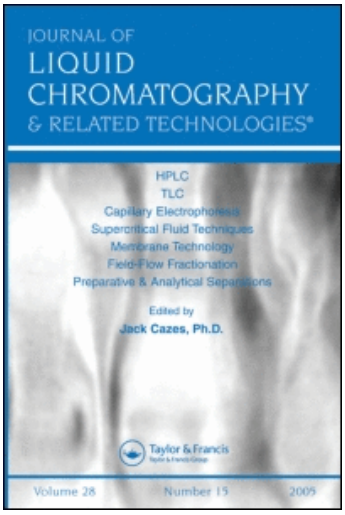

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Cyclohexanediol Fatty Acid Diesters as Model Compounds for Mechanistic Studies in Silver Ion High Performance Liquid Chromatography

Boryana Nikolova-Damyanova[a]; Gary Dobson[b]; Svetlana Momchilova[a]; William W. Christie[b]
[a] Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria [b] Scottish Crop Research Institute, Invergowrie, Dundee, UK

Online publication date: 06 September 2003

To cite this Article Nikolova-Damyanova, Boryana , Dobson, Gary , Momchilova, Svetlana and Christie, William W.(2003) 'Cyclohexanediol Fatty Acid Diesters as Model Compounds for Mechanistic Studies in Silver Ion High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 26: 12, 1905 — 1912
To link to this Article: DOI: 10.1081/JLC-120021759
URL: http://dx.doi.org/10.1081/JLC-120021759

JOURNAL OF LIQUID CHROMATOGRAPHY & RELATED TECHNOLOGIES®
Vol. 26, No. 12, pp. 1905–1912, 2003

Cyclohexanediol Fatty Acid Diesters as Model Compounds for Mechanistic Studies in Silver Ion High Performance Liquid Chromatography

Boryana Nikolova-Damyanova,[1,*] Gary Dobson,[2] Svetlana Momchilova,[1] and William W. Christie[2]

[1]Institute of Organic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Scottish Crop Research Institute, Invergowrie, Dundee, UK

ABSTRACT

Series of *cis*-1,2- and *cis*/*trans*-1,4-cyclohexanediol diesters of saturated, oleic and linoleic fatty acids were employed as model compounds in a study of the effect of the unsaturation and the position of the acyl moieties on the retention of acylglycerol molecules in silver ion high-performance liquid chromatography (HPLC). *cis*-1,2-Diunsaturated cyclohexanediol diesters were retained slightly more strongly than were the respective *cis*/*trans*-1,4-compounds, thus indicating that formation of chelate-type complexes between silver ions and the double bonds of two adjacent

*Correspondence: Boryana Nikolova-Damyanova, Institute of Organic Chemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria; E-mail: bmd@orgchm.bas.bg.

DOI: 10.1081/JLC-120021759
1082-6076 (Print); 1520-572X (Online)

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

unsaturated fatty acid residues during silver ion chromatography may occur to a limited extent.

Key Words: Silver ion HPLC; Fatty acids; Mechanistic studies; Cyclohexanediol diesters; Acylglycerols; Retention.

INTRODUCTION

Silver ion chromatography is a long-established technique for the separation of molecular species of lipids and especially of triacylglycerols, and it has been the subject of substantial reviews.[1,2] At the most basic level, separation is based on the reversible formation of a weak charge-transfer complex between a silver ion and a double bond in an alkyl chain.

Until relatively recently, there was little discussion on the detailed mechanism of the technique because most applications involved incorporation of silver nitrate into adsorbents for thin-layer chromatography, where it is difficult to control many of the factors that govern separations. However, the development of silver-ion columns for high-performance liquid chromatography (HPLC), in which the silver ions are held by ionic bonds to ion-exchange materials, has enabled detailed mechanistic studies of silver-ion (Ag-HPLC).[3] For example, in this form of the technique, the physical form and topology of the silver ions are fixed, as are the column dimensions. The composition and flow-rate of the mobile phase can be controlled accurately, as can the temperature of the column.

In studies of the separation of isomeric fatty acid derivatives, the distance between the double bond and the carboxyl group was found to be much more important than the nature of the terminal moiety in its effect on retention values.[4] Subsequent studies with esters, in which the alcohol moiety contained either electron-donating or electron-withdrawing substituents, confirmed that the silver ion interacted simultaneously with the double bond of the fatty acid and the carbonyl oxygen of the ester moiety.[5] In effect, a three-center complex between double bond, silver ion, and an electron pair from another electron-rich group in the ester moiety is formed. This explanation is consistent with x-ray crystallographic studies of stable silver ion complexes with two olefin molecules, as reviewed elsewhere.[1] Also, by means of *ab initio* calculations, it has been shown that a three-center complex between double bond, carbonyl oxygen of the ester moiety, and a silver ion is energetically favorable in solvents of moderate polarity, close to those utilized in silver ion HPLC.[6]

The retention properties of triacylglycerols on a silver ion column for HPLC have also been studied in quantitative terms.[7] Retention factors

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

were found to increase stepwise, with unsaturation. These effects are orders of magnitude stronger than is observed with simple esters. Formation of chelate-type complexes between silver ions and double bonds, with participation of the carbonyl oxygen in the complexation, was again assumed in order to explain the interactions.

In addition, the possibility was raised that complexes of the chelate type might be formed between a silver ion and, simultaneously, two double bonds in two adjacent fatty acid residues on the glycerol moiety. The increased opportunities for such interactions might help to explain the much stronger retention of triacylglycerols, as compared to simple esters.[7] To test this suggestion, we have prepared fatty acid diesters of *cis*-1,2- and *cis*/*trans*-1,4-cyclohexanediols for silver ion chromatography. The former would have the potential for simultaneous interaction of two acyl groups with a silver ion, while in latter, the fatty acids are expected to be too far apart for this to occur.

EXPERIMENTAL

Materials

Petroleum ether (bp. 40–70°C) was distilled before use. All other solvents were HPLC grade and were used without further purification. Palmitic (16 : 0), oleic (*cis* 9–18 : 1), and linoleic (*cis*, *cis* 9,12–18 : 2) acids and *cis*-1,2- and *cis*/*trans*-1,4-cyclohexanediols were purchased from Sigma-Aldrich (Poole, UK). Sunflower oil and lard were commercial samples purchased from the local market in Sofia. Oxalyl chloride was supplied by Merck (Darmstadt, Germany).

Preparation of Cyclohexanediol Diesters

Free fatty acid mixtures were produced from lard and sunflower oil as described by Christie.[11] Briefly, the lipid sample (100 mg) was hydrolysed with 1 M potassium hydroxide in 95% ethanol (2 mL), under reflux, for 1 hour. The solution was cooled, water (5 mL) was added, and the solution was extracted with diethyl ether (3 × 5 mL). The extract was washed with water (3 × 5 mL). The water washings were added to the aqueous layer, acidified with 6 M hydrochloric acid, and extracted with diethyl ether (3 × 5 mL). The extract was dried over anhydrous sodium sulphate and the free fatty acids were recovered by removing the solvent in a gentle stream of nitrogen.

Acid chlorides were prepared by adding 1 mL oxalyl chloride to 50 mg free fatty acids in a test tube. This was left at room temperature for 36 hours, before the excess reagent was removed in a stream of nitrogen. The acid chlorides were used immediately.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

The cyclohexanediol diesters were prepared by adding dichloromethane (1 mL), cyclohexanediol (20 mg), pyridine (0.2 mL), and dimethylaminopyridine (4 mg) to the acid chlorides. The test tube was left at 50°C for 6 hours and the solvents were removed in a stream of nitrogen. The residue was dissolved in hexane (5 mL), washed with water (2 × 5 mL), and dried over anhydrous sodium sulphate.

The diesters were purified by preparative silica gel G TLC by single development with hexane–acetone (100 : 8, v/v). They were detected under UV light after spraying with a fluorescent indicator. The corresponding band was scraped, transferred to a Pasteur pipette plugged with cotton wool, and the diesters were eluted with diethyl ether. The solvent was then evaporated under nitrogen and the residue was redissolved in 1,2-dichloroethane.

Ag-High Performance Liquid Chromatography of Cyclohexanediol Diesters

A Gynkotek Model 480 HPLC pump, equipped with a Gynkotek autoinjector, was used with a Varex Model III evaporative light-scattering detector (both supplied by P.S. Instruments, Sevenoaks, UK). The drift tube temperature was maintained at 50°C and the flow rate of the nebulizer gas (air) was 2.1 L/min. A column (4.6 × 250 mm) of Nucleosil^{TM} 5SA (HiChrom Ltd, Reading, UK) was converted to the silver ion form as described by Christie.[3] Samples were dissolved in either 1,2-dichloroethane or acetone and 10 μL was injected onto the column, which was maintained at a temperature of 20°C. Samples containing diesters with up to two double bonds were eluted from the column with dichloromethane–1,2-dichloroethane–acetone (1 : 1 : 0.4, v/v/v), and those with up to four double bonds were eluted with acetone–acetonitrile (98.5 : 1.5, v/v). The flow-rate was 1 mL/min. Docosane was used to measure the column hold-up time, which was found to be 2.985 ± 0.004 min.

Statistical Treatment of Experimental Data

Up to eight measurements for the monoacid CHDE species for which synthetic standards were available, and at least four measurements for the rest of the species were used to determine the respective retention factor *k*. Relative standard deviations did not exceed 5%. The mean *k* values for the pairs of 1,2- and 1,4-diesters with the same acyl chains were compared by the means of *t*-test at two levels of confidence, 95% and 99%. Values were considered significantly different only when $t_{\text{calculated}} > t_{\text{tabulated}}$ at both levels of confidence.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

RESULTS AND DISCUSSION

Cis 1,2- and the mixture of *cis*- and *trans*-1,4-cyclohexanediol fatty acid diesters (CHDDE) were assumed to be relevant models that resemble the 1,2- and 1,3-diacylglycerols, respectively. These diesters have advantages over diacylglycerols per se in that: (a) the spatial position of the acyl residues is more rigid and effects should be more clear; (b) there is no additional functionality, like the free hydroxyl group in diacylglycerols which needs derivatization, thus, introducing another parameter into retention behavior. The *cis* and *trans* isomerism of CHDE concerns the spatial position of the hydroxyl groups relative to the plane of the ring and may affect, additionally, the distance between the fatty acyl residues.

The intention of this work was to produce all possible mono- and mixed-acid *cis*-1,2- and *cis*/*trans*-1,4-diesters of cyclohexanediol with saturated (S), monoenoic (M), and dienoic (D) acyl residues, and to elucidate the effect of the fatty acid composition and the position of the acyl residues in the molecule on the retention by Ag-HPLC. Natural fatty acid mixtures obtained by alkaline hydrolysis of sunflower oil and lard were used as substrates for production of the CHDDE. Lard and sunflower oils have sufficient proportions of saturated, monoenoic and dienoic fatty acids to allow for the production of all possible combinations of 1,2- and 1,4-diesters with zero to four double bonds in reasonably high amounts. Separately, monoacid 16 : 0, 18 : 1, and 18 : 2 CHDDE were synthesised by using the appropriate pure fatty acid standards. Yields were usually low, not exceeding 20% of the starting material, the main product of the reaction being monoesters. However, sufficient amounts of the required products (diesters) were isolated from the reaction mixture by preparative silica gel TLC (the R_f values of mono- and di-esters were 0.2 and 0.6, respectively).

When subjected to Ag-HPLC, the CHDDE mixtures were clearly resolved into components depending on the degree of unsaturation. The monoacid diesters from sunflower oil and lard were identified by comparison of elution times with the monoacid CHDDE standards. The identification of the mixed acid diesters was based on the known elution order of diacylglycerol acetates in Ag-TLC.[11] SS, SM, MM, SD, and MD CHDDE were detected in the lard fatty acid mixture, and SM, MM, SD, MD, and DD species were found in the mixture obtained from sunflower oil (eluting in this order). The mobile phases used were similar to those applied for the resolution of triacylglycerols,[7] and were either dichloromethane–1,2-dichloroethane–acetone (1 : 1 : 0.4, v/v/v) for CHDDE from lard or acetone–acetonitrile (98.5 : 1.5, v/v) for CHDDE from sunflower oil.

From previous studies, it is known that the main interaction that defines retention in Ag-HPLC is the complexation with silver ions. Under comparable experimental conditions and for the same fatty acid composition, the retention factor, *k*, is a measure of the strength of the complexes formed. Table 1

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 1. Retention factors (k)[a] for *cis*-1,2- and *cis*/*trans*-1,4-cyclohexanediol diesters (CHDDE) in Ag-HPLC on a column Nucleosil™ 5SA in silver ion form; flow rate 1 mL/min; evaporative light-scattering detector.

Compound[b]	$k_{1,2\text{-CHDDE}}$	$k_{1,4\text{-CHDDE}}$	$k_{1,2\text{-CHDDE}}/k_{SM}$	$k_{1,4\text{-CHDDE}}/k_{SM}$
SM	0.65	0.71	1	1
MM	5.74*	4.94*	8.8	7.0
SD	10.22	10.14	15.7	14.3
MD	24.36*	21.32*	37.5	30.0
DD	84.42*	74.36*	129.9	104.7

[a]The k values are mean of four separate injections. All values were recalculated for the mobile phase dichloromethane–1,2-dichloroethane–acetone, 1 : 1 : 0.4 (v/v/v) by using the MM-CHDDE as an internal standard.
[b]S, saturated, M, monoenoic, D, dienoic acyl residues.
*The k values for the respective 1,2- and 1,4-CHDE pairs are significantly different.

presents the values of the retention factors for the series of *cis*-1,2- and *cis*/*trans*-1,4-CHDDE studied in this paper. The *cis* and *trans* forms of the 1,4-CHDDE were not separable under our conditions. There was no significant difference between the k values of 1,2-*cis* and 1,2-*trans* CHDDE and the values of 1,2-*cis* diesters, only, are listed in the Table. To allow the retention of all diesters to be compared, all values were recalculated for the mobile phase dichloromethane–1,2-dichloroethane–acetone (1 : 1 : 0.4, v/v/v) by using the MM-CHDDE as an internal standard. In general the chromatographic behavior of CHDDE was very similar to that found earlier for the triacylglycerols,[7] taking into account that the spatial position of the acyl moieties in CHDDE is fixed to a greater extent than that in triacylglycerols. The results presented in Table 1, confirm the rapid increase of the k values with increasing unsaturation, an observation made earlier for simple esters of fatty acids[4] and for triacylglycerols.[7] The introduction of a second monounsaturated acyl residue causes a 9-fold increase for 1,2-CHDDE and a 7-fold increase for 1,4-CHDDE in the respective k values. Also, the DD molecule is retained about 15 times more strongly than the MM molecule.

In addition, a small but measurable and statistically significant effect of the position of the acyl chains (1,2- vs. 1,4-) on the k and the relative k values (calculated as k_{CHDDE}/k_{SM} and listed in Table 1) was observed for MM, MD, and DD diesters, the 1,2- being held more strongly than the 1,4-. The k values of 1,2- and 1,4-SD- and 1,2- and 1,4-SM CHDDE differ only slightly. The t-test showed that the values are significantly different at 95% but not at 99% level of confidence and were, therefore, considered statistically equal under the experimental conditions of the present work. According to the present results,

it seems that formation of a chelate-type complex between a silver ion and simultaneously two double bonds on two adjacent unsaturated fatty acyl chains in CHDDE during silver ion chromatography, is possible but only to a limited extent; it is assumed that such an interaction is less (or not) probable with 1,4-diunsaturated CHDDE species. Additionally, the present findings are consistent with the inability of complexations of this type to occur between adjacent saturated and unsaturated chains.

In light of the results, it is possible to speculate, therefore, that retention of triacylglycerols is the result of a complicated complexation process and simultaneous participation of a double bond and the carbonyl oxygen is probably responsible for much of the strength of the interaction with silver ions, while simultaneous interaction of a silver ion with the double bonds of two unsaturated acyl chains appears to be of only minor consequence. However, it is now difficult to explain why there is a large difference in retention between, for example, SSM and SMM species.[7] There are obviously aspects of the complexation that are not understood.

It is interesting to relate the differences in retention behavior between 1,2- and 1,4-diunsaturated CHDDE species, to the ability of silver ion chromatography to differentiate between triacylglycerol species that differ by the position of the acyl moieties in the molecule. Evidently, for species that contain a single unsaturated acyl chain, the isomer that provides the easier spatial access of silver ions to this chain will be held stronger. Thus, irrespective of the silver ion separation technique used, SMS and SDS were retained less strongly than SSM and SSD.[8–10] However, in contrast to the present results, MMS was held less strongly than MSM in both Ag-TLC[8] and Ag-HPLC.[9,10] Likewise, by silver ion HPLC (mobile phase hexane–acetonitrile), DDS and DDM were retained less strongly than DSD and DMD, respectively,[10] whereas the reverse was observed in Ag-TLC (mobile phase chloroform–methanol).[8] There are two important differences between the CHDDE species studied in the present work, and triacylglycerols: (i) the 1,3-acyl chains are positioned closer together than the 1,4-acyl chains in CHDDE; and (ii) the triacylglycerol molecule is much less rigid and many conformations are possible, depending for example, on the mobile phase composition. Thus, it could be speculated that in some cases, depending on the specific conformation, acyl chains in positions 1,3 could be accessed slightly easier by a silver ion than those in positions 1,2 to form a chelate-type complex.

ACKNOWLEDGMENTS

The partial financial support of the Bulgarian National Science Fund, contract No. X-1009, and of the Scottish Executive Environment and Rural

Affairs Department are gratefully acknowledged. Thanks are due to Mrs Velikova for skillful technical assistance.

REFERENCES

1. Nikolova-Damyanova, B. Silver ion chromatography and lipids. In *Advances in Lipid Methodology–One*; Christie, W.W., Ed.; The Oily Press: Ayr (Scotland), 1992; 181–237.
2. Dobson, G.; Christie, W.W.; Nikolova-Damyanova, B. Argentation chromatography of lipids and fatty acids. J. Chromatogr. B **1995**, *671*, 197–222.
3. Christie, W.W. A stable silver-loaded column for the separation of lipids by high performance liquid chromatography. J. High Resol. Chromatogr. & Chromatogr. Commun. **1987**, *10* (3), 148–150.
4. Nikolova-Damyanova, B.; Herslof, B.; Christie, W.W. Silver ion HPLC of derivatives of isomeric fatty acids. J. Chromatogr. **1992**, *609*, 133–140.
5. Nikolova-Damyanova, B.; Christie, W.W.; Herslof, B. Mechanistic aspects of fatty acid retention in silver ion chromatography. J. Chromatogr. A **1996**, *749*, 47–54.
6. Damyanova, B.; Momchilova, S.; Bakalova, S.; Zulof, H.; Christie, W.W.; Kaneti, J. Computational probes into the conceptual basis of silver ion chromatography: I. Silver(I) ion complexes of unsaturated fatty acids and esters. J. Mol. Struct.: THEOCHEM **2002**, *589–590*, 239–249.
7. Nikolova-Damyanova, B.; Christie, W.W.; Herslof, B. Retention properties of triacylglycerols on silver ion high-performance liquid chromatography. J. Chromatogr. A **1995**, *694*, 375–380.
8. Nikolova-Damyanova, B.; Chobanov, D.; Dimov, S. Separation of isomeric triacylglycerols by silver ion thin-layer chromatography. J. Liq. Chromatogr. **1993**, *16* (18), 3997–4008.
9. Jeffrey, B.S.J. Silver-complexation liquid chromatography for fast, high-resolution separation of triacylglycerols. J. Am. Oil Chem. Soc. **1991**, *68*, 289–293.
10. Adlof, R.O. Analysis of triacylglycerol positional isomers by silver ion HPLC. J. High Resol. Chromatogr. **1995**, *18*, 105–107.
11. Christie, W.W. *Lipid Analysis*; Pergamon Press: Oxford, England, 1982.

Received November 30, 2002
Accepted January 16, 2003
Manuscript 6052

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016