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Analysis of the Active Compounds in Different Parts of the *Schisandra chinensis* Plant by Means of Pyrolysis-GC/MS

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Summary. Different parts of the *S. chinensis* tree (seeds, seed shells, fruits, leaves, and shoots) were characterized by means of analytical pyrolysis – gas chromatography/mass spectrometry. The samples were pyrolyzed at 350°C leading to the evaporation of the thermally stable lignans. Besides the quantification of the lignans deoxyschisandrin, gomisin N, schisandrin, wuweizisu C, gomisin A, and angeloylgomisin H, further information about the composition of the plant parts, such as lignin, terpene, fatty acid, and carbohydrate content, could be obtained. The results were compared to the ones obtained by supercritical fluid extraction with carbon dioxide as well as literature data and were found to match.

Keywords. Lignans; Natural products; Pyrolysis-gas chromatography/mass spectrometry; *Schisandra chinensis*.

Introduction

Schisandra chinensis is a woody, deciduous liana with round, light red berries. Its natural distribution area is Japan, China, Korea, and Russia, but the plant can also be cultivated in central Europe. Parts of it have long been used in traditional Chinese medicine. Especially to mention is the hepatoprotective effect, which is due to the high content of dibenzo[*a*,*c*]cyclooctadiene lignans [1, 2]. This group of compounds has firstly been isolated from the nonsaponifieable part of the seed oil [3] and up to now about fourty different lignans of this type have been described. The results of pharmacological investigations led to the development of the synthetic drug *DDB* (dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-

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2,2′-dicarbonate) which is derived from the natural lignan schisandrin C [4]. The lignans of *S. chinensis* have been characterized extensively by means of mass spectrometry, as they yield a stable molecular ion upon EI ionization, which makes them easy to trace and identify [5–7]. Major lignans in European seeds are reported to be deoxyschisandrin (**1**) (0.07–1.09%), gomisin N (**2**) (0.24–1.49%), schisandrin (**3**) (0.75–1.86%), wuweizisu C (**4**) (0.01–0.34%), and gomisin A (**5**) (0.13–0.90%) [5], but distribution and content of lignans strongly depend on the origin and the growth conditions of the plant [8].

A disadvantage of the GC/MS and HPLC/MS techniques used so far is that the plant material has to be extracted with a solvent prior to analysis. Thus discrimination of some compounds can occur with different solvents and therefore not all components might be transferred to the analytical process quantitatively. Analytical pyrolysis on the other hand is a technique, where the whole sample (*e.g.* a plant part) is rapidly heated up to a defined temperature and the volatile pyrolysis products are immediately transferred onto the column of a GC/MS system. This method has already been used for the characterization of wood [9–12], cellulose [13–16], lignin [17–21], and other plant materials [22]. *Galletti et al*. investigated the pyrolytic behavior of a series of 2,6-diaryl-3,7-dioxabicyclo[3.3.0]octane lignans [23], which formed mostly monoaromatic rings with a C_1 to C_3 side chain attached as known from lignin. Despite the fact that analytical pyrolysis aims at the destruction of large molecules, small, thermally stable molecules will not be fragmented but evaporated from the surrounding matrix. Therefore the method was applied to the problem of identifying lignans in different parts of *S. chinensis* and predicting their composition in an oil obtained by supercritical fluid extraction.

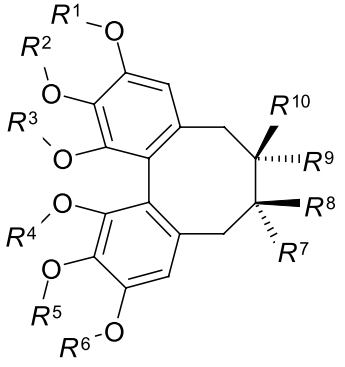
Results and Discussion

In this work the lignan composition of seeds, seed shells, fruits, leaves, and shoots from *S. chinensis* was investigated. The plants used were grown in Austria since 1991 and harvested in 2000, except for the leaves which were harvested in 1992. Table 1 shows the structure of the main lignans present in *S. chinensis*. A pyrolysis temperature of 350°C was chosen because preliminary experiments have shown that this temperature is high enough to give a reproducible profile of lignans and the decomposition of the matrix is much lower than at higher temperatures. Investigations on the plant matrix have been carried out at 500°C to achieve satisfactory pyrolytic cleavage of high molecular weight compounds (lignin, cellulose, *etc.*).

Upon chromatography the pyrolysis products can be assigned to several groups, as there are terpenes, fatty acid derivatives, lignans, and lignin derived products in the case of seed shells and shoots. Figure 1a shows the chromatogram of seeds after pyrolysis at 350°C, Fig. 1b is an enlargement of the lignan region.

The main fraction of lignans can be found in the seeds, seed shells, and fruits, where the total content of the six lignans investigated is 3.21%, 1.87%, and 1.43%. Leaves and shoots also contain the same lignans but only in very low concentrations (0.43% and 0.44%). Seeds as well as seed shells have a high amount of gomisin N (**2**) and schisandrin (**3**), whereas in fruits the content of gomisin N (**2**) is about five times higher than that of all other lignans. In leaves and shoots all lignans seem to be evenly distributed between 0.5% and 1.2%. Figure 2 shows the concentrations of

Table 1. Structure of the main lignans of *Schisandra chinensis*



	Name	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	R^9	R^{10}
1	Deoxyschisandrin	*Me*	*Me*	*Me*	*Me*	*Me*	*Me*	*Me*	H	*Me*	H
2	Gomisin N	*Me*	*Me*	*Me*	*Me*	$-CH_2-$		*Me*	H	*Me*	H
3	Schisandrin	*Me*	*Me*	*Me*	*Me*	*Me*	*Me*	*Me*	H	*Me*	OH
4	Wuweizisu C	$-CH_2-$		*Me*	*Me*	$-CH_2-$		*Me*	H	*Me*	H
5	Gomisin A	*Me*	*Me*	*Me*	*Me*	$-CH_2-$		*Me*	H	*Me*	OH
6	Angeloylgomisin H	*Me*	*Me*	*Me*	Angeloyl	*Me*	*Me*	*Me*	H	*Me*	OH

deoxyschisandrin (**1**), gomisin N (**2**), schisandrin (**3**), wuweizisu C (**4**), gomisin A (**5**), and angeloylgomisin H (**6**) as derived with analytical pyrolysis.

The distribution of lignans is very similar for all plant parts except for the fruits, where gomisin N (**2**) is by far the dominating compound and a high concentration of wuweizisu C (**4**) is present. If the results obtained from the analytical pyrolysis of shoots are compared to the results of the supercritical fluid extraction (SCFE) (Fig. 3), one can see that the ratio of deoxyschisandrin (**1**), gomisin N (**2**), schisandrin (**3**), and angeloylgomisin H (**6**) is very constant. The concentration of wuweizisu C (**4**) varies with the extraction conditions and is lower than predicted with analytical pyrolysis. Gomisin A (**5**) seems to be extracted in high yields, but it is also possible that analytical pyrolysis only shows part of the real gomisin A (**5**) concentration. This is confirmed when looking on the quantitative data obtained from SCFE. All yields are between 30 and 50% of the total amount predicted with exception of gomisin A (**5**), where the extraction yields are slightly higher than the values derived with analytical pyrolysis. A possible reason for this phenomenon could be a insufficient thermal stability of gomisin A (**5**), which would result in a partial decomposition during pyrolysis.

Besides the determination of the lignan composition analytical pyrolysis provides additional information about the composition of the plant matrix. The inner part of the seeds is characterized by a high content of unsaturated fatty acids, which arise from the fatty oil. Also terpenes such as ylangene (**16**) and others can be found indicating the presence of essential oil [24]. Seed shells on the other hand produce guaiacol (**10**) and its 4-substituted derivatives, a clear indication for the presence of lignin. Levoglucosan (1,6-anhydro-β-*D*-glucopyranose) (**18**), which

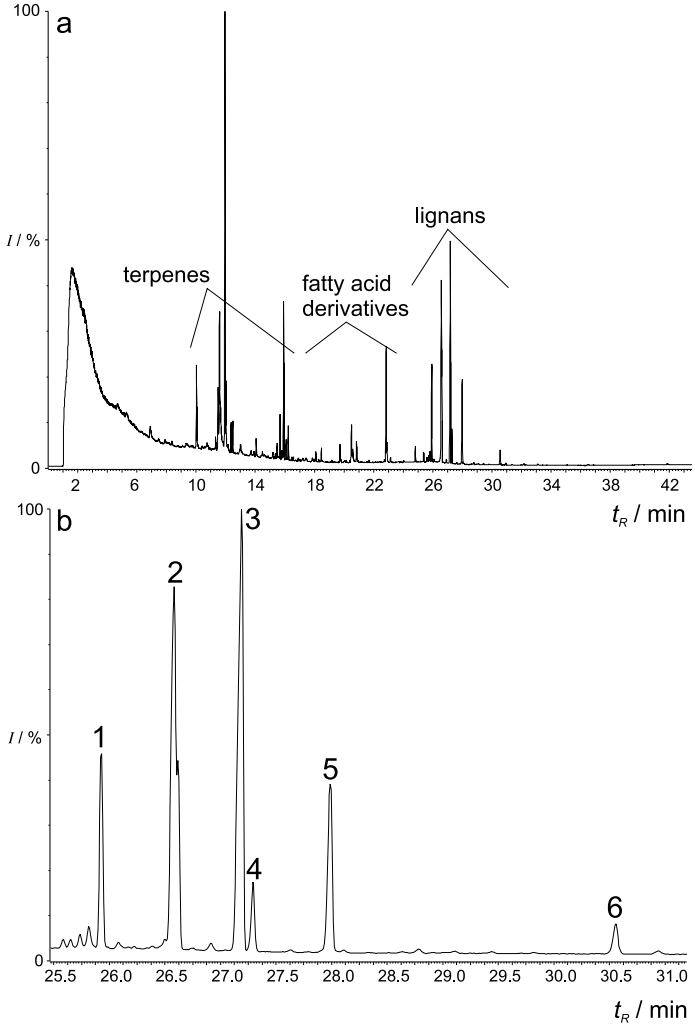


Fig. 1. a) Chromatogram of *S. chinensis* seeds obtained with pyrolysis at 350°C; b) enlargement of the lignan region; peak numbers refer to compounds listed in Table 1

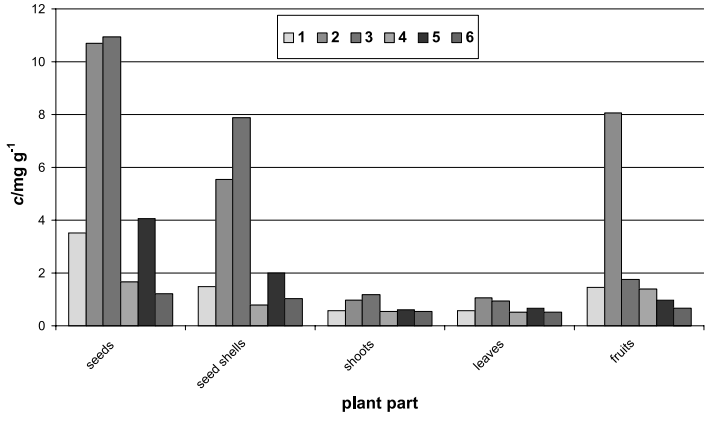


Fig. 2. Distribution of major lignans in different plant parts as derived by means of analytical pyrolysis

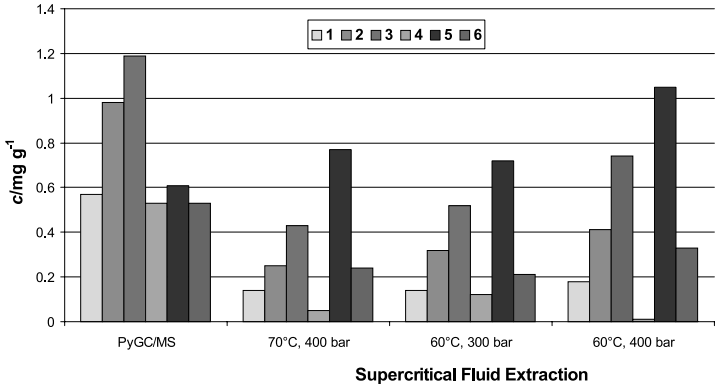


Fig. 3. Lignan distribution in shoots derived with analytical pyrolysis compared to the results of supercritical fluid extraction

can also be found in fruits, leaves, and shoots, results mainly from the pyrolytic degradation of polymeric carbohydrates, such as cellulose. In the fruits 5-hydroxymethyl-2-furfural (**13**) can be found. It is another carbohydrate marker, which is

Table 2. Main pyrolysis products of different parts of *Schisandra chinensis*

	Name	Origin[1]	Source[2]
7	Furan-2,5-dione[3]		c
8	3-Methylfuran-2,5-dione[3]		c
9	Phenol		cd
10	Guaiacol	L	b
11	Pyrocatechol[3]		bd
12	4-Methylguaiacol[3]	L	b
13	5-Hydroxymethyl-2-furfural[3]	C	cde
14	4-Ethylguaiacol[3]	L	b
15	4-Vinylguaiacol[3]	L	be
16	Ylangene[3]	T	a
17	Eugenol[3]	L	be
18	Levoglucosan	C	bcde
19	Coniferyl aldehyde[3]	L	be
20	Coniferyl alcohol[3]	L	be
21	$C_{15}H_{22}O$ (a sesquiterpene)[3]	T	abc
22	Phytol[3]	T	d
23	C16:0 Fatty acid	F	abcde
24	C18:0 Fatty acid	F	de
25	C18:2 Fatty acid	F	a
26	C18:1 Fatty acid amide[3]	F	abcde
27	C18:0 Fatty acid amide[3]	F	de
28	390, 195, 168, 167[4]		e
29	Angeloylgomisin Q[3]		ce
30	Gomisin F[3]		abce
31	Vitamin E[3]		d

[1] T . . . Terpene, F . . . Fatty oil, L . . . Lignin, C . . . Carbohydrate; [2] a . . . Seeds, b . . . Seed shells, c . . . Fruits, d . . . Leaves, e . . . Shoots; [3] Identification based on comparison of the mass spectra with library data; [4] major mass fragments of unidentified compound

more likely to arise from non polymeric sugars. The fruit acids maleic acid and citric acid can be found in the fruits as their dehydrated and decarboxylated thermally stable forms, furan-2,5-dione (**7**) and 3-methylfuran-2,5-dione (**8**) [13]. Leaves also show a carbohydrate specific profile and additionally contain phytol (**22**), a chlorophyll degradation product, and vitamin E (**31**). Shoots show the typical profile of pyrolyzed wood enriched with terpenes and fatty acid derivatives. Especially to mention is the lignan fraction, where significant concentrations of angeloylgomisin Q (**29**) and gomisin F (**30**) were detected. The pyrolysis products of plant material are listed in Table 2 and Fig. 4 shows the GC profiles of all plant parts obtained at a pyrolysis temperature of 500°C.

A very interesting fact is that the lignin of seed shells as well as of shoots contains only guaiacol type (2-methoxyphenol) pyrolysis products, whereas the physiologically active lignans are all of the syringol type (2,6-dimethoxyphenol) where all three oxygen atoms are either methylated or linked *via* a methylene bridge. In addition, the fact that *S. chinensis* is a deciduous plant would let one

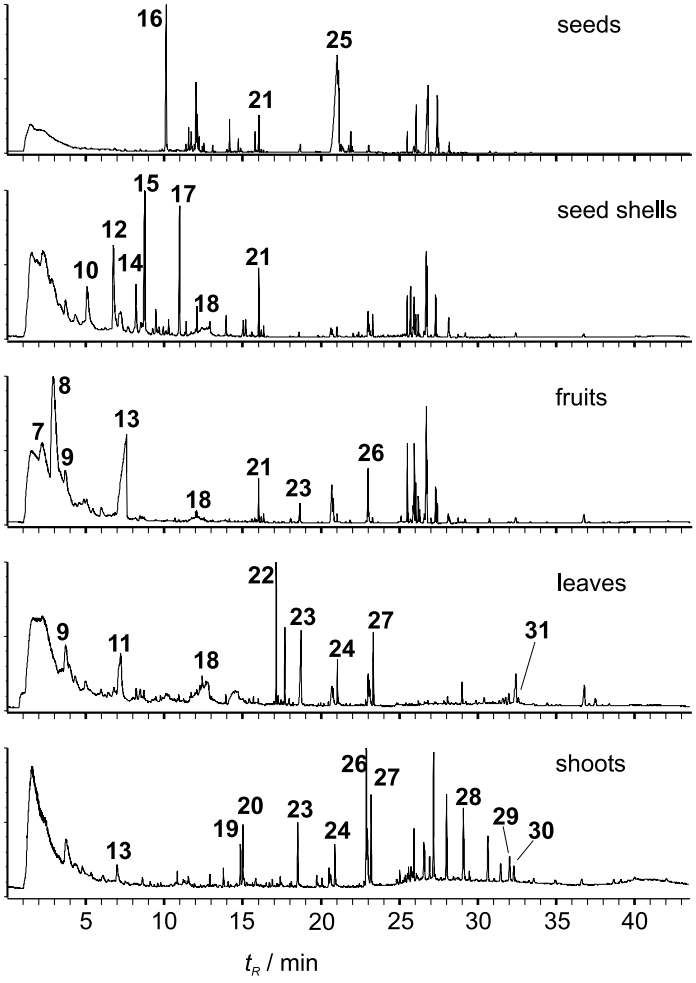


Fig. 4. Pyrolysis profiles of different parts of *S. chinensis* obtained at 500°C; peak numbers refer to compounds listed in Table 2

expect a lignin with both guaiacol and syringol units, as known from broad-leafed trees. In conclusion, analytical pyrolysis has proven to be a useful method for the determination of the distribution of lignans in different parts of *S. chinensis*. Thus it is possible to predetermine the results of an extraction process. Another potential of this method is the search for lignans in other *Schisandraceae* species or even completely different plant types.

Experimental

5α-Cholestane was purchased from Sigma. Schisandrin, gomisin A, gomisin N, and wuweizisu C were kindly provided by Dr. *J. Slanina* (Masaryk University).

Pyrolysis – Gas Chromatography/Mass Spectrometry

About 200 μg of each sample were weighed exactly, transferred into a SiO_2 tube and pyrolyzed at 350°C for 10 s, except for explicitly mentioned experiments, where the pyrolysis temperature was 500°C.

Pyrolysis experiments were carried out with a CDS Pyroprobe 2000 coil probe directly connected to a Fisons GC 8000/MD 800 system *via* a CDS 1500 interface. The volatile products were separated on a Chrompack CP-Sil 5 CB column (30 m, ID 0.32 mm, 0.25 µm film thickness) with He 4.6 as carrier gas (200 kPa) and identified by comparison of their EI mass spectra with NIST 98, Wiley, and NBS electronic libraries, literature data [2–4], or authentic standards. Pyrolysis interface, injector block, and GC/MS interface were all kept at 280°C, the GC was raised from 60 to 270°C with 8°C min^{-1}, kept at 270°C for 10 min, then raised from 270 to 290°C with 4°C min^{-1}, and kept at 290°C for 2 min. The mass spectrometer was operated in EI mode (70 eV) with a source temperature of 200°C. Quantification of the lignan fraction was carried out by calibration with an external standard of 5α-cholestane in *n*-heptane in the range of 46 to 322 ng ($R^2 = 0.9995$).

Gas Chromatography/Mass Spectrometry

Analysis of the *Schisandra chinensis* extracts was carried out under the same conditions as described above, with the only difference, that quantification was carried out using an internal standard of 5α-cholestane, which was added to the solution of extract in 2-propanol.

Supercritical Fluid Extraction

In three experiments about 150 g shoots were extracted with CO_2 at 30 MPa and 60°C, 40 MPa and 60°C, and 40 MPa and 70°C for 180 minutes and the extract precipitated at 4 MPa and 25°C in a continuous mode pilot plant.

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