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Short communication

Determination of the geometrical isomers of ethyl 2,4-decadienoate

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Abstract

Ethyl 2,4-decadienoate isomers, important aroma compounds in the Bartlett pear, were investigated. Separation and identification of isomers was performed. Separation of isomers was successful using gas chromatography (GC) with a VOCOL capillary column and high-performance liquid chromatography on a nonpolar reversed-phase column (C_{18}) with silver ions in mobile phase. Identification of the isomers was made by comparison of their retention times with the authentic ethyl *trans*-2,*cis*-4-decadienoate standard, and by NMR spectrometry. For quantitative determination of ethyl 2,4-decadienoate isomers the limit of detection and the interval of linearity for ethyl *trans*-2,*cis*-4-decadienoate were determined for both chromatographic methods.

Keywords: Geometrical isomers; Ethyl decadienoate

1. Introduction

Ethyl 2,4-decadienoate is the major aroma component of the Bartlett pear [1]. Four different geometrical isomers exist and the quantity of an individual isomer is responsible for the taste and odour of food products made from the Bartlett pear [2,3]. It is therefore important to develop methods for the separation and determination of all four possible isomers.

Silver ion (Ag) high-performance liquid chromatography (HPLC) has been recognised as a method for separating of *cis–trans* geometrical isomers. Separation is based on the reversible formation of a polar charge-transfer complex between unsaturated organic molecules and silver ions [4]. This method was first used with lipids and fatty acids [4] and later for analysis of retinoic acid photoisomers [5].

Geometrical isomers of unsaturated fatty acid esters are also successfully separated on capillary gas chromatographic columns [6,7]. However, the separation principle on such columns is quite different from HPLC columns and is based on the differences in the volatility of the isomers.

Our results show that both techniques can be used to separate ethyl 2,4-decadienoate isomers.

In the absence of pure individual standards, four geometrical isomers were identified by the comparing the retention times with one of the parent ethyl *trans*-2,*cis*-4-decadienoate. In addition, independent structural characterisation of all four isomers was made using nuclear magnetic resonance (NMR) spectroscopy.

2. Experimental

2.1. Chemicals

Ethyl *trans*-2,*cis*-4-decadienoate (90+%) was supplied by Sigma–Aldrich (Steinheim, Germany)

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and ethanol (absolute, analytical-reagent grade) was from Merck (Darmstadt, Germany). The following solvents for HPLC analysis were used: ultra-pure water (Milli-Q system, Millipore, Molsheim, France), methanol (Uvasol) and silver nitrate (analytical-reagent grade), both from Merck.

2.2. Solution preparation

Of the four possible isomers of the ethyl ester of 2,4-decadienoic acid only ethyl *trans*-2,*cis*-4-decadienoate is commercially available. To produce the other isomers 150 ml of a standard solution of ethyl *trans*-2,*cis*-4-decadienoate in ethanol (concentration 5 mg/ml) was exposed to UV irradiation from a 50 W Hg high-pressure lamp (Osram, Ultra-vitalux) for 90 min. The intensity of the incident light inside the photoreactor, measured using ferrioxalate actinometry, was $9.0 \cdot 10^{-6}$ einstein/min.

Solutions of 5 mg/ml of ethyl *trans*-2,*cis*-4-decadienoate in ethanol before and after irradiation were used for NMR analysis. For HPLC and gas chromatography–mass spectrometry (GC–MS) experiments solutions containing a hundred-times and ten-times lower concentration were used, respectively.

2.3. Gas chromatography

GC–MS analyses were performed on Hewlett-Packard gas chromatograph HP 5890 Series II coupled to a HP 5989A mass spectrometer MS engine and equipped with a VOCOL, 60 m×0.25 mm I.D., 1.50 μm film thickness, capillary column (Supelco, Bellefonte, PA, USA). Helium (5.0, Messer Griesheim, Gumpoldskirchen, Austria) was used as the carrier gas at a flow-rate of 1 ml/min. Injection (1 μl) was in the splitless mode, the purge was switched on after 1 min and the purge flow was 15 ml/min. The oven temperature program was: 120°C for 2 min, and then heated to 210°C at 30°C/min. The temperatures of the injector and GC–MS interface were 220 and 250°C, respectively. Ion source and quadrupole mass analyser temperatures were 200 and 100°C. The ionisation mode was electron impact with an electron energy of 70 eV.

2.4. NMR spectroscopy

NMR spectra were recorded on a Varian Unity Inova 600 spectrometer (^{1}H at 600.14 MHz) at the National NMR Centre of Slovenia. The samples were dissolved in ethanol, 10% of [$^2H_6$]ethanol was added for lock. Sample concentration was 7.5 mg/0.6 ml. The sample temperature was maintained at 25±0.5°C. The following parameters were used for data acquisition and processing of ^{1}H spectra: a WET solvent suppression technique was used for multiple suppression of the ethanol signals with selective SEDUCE pulse shape, 6.9 kHz sweep width, 90° pulse width, a pulse delay of 5 s, 144 scans, 32 K time domain, zero filling to −209°C and line broadening of 1 Hz. The assignments of the coupled and partly overlapped ethylene resonances in ^{1}H NMR spectra were made using selective decoupling experiments. d_H: 7.55 (dd, J_{H2H3}=15.1, J_{H3H4}=11.9 Hz, 1H, H3 of ethyl *trans*-2,*cis*-4-decadienoate), 7.31 (dd, J_{H3H4}=11.9, J_{H4H5}=15.4 Hz, 1H, H4 of ethyl *cis*-2,*trans*-4-decadienoate), 7.22 (m, H4 of ethyl *cis*-2,*cis*-4-decadienoate), 7.17 (dd, J_{H2H3}=15.5, J_{H3H4}=10.7 Hz, 1H, H3 of ethyl *trans*-2,*trans*-4-decadienoate), 6.91 (dd, 1H, H3 of ethyl *cis*-2,*cis*-4-decadienoate), 6.53 (dd, 1H, H3 of ethyl *cis*-2,*trans*-4-decadienoate), 6.16 (dd, J_{H3H4}=11, J_{H4H5}=15.4 Hz, 1H, H4 of ethyl *trans*-2,*trans*-4-decadienoate), 6.12 (m, J_{H4H5}=11 Hz, 1H, H4 of ethyl *trans*-2,*cis*-4-decadienoate), 6.04 (m, 2H, H5 of ethyl *cis*-2,*trans*-4-decadienoate and H5 of ethyl *trans*-2,*trans*-4-decadienoate), 5.95 (m, 1H, H5 of ethyl *trans*-2,*cis*-4-decadienoate), 5.82 (m, 1H, H5 of ethyl *cis*-2,*cis*-4-decadienoate), 5.81 (d, 1H, H2 of ethyl *trans*-2,*cis*-4-decadienoate), 5.73 (d, J_{H2H3}= 15.5 Hz, 1H, H2 of ethyl *trans*-2,*trans*-4-decadienoate), 5.61 (d, J_{H2H3}=11.4 Hz, 1H, H2 of ethyl *cis*-2,*cis*-4-decadienoate), 5.46 (d, J_{H2H3}=11.1 Hz, 1H, H2 of ethyl *cis*-2,*trans*-4-decadienoate), ppm.

2.5. High-performance liquid chromatography

The HPLC system (1100; Hewlett-Packard) consisted of a binary pump, a diode array detection (DAD) system and an injection valve (Model 7725; Rheodyne) with a 100-μl sample loop. The detector was set to a wavelength of 270 nm. Separations were made on a 5 μm ODS Hypersil column (250×4 mm

I.D.; Hewlett-Packard) at room temperature. Gradient elution was used at a flow-rate of 1 ml/min. The eluent comprised an aqueous solution of silver nitrate (concentration 1 g/l)–methanol in an initial ratio of (40:60, v/v), and then changed linearly to a final composition of eluent: aqueous solution of $AgNO_3$–methanol (30:70, v/v) in 60 min.

3. Results and discussion

In the present study four different ethyl 2,4-decadienoate geometrical isomers were obtained after irradiation of a standard solution of ethyl *trans*-2,*cis*-4-decadienoate for 90 min. Fig. 1 shows Ag-HPLC chromatograms of a standard solution of ethyl *trans*-2,*cis*-4-decadienoate before (a) and after irradiation for 90 min (b). A reaction time of 90 min was found optimal to reach the highest yield of isomers. With a shorter irradiation time a lower transformation of ethyl *trans*-2,*cis*-4-decadienoate to other three isomers was achieved, alternatively with increases irradiation an increased degradation of all the isomers was observed.

Separation of isomers of ethyl 2,4-decadienoate was performed both with gas and liquid chromatography. Fig. 2 depicts the separation of isomers using GC–MS analysis on a VOCOL capillary column. The elution order is: ethyl *cis*-2,*trans*-4-decadienoate, ethyl *trans*-2,*cis*-4-decadienoate, ethyl *cis*-2,*cis*-4-decadienoate and ethyl *trans*-2,*trans*-4-decadienoate. The elution pattern of isomers of ethyl 2,4-decadienoate on the VOCOL capillary GC column is identical to the elution order of isomers of

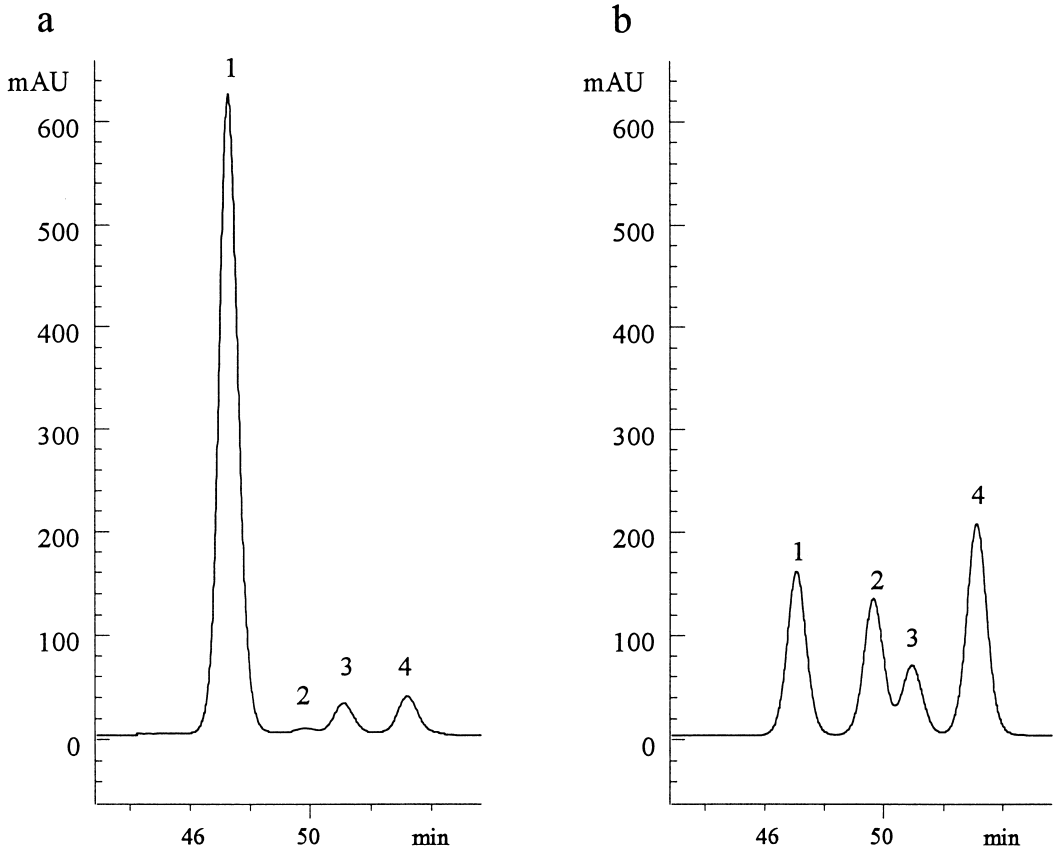

Fig. 1. Ag-HPLC chromatograms of ethyl *trans*-2,*cis*-4-decadienoate solution before (a) and after irradiation for 90 min (b). Peaks: 1=Ethyl *trans*-2,*cis*-4-decadienoate, 2=ethyl *cis*-2,*trans*-4-decadienoate, 3=ethyl *cis*-2,*cis*-4-decadienoate and 4=ethyl *trans*-2,*trans*-4-decadienoate.

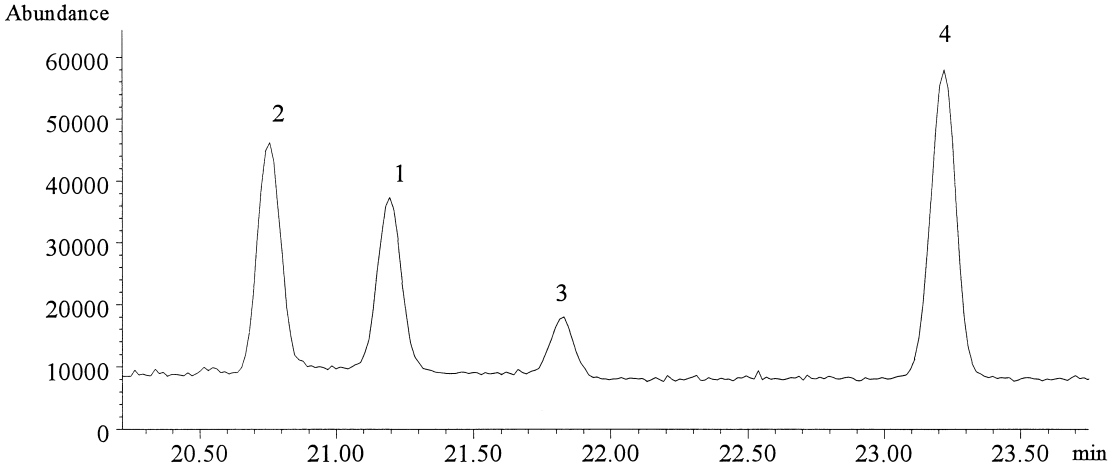

Fig. 2. GC–MS total ion current (TIC) chromatogram in the region of ethyl 2,4-decadienoate isomers. Peaks: 1=Ethyl *trans*-2,*cis*-4-decadienoate, 2=ethyl *cis*-2,*trans*-4-decadienoate, 3=ethyl *cis*-2,*cis*-4-decadienoate and 4=ethyl *trans*-2,*trans*-4-decadienoate.

9,11-octadecadienoic acid methyl esters on a SP2380 Supelco column [6] and BPX 70 SGE column [7], suggesting that the elution order of conjugated diene esters is independent of alyl chain length.

The mass spectra of the geometrical isomers are too similar, to be able to distinguish between them. However, ethyl *trans*-2,*cis*-4-decadienoate was identified by comparing the retention time of peak 1 in Fig. 2 with an authentic standard. Since the other three isomers, to the authors' knowledge, are not commercially available, identification was made using NMR analysis of the reaction mixture, which was exposed to UV light without prior separation of individual isomers. The relative ratios of isomers were determined by comparing the peak areas of individual isomers from the GC–MS total ion current (TIC) chromatograms with the integrals in the ^{1}H NMR spectrum (cf. Table 1).

Table 1
Comparison of the different methods for the quantitative analysis of ethyl 2,4-decadienoate isomers[a]

	Method		
Isomer	GC–MS	Ag-HPLC	NMR
trans-2,*cis*-4-	1	1	1
cis-2,*trans*-4-	1.2	0.8	1.1
cis-2,*cis*-4-	0.3	0.4	0.5
trans-2,*trans*-4-	1.8	1.3	1.4

[a] Amounts of individual isomer are normalised with respect to the peak area of ethyl *trans*-2,*cis*-4-decadienoate.

In the ^{1}H-NMR spectra of the parent isomer ethyl *trans*-2,*cis*-4-decadienoate four characteristic ethylene signals were observed between δ 5.5 to 7.6 ppm (Fig. 3a). The shape of the H3 of ethyl *trans*-2,*cis*-4-decadienoate (δ 7.55 ppm) was a doublet of a doublet with $^{3}J_{H2H3}$ and $^{3}J_{H3H4}$ proton–proton coupling constants of 15.1 and 11.9 Hz, respectively, which proves the *trans* configuration along C2=C3 double bond. A magnitude of $^{3}J_{H4H5}$ of 11 Hz is in agreement with the *cis* configuration across C4=C5 double bond (see Fig. 3a).

^{1}H-NMR spectrum of the reaction mixture, exposed to UV irradiation for 90 min, revealed several additional signals between δ 5.5 to 7.6 ppm (Fig. 3b) due to isomerisations along C2=C3 and C4=C5 double bonds. Resonances corresponding to all three additional geometrical isomers, ethyl *cis*-2,*trans*-4-decadienoate, ethyl *cis*-2,*cis*-4-decadienoate and ethyl *trans*-2,*trans*-4-decadienoate, could be identified together with the parent isomer ethyl *trans*-2,*cis*-4-decadienoate. A simple comparison of the two spectra shown in Fig. 3 confirmed the presence of ca. 25% of the unreacted isomer ethyl *trans*-2,*cis*-4-decadienoate. The major product of isomerization was isomer 4 in Fig. 3 (ca. 35 %, see Table 1) with C2-*trans*, C4-*trans* configurations, which was clear from the $^{3}J_{H2H3}$ and $^{3}J_{H4H5}$ proton–proton coupling constants of 15.5 and 15.4 Hz, respectively. Isomer 2 in Fig. 3 represents ca. 28% and was identified as

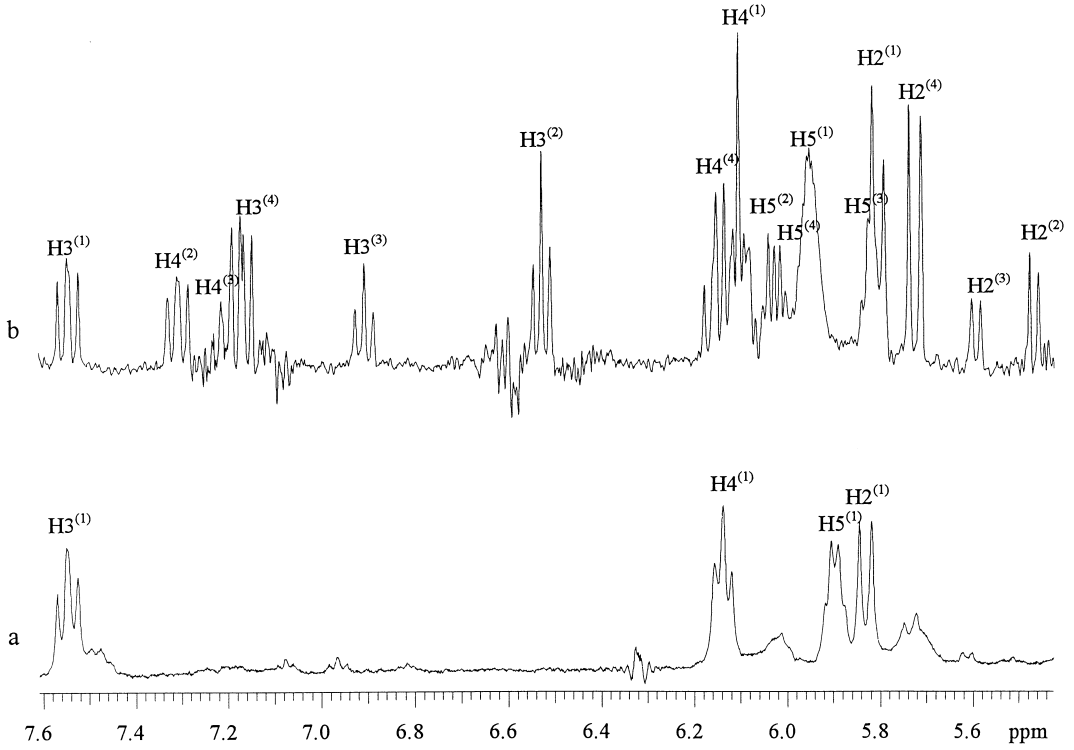

Fig. 3. NMR spectra of ethyl *trans*-2,*cis*-4-decadienoate solution before (a) and after irradiation for 90 min (b). Numbers in parentheses indicate: 1=ethyl *trans*-2,*cis*-4-decadienoate, 2=ethyl *cis*-2,*trans*-4-decadienoate, 3=ethyl *cis*-2,*cis*-4-decadienoate and 4=ethyl *trans*-2,*trans*-4-decadienoate.

C2-*cis*, C4-*trans* through $^{3}J_{H2H3}$ and $^{3}J_{H4H5}$ proton–proton coupling constants of 11.1 and 15.4 Hz, respectively. Isomer 3 in Fig. 3 represents ca. 12%.

Separation of all four isomers, less successfully than with capillary GC column, although was possible by Ag-HPLC (Fig. 1b). All four peaks were completely resolved by the gas capillary column, while with Ag-HPLC *cis*-2,*trans*-4 (peak 2) and *cis*-2,*cis*-4 isomer (peak 3) were only partially resolved (Fig. 1b).

The order of elution obtained by Ag-HPLC for the ethyl 2,4-decadienoate isomers was as follows: ethyl *trans*-2,*cis*-4-decadienoate, ethyl *cis*-2,*trans*-4-decadienoate, ethyl *cis*-2,*cis*-4-decadienoate and ethyl *trans*-2,*trans*-4-decadienoate. Identification of HPLC chromatographic peaks by UV spectra is not possible due to their similarity (UV spectra of *cis*-2,*trans*-4 and *trans*-2,*trans*-4 isomer have absorption maxima at 264.5 nm, while UV spectra of *trans*-2,*cis*-4 and *cis*-2,*cis*-4 isomer have absorption maxima at 268.4 nm). This is the reason why the eluent was collected in fractions and the peaks were identified using gas chromatograms according to their retention times.

The elution profile of ethyl 2,4-decadienoate isomers obtained by Ag-HPLC on reversed-phase column with silver ions in the mobile phase differ from the elution order of isomers of 9,11-octadecadienoic acid methyl ester obtained using silver ions attached to cation exchangers [6,8,9]. The reason could be the different mode of Ag-HPLC we used since hydrophobic interactions are the predominant separation mechanism in Ag reversed-phase-HPLC, while in the Ag-normal phase-HPLC the interactions are based on charge-transfer complex formation. The complexation mechanism between the Ag^{+} ion and the unsaturated ester includes dual interaction of a silver ion with the π electrons of a double bond on the alyl chain and the electron pair of the ester carbonyl group [4]. Regarding this, it is obvious that the complex formation constants depend on chain length

[4] and the distance of the double bond from the carbonyl group [10].

Analyses of the irradiated solution of ethyl 2,4-decadienoate by GC–MS, Ag-HPLC and NMR are presented in Table 1. The variation in the relative areas obtained for the same irradiated solution is due to the different detection characteristics of the methods. The GC–MS signal is mainly influenced by the ionisation of an individual isomer, whereas DAD of the Ag-HPLC is influenced by the absorption coefficient, which is different for different isomers. The integration of NMR signals is hampered by the partial overlapping of H2–H4 resonances. These effects will be the subject of future studies.

Both chromatographic methods are appropriate for quantitative analysis of ethyl 2,4-decadienoate isomers. Linear range and limit of detection (LOD) (defined as triple standard deviation of noise peak areas expressed in concentration units) of the chromatographic methods used were determined by analysing standard solutions of the only commercially available ethyl *cis*-2,*trans*-4-decadienoate. GC–MS response is linear in concentration range from 20 μg/ml to 800 μg/ml and LOD was found to be 5 μg/ml, while Ag-HPLC response is linear from 0.5 μg/ml to 15 μg/ml and LOD is 0.1 μg/ml.

4. Conclusions

Geometrical isomers of ethyl 2,4-decadienoate were produced in a UV-reactor and the reaction mixture was subsequently analysed by NMR, GC and Ag-HPLC. It was found that NMR is suitable for qualitative analysis, while identification in chromatography could only be done by comparison of qualitative data obtained from NMR.

Furthermore, we showed that the separation of ethyl 2,4-decadienoate isomers is possible by gas and liquid chromatography, although the isomers are no eluted in the same order, i.e., with capillary GC ethyl *cis*-2,*trans*-4-decadienoate elutes before the *trans*-2,*cis*-4-isomer. However, GC separation is more efficient and time of analysis is shorter.

In the future, using the developed chromatographic methods, we will be able to characterise, qualitatively and quantitatively, the important aroma compounds in the Bartlett pear.

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