*Journal of Chromatography*, 603 (1992) 165–173
Elsevier Science Publishers B.V., Amsterdam

CHROM. 24 117

# High-performance liquid chromatographic investigations of stillingia oil

K. Aitzetmüller, Yaonian Xin*, Gisela Werner and Margaretha Grönheim

*Institute for Chemistry and Physics of Lipids, Federal Centre for Cereals, Potato and Lipid Research, Piusallee 76, W-4400 Münster (Germany)*

(First received December 3rd, 1991; revised manuscript received February 10th, 1992)

ABSTRACT

Stillingia oil is a Chinese raw material containing biosynthetically intriguing estolides. Oil extracted from the kernels of the seeds of *Sapium sebiferum* Roxb. was investigated using various high-performance liquid chromatographic (HPLC) techniques such as reversed-phase HPLC of the oil itself, its triacylglycerol and estolide fractions, both with refractive index and with UV detection at two different wavelengths, and using normal-phase (silica) HPLC of the tocopherols. Qualitative fingerprints and semi-quantitative results on triacylglycerols and estolides and on tocopherols are given. In the tocopherol fraction, stillingia oil contains nearly pure (92%) γ-tocotrienol.

## INTRODUCTION

The Chinese tallow tree *Sapium sebiferum* Roxb. is a "renewable resource" of local importance from which two totally different fats can be produced. Chinese tallow is a potential raw material for edible fats because it contains mostly symmetrical 1,3-dipalmitoyl-2-oleoylglycerol (POP)** from wich cocoa butter substitutes can be produced [1–4].

Stillingia oil is a liquid drying oil produced from seed kernels of the tree. In China it is used as a raw material for the local lacquer and paint industry [1,5–13]. Stillingia oil is interesting because it contains, in addition to a range of highly unsaturated triacylglycerols (TG) [6–9] also a range of estolides (Fig. 1). Because of this remarkable feature a number of studies have been published on stillingia oil, using more conventional methods of lipid analysis [5,10–15].

Quantitative studies using HPLC with IR detection on a silica column showed that estolides amount to about 25 mol% of the oil [10]. The estolide moiety occurs exclusively in the *sn*-3-position of the glycerol [5,11].

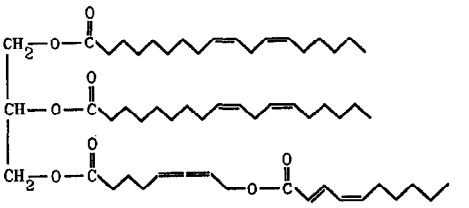

Fig. 1. A typical *Sapium sebiferum* estolide. The *sn*-3-position is esterified with the allenic ω-hydroxy-5,6-octadienoic acid, which itself is esterified with *trans*-2,*cis*-4-decadienoic acid. Positions *sn*-1 and *sn*-2 are occupied by normal unsaturated fatty acids (O,L,Ln).

*Correspondence to:* K. Aitzetmüller, Institute for Chemistry and Physics of Lipids, Federal Centre for Cereals, Potato and Lipid Research, Piusallee 76, W-4400 Münster, Germany.

* Present address: North Laodong Street 56, Xian, Shaanxi Province, China.

** Fatty acids and triacylglycerols are abbreviated {as usual in high-performance liquid chromatography (HPLC) of lipids [21,22]} using S, P, O, L and Ln for stearoyl, palmitoyl, oleoyl, linoleoyl and linolenoyl residues, respectively. E stands for estolide.

0021-9673/92/$05.00 © 1992 Elsevier Science Publishers B.V. All rights reserved

## EXPERIMENTAL

Seeds of *Sapium sebiferum* were obtained from Sichuan Province, China. After removal of the seed coat fat (Chinese tallow) [1], the seeds were ground and stillingia oil (kernel oil) was extracted with hexane.

The oil as extracted was used directly for reversed-phase HPLC of the intact oil and for HPLC of the tocopherols. For further investigation, the oil was separated into two zones by preparative thin-layer chromatography (TLC) on plates of 0.5 mm thickness [20 × 20 cm, Kieselgel 60 (E. Merck, Darmstadt, Germany)] using hexane–diethyl ether (65:35, v/v). Zone I (triacylglycerols) migrated to a higher $R_F$ value than zone II (estolides). After detection with phloxin (0.02% in ethanol), both zones were scraped off and the lipids extracted from the silica with diethyl ether.

### *Gas chromatography (GC)*

The GC system used was a Perkin-Elmer F 22 + AS 41 with flame ionization detection and a Silar 5 CP WCOT fused-silica (Chrompack, Middelburg, Netherlands) column (50 m × 0.22 mm I.D., film thickness 0.21 μm). The column temperature was programmed from 160 to 200°C at 1°C/min; the detector and injector temperatures were 230°C. A D-2000 chromato-integrator (Merck–Hitachi) was used.

Fatty acid methyl esters were prepared using boron trifluoride–methanol.

### *Optical activity*

Measurements of the specific rotation of stillingia oil, zone I and zone II were carried out in chloroform using a polarimeter (Perkin-Elmer 241 MC; chloroform blank, sodium lamp, 1.0-cm micro-cuvettes).

### *Mass spectrometry*

After hydrogenation (Walter F. C. Ebel 2410 Müller, hydrogen generator), zone II was investigated by mass spectrometry (Varian MAT CH 7 mass spectrometer, source temperature 250°C, $10^{-6}$ Torr) to verify its structure.

### *HPLC*

HPLC was carried out using two separate reversed-phase (RP) systems, one with two columns and a refractive index (RI) detector, the other with only one column and a short-wavelength UV detector. HPLC of tocopherols was carried out in the usual way using a silica column with a fluorescence detector.

HPLC system 1, used for oils, triacylglycerols and estolides, with UV and RI detection, consisted of a Merck–Hitachi 655-12 pump and LC 5000 gradient controller, Superspher RP-18 end-capped column (E. Merck) (250 × 4 mm I.D., 4 μm), Rheodyne Model 7125 injection valve (20 μl) and Model 655-A-22 variable-wavelength UV monitor set at 210, 235 and 260 nm. Alternatively, an ERC-7510 RI detector was also used with HPLC system 1. The UV-transparent mobile phase used was acetonitrile–propanol–2-hexane (70:20:10, v/v/v) at 0.8 ml/min.

HPLC system 2, used for oils, triacylglycerols and estolides, with RI detection only, consisted of a Waters Model 510 pump, Rheodyne Model 7125 injector (20 μl), two Nucleosil 100 $C_{18}$ columns (250 mm × 4 mm I.D., 5 μm) (Knauer, Berlin, Germany) and a Knauer RI detector. The mobile phase was acetonitrile–acetone (38:62, v/v) at 0.9 ml/min.

HPLC system 3, used for tocopherols in oils with fluorescence detection, consisted of a Merck–Hitachi L-6000 pump, Rheodyne Model 7125 injection valve (20 μl), a Nucleosil 50 silica column (250 × 4 mm I.D., 5 μm) (Knauer) and a Merck–Hitachi F-1000 fluorescence detector set at 295/330 nm. The mobile phase was hexane–dioxane (95:5, v/v) at 1.0 ml/min. Dilute solutions of chinese tallow and stillingia oil were injected directly.

Peak integration was performed with a Merck–Hitachi D-2500 chromato-integrator [a drawback of such an integrator for applications of this type, however, is that they are unable to recalculate relative retention times (RRT) after one peak has been designated as having RRT = 1.000].

Palm oil, soyabean oil, linseed oil and interesterified mixtures of LnLnLn, LLL, OOO and PPP (see footnote in Introduction) and saturated TG ($C_{30}$–$C_{54}$) were used to identify individual peaks in TG HPLC.

## RESULTS AND DISCUSSION

Stillingia oil and tung oil are both classical Chi-

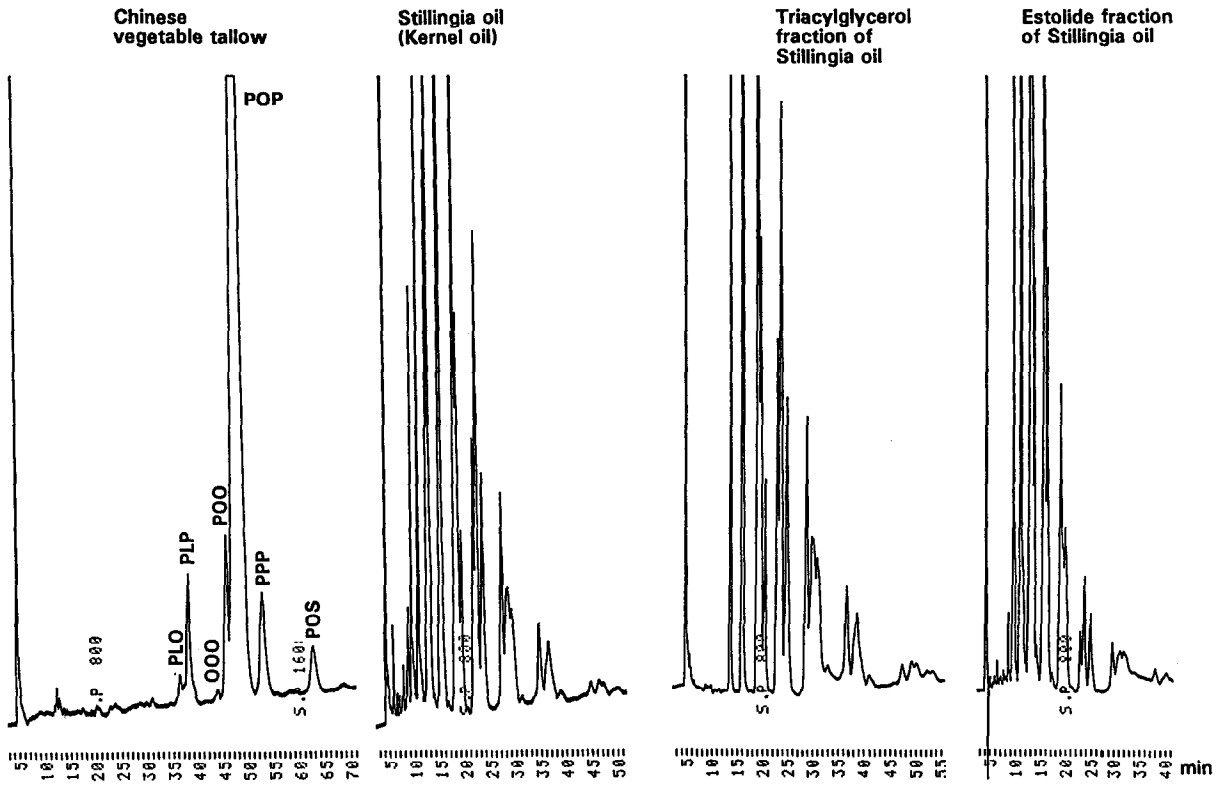


Fig. 2. RP-HPLC–RI fingerprints of Chinese tallow, stillingia oil and its triacylglycerol and estolide fractions. HPLC system 2 was used with RI detection; for HPLC conditions, see text. S, P, O, L: stearic, palmitic, oleic and linoleic acid residues, respectively.

nese drying oils. Although the content of highly unsaturated and conjugated fatty acids in tung oil is higher than in stillingia oil, the latter is the better drying oil [16]. A potential explanation for this is the presence of estolides in stillingia oil which are unstable and easily oxidized.

In conventional fingerprint RP-HPLC–RI traces of Chinese tallow, stillingia oil and its triacylglycerol and estolide fractions (Fig. 2), one notices immediately that stillingia oil and its two fractions contain much more highly unsaturated triacylglycerol and estolide molecules than Chinese tallow, which contains mostly POP.

By comparison with known oils and triacylglycerol reference mixtures, the triacylglycerol peaks in the RP-HPLC traces (UV at 210 nm and RI) were further identified as shown in Fig. 3. The identification of the estolides (--E) is tentative. Because of the different mobile phases, the separation selectivity is slightly different (compare Fig. 3a and b).

The relative contribution of one estolide (E) moiety towards reducing retention in RP-HPLC is large, although this is caused primarily by the additional (fourth) ester group. The four double bonds are not fully available for interaction with the chromatographic system, *i.e.*, they are not contributing fully to retention time reduction in RP-HPLC, two of them because they form an allene and the other two because they are conjugated. However, exchange of an L by an E residue, as in LLE *vs.* LLL peaks, causes a dramatic decrease in retention time.

The results of the optical rotation analysis of stillingia oil (Table I) show that the optical activity is clearly caused by zone II. It has been discussed [5,11] that not the estolide in the *sn*-3-position as such but rather the allene in the $C_8$ hydroxy fatty

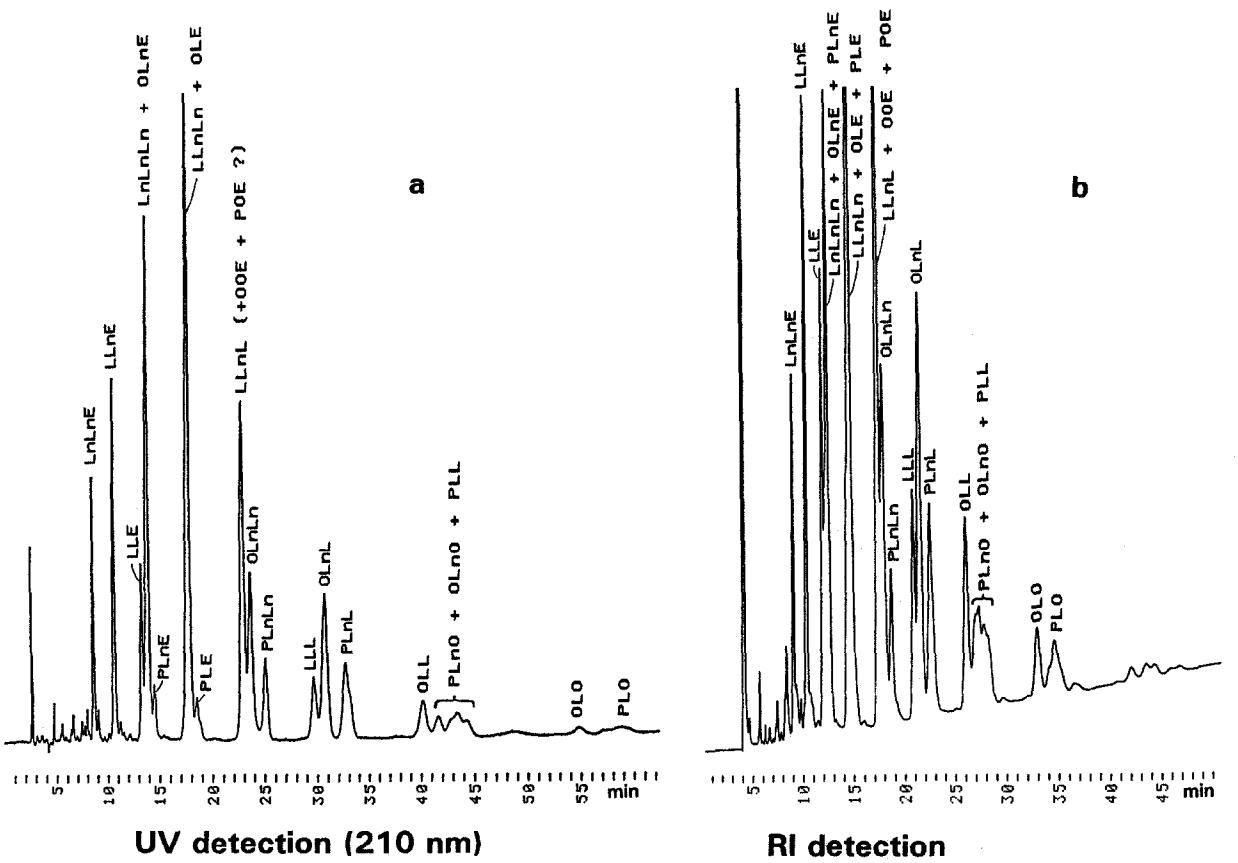


Fig. 3. RP-HPLC of intact stillingia oil: (a) with UV detection, HPLC system 1; (b) with RI detection, HPLC system 2. Triacylglycerol peaks are identified by comparison with known oils and triacylglycerol standards. Identification of estolide peaks (–E) is tentative. For HPLC conditions, see text. P, O, L, Ln: palmitic, oleic, linoleic, linolenic acid residues, respectively; E = estolide.

acid moiety of the estolides is the major cause of optical activity.

Mass spectrometry of the hydrogenated zone II showed $M^+$ at *m/z* 920 and fragments at *m/z* 297, 371, 425 and 637. This result confirms the presence of estolides [5].

In the estolides, apparently the moiety consisting of 2,4-decadienoic acid and ω-hydroxy-5,6-octadienoic acid (the allene causing the major part of the optical activity) is constant in the *sn*-3 position [5,9,11]. In RP-HPLC, the observed differences in the retention of the individual estolides are introduced by the various fatty acids (O, L, Ln) esterified at positions 1 and 2.

Fig. 4 shows GC traces of the oil and its two fractions. Table II shows the area composition found for the fatty acids; however, no precautions were taken against losses of the $C_8$ and $C_{10}$ acids [11]. Compared with the results of Sprecher *et al.* [5]

TABLE I

SPECIFIC ROTATION OF STILLINGIA OIL AND ITS FRACTIONS

| Component | Concentration (g per 100 ml) | Value measured | $[\alpha]_D^{20}$ (°) |
|---|---|---|---|
| Stillingia oil | 1.02 | −0.056 | −5.49 |
| Stillingia oil | 4.98 | −0.263 | −5.28 |
| Zone I | 2.92 | −0.011 | −0.38 |
| Zone II | 1.00 | −0.212 | −21.20 |
| Stillingia oil [13] | | | −5.01 |

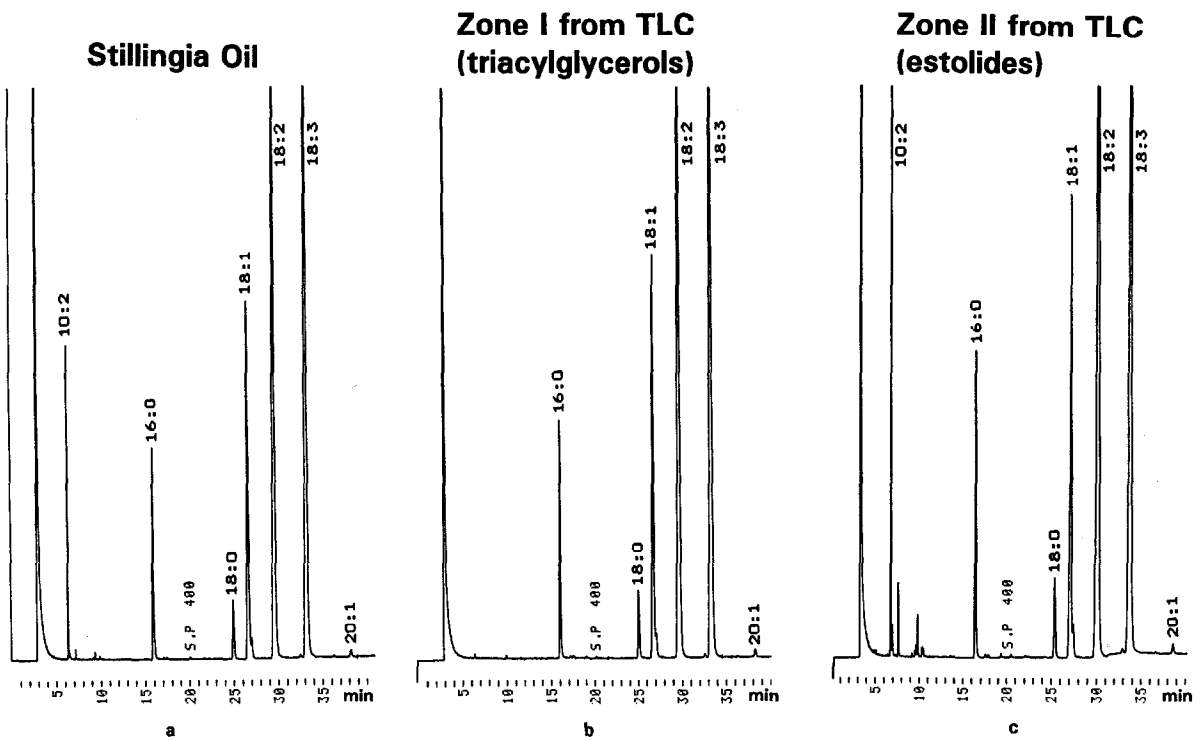


Fig. 4. GC of fatty acid methyl esters obtained from (a) stillingia oil and its (b) triacylglycerol and (c) estolide TLC-fractions. For GC conditions, see text. 16:0, 18:0, 18:1, 18:2, 18:3 and 20:1 are the usual fatty acid shorthand notations; 10:2 = 2*t*,4*c*-decadienoic acid.

and Narang and Sadgopal [8], our samples had a significantly higher linolenic acid content (38%). However, up to 50% or more linolenic acid has been found in samples from Pakistan [7] and elsewhere [11]. Apart from the $C_8$ and $C_{10}$ acids, which have been reported to be missing in samples from India and Pakistan [7,8], the fatty acid compositions of both the TG and estolide fractions are very similar.

TABLE II

FATTY ACID COMPOSITION (AREA%) OF STILLINGIA OIL AND ITS TRIACYLGLYCEROL (ZONE I) AND ESTOLIDE (ZONE II) FRACTIONS

| Sample | Fatty acids[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10:2 | 16:0 | 18:0 | 18:1 *n*−9 | 18:1 *n*−7 | 18:2 *n*−6 | 18:3 *n*−3 | 20:1 *n*−9 | Unidentified[b] |
| Stillingia oil: | | | | | | | | | |
| Sample 1 | 3.3 | 5.9 | 2.3 | 13.8 | 0.8 | 36.1 | 37.3 | 0.3 | 0.2 |
| Sample 2 | 2.8 | 5.6 | 2.3 | 13.9 | 0.8 | 35.5 | 38.3 | 0.3 | 0.5 |
| Zone I (triacylglycerols) | – | 6.0 | 2.4 | 14.8 | 0.8 | 35.9 | 39.0 | 0.3 | 0.8 |
| Zone II (estolides[a]) | 7.6 | 5.6 | 2.0 | 12.1 | 0.8 | 36.5 | 32.6 | 0.3 | 2.5 |

[a] ω-Hydroxy-5,6-octadienoic acid was not determined and no special efforts were made to secure a quantitative recovery of 10:2.
[b] Traces of $C_{12}$ and $C_{14}$ (unsaturated) acids.

TABLE III

TENTATIVE TRIGLYCERIDE AND ESTOLIDE COMPOSITION OF *SAPIUM SEBIFERUM* FATS AND THEIR FRACTIONS

Area% (RI) from RP-HPLC with RI detection (uncorrected). *PN* = partition number, defined in the usual way [19,26] as carbon number (*CN*) minus twice the double bond number (*DB*): *PN* = *CN*-2*DB*. Although PN is not applicable as such to the estolides (--E), with their experimentally observed equivalent partition numbers *EPN* [26] they fall within the TG *PN* ranges as indicated in the table (vertical columns).

| Sample (HPLC-system/detector) | Triglycerides and estolides (--E) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LnLnE | LLnE | LLE | *PN* = 36 | | | *PN* = 38 | | | *PN* = 40[a] | | |
| | | | | LnLnLn | OLnE | PLnE | LLnLn | OLE | PLE | LLnL | OLnLn | PLnLn |
| *Chinese tallow* | | | | | | | | | | | | |
| Sample 1 (HPLC-2/RI) | | | | | | | | | | | | |
| Sample 2 (HPLC-2/RI) | | | | | | | | | | | | |
| *Stillingia oil* | | | | | | | | | | | | |
| Sample 1 (HPLC-2/RI) | 3.4 | 6.7 | 4.6 | | 10.5 | | | 18.5 | | 11.9 | 6.3 | 2.8 |
| Sample 2 (HPLC-2/RI) | 3.0 | 6.9 | 4.6 | | 10.3 | 0.2 | | 18.0 | 0.1 | 11.5 | 6.6 | 2.7 |
| Sample 1 (HPLC-1/RI) | 2.8 | 6.3 | 4.7 | | 8.7 | 1.5 | | 15.7 | 2.1 | 11.6 | 6.4 | 2.5 |
| *TLC zone I* | | | | | | | | | | | | |
| (*triglycerides*) | | | | | | | | | | | | |
| Plate 1 (HPLC-2/RI) | – | – | – | 7.3 | – | – | 16.5 | – | – | 15.0 | 7.0 | 3.3 |
| Plate 2 (HPLC-1/RI) | – | – | – | 8.4 | – | – | 17.7 | – | – | 15.8 | 7.2 | 3.5 |
| *TLC zone II* | | | | | | | | | | | | |
| (*estolides*) | | | | | | | | | | | | |
| Plate 1 (HPLC-2/RI) | 8.0 | 17.5 | 14.3 | – | 9.7 | 4.3 | – | 13.1 | 6.2 | | [f] | |
| Plate 2 (HPLC-1/RI) | 12.7 | 25.5 | 20.1 | – | 9.7 | 5.8 | – | 11.1 | 7.8 | | (4.4)[a] | |
| *TLC zone II*[g] | | | | | | | | | | | | |
| (*estolides, UV*) | 11.2 | 24.6 | 18.5 | – | 9.9 | 5.6 | – | 11.1 | 8.7 | | (5.5)[a] | |
| Plate 2 (HPLC-1/UV) | | | | | | | | | | | | |

[a] Including OOE, POE and PPE.
[b] Including SLnLn, SOE and PSE.
[c] Including PLnO and SLnL.
[d] Including PLnS.
[e] Including PLS.
[f] Various smaller peaks and contaminants, possibly OOE + POE + PPE, together max. 10%, in *PN* group 40, and containing SOE + PSE, together max. 4%, in *PN* group 42.
[g] Using 260 nm UV, area% (uncorrected).

Table III shows the triacylglycerol and estolide compositions as found by RP-HPLC–RI for chinese tallow, stillingia oil and its two major fractions, triacylglycerols (zone I from TLC) and estolides (zone II), calculated as area% (RI detection), uncorrected. For the estolides, this is also compared with area% (UV detection at 260 nm).

In HPLC with UV detection, stillingia oil chromatograms obtained at a wavelength of 232 or 260 nm are different from those obtained at 210 nm. The unspecific absorption of C=C double bonds and ester groups at 210 nm gives rise to chromatograms showing peaks for all the triacylglycerols and estolides present. Fig. 5 shows chromatograms at a UV detection wavelength of 210 nm for stillingia oil and its triacylglycerols (zone I) and estolides (zone II).

The same is shown in Fig. 6 for a UV detection wavelength of 260 nm. At 232 nm the conjugated diene absorption (*e.g.*, in oxidation products, if present) would predominate. At 260 nm, extended conjugation (conjugated diene at the 2,4-position, *i.e.*,

| *PN* = 42[b] | | | *PN* = 44[c] | | | | *PN* = 46[d] | | | *PN* = 48[e] | | | | *PN* = 50 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LLL | OLnL | PLnL | OLL | OLnO | PLL | PLnP | OLO | PLO | PLP | OOO | POO | POP | PPP | POS | PPS |
| | | | | | | | – | 0.6 | 4.4 | 0.4 | 4.6 | 81.3 | 4.7 | 2.6 | 0.7 |
| | | | | | | | – | 0.4 | 3.6 | 0.2 | 5.2 | 83.1 | 4.7 | 2.1 | |
| 3.7 | 8.4 | 5.0 | 4.1 | 3.7 | 3.2 | 1.9 | 2.5 | 0.3 | 0.5 | 0.1 | | | | | |
| 3.5 | 8.5 | 5.1 | 4.1 | 3.6 | 3.2 | 1.8 | 2.5 | 0.3 | 0.4 | 0.4 | 0.4 | 0.3 | | | |
| 3.7 | 7.9 | 4.8 | 4.6 | 2.8 | 4.2 | 2.5 | 2.2 | | 3.1 | | | | | | |
| 5.1 | 11.3 | 6.8 | 5.7 | 5.6 | 4.8 | 1.1 | 2.9 | 3.7 | 0.4 | 0.6 | 1.6 | 1.0 | | | |
| 5.4 | 11.4 | 7.0 | 6.3 | 3.0 | 5.1 | 2.0 | 2.9 | 3.0 | | | | | | | |

[f]

conjugated with the carbonyl oxygen [6]) found only in the estolides containing the 2,4-decadienoic acid moiety, makes these peaks much more prominent (Fig. 6). In semi-quantitative (peak area %) calculations, it can be seen that the data for UV detection at 260 nm compare well with RI data for the estolide fraction of plate 2 (Table III). However, for truly quantitative work, individual calibration of all the peaks would be necessary.

The HPLC of tocopherols showed that chinese tallow was nearly free from tocopherols. Stillingia oil contained 700 mg/kg of total tocopherols, of which 91.8% was γ-tocotrienol, identified by co-chromatography with palm oil tocotrienols (Fig. 7). Minor tocopherols also identified include α-tocotrienol (1.2%), β-tocopherol (5.5%) and δ-tocotrienol (0.5%).

Considerable experience has been accumulated over the years regarding experimental techniques, separation mechanisms and selectivities in the RP-HPLC of triacylglycerols using different column–solvent systems [17–25]. This work extended this to estolides, although further work is needed to draw more quantitative conclusions with regard to separation effects and retention characteristics of these and other estolides. The estolides (--E), although a

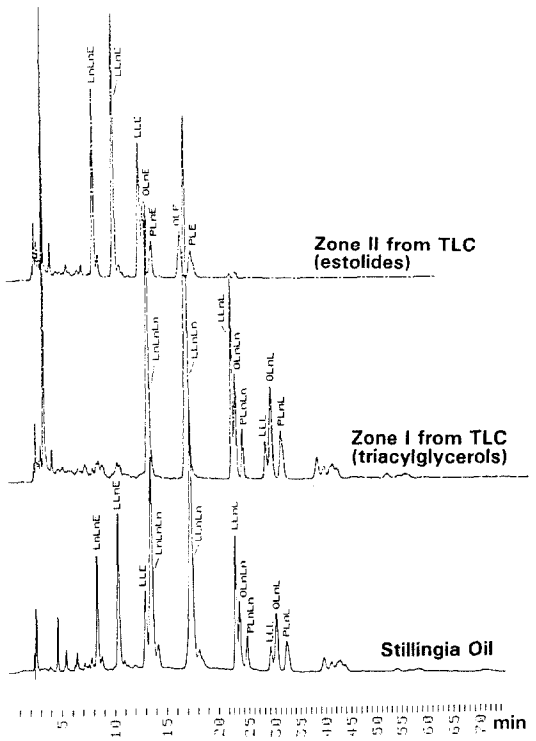


Fig. 5. Superimposed RP-HPLC traces of stillingia oil, zone I and zone II with UV detection at 210 nm. At 210 nm, by way of unspecific absorption all triacylglycerols and estolides present are seen. HPLC system 1; for conditions, see text. Abbreviations as in Fig. 3.

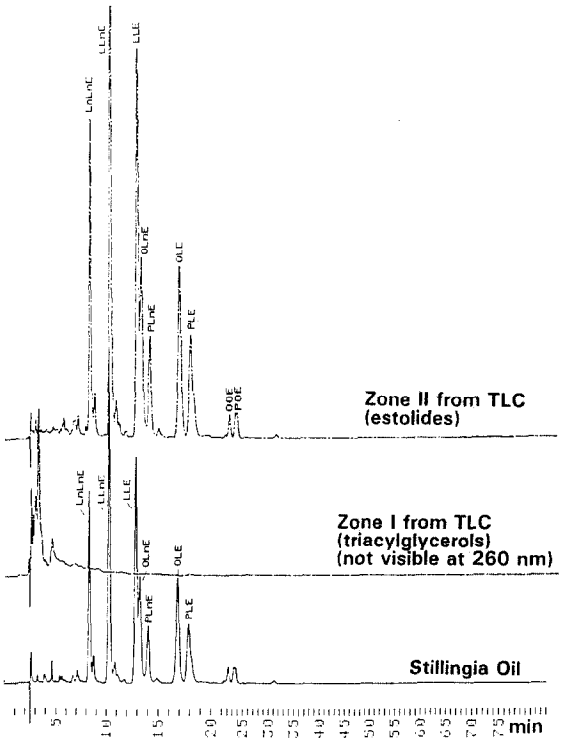


Fig. 6. RP-HPLC of stillingia oil, zone I and zone II with UV detection at 260 nm. Separately obtained chromatograms were subsequently superimposed. At 260 nm peaks containing the estolide moiety appear prominent, regular triacylglycerols are invisible. HPLC system 1; for conditions, see text. Abbreviations as in Fig. 3.

partition number as defined by Litchfield [26] is not applicable to them because of the additional ester linkage, fall into partition number groups [21] as indicated in Table III.

The observations and techniques described in this paper will also be useful for further characterization of stillingia oils and other technical drying oils. The reported significant variation [5,7,8,11] in the fatty acid composition of stillingia oils of different origin should be investigated and verified by HPLC of their intact TG and estolide molecules. Moreover, it would be interesting to investigate Indian [8] and Pakistani [7] reports that 2,4-decadienoic acid is missing in stillingia oils from the Indian subcontinent. If this is true, no estolides should be found in Indian and Pakistani oils with the HPLC methods described here.

It is interesting to speculate what the biosynthetic pathway leading to the constant estolide moiety (shown in the *sn*-3 position in Fig. 1) might be. The sum of the two medium-chain acids forming the estolide residue adds up to 18 carbons. Is it only a functional requirement that causes the formation of a "$C_{18}$ chain" from a combination of two smaller fragments? Or could it possibly be that an original polyunsaturated $C_{18}$ chain was split in half by some peculiar enzyme system? If the latter were found to be true, a more detailed investigation could be warranted. Any such enzyme system, if it existed at all, could become of major importance in biotechnology, and later possibly in gene transfer, for the production of oleochemicals.

ACKNOWLEDGEMENTS

The authors thank Ms. B. Bielefeld for the to-

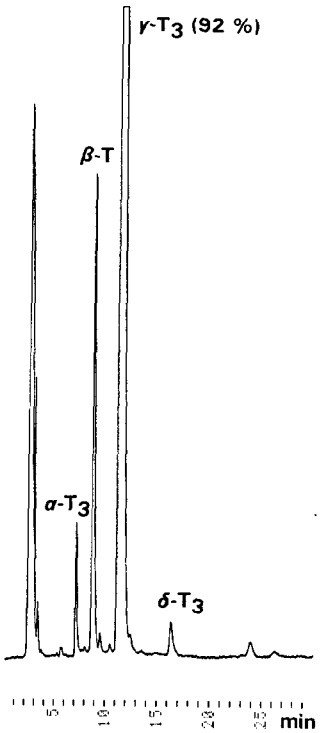


Fig. 7. HPLC of tocopherols of stillingia oil. The prominent peak (92% of total tocopherols) was tentatively identified as γ-tocotrienol. HPLC system 3 (silica column, fluorescence detection). $\alpha$-$T_3$, $\gamma$-$T_3$, $\delta$-$T_3$ = $\alpha$-, $\gamma$-, $\delta$-tocotrienols, respectively; $\beta$-T = $\beta$-tocopherol.

copherol data. Y.X. is indebted to Carl Duisberg Gesellschaft, Cologne, for financial support during his stay in Germany.

## REFERENCES

1 Y. Xin and K. Aitzetmüller, *China Oils Fats (Xian)*, 1 (1991) 23.
2 K. Itagaki, T. Yanagihara, S. Maruzeni and N. Yasuda, *Jpn. Pat.*, 62210951 (1987); *C.A.*, 107 (1987) 235186q.
3 Z. Xing, *Faming Zhuanli Shenqing Gongkai Shuomingshu*, 1988; *C.A.*, 111 (1988) 152446y.
4 J. Wang, F. Yu, J. Li and X. Shan, *Zhiwu Xuebao*, 30 (1988) 619; *C.A.*, 111 (1988) 230926w.
5 H. W. Sprecher, R. Maier, M. Barber and R. T. Holman, *Biochemistry*, 4 (1965) 1856.
6 A. Crossley and T. P. Hilditch, *J. Chem. Soc.* (1949) 3353.
7 M. Y. Raie, S. Zaka, S. Iqbal, A. W. Sabir and S. A. Khan, *Fette Seifen Anstrichm.*, 85 (1983) 359.
8 S. A. Narang and Sadgopal, *J. Am. Oil Chem. Soc.*, 35 (1958) 68.
9 R. Maier and R. T. Holman, *Biochemistry*, 3 (1964) 270.
10 K. Payne-Wahl and R. Kleiman, *J. Am. Oil Chem. Soc.*, 60 (1983) 1011.
11 W. W. Christie, *Biochim. Biophys. Acta* 187 (1969) 1.
12 H. P. Kaufmann and B.-W. King, *Fette Seifen*, 46 (1939) 388.
13 P. T. Huang, R. T. Holman and W. M. Potts, *J. Am. Oil Chem. Soc.*, 26 (1949) 405.
14 Y. C. Chen, A. Zlatkis, B. S. Middleditch, J. Cowles and W. Scheld, *Chromatographia*, 23 (1987) 240.
15 W. H. Heimermann and R. T. Holman, *Phytochemistry*, 11 (1972) 799.
16 T. P. Hilditch, *J. Oil Colour Chem. Assoc.*, 17 (1949) 5.
17 A. H. El Hamdy and E. G. Perkins, *J. Am. Oil. Chem. Soc.*, 58 (1981) 867.
18 J. P. Goiffon, C. Reminiac and D. Furon, *Rev. Fr. Corps Gras*, 28 (1981) 199.
19 K. Aitzetmüller, *Prog. Lipid Res.*, 21 (1982) 171.
20 A. Seher and H.-J. Fiebig, *Fette Seifen Anstrichm.*, 85 (1983) 333.
21 E. Deffense, *Rev. Fr. Corps Gras*, 31 (1975) 454.
22 K. Aitzetmüller, in H. Engelhardt (Editor), *Practice of High Performance Liquid Chromatography*, Springer, Berlin, 1986, pp. 287–321.
23 W. W. Christie, *High Performance Liquid Chromatography and Lipids*, Pergamon Press, Oxford, 1987.
24 K. Aitzetmüller, *Chem. Ind. (London)*, (1988) 452.
25 O. Podlaha and B. Töregard, *J. Chromatogr.*, 482 (1989) 215.
26 C. Litchfield, *Analysis of Triglycerides*, Academic Press, New York, 1972.