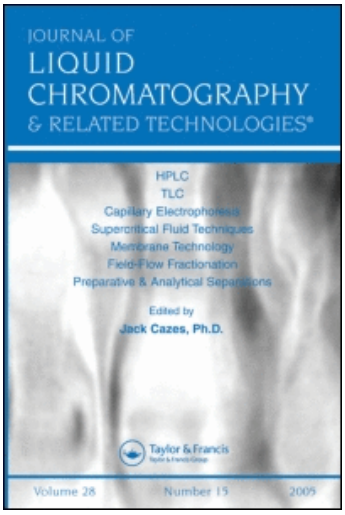

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Relative Retention Times of Molecular Species of Acylglycerols, Phosphatidylcholines, and Phosphatidylethanolamines Containing Ricinoleate in Reversed-Phase HPLC

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ABSTRACT

The relative retention times (RRT) of twenty one molecular species of diacylglycerols (DAG), forty two molecular species of triacylglycerols (TAG), thirty six molecular species of phosphatidylcholines (PC), and thirty six molecular species phosphatidylethanolamines (PE) in reversed-phase HPLC are reported. They were derived from incorporation of six [^{14}C]-labeled fatty acids, ricinoleate (R), stearate (S), oleate (O), linoleate (L), linolenate (Ln), and palmitate (P) in castor microsomal incubations. Most of these RRT were not reported earlier because the standards were

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not available, including the molecular species containing R. In general, DAG eluted earlier than TAG on a C_{18} HPLC used for the separation of the molecular species of acylglycerols (AG). When only one acyl chain differed in the molecular species of both DAG and TAG, the elution order was: R, Ln, L, P, O, and S. Two C_8 HPLC systems were used for separation of the molecular species of PC and PE, respectively, with a more polar eluent used to elute the less polar PE. The elution order for the molecular species of both PC and PE, when one acyl chain was different, was the same as that of AG. The pairs of positional isomers of the molecular species of AG, PC, and PE elute closely. The RRT and elution characteristics reported are useful for the preliminary identification of molecular species of AG, PC, and PE, the important lipid classes in oil biosynthesis and membrane formation.

Key Words: HPLC; Relative retention times; Ricinoleate; Molecular species; Acylglycerols; Phosphatidylcholines; Phosphatidylethanolamines.

INTRODUCTION

Lipids are important components in living systems and have many uses in medicine and industry. The HPLC separation of lipid classes[1] and HPLC separations of the molecular species of lipid classes, triacylglycerols (TAG),[2] and phosphatidylcholines (PC),[3] have recently been reviewed. We have also reported the reversed-phase HPLC separations of the molecular species of lipid classes, fatty acids (FA),[4] acylglycerols (AG),[5] PC,[6] and phosphatidylethanolamines (PE),[7] as well as their relative retention times (RRT). Relative retention times is the retention time (RT) normalized to the RT of a standard in an HPLC run, e.g., triolein (OOO) at 32.8 min reported earlier,[5] and we have found it to be useful in comparing HPLC separations carried out at different times. These HPLC systems[4–7] can be used for the identification of [^{14}C]metabolites in lipid biosynthesis by co-chromatography with standards, and by matching the RT of absorbance detector (205 nm) and flow scintillation analyzer in sequence. The eluents used are the mixtures of methanol, 2-propanol and/or water, which have low A_{205}, do not quench in scintillation counting, and are among the least toxic of elution solvents.

These methods have been used in establishing the biosynthetic pathway of castor oil [TAG containing high level of ricinolate (R)] by identifying the [^{14}C]-labeled metabolites.[8,9] We have recently expanded this pathway of lipid classes to their molecular species by using these HPLC methods to identify and quantify many molecular species of [^{14}C]AG, [^{14}C]PC, and [^{14}C]PE that incorporate each of six [^{14}C]FA in castor microsomal incubations.[10,11] Molecular species of lipid classes containing R are not commercially available

as standards. We report here the RRT of the molecular species of AG, PC, and PE incorporating these six [^{14}C]FA, which can aid in the identification of molecular species of AG, PC, and PE, especially the molecular species containing R.

EXPERIMENTAL

HPLC was carried out on a liquid chromatograph (Waters Associates, Milford, MA), using an absorbance detector (Waters 2487) at 205 nm and a flow scintillation analyzer (150TR, Packard Instrument Co., Downers Grove, IL) to detect [^{14}C]-labeled compounds. The flow-rate of liquid scintillation fluid (Ultima Flo M, Packard Instrument Co.) of the flow scintillation analyzer was 3 mL/min. A 200 μL scintillation flow cell was used. Molecular species of AG were separated as previously reported,[5] using a C_{18} column (25 × 0.46 cm, 5 μm, Ultrasphere C_{18}, Beckman, Fullerton, CA) with a linear gradient of 100% methanol to 100% 2-propanol in 40 min. Molecular species of PC were separated as previously reported,[6] using a C_8 column (25 × 0.46 cm, 5 μm, Luna C_8, Phenomenex, Torrance, CA) with a linear gradient of 90% aqueous methanol to 100% methanol, both containing 0.1% of conc. NH_4OH, in 40 min. Molecular species of PE were separated as previously reported,[7] using the same C_8 column with a linear gradient of 88% aqueous methanol to 100% methanol, both containing 0.1% of conc. NH_4OH, in 40 min. NH_4OH was used as the silanol suppressing agent.[3]

RESULTS AND DISCUSSION

Six [1-^{14}C]-labeled FA, ricinoleate (R), stearate (S), oleate (O), linoleate (L), linolenate (Ln), and palmitate (P) were incubated in castor microsomes individually.[10,11] The lipids incorporating label, [^{14}C]AG, [^{14}C]PC, and [^{14}C]PE, were fraction collected after separation on silica HPLC[12] of the total lipid extracts separating lipid classes. The fractions of [^{14}C]AG, [^{14}C]PC, and [^{14}C]PE were then separated into the molecular species by reversed-phase HPLC and co-chromatographed with standards to identify the [^{14}C]peaks. The chromatograms have been shown earlier.[10,11] When the standards were not available, the designation of molecular species was done by the elution characteristics of each lipid class.[5–7] In addition, the designation of molecular species of TAG was also done using the RRT prediction method.[13]

Sixty-three molecular species of AG, thirty-six molecular species of PC, and thirty-six molecular species of PE, incorporated from six [^{14}C]FA, were identified and designated by HPLC.[10,11]

Their RRT in order of elution are shown in Tables 1–3. Among them, RRT of forty-eight AG (Table 1), twenty-one PC (Table 2), and twenty-eight PE (Table 3) were not previously reported.[5–7] The FA constituents of AG, PC, and PE in Tables 1–3 are limited to the six FA used in the incorporation studies,[10,11] while the lipid standards used in our previous reports of RRT[5–7] contained other FA. In the reversed-phase HPLC system, the elution order depends on polarity of their FA constituents with elution time increasing as polarity decreases.

Diacylglycerols (DAG) eluted earlier than TAG, in general, because DAG contains a polar hydroxyl group on glycerol backbone and one less acyl chain (Table 1). However, some TAG containing R (with a hydroxyl group on acyl chain) eluted earlier than DAG containing no R. The elution of molecular species of DAG with one identical acyl chain and one different acyl chain, as shown in Table 1, were in order of increasing RT: R, Ln, L, P, O, S, e.g., RR, RLn, RL, RP, RO, RS. The RRT of seven DAG containing P, O, and S were previously reported.[5] We report here the RRT of twenty-one molecular species of DAG, among them seventeen that have not previously been reported[5] and are not available commercially. The elution order of the molecular species of TAG with two identical acyl chains and one different acyl chain were in order of increasing RT: R, Ln, L, P, O, S, the same as that of DAG. This order was the same as the elution orders of free FA and methyl esters of FA reported in C_{18} HPLC.[4] The elution orders of the molecular species of DAG and TAG containing the FA other than these six FA, are likely similar to the elution order of FA reported earlier on a C_{18} HPLC.[4] We do not know the arrangement of acyl chains on AG containing R shown in Table 1. The HPLC system is capable of separating the lipids of differing regiospecific isomers slightly, while enantiomers are not separated.

Tables 2 and 3 show the RRT of the molecular species of PC and PE, respectively, that incorporate one of six [^{14}C]FA in castor microsomal incubations. The structures of PC and PE in Tables 2 and 3 were stereospecific, since the incorporation of FA were almost exclusively in the *sn*-2 position of PC and PE.[11] Isomers of *sn*-1,2, in general, could not be separated well (Tables 2 and 3). The elution orders of the molecular species of PC and PE depend on their FA constituents. As shown in Tables 2 and 3, it was: R, Ln, L, P, O, and S consistently, when one acyl chain on PC (or PE) was the same. This order is the same as that of AG on C_{18} HPLC reported here and the same as PC[6] and PE[7] previously reported on C_8 HPLC. The elution orders of PC and PE containing FA, other than these six FA, are likely similar to the elution order of FA reported earlier on a C_{18} HPLC.[4] The elution characteristics of PC and PE obtained here are similar.

Relative retention times of seventeen molecular species of DAG, thirty-one molecular species of TAG, twenty-one molecular species of PC, and

Table 1. Relative retention times (min) of AG incorporating label R, S, O, L, Ln, and P in castor microsomal incubations on C_{18} HPLC.[a]

AG	RRT	AG	RRT	AG	RRT	AG	RRT	AG	RRT
RR	3.2	RRL	12.9	OS	18.3	RLS	23.4	LLO	28.9
RLn	4.8	LP	13.7	RLLn	18.3	LnLLn	24.1	LSLn	29.5
RL	6.0	OL	14.0	RLnP	19.6	RSP	24.5	LOP	29.7
RP	7.0	RRP	14.1	ROLn	19.7	LLnLn	24.5	LOO	30.5
RO	7.4	RRO	14.5	RLL	19.8	ROS	24.9	LLS	30.7
RRR	7.4	LnS	14.8	SS	20.5	LLLn	26.4	LSL	30.8
LnLn	9.1	PP	15.6	RLP	20.9	RSS	26.8	LSP	31.4
RS	9.7	OP	15.9	RLO	21.4	LnLP	26.9	OOO	31.8
LLn	10.7	OO	16.0	RLnS	21.9	LOLn	27.0	OLS	32.2
RRLn	11.6	LS	16.6	RPP	22.3	LLL	27.5	LSO	32.3
LnP	12.1	RRS	16.6	ROP	22.6	LLP	28.2		
OLn	12.3	RLnLn	16.7	LnLnLn	22.7	LnSLn	28.2		
LL	12.5	SP	18.1	ROO	22.8	OOLn	28.3		

[a]For HPLC conditions, see Experimental. Relative retention times given here are normalized to RT (14.5 min) of RRO reported previously.[5] Triacylglycerols containing R and DAG are not regiospecific.

Table 2. Relative retention times (min) of PC incorporating [^{14}C]FA in castor microsomes in C_8 HPLC.[a]

PC	RRT	PC	RRT	PC	RRT	PC	RRT
RR-PC	9.4	RS-PC	22.6	OLn-PC	28.2	PO-PC	32.1
LnR-PC	14.7	SR-PC	22.7	LP-PC	29.2	LS-PC	33.5
RLn-PC	16.0	LnLn-PC	23.2	PL-PC	29.6	OO-PC	33.6
LR-PC	17.1	LnL-PC	25.1	LO-PC	30.5	SL-PC	33.7
RL-PC	17.2	LLn-PC	25.7	OL-PC	30.7	SP-PC	35.0
PR-PC	18.6	LnP-PC	26.6	LnS-PC	30.8	PS-PC	35.2
RP-PC	18.7	PLn-PC	27.1	PP-PC	30.8	OS-PC	36.3
OR-PC	19.6	LnO-PC	27.8	SLn-PC	30.9	SO-PC	36.4
RO-PC	19.7	LL-PC	27.9	OP-PC	31.9	SS-PC	39.1

[a]For HPLC conditions, see Experimental. Relative retention times given here are normalized to RT (32.1 min) of PO-PC reported previously.[6]

twenty-eight molecular species of PE, which were not previously reported, nor have standards commercially available, are reported. Most of these were the RRT of molecular species containing R produced in the course of our studies on castor oil biosynthesis.[10,11] The RRT and elution characteristics obtained are useful for the preliminary identification of the molecular species of these lipid classes, AG, PC, and PE, and we have found this information very helpful in our metabolic profiling of lipid biosynthesis.

Table 3. Relative retention times (min) of PE incorporating [^{14}C]FA in castor microsomes in C_8 HPLC.[a]

PE	RRT	PE	RRT	PE	RRT	PE	RRT
RR-PE	9.7	LnLn-PE	21.8	LL-PE	27.4	PO-PE	31.6
LnR-PE	15.2	SR-PE	22.3	PL-PE	28.8	OO-PE	32.5
RLn-PE	15.3	RS-PE	22.5	LP-PE	28.9	SL-PE	32.7
LR-PE	17.7	LLn-PE	24.4	LO-PE	29.8	LS-PE	32.8
RL-PE	18.3	LnL-PE	24.8	OL-PE	29.9	PS-PE	34.2
PR-PE	19.1	PLn-PE	25.9	PP-PE	30.1	SP-PE	34.2
RP-PE	19.2	LnP-PE	26.5	LnS-PE	30.8	SO-PE	35.3
RO-PE	19.8	OLn-PE	27.2	SLn-PE	30.8	OS-PE	35.3
OR-PE	20.4	LnO-PE	27.4	OP-PE	31.4	SS-PE	38.7

[a]For HPLC conditions, see Experimental. Relative retention times given here are normalized to RT (31.6 min) of PO-PE reported previously.[7]

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