Chemistry and Physics of Lipids, 24 (1979) 265–276

SYNTHESIS AND ENZYMATIC CONVERSION OF AN ETHER ANALOGUE OF MONOGALACTOSYL DIACYLGLYCEROL

E. HEINZ, H.P. SIEBERTZ and M. LINSCHEID

Institutes of Botany and Organic Chemistry, University of Cologne, D-5000 Köln 41, Gyrhofstr. 15 and Greinstr. 4, (F.R.G.)

Received December 14th, 1978 accepted February 26th, 1979

The synthesis of 1,2-di-*O*-9′-octadecenyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol is described, including a detailed discussion of mass spectrometric fragmentation patterns of this and related substances. Enzymatic experiments showed that this compound is converted by plant enzymes to the 6-*O*-acyl derivative. The availability of the di-9-octadecynyl compound for tritium reduction will provide a substrate for studies on direct desaturation of lipid-linked acyl or alkyl chains.

Introduction

Evidence is increasing of the desaturation of lipid-linked acyl groups. Oleoyl groups at the *sn*-1- and *sn*-2-positions of phospholipids such as phosphatidyl choline and -ethanolamine have been shown to be converted to linoleoyl groups by microsomal fractions prepared from yeasts and rat liver [1–5].

The reactions resembled microsomal stearoyl-CoA desaturation in their requirement for molecular oxygen and NAD(P)H and their inhibition by cyanide but not by carbon monoxide pointing to the involvement of cytochrome b_5. But in contrast to stearoyl-CoA desaturation, saturated acyl groups in phospholipids could not be desaturated [1,4]. It has been suggested that similar reactions take place in the plant kingdom. In addition to phospholipids, among which phosphatidyl choline is the most favoured candidate [6–9], galactolipids have also been proposed as direct substrates for desaturations, particularly in organisms such as blue-green algae, which do not contain phosphatidyl choline [10]. Our own experiments [11] on changing labelling patterns of molecular species of leaf galactolipids were interpreted in two ways, since experimental evidence did not allow a decision between two alternatives. At early sampling times the more saturated lipid species predominated, whereas with longer incubation times increasing proportions of radioactivity accumulated in the more unsaturated species. These patterns could be due either to direct and consecutive desaturation of a nearly saturated galactolipid species, or they could be explained by assuming that various acyl thioester pools of increasing degrees of unsaturation drained off minor proportions of acyl residues into lipids without further desaturation.

A radioactive galactolipid having alkyl ether instead of ester groups should be an interesting compound for investigating a direct desaturation of galactolipids in plants, since hydrolysis as possible with acyl groups followed by classical CoA-dependent desaturation, and reincorporation into lipids can be excluded. In this paper we describe the synthesis of ether analogues of monogalactosyl diacylglycerol and some enzymatic experiments which show that these compounds are accepted by plant enzymes which metabolize galactolipids.

Materials and methods

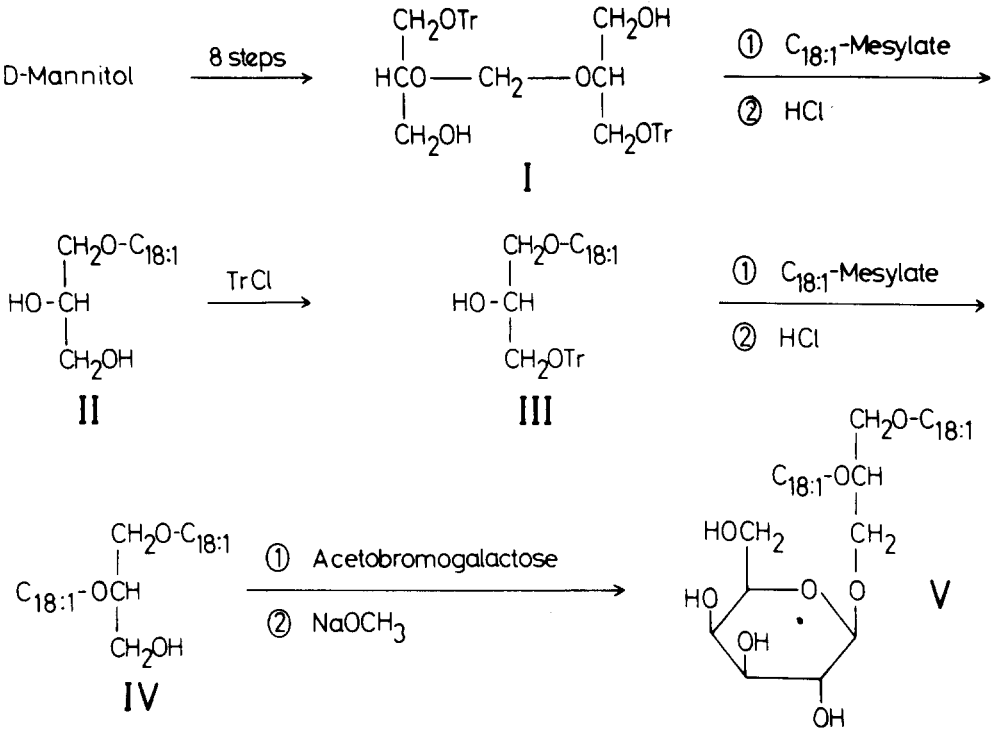
Solvent mixtures used for chromatography (Table I) are characterised by volume ratios. Petroleum ether had a boiling range of 40–60°C. When necessary, solvents were dried over molecular sieves. Oleic and palmitoleic acid were purchased from Roth, diazabicycloundecene (DBU) was obtained from Aldrich, acetobromogalactose from Koch & Light. Optical rotations were measured with a Zeiss Lichtelektrisches Präzisionspolarimeter 0.005°, NMR spectra recorded at 90 MHz with a Varian EM 390 90 MHz instrument and mass spectra taken on a MAT 731 spectrometer. Elemental analyses were carried out by the A. Bernhardt Analytical Laboratory. For GLC a Hewlett Packard gaschromatograph model 5700 A with FIDs was used. Methyl esters were separated on 15% Reoplex on chromosorb W (steel column, 2.5 m × 0.3 cm), trimethylsilyl ether derivatives of galactolipids on 2% SE 30 on Gaschrom P (steel column 1 m × 0.3 cm).

9-Octadecynyl methanesulfonate

Oleic acid (10 g) was esterified by refluxing in HCl/MeOH. The resulting methyl oleate (10.3 g) was dissolved in CH_2Cl_2 (100 ml) and converted to methyl 9,10-dibromostearate (15 g) by addition of 2.1 ml of Br_2 in CH_2Cl_2 (50 ml). Dehydrobromination [12] by heating for 5 h at 120°C with DBU (15 g) gave pure methyl 9-octadecynoate (9 g) as checked by GLC. On Reoplex it had a retention time very close to that of methyl linolenate. Reduction with $LiAlH_4$ in refluxing diethyl ether gave 9-octadecynol (7 g), which was subsequently reacted overnight [13] with methanesulfonyl chloride (4 ml) in pyridine to give octadecynyl methanesulfonate (7.8 g), which after the usual work up was dissolved in dry xylene.

1,2-di-O-9'-octadecynyl-3-O-β-D-galactopyranosyl-sn-*glycerol* V

Starting with D-mannitol 2,2'-methylene-bis-(3-*O*-triphenylmethyl-*sn*-glycerol) *I* (Scheme 1) was prepared via 2,5-methylene-D-mannitol according to established procedures [14,15]. *I* (6.5 g) was refluxed in xylene (100 ml) over powdered KOH (6.5 g) using a phase separation head for water removal [13]. After 2 h 9-octadecynyl methanesulfonate (6.9 g) in xylene (50 ml) was added dropwise and refluxing



Scheme 1. Steps in the synthesis of an ether analogue *V* of monogalactosyl diacylglycerol.

continued for 7 h. After cooling and addition of diethyl ether/petroleum ether (1 : 1, 400 ml), the organic phase was washed with water and dried over Na_2SO_4. The product obtained after solvent removal, presumably 2,2′-methylene-bis-(1-*O*-9′-octadecynyl-3-*O*-triphenylmethyl-*sn*-glycerol), was hydrolysed by refluxing in 1 N HCl in MeOH (150 ml). After 3 h the reaction mixture was diluted, washed with bicarbonate and water and dried as described above. The crude product was purified by column chromatography on SiO_2 using petroleum ether/diethyl ether/$CHCl_3$ 50 : 50 : 1 for elution of 1-*O*-9′-octadecynyl-*sn*-glycerol *II* (4 g). This compound was dissolved in pyridine and an excess of triphenylmethyl chloride (8 g) added with stirring. After two days at room temperature the reaction mixture was diluted, washed and dried as above. Column chromatography on SiO_2 gave pure 1-*O*-9′-octadecynyl-3-*O*-triphenylmethyl-*sn*-glycerol *III* (3.8 g), which was eluted with petroleum ether/diethyl ether 9 : 1. This compound was again reacted with 9-octadecynyl methanesulfonate (3 g) exactly as described above. Hydrolysis and column chromatography on SiO_2 gave 1,2-di-*O*-9′-octadecynyl-*sn*-glycerol *IV* (2.4 g), which was eluted with petroleum ether/diethyl ether 94:6. $[\alpha]_D^{26} = -6.6°$ (*c* 3.5 in $CHCl_3$). Reported values [16–19] for compounds differing in degree of

unsaturation (−7.5° and −6.9° for dioctadecyl and −8.8° for dioctadecenyl derivatives) or having opposite configuration (+ 7.4° for dioctadecenyl and + 6.7° for 2-octadecyl-3-octadecenyl-*sn*-glycerol) are of the same magnitude (Found: C 79.28; H 12.16. Calc. for $C_{39}H_{72}O_3$: C 79.53; H 12.32). An aliquot of *IV* was acetylated in pyridine with acetic anhydride and purified by preparative TLC on Kieselgel G with petroleum ether/diethyl ether, 2 : 1, for mass spectrometric (Fig. 1) analysis.

For Königs-Knorr galactosylation *IV* was dissolved in CH_2Cl_2 (7 ml). Then Ag_2O (2.5 g) was added followed by acetobromogalactose (2.8 g in 5 ml of CH_2Cl_2). After three days stirring in the dark in the presence of molecular sieves (4Å, 2 mm pearls) the reaction mixture was diluted with $CHCl_3$, filtered and washed with NaCl-solution (0.5%). Evaporation of solvents and deacetylation in 0.1 M sodium methoxide (150 ml, 1 h at 40°C) gave a crude product which was fractionated by SiO_2 column chromatography to give 1,2-di-*O*-9′-octadecynyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol *V* (1.3 g), which was eluted with $CHCl_3$/acetone 88 : 12. This compound could be obtained as an amorphous white powder by precipitation from warm ethyl acetate. Sintering point 55°C, m.p. 76°C. $[\alpha]_D^{27}$ = −5.1° (*c* 4.5 in $CHCl_3$) as compared to approx. −2° for ester analogues (Found: C 71.87; H 10.81.Calc. for $C_{45}H_{82}O_8$: C 71.96; H 11.06).

TABLE I

R_F-VALUES AND YIELDS OF INTERMEDIATES

For TLC on Kieselgel G the following solvent mixtures were used: 1, petroleum ether/diethyl ether, 90 : 3; 2, $CHCl_3$/acetone, 85 : 15; 3, petroleum ether/diethyl ether, 2 : 1; 4, $CHCl_3$/MeOH, 85 : 15. Compounds were detected by spraying with 50% aqueous H_2SO_4 followed by charring at 130°C. Triphenylmethyl derivatives became yellow during spraying and turned brown after heating. In each case yields are calculated in relation to the preceeding intermediate (taken as 100%), for which a yield is given. R_F-values in brackets refer to chromatography on $AgNO_3$-impregnated plates.

Compound	Solvent	R_F	Yield (%)
Octadecenoic acid	1	0.08	100
Methyl octadecenoate	1	0.35 (0.24)	98
Methyl 9,10-dibromooctadecanoate	1	0.28 (0.35)	95
Methyl octadecynoate	1	0.28 (0.24)	93
Octadecynol	1	0.07	86
Octadecynyl methanesulfonate	1	0.09	86
2,2′-Methylene-bis-(3-trityl-*sn*-glycerol) *I*	2	0.36	100[a]
2,2′-Methylene-bis-(1-octadecynyl-3-trityl-*sn*-glycerol)	2	0.83	–
1-Octadecynyl-*sn*-glycerol *II*	3	0.15	59
1-Octadecynyl-3-trityl-*sn*-glycerol *III*	3	0.38	57
1,2-Dioctadecynyl-3-trityl-*sn*-glycerol	3	0.78	–
1,2-Dioctadecynyl-*sn*-glycerol *IV*	3	0.33	61
1,2-Dioctadecynyl-3-galactopyranosyl-*sn*-glycerol *V*	4	0.51	44

[a] Also set as 100%.

For mass spectrometry (Fig. 2) and NMR spectrocopy an aliquot was acetylated, and purified by preparative TLC on Kieselgel G in diethyl ether/petroleum ether, 2 : 1. For GLC as trimethylsilyl ether an aliquot was dissolved in pyridine, reacted with excess of trimethylsilyl chloride and extracted into petroleum ether.

During column chromatography of the galactolipid ether some unreacted dioctadecynylglycerol *IV* (780 mg) was recovered by elution with $CHCl_3$/acetone, 96 : 4. In addition, the Königs-Knorr reaction repeatedly produced a small proportion of a more polar byproduct, which was isolated by preparative TLC on Kieselgel G in $CHCl_3$/MeOH 85 : 15 and analysed by mass spectrometry as acetyl and trimethylsilyl derivative.

In addition to *V* the following compounds were synthesised by using different methanesulfonates for alkylation: 1,2-Di-*O*-9′-octadecenyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol *VI* $[\alpha]_D^{23} = -4.7°$ (*c* 3.2 in $CHCl_3$).

1-*O*-9′-octadecenyl-2-*O*-9′-hexadecenyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol *VII* $[\alpha]_D^{26} = -7.4°$ (*c* 3.5 in $CHCl_3$).

1,2-Di-*O*-hexadecyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol *VIII* $[\alpha]_D^{23} = -4.2°$ (*c* 2.5 in pyridine), sintering point 85–91°C, m.p. 105–123°C after precipitation from $CHCl_3$/MeOH.

Enzymatic formation of the 6-O-acyl derivative of VI = IX

For the preparative enzymatic formation of this compound protein solution (6 ml, purified as described before [20]), acetate buffer (6 ml, 1 M, pH 5.3) and substrate dispersion (6 ml), containing a mixture of 32 mg of synthetic [21] 1-*O*-oleoyl-2-*O*-palmitoleoyl-3-*O*-(6-*O*-α-D-galactopyranosyl-β-D-galactopyranosyl)-*sn*-glycerol and 24 mg of *VI* dispersed in 20 mM sodium desoxycholate (pH 7.3) as described before [20], were incubated in a separation funnel at room temperature. After 30 min $CHCl_3$/MeOH(2 : 1, 200 ml) was added and the organic phase washed with 0.5% NaCl solution (30 ml). The product *IX* recovered from the organic phase was acetylated for mass spectrometry (Fig. 4).

Mass spectrometry of synthesised compounds

The mass spectra to be discussed in the following were recorded for acetylated compounds, each of which gave molecular and fragment ions for glycerol and galactose moieties (if present), which were most important for structural considerations. In the case of acetylated *IV* (Fig. 1) the signal of the molecular ion is at *m/e* 630. The mass region up to 200 amu is dominated by intensive fragments originating from the alkynyl chains (*m/e* 109 is the most frequent ion from 100 to 700 amu). The loss of alkyl radicals from the molecular ion is determined by the triple bonds; starting with $(M-C_2H_5)^+$ the series of ions ceases with $(M-C_8H_{17})^+$ at *m/e* 517. The peak at *m/e* 365 may be due to an ion of cyclic structure similar to the known ones [22] formed by loss of $C_{18}H_{33}$. Cleavage between C-1 and C-2 of the glycerol

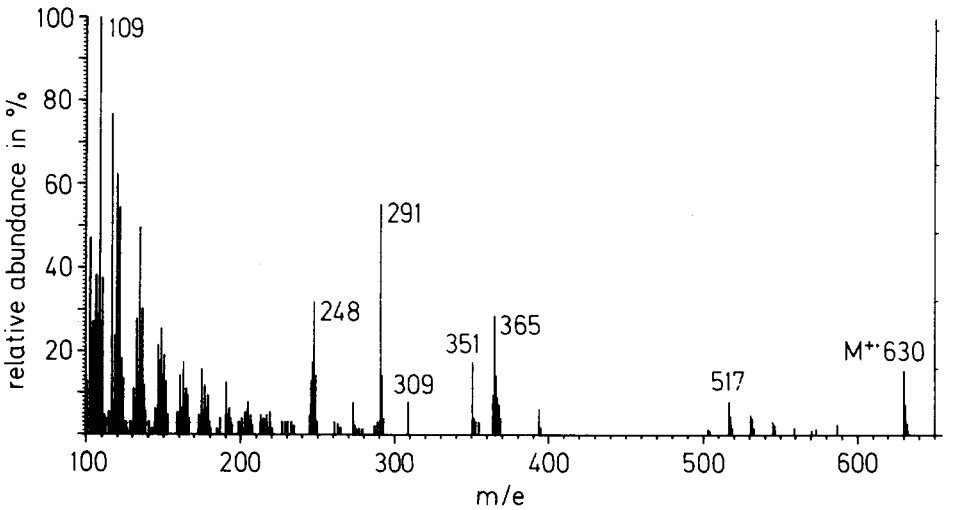
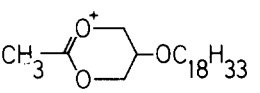
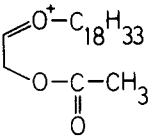


Fig. 1. Mass spectrum of acetylated 1,2-di-*O*-9′-octadecynyl-*sn*-glycerol *IV*.

moiety produces ions at *m/e* 351, which are able to lose CH_3COOH or C_2H_2O (*m/e* 291 or 309):

m/e 365 

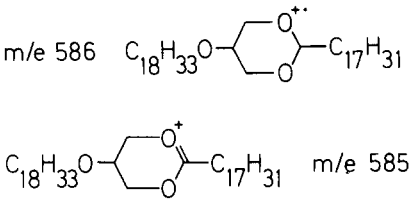


The base peak in the mass spectrum of acetylated *V* (Fig. 2) is *m/e* 331 and represents the galactose moiety [22]. The known fragmentation pathway of peracetylated hexoses leads to *m/e* 271 (331–CH_3COOH), *m/e* 229 (–CH_3COOH, –C_2H_2O), *m/e* 211 (–2 × CH_3COOH) and *m/e* 169 (–2 × CH_3COOH, –C_2H_2O). The complementary part of the molecule can be recognized by peaks at *m/e* 587 (M–331) +, *m/e* 571 (M–331–16) + and *m/e* 557 (M–331–30) +. Cleavage between C-1 and C-2 of glycerol or between C-1 and C-2 of the ether side chain yields two different sets of fragments formed by successive or competing loss of CH_3COOH and C_2H_2O:

M–$C_{18}H_{33}OCH_2$ (*m/e* 639, 579, 537, 519, 477, 459, 417, 399) and M–$C_{17}H_{31}$ (*m/e* 683, 641, 599, 563, 503, 443).

A closer examination of the spectrum reveals other features of these compounds in contrast to their acyl analogues [22]: the loss of H_2O from molecular ions in some cases (see Figs. 3,4) and the elimination of the sugar moiety to form ions at *m/e* 586 and 585, which can lose alkyl and alkynyl radicals, respectively. The stability

of these may be explained again by cyclisation [23,24] :



The same fragmentation pattern is observed in the mass spectrum of acetylated *VII* (Fig. 3). The presence of two different alkenyl chains allows a positional analysis. The C-1 linked octadecenyl group dominates the fragmentation of the glycerol part of the molecule. Therefore only the loss of $C_{18}CH_{35}$–O–CH_2 (*m/e* 613) from the molecular ion (*m/e* 894) and from fragments formed by elimination of acetic acid or ketene is recorded. Again elimination of 332 and 333 amu and

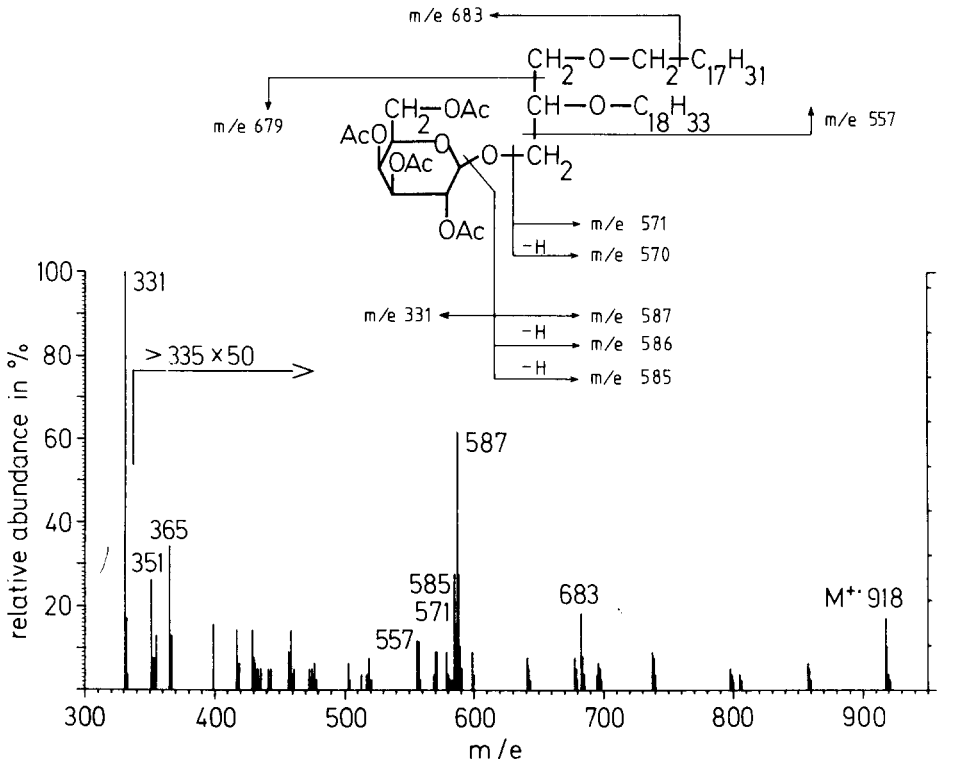
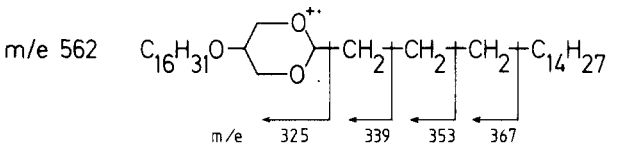


Fig. 2. Mass spectrum of acetylated 1,2-di-*O*-9′-octadecynyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol *V*.

subsequent loss of alkenyl radicals up to $C_{17}H_{33}$ from the side chains reconfirms the structures proposed for these ions:



The peaks at *m/e* 525 and 553 are the most abundant ones in the spectrum of enzymatically-produced and subsequently acetylated *IX* (Fig. 4). They represent the sugar moiety carrying oleic or palmitoleic acid residues at C-6 of galactose. The acylium ions of the acids (*m/e* 237 and 265) and the signals due to loss of acetic acids or ketene from *m/e* 525 and 553 form the lower part of the spectrum. The peaks due to elimination of galactose at *m/e* 591, 590 and 589 as well as at *m/e* 576, 575 and 574 reflect the glycerol part in the way discussed above. The molecular ions *m/e* 1144 and *m/e* 1116 and the elimination products $(M-n \times 600)^+$ are recorded and confirm the structure of the enzymatically formed compound.

The byproduct isolated after Königs-Knorr galactosylation was analysed as acetyl and trimethylsilyl derivatives. It turned out to be a galactoside carrying a long chain ether glycosidically linked to C-1. The nature of the aglycone depended on the methanesulfonate used during the synthesis. The byproduct isolated after synthesis of *VII* was a mixture of octa- and hexadecenyl galactoside. The mass spectra showed in each case the molecular ion and ions resulting from cleavage

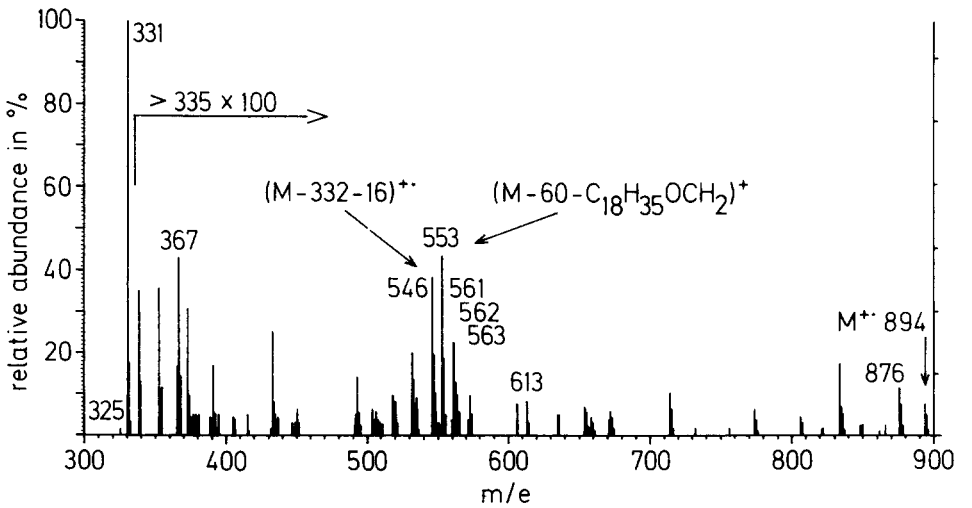


Fig. 3. Mass spectrum of acetylated 1-*O*-9′-octadecenyl-2-*O*-9′-hexadecenyl-3-*O*-β-D-galactopyranosyl-*sn*-glycerol *VII*.

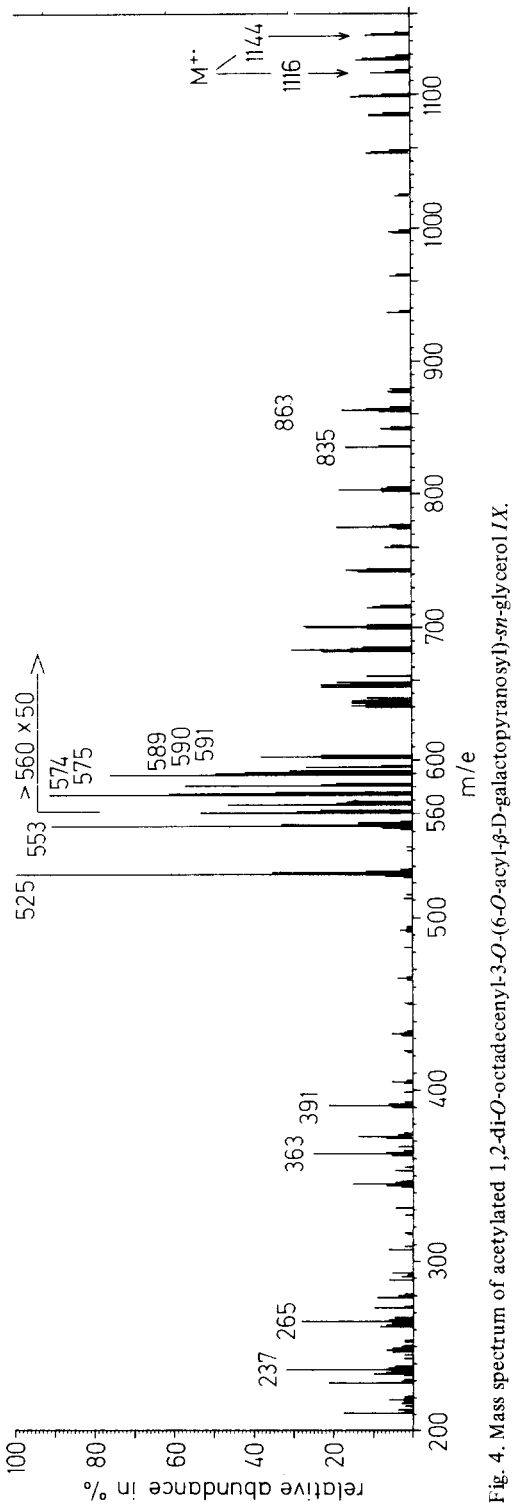


Fig. 4. Mass spectrum of acetylated 1,2-di-*O*-octadecenyl-3-*O*-(6-*O*-acyl-β-D-galactopyranosyl)-*sn*-glycerol *IX*.

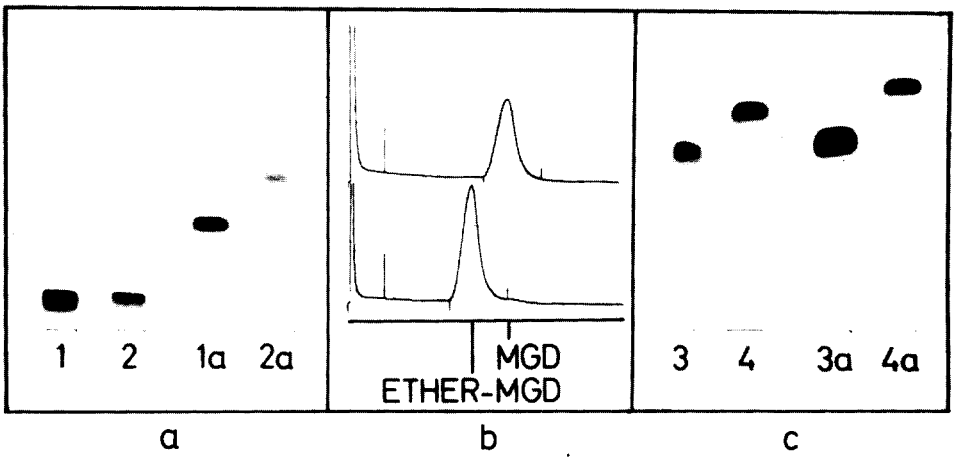


Fig. 5. Chromatographic comparison of monogalactosyl diacylglycerols and -dialkylglycerols (a) TLC of ester (1) and ether (2) before and after acetylation (1a, 2a) on Kieselgel G in diethyl ether/MeOH/isopropanol 100 : 2.5 : 4 for (1,2) and in diethyl ether/petroleum ether 1 : 1 for (1a, 2a). (b) GLC of trimethylsilyl derivatives of di-hexadecyl ether *VIII* and di-hexadecanoyl ester galactolipid. (c) TLC of enzymatically formed 6-*O*-acyl derivative of the di-ester (3) or di-ether (4) analogue *IX* before and after acetylation (3a, 4a) in solvents as in (a).

of the glycosidic bond. In the mass spectrum of the silylated compound the intense peaks at *m/e* 204 and 217 and a smaller one at *m/e* 305 are due to the well-known fragments of silylated hexose moieties.

Results and discussion

To arrive at the desired *sn*-1,2-di-alkyl glycerol backbone the synthetic sequence was started with D-mannitol following established procedures [14,15]. After appropriate blocking and chain cleavage two identical, asymmetrically substituted glycerol units I (Scheme 1) are obtained providing free *sn*-1-hydroxyl groups for the first alkylation. Ether linkages were formed by making use of the versatile methanesulfonates [13]. Hydrolysis, selective triphenylmethylation of the primary *sn*-3-hydroxyl group and introduction of a second and, if intended, different alkyl group into the *sn*-2 position (see *VII*, Fig. 3) followed a previously described way [17,25], which resulted, after final hydrolysis and purification, in 1,2-di-*O*-alkyl-*sn*-glycerols *IV*. The structure of this intermediate was confirmed for 1,2-di-*O*-9'-octadecynyl-*sn*-glycerol by mass spectrometry (Fig. 1) and elemental analysis. Its optical rotation demonstrates chirality and is of the same magnitude as reported for glycerol ethers of identical or enantiomeric configuration but differing in the nature of alkyl groups [16–19]. The final Königs-Knorr reaction yielded the desired β-galactosidic linkage as evident from the low and negative optical rotation

and the NMR spectrum of the acetylated compound, which showed the expected doublet at 4.55 ppm with $J = 8$ Hz characteristic for H-1 of galactose in β-glycosidic linkage. A signal for an α-anomeric proton with a small coupling constant in the region of 4.9 ppm was missing. The overall structure was confirmed by mass spectrometry (Fig. 2).

TLC did not differentiate between ether and ester galactolipids in underivatized form but, after acetylation, the ether had a slightly higher mobility than the ester (Fig. 5). Apparently the slight difference in polarity between both compounds becomes more effective in the rather apolar conditions used to chromatograph acetates. GLC of the trimethylsilyl derivatives of ester and ether galactolipids resulted in the expected separation due to the lower molecular weight of the ether and an accordingly lower elution temperature (Fig. 5).

Acceptance of ether galactolipids by plant enzymes was tested by assaying enzymatic acylation of *VI*. In this reaction acyl groups from the glycerol part of an acyl donor lipid (in this case digalactosyl diacylglycerol) are transferred to C-6 of the galactose moiety of monogalactosyl diacylglycerol resulting in the formation of acylgalactosyl diacylglycerol [26]. This reaction can be carried out with an enriched protein fraction containing the enzyme. This enzyme also accepts the ether analogue *VI* as acyl acceptor. Mass spectrometry (Fig. 4) of the isolated (Fig. 5) and acetylated product confirmed its structure as being 1,2-di-*O*-octadecenyl-3-*O*-(6-*O*-acyl-β-D-galactopyranosyl)-*sn*-glycerol. By using a synthetic [21] digalactosyl diacylglycerol as acyl donor with oleoyl groups at C-1 and palmitoleoyl groups at C-2, previous results were confirmed which had shown that the enzyme transfers acyl groups from both positions of the donor molecules [20]. Therefore, the acylated ether contained equimolar proportions of oleic and palmitoleic acid residues at C-6 of the galactose moiety (Fig. 4).

After this positive results we thought it justified to prepare a radioactive ether galactolipid for investigating direct desaturations. Considering the slow appearance of radioactivity in polyunsaturated fatty acids during labelling of leaves and the need for a compound of very high specific activity we decided to prepare the ether galactolipid *V* with two triple bonds. These are suitable for reduction with tritium gas leading to *cis*-double bonds. In this way an ether analogue having two 9-*cis*-octadecenyl groups and the desired high specific radioactivity will be available for further experiments. In this connection, naturally occurring compounds should be mentioned, which have structures similar to the substances we have synthesised. Diether analogues of glycolipids have so far only been detected in *Halobacterium* where phytanyl groups occupy the *sn*-2-and -3 position of glycerol with a sulfated trisaccharide chain occupying the *sn*-1 position [27]. Ether galactolipids are more abundant in the animal kingdom, where they have only one ether group, usually localized at *sn*-1, an ester at *sn*-2 and, occasionally, a sulfated β-galactopyranosyl group at *sn*-3 [28–32]. In human gastric content a similar ether ester glycolipid with a sulfated triglucosyl chain has been found [33]. Plant lipids with alkyl groups at the glycerol part have not been demonstrated with certainty, although alk-1-enyl ether residues occur in various lipids of plant origin [34].

Acknowledgements

We gratefully acknowledge the expert technical assistance by Miss K. Wrage and financial support by the Deutsche Forschungsgemeinschaft. We also thank Professors H. Budzikiewicz and H. Reznik for their continuous interest.

References

1 N. Baker and F. Lynen, Eur. J. Biochem., 19 (1971) 200.
2 B. Talamo, N. Chang and K. Bloch, J. Biol. Chem., 248 (1973) 2738.
3 E.L. Pugh and M. Kates, Biochim. Biophys. Acta, 316 (1973) 305.
4 E.L. Pugh and M. Kates, Biochim. Biophys. Acta, 380 (1975) 442.
5 E.L. Pugh and M. Kates, J. Biol. Chem., 252 (1977) 68.
6 M.I. Gurr, M.P. Robinson and A.T. James, Eur. J. Biochem., 9 (1969) 70.
7 M.I. Gurr, Lipids, 6 (1971) 266.
8 C. Willemot and G. Verret, Lipids, 8 (1973) 588.
9 P.G. Roughan, Lipids, 10 (1975) 609.
10 R.S. Appleby, R. Safford and B.W. Nicholas, Biochim. Biophys. Acta, 248 (1971) 205.
11 H.P. Siebertz and E. Heinz, Z. Naturforsch., 32c (1977) 193.
12 F.D. Gunstone and G.M. Hornby, Chem. Phys. Lipids, 3 (1969) 91.
13 W.J. Baumann and H.K. Mangold, J. Org. Chem., 29 (1964) 3055
14 A.T. Ness, R.M. Hann and C.S. Hudson, J. Am. Chem. Soc., 65 (1943) 2215.
15 B. Wickberg, Acta Chem. Scand., 12 (1958) 1187.
16 M. Kates, T.H. Chan and N.Z. Stanacev, Biochemistry, 2 (1963) 394.
17 B. Palameta and M. Kates, Biochemistry, 5 (1966) 618.
18 G.K. Chacko and D.J. Hanahan, Biochim. Biophys. Acta. 164 (1968) 252.
19 J.S. Chen and P.G. Barton, Can. J. Biochem., 48 (1970) 585.
20 E. Heinz, H.P. Dieler and J. Rullkötter, Z. Pflanzenphysiol., 75 (1975) 78.
21 E. Heinz, Biochim. Biophys. Acta, 231 (1971) 537.
22 H. Budzikiewicz, J. Rullkötter and E. Heinz, Z. Naturforsch., 28c (1973) 499.
23 A.J. Aasen, W.M. Lauer and R.T. Holman, Lipids, 5 (1970) 869.
24 H.J. Drexler, H. Schiller and A. Seher, Chem. Phys. Lipids, 20 (1977) 71.
25 W.J. Baumann and H.K. Mangold, J. Org. Chem., 31 (1966) 498.
26 E. Heinz, Z. Pflanzenphysiol., 69 (1973) 359.
27 M. Kates and P.W. Deroo, J. Lipid Res., 14 (1973) 438.
28 W.T. Norton and M. Brotz, Biochem. Biophys. Res. Commun., 12 (1963) 198.
29 M.G. Rumsby, Neurochem., 14 (1967) 733.
30 J. Ishizuka, M. Suzuki and T. Yamakawa, J. Biochem., 73 (1973) 77.
31 M. Levine, M.J. Kornblatt and R.K. Murray, Can. J. Biochem., 53 (1975) 679
32 J. Ishizuka, M. Inomata, K. Ueno and T. Yamakawa, J. Biol. Chem., 253 (1978) 898.
33 B.L. Slomiany, A. Slomiany and G.B.J. Glass, Eur. J. Biochem., 78 (1977) 33.
34 H.K. Mangold, in F. Snyder (Ed.), Ether Lipids, Chemistry and Biology, Academic Press, New York, 1972, p. 399.