



ELSEVIER

Journal of Chromatography A, 693 (1995) 235–239

JOURNAL OF
CHROMATOGRAPHY A

Silver ion high-performance liquid chromatography of esters of isomeric octadecenoic fatty acids with short-chain monounsaturated alcohols

Boryana Nikolova-Damyanova^{a,1}, William W. Christie^{a,*}, Bengt Herslöf^b

^aThe Scottish Crop Research Institute, Invergowrie, Dundee, Scotland DD2 5DA, UK

^bKarlshamns LipidTeknik AB, P.O. Box 6686, S-11384 Stockholm, Sweden

First received 15 August 1994; revised manuscript received 1 December 1994; accepted 5 December 1994

Abstract

The retention characteristics of a series of positionally isomeric octadecenoic acids, derivatized with allyl, butenyl, pentenyl and hexenyl alcohols, were studied by high-performance liquid chromatography on an ion-exchange column loaded with silver ions. For a given isomer the retention factor, k' , increased with the distance of the double bond in the alcohol moiety from the ester group. The general retention patterns of butenyl, pentenyl and hexenyl esters were similar to those of methyl and phenacyl esters of the same series of isomeric fatty acids. Allyl esters differed in that the effect of double bond position in the fatty acid backbone on retention was weak. The results support the earlier suggestion for simultaneous interaction between one silver ion and one double and one electron-donating atom or group, presumed to be oxygen co-ordinating with silver ions through its free electron pairs.

1. Introduction

In previous papers [1,2], we described the retention characteristics of methyl, phenacyl and phenethyl derivatives of unsaturated fatty acids on high-performance liquid chromatography in either the silver ion (Ag-HPLC) or the combined silver ion and reversed-phase modes. With the former, the results were broadly comparable to those given by silver ion thin-layer chromatography (Ag-TLC) [3,4] in that a distinctive effect of double bond position on retention of isomeric

octadecenoates was established. Appreciable differences in retention of positionally isomeric octadecenoates in dependence of the nature of the derivative were also observed. The part of the molecule which contains the ester moiety is more important than the terminal end for resolution. Based on these results some interesting practical separations were achieved of the phenacyl esters of the three naturally occurring octadecenoate isomers, petroselinic (6–18:1), oleic (9–18:1) and vaccenic (11–18:1), by Ag-TLC as well as HPLC [1,5].

Three possible interactions were proposed for the retention mechanism: (i) the ability of a silver ion to interact with more than one coordination centre [6] —these could be either two

* Corresponding author.

¹ Present address: Institute of Organic Chemistry, Centre of Phytochemistry, Sofia 113, Bulgaria.

double bonds in the fatty acid molecule or one double bond and free electron pairs on an oxygen (or another electron-rich part) of the ester moiety; (ii) different and specific conformations of the fatty acid molecule which expose the double bond(s) in a more favourable way to complex with a silver ion; (iii) participation of the ester moiety in a separate polar interaction with the residual silanol groups on the support material. All three mechanisms are possible and could act simultaneously, although the magnitudes of each may be very different. In order to understand these interactions better we extended the range of derivatives and studied the retention of their esters with monounsaturated short-chain fatty alcohols under the conditions of Ag-HPLC.

2. Experimental

2.1. Materials

Reagents were analytical grade and were purchased from Sigma (Poole, UK). HPLC-grade or analytical-grade solvents (FSA Scientific Apparatus, Loughborough, UK) were used without further purification. The isomeric *cis*-octadecenoates had been prepared earlier [7] by total synthesis. Allyl alcohol, 3-buten-1-ol, 4-penten-1-ol and 5-hexen-1-ol were purchased from Aldrich (Gillingham, UK).

2.2. Derivatization

To produce the various derivatives, the free fatty acids (20 mg) were first converted into the corresponding acid chlorides by reaction with 0.5 ml of oxalyl chloride for 36 h at room temperature. The excess reagent was then evaporated, first under nitrogen and then under vacuum. The residue was dissolved in toluene (1 ml), and 0.25 ml (5 mg of the fatty acid chloride) was immediately reacted with 5 mg of each unsaturated alcohol in toluene (0.5 ml) and pyridine (0.1 ml) at 50°C overnight. The excess solvent and pyridine were removed in a rotary evaporator. Hexane (5 ml) was added to the residue and the

solution was washed with water (2 × 5 ml). Finally, the products were purified by elution through a Florisil column (Pasteur pipette) with hexane–acetone (99:1, v/v). The purity of each derivative was checked by TLC (Alufolio silica gel 60; Merck, Darmstadt, Germany) with a mobile phase of hexane–acetone (100:8, v/v) and detection with iodine vapour.

2.3. High-performance liquid chromatography

A Hitachi L-6200A HPLC pump was used with a Varex Model IIA evaporative light-scattering detector (P.S. Instruments, Sevenoaks, UK). A Nucleosil 5SA column (25 cm × 4.6 mm I.D.) was converted to the silver form as described earlier [8]. The temperature of the column was maintained at 20.0 ± 1.0°C by fitting it into a water jacket through which water was pumped from a temperature control unit. Sample (5 μl) was injected as a solution in dichloroethane. A mixture of dichloroethane–dichloromethane–acetonitrile (50:50:0.25, v/v/v) was used as mobile phase at a flow-rate of 1.5 ml/min. The temperature of the drift tube in the detector was maintained at 80°C. The dead volume of the column was determined by repeated injection of docosane. The mean retention time was 2.847 ± 0.006 min (6 injections). Retention (capacity) factors (k') were determined as a mean of three parallel measurements with relative standard deviations not exceeding 3.2%.

3. Results and discussion

Compared to derivatives studied previously, the esters examined here had a distinctive new feature—a double bond in the alcohol moiety that was a second site for a π -electron interaction with silver ions. Thus, the octadecenoic ester species comprised a variety of combinations of two double bonds at different distances from the ester group and different distances apart, as illustrated for oleic acid in Fig. 1.

It was possible to elute all four derivatives using the same mobile phase of dichloroethane–dichloromethane–acetonitrile (50:50:0.25, v/v/v)

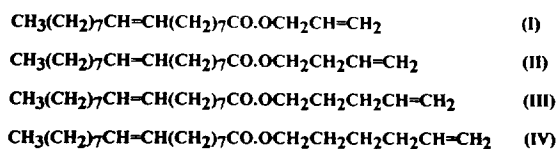


Fig. 1. Esters of oleic acid (9–18:1) with short-chain monoenoic fatty alcohols, allyl (I), butenyl (II), pentenyl (III) and hexenyl (IV).

v). The proportion of acetonitrile in the solvent mixture was higher than that used for the methyl and the phenacyl derivatives (0.01%), and was close to that used earlier to elute the dienoic fatty acids and the unsaturated long-chain fatty acid alcohols (0.35% acetonitrile) [1]. Fig. 2 reveals the distinctive influence of double bond position in the fatty acid backbone on the retention factor. The general pattern for a given ester moiety resembled that obtained for the phenacyl derivatives, e.g. there were maxima for

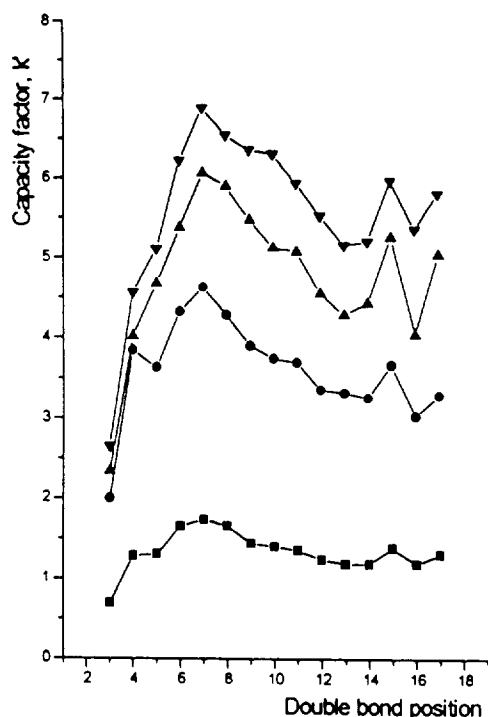


Fig. 2. Retention factors, k' , for silver ion HPLC of allyl (■), butenyl (●), pentenyl (▲) and hexenyl (▼) esters of the series of *cis*-octadecenoates at 20°C on a Nucleosil 5SA column with a mobile phase of dichloroethane–dichloromethane–acetonitrile (50:50:0.25, v/v/v).

7- and 15–18:1 isomers. As with all fatty acid derivatives studied so far, a double bond in the acyl chain positioned near the ester group reacted only weakly with the silver ions; hence these derivatives (from 3–18:1 to 5–18:1) had lower k' values. When this bond was located at a distance from the ester group, its complexation ability increased and even the 17–18:1 isomer had a higher k' value than 3–18:1. This may be due to partial delocalization of the π -electrons of the double bond under the influence of the inductive effect of the ester group.

This conclusion is confirmed by the behaviour of the second double bond, positioned in the alcohol moiety, which shows the same effect: the retention factors for a given fatty acid isomer depend on the alcohol, the order being: allyl < butenyl < pentenyl < hexenyl. The same order was observed for the stearic acid esters, where retention is affected only by the double bond of the alcohol, the respective values being 0.0, 0.09 ± 0.01 , 0.26 ± 0.01 , 0.41 ± 0.01 . These values can be considered as the contribution of the alcohol double bond to retention. It is evident that retention factors of ester species are not a simple sum of the fractional values of the two parts of the molecule which can interact with silver ion, thus confirming our earlier observation [1].

Retention tended to increase initially with increasing distance between the two double bonds, the highest k' value established in this work, that of 7–18:1 hexenyl ester ($k' = 6.86$), was about 3 to 7 times lower than those of any of the isomeric octadecadienoates with double bonds separated by more than one methylene group studied previously [1]. This indicates that the existence of a second double bond is not the only factor affecting retention, and presumably the distance between them is also important. Depending on the distance one silver ion could interact either with two double bonds simultaneously or with each double bond separately, or two silver ions could in theory interact with each double bond separately. It seems that there is an optimum distance, and this was found to be the 1,5-diene configuration (as in 1,5-hexadiene [6]).

Allyl esters were held least strongly of all the derivatives examined and showed the smallest differences between the retention factors of the isomers, i.e. the double bond position in the fatty acid backbone has much a weaker effect on retention than usual (Fig. 2). Evidently, the double bond of the alcohol when positioned near the ester group is interacting either very weakly or not at all with silver ions, and this conclusion is confirmed as allyl stearate like methyl stearate has $k' = 0$. In addition, a double bond in the alcohol moiety adjacent to the carboxylic group seems to interfere with the general retention of the molecule, or at least with those factors responsible for the differences in the retention factors of the various fatty acid isomers. In contrast, propyl esters (with the same number of carbon atoms in the alcohol moiety) permit excellent separations of octadecenoate isomers [9]; k' values for propyl esters are greater than those for allyl esters.

Although steric factors may play a part, we believe that the behaviour of allyl ester is a confirmation that the carboxyl moiety has a specific role in the interaction with the silver ions, probably through free electron pairs at the oxygen atom. These may react separately with the silver ion, but could co-ordinate jointly with the double bond in the fatty acid. In the latter case, the distance between the ester moiety and the double bond would be of significance for the interaction and some configurations would be preferable. This may explain the strong retention of phenacyl esters, which have a second carbonyl oxygen attached to the electron-rich phenyl moiety. Evidence that such an interaction is possible has been shown already for unsaturated alcohols [10,11]. It was also observed that mono-unsaturated alcohols were retained very strongly under the conditions of Ag-HPLC, having k' values of the magnitude of those of fatty acids with two separate double bonds, decreasing with the distance of the double bond from the oxygen atom [1].

By participation of the carbonyl oxygen in the interaction with silver ions, it is possible to explain the differences in retention of positional-ly isomeric monoenoic fatty acids and the fact

that the methyl, phenacyl and unsaturated alcohol esters, while providing different absolute retention factors of the isomers, have broadly similar relative patterns with respect to double bond position in the fatty acid. The exception is allyl esters in which the double bond in the alcohol moiety is close to the carbonyl oxygen. Although simple electronic or steric effects are possible in this instance, some kind of mutual interference via a field effect appears more probable. This hinders formation of a favourable structure for the complex, and decreases both the general retention of the isomeric species and the effects of the double bond position in the fatty acid.

4. Conclusions

In general, butenyl, pentenyl and hexenyl esters behaved similarly on Ag-HPLC to methyl and phenacyl esters of the same series of isomeric fatty acids. Allyl esters differed in that the interaction with the silver ions was very weak. This investigation supports an earlier conclusion that a silver ion may interact both with the fatty acid double bond and with other electron-donating parts of the molecule such as the carbonyl oxygen.

Acknowledgements

This research was supported by the Karlshamns Research Board, Sweden, the Scottish Office Agriculture and Fisheries Department and the Bulgarian National Research Foundation.

References

- [1] B. Nikolova-Damyanova, B.G. Herslöf and W.W. Christie, *J. Chromatogr.*, 609 (1992) 133.
- [2] B. Nikolova-Damyanova, W.W. Christie and B.G. Herslöf, *J. Chromatogr. A*, 653 (1993) 15.
- [3] L.J. Morris, D.M. Wharry and E.W. Hammond, *J. Chromatogr.*, 31 (1967) 69.

- [4] F.D. Gunstone, I.A. Ismail and M.S.F. Lie Ken Jie, *Chem. Phys. Lipids*, 1 (1967) 376.
- [5] B. Nikolova-Damyanova, W.W. Christie and B. Herslöf, *J. Planar Chromatogr.*, 7 (1994) 382.
- [6] *Gmelin Handbuch der Anorganischen Chemie, Silver*, Vol. 61, Part B5, Springer, Berlin, 1975, pp. 26–42.
- [7] A. Valicenti, F.J. Pusch and R.T. Holman, *Lipids*, 20 (1985) 234.
- [8] W.W. Christie, *J. High Resolut. Chromatogr. Chromatogr. Commun.* 10 (1987) 148.
- [9] B. Nikolova-Damyanova, unpublished results.
- [10] C.D.M. Beverwijk, G.J.M. van der Kerk, A.J. Leusink and J.G. Moltes, *Organometal. Chem. Rev.*, A5 (1970) 215.
- [11] M. Novak, D.A. Aikens and W.D. Closson, *Inorg. Nucl. Chem. Lett.*, 10 (1974) 1117.