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SHORT REPORTS

THERMAL PROPERTIES OF 1-HEXADECANOYL-2-*TRANS*-3-HEXADECENOYL PHOSPHATIDYLGLYCEROL

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Key Word Index—Phosphatidylglycerol; *trans*-3,hexadecenoic acid; parinaric acid; thermal properties.

Abstract—Techniques are described for the isolation of 1-hexadecanoyl-2-*trans*-3-hexadecenoyl phosphatidylglycerol and *trans*-3-hexadecenoic acid, using reversed phase HPLC. The phase transition temperature of 1-hexadecanoyl-2-*trans*-3-hexadecenoyl phosphatidylglycerol is 32° (Na^+ form) and 43° (Mg^{2+} form) while the melting point of *trans*-3-hexadecenoic acid is 54–55°. These values are *ca* 10° lower than those of the fully saturated analogues and it is concluded that 1-hexadecanoyl-2-*trans*-3-hexadecenoyl phosphatidylglycerol cannot be considered as a saturated lipid in terms of its proposed role in the chilling sensitivity of plants.

INTRODUCTION

The concept that phase separations in membrane lipids are primary events in inducing chilling injury in some higher plants has met with broad, although not complete, acceptance in recent years [1–3]. However, proposals that the fatty acid composition of plastid phosphatidylglycerol (PG) may be a determining factor in chilling sensitivity [4–7] has focussed attention on molecular species of PG which possess a comparatively high phase separation temperature. Such molecular species include 16:0, 16:0 PG and 16:0, 16:1 (*trans*-3) PG. The latter has been considered to have thermal properties very similar to those of 16:0, 16:0 PG [4, 6] by virtue of the fact that the double bond of 16:1 is a *trans* configuration and is located close to the carboxyl group of the fatty acid. However, no data exists on the thermal properties of 16:0, 16:1 (*trans*-3) PG and the assumption that its physical properties are very similar to those of the corresponding fully saturated PG has been questioned [8].

This communication describes HPLC techniques for the isolation of 16:0, 16:1 (*trans*-3) PG and 16:1 (*trans*-3) from leaves of higher plants and reports some of their thermal properties.

RESULTS AND DISCUSSION

Fatty acid analysis of 16:0, 16:1 (*trans*-3) PG prepared as described showed that the composition was 16:0, 46%; 16:1 (*trans*-3) 48%; 18:0, 1%; 18:1, 4% and 18:2, 1%. Molecular species analysis revealed that 16:0; 16:1 (*trans*-3) was the major species present (94%) with small amounts of 18:1, 16:1 (*trans*-3) (3%) and 16:0, 16:0 (2%) (Fig. 1B). The influence of the *trans*-3 double bond on the chromatographic properties of the *p*-methoxybenzoyl derivatives of diacylglycerols (DAG) derived from PG is clearly shown in Fig. 1. The R_f of the 16:0, 16:1 (*trans*-3) molecular species (Fig. 1B) is quite distinct from that of the 1,2 di-16:0 molecular species (Fig. 1A) and from 16:1, (*cis*-9) 16:0 (Fig. 1C) which is the major molecular species of PG from the cyanobacterium *Anacystis nidulans* [9].

The phase transition temperature of the Na^+ and Mg^{2+} salts of 16:0, 16:1 (*trans*-3) PG were determined by fluorescence polarization of *trans*-parinaric acid, and compared with that of the Na^+ salt of 16:0, 16:0 PG. This latter compound has been reported to have a phase transition temperature of 41° [10], and the results obtained by the present technique are in excellent agreement (Fig. 2A). In contrast, the phase transition temperature of the Na^+ salt of 16:0, 16:1 (*trans*-3) PG is 32°, *ca* 10° lower than that of the fully saturated analogue. The phase transition temperature of the Mg^{2+} salt of 16:0, 16:1 (*trans*-3) PG is 43° as compared to a value of 54° for that of 16:0, 16:0 PG [10]. It is thus apparent that the presence of the unsaturated acid in a PG molecule does have a significant effect in depressing the phase transition temperature as compared to the corresponding saturated one although not as great as that caused by 16:1 (*cis*-9) [9]. It is not envisaged that the small amounts of other molecular species present in the sample of 16:0, 16:1 (*trans*-3) PG would cause the measured transition temperature to vary significantly from the value of a pure sample, especially when it is considered that one of these components (16:0, 16:0 PG) would tend to elevate the measured transition temperature whereas the other (18:1, 16:1 (*trans*-3) PG) would tend to depress it.

The thermal properties of long chain unsaturated fatty acids are markedly affected by the configuration of the double bond and its position in the chain [11]. The melting point of purified 16:1 (*trans*-3) (>98% pure) was 54–55°, compared with a value of 63–64° for its saturated

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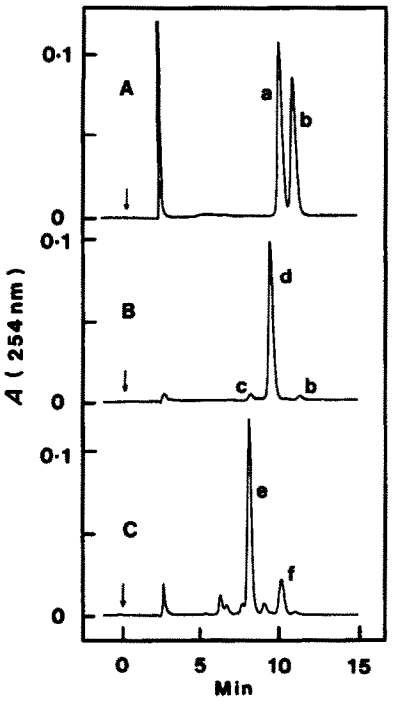

Fig. 1. HPLC analysis of the *p*-methoxybenzoyl derivatives of (A), dipalmitin; (B), DAG derived from 16:0, 16:1 (*trans*-3) PG; (C), DAG derived from *Anacystis* PG. Peak identification is as follows; (a), 16:0, 16:0 (1,3-isomer); (b), 16:0, 16:0 (1,2-isomer); (c), 18:1 (*cis*-9), 16:1 (*trans*-3); (d), 16:0, 16:1 (*trans*-3); (e), 16:1 (*cis*-9). 16:0; (f), 18:1 (*cis*-9), 16:0.

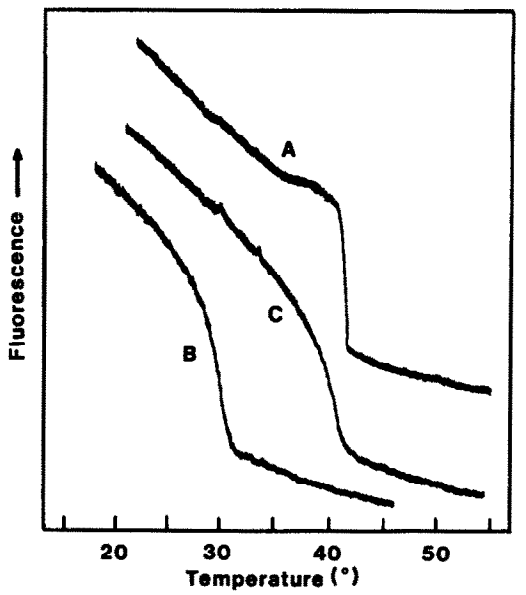

Fig. 2. Phase transition temperatures of PG measured by fluorescence polarisation of *trans*-parinaric acid. The tracings represent the fluorescence intensity of polarised light in an ascending temperature scan. (A), 16:0, 16:0 PG (Na^+ salt); (B), 16:0, 16:1 (*trans*-3) PG (Na^+ salt); (C), 16:0, 16:1 (*trans*-3) PG (Mg^{2+} salt).

analogue, and 0.5° for 16:1 (*cis*-9) [12]. The depression of melting point caused by the introduction of double bonds into 16:0 is thus similar to that reported for C_{18} fatty acids [11].

The biological role of 16:1 (*trans*-3), which occurs only in thylakoids of algae and higher plants and is specifically located at the *sn*-2 position of PG, is unknown. Its production is affected in some higher plants by factors such as growth temperature, light intensity and oxygen tension and the double bond is apparently synthesised in a light-dependent reaction after assembly of the PG molecule [8]. The results presented here do indicate however, that the introduction of the *trans*-3 double bond has a significant effect on the thermal properties of the PG molecule. Considering that the content of 16:1 (*trans*-3) in PG may vary from 0 to over 40% of the total fatty acids [13, 14] and that these variations are at the expense of 16:0, it seems unlikely that a simple summation of the content of PG molecular species containing 16:0, 16:0, 18:0, 16:0 and 16:0, 16:1 (*trans*-3) will provide an accurate guide to the thermal properties of PG from a specific source, or be a reliable index of the chilling sensitivity of a plant.

EXPERIMENTAL

PG was isolated from lipid extracts of mature leaves of field-grown squash (*Cucurbita moschata* Durch) by a combination of DEAE-Sepharose chromatography and TLC [6]. The purified PG was then fractionated into molecular species by HPLC on a 10 μm ODS-II reverse phase column, 25 × 1 cm (Regis Chemical Co.). The solvent used was MeOH–H_2O–MeCN (181:14:5) [15] at a flow rate of 2.5 ml/min and up to 40 mg PG could be injected at one time. Fractions (5 ml) were collected and aliquots analysed by TLC and GC of fatty acid Me esters [16]. Fractions enriched in the 16:0, 16:1 (*trans*-3) molecular species were combined and refractionated on the same column at a flow rate of 1.25 ml/min. Twenty fractions (2.5 ml) were collected and it was found that fractions 11–14 contained almost pure 16:0, 16:1 (*trans*-3) PG.

16:1 (*trans*-3) was prepd from lipid extracts of *Beta vulgaris* enriched in PG. After saponification, the free fatty acids were extd and converted to their Me esters with BF_3–MeOH [17]. The esters were fractionated by HPLC on 10 μm ODS-II reversed phase columns. For the initial sepn, 200 mg of ester were injected onto a 5 × 2.1 cm column using MeOH as solvent and a flow rate of 12.5 ml/min. Fractions (25 ml) enriched in Me 16:1 (*trans*-3) were identified by GC, pooled and freed from contamination (mainly 18:2) by a second HPLC sepn on a 25 × 1 cm column using MeCN as solvent. The purity of the isolated Me 16:1 (*trans*-3) was confirmed by GC and by $AgNO_3$-silica gel TLC [18] developed twice at −20° in toluene–hexane, (9:1). No *cis*-16:1 acids could be detected in the sample by the latter technique. Finally, Me 16:1 (*trans*-3) was converted to the free acid by saponification.

Molecular species analysis of PG was carried out by HPLC of *p*-methoxybenzoyl derivatives of diacylglycerols (DAG) released from PG by phospholipase C hydrolysis [19].

Phase transition temps of PG samples were measured by fluorescence polarization of *trans*-parinaric acid [20]. Samples were prepared as described in ref [9] in 20 mM HEPES buffer pH 7.5 containing either 2 mM EDTA or 2 mM $MgCl_2$. The

excitation beam (313 nm) was passed through a polarizing prism (Carl Zeiss) and a film polarizer was oriented parallel to the emission polarizer. The intensity of emitted fluorescence was measured at 420 nm. The slit width for both excitation and emission beams was 10 nm. The thermo-regulated cuvette was stirred and the temp monitored with a copper-constantan thermocouple. Measurements were made in both heating and cooling modes but the temp of the completion or onset of the transition did not differ by more than one degree in any sample.

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A NOVEL OXO FATTY ACID IN *PLANTAGO OVATA* SEED OIL

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Key Word Index—*Plantago ovata*; Plantaginaceae; seed oil; 9-oxooctadec-*cis*-12-enoic acid.

Abstract—*Plantago ovata* seed oil contains two oxygenated fatty acids one of which is the known 9-hydroxyoctadec-*cis*-12-enoic acid. The other is 9-oxoactadec-*cis*-12-enoic acid a new acid. The structural elucidation of this novel compound is described.

INTRODUCTION

The occurrence of keto fatty acids in natural seed oils is a rarity [1], although naturally occurring long chain hydroxy acids are widely distributed in plants [2] and microorganism [3]. *Plantago major* [4], a member of Plantaginaceae contains an unusual hydroxy fatty acid, 9-hydroxy-*cis*-11-octadecenoic, an isomer of ricinoleic acid. This prompted us to analyse another *Plantago* species, *P. ovata*, to examine the presence of any unusual fatty acid in its oil. An earlier report [5] on this seed oil indicated that it contained only the usual fatty acids. However, we identified isoricinoleic acid (**2a**) and its corresponding keto derivative (**1a**) having the same positions of double bond and oxygenated group. Identification of these two acids is described herein and is based upon spectral and chemical evidences.

RESULTS AND DISCUSSION

P. ovata oil responded to the DNP test [6], indicating the presence of an oxo function. The IR and UV spectra of the oil exhibited no absorption bands for the presence of