The Structure of the Triacylglycerols, Containing Punicic Acid, in the Seed Oil of *Trichosanthes kirilowii*

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ABSTRACT: Triacylglycerols of the seed oil of *Trichosanthes kirilowii* have been resolved by high-performance liquid chromatography (HPLC) in the silver-ion and reverse-phase modes. The fatty acids were identified by gas chromatography/mass spectrometry as the picolinyl esters. The main components are *cis,cis*-C18:2n-6 (38.2 mole%), C18:$3_{9c,11t,13c}$ (punicic acid, 38.0 mole%), and *cis*-C18:1n-9 (11.8 mole%). Small amounts of C18:$3_{9c,11t,13t}$ (α-eleostearic acid) and C18:$3_{9t,11t,13c}$ (catalpic acid) were detected. Silver-ion HPLC exhibited excellent resolution in which fractions were resolved on the basis of the number and configuration of double-bonds. In this instance, the strength of interaction of a conjugated trienoic double-bond system with silver ions seemed to be between that of dienoic and monoenoic double bond systems. The triacylglycerols were also separated into 20 fractions by reverse-phase HPLC, and the fractionation was achieved according to the partition number in which a conjugated trienoic double bond was not equivalent to three monoenoic double bonds in a molecule with a given chainlength. The principal triacylglycerol species are (C18:2n-6)(C18:$3_{9c,11t,13c}$)$_2$ (28.3 mol% of total), (C18:1n-9)(C18:2n-6)(C18: $3_{9c,11t,13c}$) (19.0 mol%), and (C18:2n-6)$_2$(C18:$3_{9c,11t,13c}$) (16.2 mol%), while simple triacylglycerols such as (C18:$3_{9c11t,13c}$)$_3$ and (C18:2n-6)$_3$ were present as minor components only (<1.0 mol%). Species esterified with conjugated trienoic acids comprise more than 96% of the total.

Stereospecific analysis gave rise to insurmountable difficulties, but it is evident that there is some asymmetry in the distribution of fatty acids in the molecules.
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KEY WORDS: α-Eleostearic acid, punicic acid, reverse-phase HPLC, silver-ion HPLC, stereospecific analysis, *Trichosanthes kirilowii*.

The seed oils of *Fevillea trilobata* and *Trichosanthes anguina* of the Cucurbitaceae family contain punicic acid (C18:$3_{9c,11t,13c}$) as a major component with a small amount of α-eleostearic acid (C18:$3_{9c,11t,13t}$) (1,2). The conjugated trienoic fatty acids also are present in the seed oils of some plants belonging to the Bignoniaceae, Punicaceae, Rosaceae, and Euphorbiaceae families (3). These unusual fatty acids, which are limited to triacylglycerols, can be easily oxidized and polymerized to viscous oils, even at ordinary temperatures (3), and give various characteristics—desirable or undesirable—to the oil. However, relatively little is known of the detailed chemical and physical properties, as well as the biosynthetic mechanism for formation of the triacylglycerols. One approach to the problems is to clarify the structure of the triacylglycerols in the oils. Recently, Comes *et al.* (4) separated the triacylglycerols of cherry seeds containing α-eleostearic acid on the basis of molecular species by reverse-phase high-performance liquid chromatography (RP-HPLC).

In this study, the structural analysis of the triacylglycerols in the seed oil of *Trichosanthes kirilowii*, which contains mainly punicic acid, has been performed for the first time by HPLC in both reverse-phase and silver-ion modes.

EXPERIMENTAL PROCEDURES

Lipid samples and reagents. The seeds were taken from ripened fruits of *T. kirilowii* grown in the suburbs of Pusan, Korea, and air-dried in a shady place. All solvents and reagents were Analar- or HPLC-grade and were supplied by Fisons Ltd. (Poole, United Kingdom). The total lipids were extracted under an atmosphere of nitrogen according to the method of Bligh and Dyer (5). Triacylglycerols were purified on Isolute™ silica solid-phase extraction columns (International Sorbent Technology Ltd., Mid Glamorgan, United Kingdom) with hexane/acetone (99:1, vol/vol; 30 mL). The eluants were evaporated to dryness under vacuum. All the extraction procedures were performed in a darkened room.

RP-HPLC separation of methyl esters of fatty acids. A Spectra-Physics (St. Albans, United Kingdom) model SP 8770 isocratic pump and a Pye Unicam (Cambridge, United Kingdom) model PU 4025 ultraviolet detector were used with a Hewlett-Packard (Stockport, Cheshire, United Kingdom) HP3395 integrator. Methyl esters (10 μL of a 1% solution in acetonitrile) were injected onto a column of ChromSpher™ C18 (3 μm particles, 100 × 4.6 mm i.d.; Chrompack U.K. Ltd., London, United Kingdom), eluted with acetonitrile/water (80:20, vol/vol) at a flow rate of 1 mL/min with detection at 205 nm.

Silver-ion HPLC analysis of triacylglycerols. HPLC analysis was carried out with a Spectra-Physics model 8700 solvent delivery system, a Cunow (Cergy St. Christophe, France)

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Model DDL 21 light-scattering detector and a Hewlett-Packard HP 3395 integrator. A stream-splitter (approx. 8:2) was placed between the column and the detector. A silver-ion column (Nucleosil™ 5SA, 250 × 4.6 mm i.d.; HPLC Technology, Macclesfield, United Kingdom) was prepared as described elsewhere (6). The resolution of triacylglycerols was conducted with a ternary gradient system, which consisted of 1,2-dichloroethane/dichloromethane (1:1, vol/vol) (A), acetone (B), and acetone/acetonitrile (9:1, vol/vol) (C). The mobile phase composition was changed linearly from 100% solvent A to 50% solvent A/50% solvent B over 5 min, then to 20% solvent A/50% solvent B/30% solvent C over 60 min, and finally 50% solvent B/50% solvent C was eluted for 5 more min. The column was kept at ambient temperature, and the flow rate was 1.0 mL/min. Sample (1–1.2 mg) was dissolved in 1,2-dichloroethane (1 mL), and aliquots (10 μL) were injected onto the column.

RP-HPLC analysis of triacylglycerols. The instruments used for RP–HPLC were as above. Triacylglycerols were fractionated on the ChromSpher™ C18 column with a binary solvent gradient system composed of dichloromethane/acetonitrile (20:80, vol/vol) (A) and dichloromethane/acetonitrile (30:70, vol/vol) (B). The mobile phase profile was 100% solvent A for 20 min and was then linearly programmed from 100% solvent A over a further 20 min. The column was kept at room temperature, and the flow rate was 1.0 mL/min.

Gas chromatography (GC) of fatty acid methyl esters. Methyl nonadecanoate (internal standard, 25 μL of a solution of 5.8 mg in 25 mL hexane) and 1 mL of 0.01% butylated hydroxytohune (BHT) in hexane were added to the fractions collected from a single HPLC run. The triacylglycerols of each fraction were transmethylated with sodium methoxide–methanol solution for 5 min at ambient temperature, and the methyl esters were recovered with hexane. Care was taken to protect it from light and to remove the excess solvent gently in a stream of nitrogen at 30°C. A Hewlett-Packard Model 5890 Series II gas chromatograph with a split/splitless injection system was equipped with a capillary column (25 m × 0.22 mm i.d.) of fused silica coated with Carbowax 20M. The initial temperature of 170°C was held for 3 min and then programmed up to 210°C at 4°C/min, with a final hold for 10 min. Hydrogen was the carrier gas, and the area percentage of each peak was calculated by electronic integration.

Preparation of picolinyl esters. Picolinyl derivatives of fatty acids were prepared essentially according to the method recommended by Balazy and Nies (7), i.e., a solution of 1,1′-carbonyl diimidazole in dichloromethane, freshly prepared (100 μL, 100 mg/mL), was added to free fatty acids (1–5 mg) dissolved in dichloromethane (100 μL). The mixture was left at 37°C for 10 min, and the excess solvent was then removed in a stream of nitrogen. Hexane (5 mL) and water (2 mL) were added to the product, which was vortexed and centrifuged. The hexane layer recovered was concentrated before loading on a Pasteur pipette Florisil™ column, and the products were purified by elution with hexane/acetone (8:2, vol/vol).

GC/mass spectrometry (GC/MS). The instrument used is a Kratos Model 8/9 Double Focusing Magnetic Sector Mass Spectrometer (Carlo Erba Mega Gas Chromatograph; Fisons Instruments, Crawley, Sussex, United Kingdom). A fused-silica capillary column DB™-5ms (30 m × 0.25 mm, i.d., 0.25 μm film) coated with a cross-linked 5% phenylmethylpolysiloxane stationary phase (J&W Scientific, Folsom, CA) was connected directly to the ion source. The column temperature was programmed from 80 to 220°C at 30°C/min, and then to 270°C at 4°C/min. The spectra were recorded at an electron-impact ionization energy of 70 eV and a trap current of 100 μA. The instrument was scanned over a mass range of 18–700 amu at 0.6 s/decade. The source temperature was held at 250°C, and helium was used as carrier gas.

RESULTS AND DISCUSSION

The seed oil of *T. kirilowii* contains four unusual fatty acids, in addition to conventional monoenoic and dienoic fatty acids (which all have the *cis* configuration), as listed in Table 1. The three most abundant of the unusual fatty acids, eluting later on GC [equivalent chainlengths (ECL) = 21.42, 21.56, and 21.76], were analyzed by GC/MS for elucidation of their structures. Total fatty acids were converted into the picolinyl esters prior to injection onto the GC/MS, because these derivatives of unsaturated fatty acids give spectra that readily indicate the location of the double bonds in the molecules. The spectra of the unusual fatty acids gave identical fragmentation patterns and, therefore, have the same number of double bonds in the same positions. Figure 1 is the spectrum of the last eluted (ECL = 21.76) of these three conjugated fatty acids. The molecular ion is m/z = 369, and other diagnostic ions, such as m/z = 234, 260, 286, and 312 (26 amu between two major peaks) and at m/z = 277 $[M - 92]^+$ are clearly observed. The two conjugated trienoic fatty acids eluted earlier on the Carbowax 20M stationary phase (ECL = 21.42 and 21.56) were identified as C18:$3_{9c11t13c}$ and C18:$3_{9c,11t,13t}$ by nuclear magnetic resonance techniques (8).

The component acids (as the methyl esters) were also separated by RP-HPLC for structural analyses. The last one (ECL = 21.76 by GC) was resolved from C18:$3_{9c11t,13c}$, but coeluted with C18:$3_{9c,11t,13t}$, as shown in Figure 2, and was tentatively identified as C18:$3_{9t,11t,13c}$ by comparison of relative retention time of GC and HPLC with those published before (2). The other unknown fatty acid was separated from the C18:2_{9c12c} acid with a slight difference of retention time, but was well resolved between C18:2_{9c12c} and the C18:$2_{9t,12t}$ by RP-HPLC (Fig. 2) (9). By treatment of the total fatty acids with nitrous acid (10), the level of this component as well as of the C18:$2_{9t,12t}$ acid increased. This fatty acid was identified tentatively as a geometrical isomer (C18:2_{9c12t} and/or C18:$2_{9t,12c}$) of the C18:$2_{9c,12c}$ acid.

Recently, silver-ion HPLC has been extensively used for analysis of triacylglycerols (11,12–14), because this approach is a powerful tool to separate the molecular species of triacylglycerols by degree of unsaturation alone; it gives clean frac-

TABLE 1

Fatty Acid Composition (mol% of the total fatty acids) and Proportions of Triacylglycerol Fractions Obtained by Silver-Ion High-Performance Liquid Chromatography from the Seed Oil of *Trichosanthes kirilowii*

Fatty acid	Total	Check[a]	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction10	Fraction 11
16:0	4.8	3.4	31.0			18.7						
18:0	3.1	2.4				14.6						
18:1n-9	11.8	10.9	39.9	63.2	39.4	1.9	34.6	23.2				
18:1n-7	0.7	0.6		1.8			2.1	2.1				
18.2n-6	38.2	38.1				33.0	35.0	34.7	34.7	64.2	66.6	100.0
18:2(*iso*)	0.7	0.8				1.0	1.0		1.4	5.2	1.0	
18:3*c,t,c*[b]	38.0	37.9	29.2	22.7	46.8	29.4	9.8	29.3	59.5	16.1	29.1	
18:3*c,t,t*	2.1	3.5		7.9	8.8	0.9	12.4	6.4	2.2	13.2	1.5	
18.3*t,t,c*	0.4	2.3		4.5	5.0	0.5	5.0	3.5	2.2	1.3	1.7	
20:1n-9	0.2	0.2						0.9				
Mole% fraction			1.2	3.8	5.3	16.3	1.9	21.2	28.4	0.1	21.0	0.8

[a]Reconstituted from the relative proportions in each of the fractions.

[b]18:3*c,t,c*, 18:39*c*,11*t*,13*c*; 18:3*c,t,t*, 18:39*c*,11*t*,13*t*; and 18:3*t,t,c*, 18:39*t*,11*t*,13*c*.

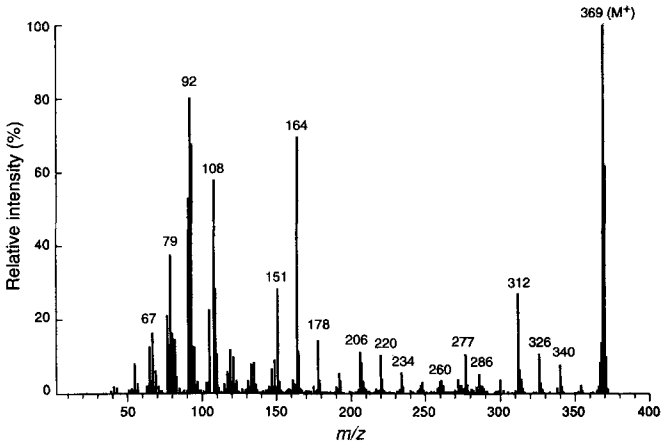

FIG. 1. Mass spectrum of the picolinyl ester of C18:$3_{9t,11t,13c}$ fatty acid.

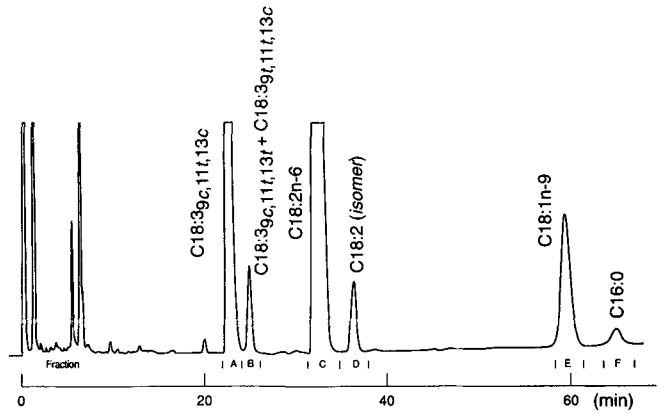

FIG. 2. Fractionation of the fatty acid methyl esters from the triacylglycerols of the seed oil of *Trichosanthes kirilowii* by reverse-phase high-performance liquid chromatography.

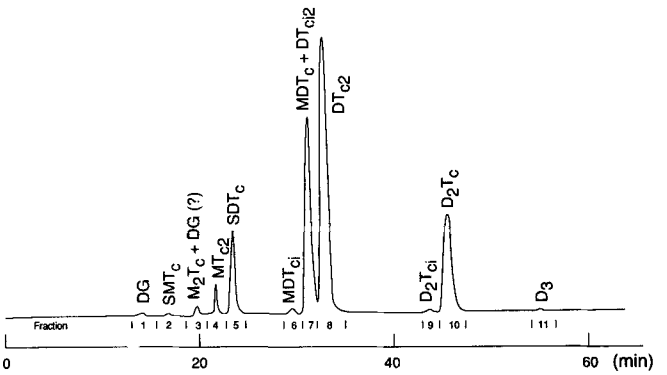

FIG. 3. Fractionation of the triacylglycerols from the seed oil of *Trichosanthes kirilowii* by silver-ion high-performance liquid chromatography; DG, diacylglycerol; M, monoenoic acid; S, stearic acid; T, trienoic acid; D, dienoic acid; subscript i, denotes configurational isomers of the main conjugated acid mentioned.

tions without contamination by silver ions and other artifacts often experienced when thin-layer chromatography (TLC) is used in conjunction with silver ions. When this technique was applied to analysis of the triacylglycerols that contained fatty acids with conjugated double bonds, excellent resolution was achieved, as illustrated in Figure 3. The abbreviations S, M, D, and $T_{c(i)}$ stand for saturated, monoenoic, dienoic, and conjugated trienoic acids, respectively, and the subscript i of Tc(i) denotes configurational isomers of the main conjugated trienoic acid (C18:$3_{9c,11t,13c}$). Each fraction collected from silver-ion HPLC *via* the stream-splitters was transmethylated in the presence of an internal standard, and the methyl esters recovered were analyzed by GC for identification and quantitation of the molecular species in the fraction. The results are listed in Table 1. As a check on the recoveries, the fatty acid composition of the whole was reconstituted from the relative proportions in each of the fractions. The fatty acid composition calculated in this way was in good agreement, in general, with that of the intact triacylglycerols, although the proportion of the 18:3 isomers (C18:$3_{9c,11t,13t}$ and C18:$3_{9t,11t,13c}$) appeared to have increased. Although the presence of such isomers in this oil could arise through isomerization induced by light and other factors during experimental procedures, the possibility that they are present in the seed as metabolites *per se* cannot be ruled out, because they have all been found as main components in the fatty acids of the seed oils of *Mo-*

mordica charantia (2,8), *Catalpa ovata* (2,15), and tung (2,15).

With silver-ion HPLC, eleven components were separated, as shown in Figure 3, and comprised eight main groups as follows: SMT_c, M_2T_c, MT_{c2}, SDT_c, MDT_c, DT_{c2}, D_2T_c, and D_3, according to the number of double bonds. Fraction 1 (not listed) is considered to be residual diacylglycerols, and Fraction 2 (SMT_c) can be represented as (C16:0) (C18:1n-9) ($C18:3_{9c11t,13c}$). Fraction 3 (M_2T_c) contains mainly (C18:1n-9) (C18:1n-9)($C18:3_{9c,11t,13c}$). Fraction 4 (MT_{c2}) is composed of (C18:1n-9) ($C18:3_{9c11t,13c}$)($C18:3_{9c,11t,13c}$), while Fraction 5 (SDT_c) is a mixture of (C16:0)(C18:2n-6)($C18:3_{9c,11t13c}$) and (C18:0)(C18:2n-6)($C18:3_{9t,11t,13c}$). Fraction 6 contained the molecular species represented as MDT_{ci} in which two isomers of ($C18:3_{9c,11t,13c}$) and the C18:2(isomer) were concentrated. Fraction 7 (MDT_c) was one of the main species (C18:1n-9)(C18:2n-6)($C18:3_{9c11t,13c}$) with some DT_{ci2}. Fraction 8 (DT_{c2}) is the most prominent fraction comprising the molecular species of (C18:2n-6)$(C18:3_{9c,11t,13c})_2$. Fraction 9 (D_2T_{ci}) is a minor fraction (0.1 mol%) that contains the isomeric species of D_2T_{ci} with some D_2T_c (because the precise end-point for collecting fractions is not always easy to judge). Fraction 10 {D_2T_c or (C18:2n-6)(C18:2n-6)($C18:3_{9c11t,13c}$)}, is also one of the main species, while D_3 species emerged in the last fraction.

With silver-ion HPLC of the triacylglycerols that contain conjugated trienoic acids, the elution order of the molecular species of triacylglycerols is easily defined; for instance, the simple species composed of conjugated trienoic acids eluted earlier than that of dienoic acids in spite of having larger numbers of double bonds. It means that the interaction of conjugated double bonds with silver ions is weaker than that of nonconjugated double bonds, presumably because of the delocalization of π-electrons in conjugated double bonds. In this context, it is evident that the main triacylglycerols are separated in the order of SDT_c, MDT_c, DT_{c2}, and D_2T_c. This is supported in part by reports that an acetylenic (octadec-9-ynoic) acid was eluted ahead of oleate, while *cis,trans-* and *trans,trans*-conjugated dienoic acids eluted with *cis*-monoenoic acids (16). The distribution of an unsaturated fatty acid within a triacylglycerol molecule does not affect the elution order. The *trans*-configurational isomers of triacylglycerols, such as D_2T_{ci}, DT_{ci2}, and MDT_{ci} species, were separated earlier than the normal species of D_2T_c, DT_{c2}, and MDT_c, respectively. A similar elution pattern can be seen in the elution order of methyl esters of unsaturated fatty separated by silver-ion HPLC.

Conjugated trienoic acids are easily oxidized and are decomposed when they are separated on a silica gel TLC plate or a column impregnated with silver nitrate (1,8). However, no detectable decomposition of the conjugated trienoic acids was found in the separation of the triacylglycerols by silver-ion HPLC. Excess silver ions, which do not complex with double bonds of molecules, can be reduced rapidly to metal when exposed to light and, concurrently, the oxidation of conjugated trienoic acids could occur. In contrast, in a silver-ion HPLC column, the silver ions are protected from light and dissolved oxygen, and any damage to the structures of the components is minimal (16).

The intact triacylglycerols were also analyzed by RP-HPLC with an octadecylsilyl packing material and a linear gradient elution system. They were well-resolved into 20 fractions, which could be categorized in 14 groups: T_{c3}, DT_{c2}, D_2T_c, MT_{c2}, D_3, two MDT_c, two SDT_c, MD_2, M_2T_c, M_2D, SD_2, and SMT_c, as represented in Figure 4. Each of the fractions was identified and quantitated as described above, and the results are summarized in Table 2.

The first fraction (Fraction 1) contains a simple triacylglycerol, i.e., composed of only $C18:3_{9c,11t,13c}$. The group of DT_{c2} was resolved into two fractions (Fractions 2 and 3); Fraction 2 is the most abundant one (28.3 mol%) and consisted of (C18:2n-6)($C18:3_{9c11t,13c}$)($C18:3_{9c,11t,13c}$), and Fraction 3 (4.1 mol%) mainly consisted of configurational isomers of the fatty acid found in Fraction 2 and was inevitably contaminated with the components of Fraction 2, due to incomplete resolution of the peaks.

Similar separation features are seen with the D_2T_c, MT_{c2}, and two SDT_c groups of peaks, i.e., the main peaks of molecular species comprising conjugated trienoic acids and dienoic acids, which were well-resolved with their isomer peaks differing in the configuration of double bonds in the tri- and/or dienoic acids (as experienced in the silver-ion fractionation in which the elution order of both peaks was reversed). Fraction 4 (D_2T_c, 16.1 mol%) is one of the main fractions and contained the species of $(C18:2n-6)_2(C18:3_{9c,11t,13c})$. As a minor component, the simple triacylglycerol (D_3) was isolated at a level of <1.0 mol% (Table 2).

Separations by the chainlength of the molecule were achieved for the MDT_c and SDT_c groups; the MDT_c group was partitioned into the species of (C18:1n-9)(C18:2n-6) ($C18:3_{9c11t,13c}$) (Fraction 9) and (C20:1n-9)(C18:2n-6) ($C18:3_{9c11t,13c}$) (Fraction 14), and the SDT_c group was divided into the fractions of (C16:0) (C18:2n-6)($C18:3_{9c,11t,13c}$) (Fraction 11) and (C18:0)(C18:2n-6)($C18:3_{9c,11t,13c}$) (Fraction 16). Other small fractions were identified in this way, and

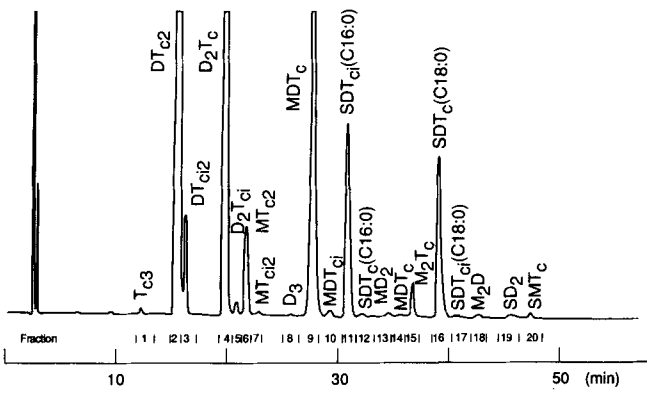

FIG. 4. Fractionation of the triacylglycerols from the seed oil of *Trichosenthes kirilowii* by reverse-phase high-performance liquid chromatography. See Figure 3 for abbreviations.

TABLE 2
Fatty Acid Compositions (mol% of the total fatty acids) and Proportions of Triacylglycerol Fractions Obtained by Reverse-Phase High-Performance Liquid Chromatography from the Seed Oil of *Trichosanthes kirilowii*

Fatty acid	Total	Check[a]	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10	Fraction 11
16:0	4.8	4.1											33.8
18:0	3.1	2.6											
18:1n-9	11.8	10.3						32.7	33.0		30.3	29.9	
18:1n-7	0.7	0.6									2.8		
18:2n-6	38.2	37.1		33.9	28.9	66.7	54.7			100.0	35.3	32.7	34.3
18:2(*iso*)	0.7	0.5			4.4		13.2					3.8	
18:3*c,t,c*[b]	38.0	39.8	100.0	59.9	45.1	29.5	14.0	63.9	40.1		29.8	25.5	28.2
18:3*c,t,t*	2.1	2.8		3.2	16.5	2.0	10.9	1.8	19.9		1.1	4.2	2.0
18:3*t,t,c*	0.4	2.0		3.1	5.1	1.8	7.2	1.7	7.0		0.7	4.0	1.7
20:1n-9	0.2	0.2											
Mole % fraction			0.6	28.3	4.1	16.1	1.1	5.2	0.5	0.8	19.0	0.8	7.9

Fatty acid	Fraction 12	Fraction 13	Fraction 14	Fraction 15	Fraction 16	Fraction 17	Fraction 18	Fraction 19	Fraction 20
16:0	31.6				10.4	16.9		24.6	8.9
18:0	4.8				25.3	20.5		16.3	28.1
18:1n-9		28.3	43.6	54.1			61.9		31.7
18:1n-7		1.6	7.2				1.4		
18:2n-6	26.8	66.6		10.3	31.9	32.6	36.7	59.2	
18:2(*iso*)	5.5			2.8					
18:3*c,t,c*[b]	19.8		26.5	26.2	32.3	25.0.			31.3
18:3*c,t,t*	8.9		4.8	3.4		4.0			
18:3*t,t,c*	2.7		4.8	3.2		1.1			
20:1n-9		3.6	13.2						
Mole %	0.9	1.1	0.7	2.0	8.3	0.4	0.7	0.6	1.1

[a]Reconstituted from the relative proportions in each of the fractions.
[b]18:3*c,t,c*, 18:39*c*,11*t*,13*c*; 18:3*c,t,t*, 18:39*c*,11*t*,13*t*; and 18:3*t,t,c*, 18:39*t*,11*t*,13*c*.

the results obtained are listed in Table 3. When the data from the silver-ion HPLC technique were used to compute relative proportions of different components, similar results were obtained (data not shown).

With the reverse-phase HPLC, the molecular species that contained conjugated trienoic acids did not merely follow the elution order that is determined by partition number, defined as the effective number of carbon atoms in all acyl residues minus twice the number of double bonds in a molecule. For instance, the species D_2T_c and MT_{c2} have the same partition number $40[(3 \times 18) - (2 \times 7)]$, but the D_2T_c fraction eluted much earlier than MT_{c2}.

Simple triacylglycerols, composed of only one of the fatty acids that occupied over 30 mol% in the composition, did not exceed 1 mol%, and this poses an interesting biosynthetic question.

It is not possible to make a comparison of the relative merits of both HPLC systems in a general manner when unusual samples are analyzed. In this instance, both methods gave excellent results, although RP–HPLC gave more distinct fractions, and these were relatively easy to identify.

Attempts to perform stereospecific analysis of triacyl-*sn*-glycerols from the seed oil of *T. kirilowii* were not successful for various reasons. They were first subjected to partial hydrolysis by either pancreatic lipase (17) or a Grignard reagent. However, the ester linkages of conjugated trienoic acids in the molecules were immune to attack by the lipase (checked by TLC, data not shown); it is possible that a bacterial lipase might have been more successful (18). Diacylglycerols from the hydrolytic mixture produced by treatment of a Grignard reagent were therefore used for resolution by chiral chromatography (11,12,19). However, the conjugated trienoic acids were almost completely isomerized into $C18:3_{9t,11t,13t}$,

TABLE 3
The Main Molecular Species of Triacylglycerols of the Seed Oil of *Trichosanthes kirilowii* as Resolved by Reverse-Phase High-Performance Liquid Chromatography (mol%)

Fraction number	Species	Amount
1	$(C18:3_{9c,11t,13c})_3$	0.6
2	$(C18:2n\text{-}6)(C18:3_{9c,11t,13c})_2$	28.3
3	Configurational isomers of Fraction 2 + Fraction 2	4.1
4	$(C18:2n\text{-}6)_2(C18:3_{9c,11t,13c})$	16.1
5	Configurational isomers of Fraction 4 + Fraction 4	1.1
6	$(C18:1n\text{-}9)(C18:3_{9c,11t,3c})_2$	5.2
7	Configurational isomers of Fraction 6 + Fraction 6	0.2
8	$(C18:2n\text{-}6)_3$	0.8
9	$(C18:1n\text{-}9)(C18:2n\text{-}6)(C18:3_{9c,11t,13c})$	19.0
10	Configurational isomers of Fraction 9 + Fraction 9	0.8
11	$(C16:0)(C18:2n\text{-}6)(C18:3_{9c,11t,13c})$	7.9
12	Configurational isomers of Fraction 11 + Fraction 11	0.9
13	$(C18:1n\text{-}9)(C18:2n\text{-}6)_2$	1.1
14	$(C20:1n\text{-}9)(C18:2n\text{-}6)(C18:3_{9c,11t,13c})$	0.7
15	$(C18:1n\text{-}9)_2(C18:3_{9c,11t,13c})$	2.0
16	$(C18:0)(C18:2n\text{-}6)(C18:3_{9c11t13c})$ + others	8.3
17	Configurational isomers of Fraction 16	0.4
18	$(C18:1n\text{-}9)_2(C18:2n\text{-}6)$	0.7
19	$(16:0)(C18:2n\text{-}6)_2$	0.6
20	$(C18:0)(C18:1n\text{-}9)(C18:3_{9c,11t,13t})$	1.1

and the diastereomeric components were not easily recognized. It appeared that there is indeed an asymmetric distribution of fatty acids in the molecules, but an improved stereospecific analysis procedure is necessary if this is to be established properly.

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