minutes. Injector temperature: 270°C; detector temperature: 280°C; carrier gas: hydrogen; splitting ratio; 1:30. Retention indices were calculated according to *Kovats* [60] with *n*-alkanes ($C_{10}H_{22}$-$C_{30}H_{62}$) as reference compounds. The integrator was a Shimadzu CR-3A model.

Gas chromatography/mass spectrometry

Measurements were preformed on a double focussing mass spectrometer Finnigan MAT 95 running under electron impact conditions at 70 eV. A HP 5980 series II gas chromatograph with a 30m × 0.3 mm DB-1 fused-silica column was used for sample separation. Carrier gas: hydrogen temperature program: see above.

Trimethylsilyl derivatives

α- and β-*sitosterolepoxide*: GC (DB-1): $R_i = 3425$ (α-isomer), 3412 (β-isomer); GC-MS (70 eV): $m/z(\%) = 502$ (M^+, 90), 73 (85), 95 (66), 412 (65), 123 (55), 394 (42), 384 (33), 487 (20), 253 (19),

357 (10). α- and *β-compesterolepoxide*: GC (DB-1): $R_i = 3315$ (β-isomer); 3307 (β-isomer); GC-MS (70 eV): $m/z(\%) = 488$ (M^+, 90), 73 (100), 398 (60), 380 (40), 473 (20), 470 (18), 253 (20), 211 (21).

Lupeolepoxide: GC (DB-1): $R_i = 3590$; GC-MS (70 eV): $m/z(\%) = 514$ (M^+, 2), 189 (100), 75 (60), 190 (55), 456 (42), 109 (41), 95 (38), 135 (35), 123 (33), 175 (32).

14-Methoxy-15,16-dihydroxy-octadeca-9,12-dienoic acid methylester: GC (DB-1): $R_i = 2612$; GC-MS (70 eV): m/z (%) = 500 (M^+, 1), 233 (100), 73 (92), 143 (58), 131 (39), 44 (38), 340 (21), 378 (15), 55 (17), 267 (10), 147 (17).

15-Methoxy-9,16-dihydroxy-octadeca-10,12-dienoic acid methylester: GC (DB-1): $R_i = 2610$; GC-MS (70 eV): m/z (%) = 500 (M^+, 8), 131 (100), 73 (81), 337 (42), 175 (23), 259 (19), 378 (19), 410 (16), 143 (18), 468 (11).

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References

[1] Galliard T (1970) Phytochemistry **9**: 1725
[2] Gardner HW (1989) Biochim Biophys Acta **1001**: 274
[3] Gardner HW, Jursinic PA (1981) Biochim Biophys Acta **665**: 100
[4] van Os CPA, Vliegenthart JFG, Crawford CG, Gardner HW (1982) Biochim Biophys Acta **713**: 173
[5] Dix TA, Marnett LJ (1985) J Biol Chem **260**: 5351
[6] Dick BA, Zimmerman DC (1983) Biochem Biophys Res Comm **111**: 470
[7] Creelman RA, Tierney ML, Mullet JE (1992) Proc Natl Acad Sci USA **89**: 4938
[8] Farmer EE, Ryan CA (1990) Proc Natl Acad Sci USA **87**: 7713
[9] Choi D, Bostock RM, Avdiushko S, Hildebrand DF (1994) Proc Natl Acad Sci USA **91**: 2329
[10] Skriver K, Mundy J (1990) Plant Cell **2**: 503
[11] David WJ, Jones HG (1991) Abscisic Acid. Scientific Publishers, Oxford UK
[12] Hughes JC, Swain T (1960) Phytopathology **50**: 398
[13] Rhodes MJC, Hill ACR, Wooltorton LS (1976) Phytochemistry **15**: 707
[14] Tanaka Y, Uritani I (1977) Eur J Biochem **73**: 255
[15] Hammerschmidt R (1984) Plant Pathol **24**: 33
[16] Graveland A (1973) Lipids **8**: 599
[17] Sessa DJ, Gardner HW, Kleiman R, Weisleder D (1977) Lipids **12**: 613
[18] Arens D, Grosch W (1974) Z Lebensm-Unters Forsch **156**: 292
[19] Gardner HW, Weisleder D, Kleiman R (1978) Lipids **13**: 246
[20] Gardner HW, Weisleder D, Nelson EC (1984) J Org Chem **49**: 508
[21] Meyer W, Spiteller G (1993) Liebigs Ann Chem **1993**: 1253
[22] Meyer W, Spiteller G (1996) Z Naturforschung (in press)
[23] Spreitzer H, Spiteller G (1989) Monatsh Chem **120** 1139
[24] Bergers M, Verhagen AR, Jongerius M, Mier PD (1986) Biochim Biophys Acta **876**: 327
[25] Bligh EG, Dyer WJ (1959) Can J Biochem Physiol **37**: 911
[26] Minnikin DE, Patel DV (1979) Chem Phys Lipids **23**: 173

[27] Ryhage R, Stenhagen E (1960) Arkiv Kemi **15**: 545
[28] Woollard PM, Mallet Al (1984) J Chromatogr **306**: 1
[29] Lehmann WD, Stephan M; Fuerstenberger G (1992) An Biochem **204**: 158
[30] Toyoda I, Terao J, Matsushita S (1982) Lipids **17**: 84
[31] Esterbauer H, Schauenstein E (1977) Monatsh Chem **108**: 963
[32] Kato T, Yamaguchi Y, Abe N, Uyehara T, Namai T, Kodama M, Shiobara Y (1985) Tetrahedron Lett **26**: 2357
[33] Masui H, Kondo T, Kojima M (1989) Phytochemistry **28**: 2613
[34] Panossian AG, Avetissian GM, Mnatsakanian VA, Batrakov SG, Vartanian SA, Gabrelian ES, Amroyan, EA (1983) Planta Med **47**: 17
[35] Hahlbrock K, Scheel P (1987) Biochemical Responses of Plants to Pathogens. In: Chet I (ed) Innovation Approaches to Plant Disease Control. Wiley, New York, p 229
[36] Dixon RA (1986) Biol Rev Cambridge Philos Soc **61**: 239
[37] Lyons PC, Wood KV, Nicholson RL (1990) Phytochemistry **29**: 97
[38] Keen NT, Littlefield LJ (1979) Physiol Plant Pathol **14**: 265
[39] Halliwell B (1993) Oxygen Radicals as Key Mediators in Human Disease: Fact or Fiction? In: Parke DV, Ioannides R, Walker R (eds) Food, Nutr Chem Toxic [Int Conf 1st, Meeting Date 1991]. Smith-Gordon Ltd, London, p 129
[40] Rubbo H, Radi R, Trujillo M, Telleri R, Kalyanaraman B, Barnes S, Kirk M, Freeman BA (1994) J Biol Chem **269**: 26066
[41] Mlakar A, Spiteller G (1994) Biochim Biophys Acta **1214**: 209
[42] Dudda A, Spiteller G (1996) Chem Phys Lipids (in press)
[43] Bohlmann F, Jakupovic J, Schuster A, King RM, Robinson H (1984) Phytochemistry **23**: 1445
[44] Bohlmann F, Jakupovic J (1979) Phytochemistry **18**: 1189
[45] Corbett RE, Cong ANT, Holland PT, Wilkins AL (1987) Aust J Chem **40**: 461
[46] Nourooz-Zadeh J, Appelqvist LA (1992) J Am Oil Chem Soc **69**: 288
[47] Della Greca M, Fiorentino A, Molinaro A, Monaco P, Previtera L (1993) Nat Prod Lett **2**: 27
[48] Lee K, Herian AM, Richardson T (1984) J Food Prot **47**: 340
[49] Lee K, Herian AM, Higley N (1985) J Food Prot **48**: 158
[50] Walther U (1992) Thesis, University of Bayreuth
[51] Aringer L (1980) Lipids **15**: 563
[52] Johansson G (1971) Eur J Biochem **21**: 68
[53] Watabe T, Tsubaki A, Isobe M, Ozawa N, Hiratsuka A (1984) Biochim Biophys Acta **795**: 60
[54] Kayganich-Harrison KA, Rose DM, Murphy RC, Morrow JD, Roberts LJII (1993) J Lipid Res **34**: 1229
[55] Karara A, Dishman E, Blair I, Falck JR, Capdevila JH (1989) J Biol Chem **264**: 19822
[56] VanRollins M, Baker RC, Sprecher HW, Murphy RC (1984) J Biol Chem **259**: 5776
[57] Yanishlieva N, Schiller H (1994) J Sci Food Agric **35**: 219
[58] Yuanishlieva N, Marinova E, Schiller H, Seher A (1985) Dev Food Sci 11 (Fat Sci 1983, Pt B): 619
[59] Cornwell DG, Morisaki N (1984) Fatty Acid Paradoxes in the Control of Cell Proliferation: Prostaglandins, Lipid Peroxides, and Cooxidation Reactions. In: Pryor WA (ed) Free Radicals Biol 6. Academic Press, Orlando, p 95
[60] Kovats E (1958) Helv Chim Acta **41**: 1915

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