Isolation of New Alkylthiosulfides from the Essential Oil and Extracts from the Bark of *Scorodophloeus zenkeri* Harms

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2,3,5-trithiahexane, 2,3,4,6-tetrathiaheptane, 2,4,5,7-tetrathiaoctane, two pentathianonanes, 2,4,5,7,9-pentathiadecane and two hexathiaundecanes were isolated from the essential oil and extracts from the bark of *Scorodophloeus zenkeri* Harms. Four other thioalkanes were found in small amounts in the essential oil.

Introduction

Scorodophloeus zenkeri Harms is an endemic tree of Central Africa. It is of restricted height and its diameter exceeds rarely 80 cm (Aubreville and Leroy, 1970). The tree has a garlic-like odor. It is known that this odor comes from sulfur containing compounds as also found in garlic (*Allium sativum*) (Amvam *et al.*, 1995).

The bark, seeds and wood of *Scorodophloeus zenkeri* are used as spices in some traditional foods such as "Nà-pôô", "Nkuii" and "Bongo-tjobi" in Cameroon. In Gabon, the bark and the yellow leaves are used as condiments. The bark delivers the so-called "Bubimbi-bark" drug (Hegnauer, 1996). Many healing powders in Central Africa contain part of this plant.

Until now only little work has been carried out on *S. zenkeri* Harms: determination of the fatty acid profile of the spice and chemical analysis of the bark essential oil (Mbofung *et al.*, 1994; Amvam *et al.*, 1995).

In the present work, we have analysed and isolated the main compounds of the essential oil from the bark of *S. zenkeri*. We also isolated some other sulfur-rich compounds from the extracts of the bark.

Results and Discussion

The essential oil and the dichloromethane and methanol extracts of the bark were fractioned using vacuum liquid chromatography (VLC), size exclusion chromatography (SEC), TLC and HPLC. The fractions and isolated compounds were analysed by GC-MS and NMR spectroscopy.

Isolated compounds

Compound **1** showed the molecular ion peak in the EIMS at *m/z* 140. The mass peak at *m/z* 46 indicated the presence of a CH_2S^+ fragment. The base peak, *m/z* 61 , represented a $CH_3SCH_2^+$ fragment. Another important peak at *m/z* 93 was attributed to a $C_2H_5S_2^+$ fragment ($CH_3SSCH_2^+$ or $CH_3SCH_2S^+$). The ^1H and ^{13}C NMR spectra showed signals for two methyls (δ_H 2.21, δ_C 15.2, CH_3S; δ_H 2.48, δ_C 23.4, CH_3SS) and a methylene group (δ_H 3.83, δ_C 44.3, SCH_2S). These data led to the structure of 2,3,5-trithiahexane, which where in good accordance to the literature data (Block and O'Connor, 1973; Dubs and Stüssi, 1978; Moir *et al.*, 1980; Baerlocher *et al.*, 1999).

The molecular formula of **2**, $C_3H_8S_4$, was obtained from the EIMS (*m/z* 172). Its ^1H NMR spectrum showed three singlets at δ_H 2.23 (3H, CH_3S), 2.57 (3H, CH_3SS) and 3.98 (2H, SCH_2S). The presence of three signals in the ^1H NMR spectrum implied that the structure of **2** is asymmetrical and therefore led to 2,3,4,6-tetrathiaheptane, which was supported by comparison of its mass spectrum with that reported in literature (Rapior *et al.*, 1997).

The mass spectrum of compound **3** showed the molecular ion peak at *m/z* 186, in agreement with the molecular formula $C_4H_{10}S_4$. The base peak

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was at *m/z* 61 and represented a $CH_3SCH_2^+$ fragment. The ^1H and ^{13}C NMR spectra showed signals for a methyl (δ_H 2.22, δ_C 15.2, CH_3S) and a methylene group (δ_H 3.92, δ_C 45.1, SCH_2S). The spectroscopic data implied that **3** was symmetrical with two $CH_3SCH_2^+$ fragments surrounding 2 sulfurs. The compound was identified as 2,4,5,7-tetrathiaoctane, in agreement with MS and ^1H NMR data reported by Weissflog (1983).

Compound **4** was found in a fraction containing **3** as a minor component. The ^1H and ^{13}C NMR spectra of **4** were very close to those of **3**. However its EIMS showed the molecular ion peak at *m/z* 218 indicating one more sulfur than compound **3**. Thus, the additional sulfur could only be located in the center of the molecule and therefore compound **4** was identified as 2,4,5,6,8-pentathianonane.

Compound **5**, 2,3,4,6,8-pentathianonane, with *m/z* at 218 in the EIMS could be deduced as an isomer of **4** . Its ^1H and ^{13}C NMR spectra showed signals for two methyls (δ_H 2.26, δ_C 15.6, CH_3S; δ_H 2.41, δ_C 17.4, CH_3SS) and two methylenes (δ_H 4.28, δ_C 42.7, SCH_2S; δ_H 4.34, δ_C 67.3, SCH_2SSS).

Compound **6** showed the molecular ion peak at *m/z* 232 in the EIMS indicative of a compound with one more methylene group than the pentathianonanes **4** or **5**. The comparison of its MS and ^1H NMR spectra with those of known pentathiadecanes from the literature (Block *et al.*, 1994; Rapior *et al.*, 1997) led to the identification of 2,4,5,7,9-pentathiadecane as the structure of **6**.

Compound **7** was assigned the molecular formula $C_5H_{12}S_6$ (EIMS, *m/z* 264). The ^1H NMR spectrum showed three singlets at δ_H 2.22 (6H, 2 × CH_3S), δ_H 3.94 (4H, 2 × SCH_2SS) and δ_H 4.20 (2H, $SSCH_2SS$) indicating that **7** was symmetrical. This led to the identification of **7** as 2,4,5,7,8,10-hexathiaundecane.

Compound **8** (EIMS, *m/z* 264) was found to be another hexathiaundecane. The ^1H and ^{13}CNMR spectra revealed an asymmetrical structure for **8** with two methyls (δ_H 2.22, δ_C 15.1, CH_3S and δ_H 2.40, δ_C 16.4, CH_3SS) and three methylene groups (δ_H 3.99, δ_C 52.9, $CH_3SC\mathbf{H}_2SCH_2$; δ_H 4.03, δ_C 44.9, $CH_3SC\mathbf{H}_2SSCH_2$; δ_H 4.40, δ_C 56.9, $SSC\mathbf{H}_2SS$). Accordingly, **8** was identified as 2,3,5,6,8,10-hexathiaundecane.

Headspace-SPME (solid phase microextraction)/*GC-MS*: A single peak was detected and its EIMS showed a molecular ion peak at *m/z* 140. The corresponding compound was identified as 2,3,5-trithiahexane by comparison with the literature (see compound **1**).

The essential oil: We obtained a yellow essential oil with a density of 1,3. It expressed a distinctive garlic-like odor. Taken together, the ether fraction (EF) and the pentane fraction (PF) represented a yield of 0.1% of the total plant material. Amvam *et al.* (1995) obtained the essential oil from *S. zenkeri* with yields ranging between 0.02 and 0.14%.

The chemical composition was as summarized in Table I.

The identification of compounds **9**–**12** of the essential oil was by comparison of their mass spectra with those found in the literature (Rapior *et al.*, 1997).

The essential oil was composed of sulfides and alkylthiosulfides. The major compounds were 2,4,5,7-tetrathiaoctane (**3**), 2,3,5-trithiahexane (**1**), 2,3,4,6-tetrathiaheptane (**2**) and 2,4,5,6,8-pentathianonane (**4**) found in both fractions (EF and PF). **1** and **3** had been also reported as the main constituents of the essential oil of *S. zenkeri* with 8 and 34.2% respectively (Amwam *et al.*,1995).

This is the first report for 2,4,5,7,8,10-hexathiaundecane (**7**), 2,3,5,6,8,10-hexathiaundecane (**8**) and 2,4,5,6,8-pentathianonane (**4**).

The alkylthiosulfides constitute a group of compounds which are not widely distributed in higher plants. Until now, only **1** and **3** have been reported from *Scorodocarpus borneensis* Becc., Olacaceae (Kubota *et al.*, 1994). A GC-MS analysis of the fungus *Marasmius alliaceus* has shown the pres-

Table I. Chemical composition of the essential oil from the bark of *S. zenkeri* Harms.

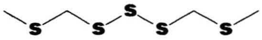
Compound	PF	EF	RT* [min]
1,2,4-Trithiolane (**9**)	0	3.4	4.88
2,3,5-Trithiahexane (**1**)	43.2	46.6	5.45
Dimethyltetrasulfide (**10**)	0	0.4	6.42
Tris-methylthiomethane (**11**)	0	1.7	6.93
2,3,4,6-Tetrathiaheptane (**2**)	4.4	6.3	7.93
2,3,5,7-Tetrathiaoctane (**12**)	0	1.2	9.15
2,4,5,7-Tetrathiaoctane (**3**)	50.0	35.5	9.37
2,4,5,6,8-Pentathianonane (**4**)	2.4	3.8	11.15
2,4,5,7,9-Pentathiadecane (**6**)	0	1.1	12.10

* PF = pentane fraction, EF = ether fraction, RT = Retention time.

1

2

3



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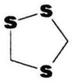

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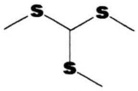

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12

ence of **1**, **2**, **3**, **5**, **9**, **10**, **11**, **12**, and a pentathiadecane (Rapior *et al.*, 1997).

S. zenkeri can thus be considered as an important source of alkylthiosulfides.

Experimental

Plant material

The bark was purchased from the central local market in Yaounde, Cameroon, in March 1999. It was kept at −20 °C until the experiments were carried out. A voucher specimen is retained in the collection of the "Fachrichtung Pharmakognosie und Analytische Phytochemie", Universität des Saarlandes, D66041 Saarbrücken.

Steam distillation

Plant material (1057 g) was ground and subjected to steam distillation according to the European Pharmacopeia (Europäisches Arzneibuch, 1997). Instead of xylol, we used pentane (because it does not disturb the GC-MS analysis) to trap the essential oil. Only part of the essential oil could be collected because of its partial solubility in water. Diethyl ether was therefore used to extract the remaining part from water. Both the pentane fraction (PF) and the ether fraction (EF) were dried on a column of anhydrous sodium sulfate and analysed separately. The pentane and the ether fractions were 308 mg and 727 mg respectively.

Extraction

Dichloromethane was used first to extract the lipophilic substances from bark powder (687 g). Then, the powder was dried and extracted with methanol. The methanol extract was suspended in water and subjected to a liquid-liquid extraction using ethyl acetate to separate the most non-polar compounds.

Fractionation, isolation and identification

The following techniques were used:

-VLC on silica gel with gradients of ethyl acetate in *n*-hexane,

-TLC on silica gel with mixtures of ethyl acetate and n-hexane as eluents. The TLC plates were sprayed with anisaldehyde/sulfuric acid reagent and heated. The sulfur-rich compounds appeared as yellow spots which faded within one minute.

-SEC (size exclusion chromatography) was carried out on Sephadex LH-20 with mixtures of dichloromethane and methanol as mobile phases to remove the lipids.

-HPLC: we used a silica gel column (Lichrospher Si 100, 4 mm*250 mm, 5 µm packing material from Merck, Darmstadt) and mixtures of ethyl acetate and n-hexane as mobile phases; a differential refractometer for the detection (RI-8110, Bischoff, Leonberg) and a Knauer HPLC-pump 64 (Knauer, Berlin). The flow rate was 1.5 ml/min.

GC/MS :The bark powder was first submitted to a headspace SPME-GC/MS analysis according to Arthur and Pawlyszin (1990):1 g of powder was equilibrated in a 20 ml vial at 23 °C for 60 min on a polydimethylsiloxane phase. The GC conditions were the same as for the essential oil. The essential oil and the isolated compounds were analysed using a HP G1800A gas chromatograph from Hewlett Packard, Palo Alto, coupled to an electron ionization detector (at 280 °C) with a HP-5 capillary column (0.25 mm*15 m, 0.25 µm film thickness). The oven temperature was programmed from 50 °C to 325 °C for a total time of 33.3 min. The vector gas was helium at a rate of 1.0 ml/min and the injection mode was either split or splitless at 250 °C.

NMR: The one-dimensional NMR-spectra were recorded with a AM 400 NMR-spectrometer from Bruker, Karlsruhe, with 400 MHz (1H-NMR) and 100 MHz (13C-NMR). The two dimensional NMR spectra were recorded with a DRX-500-NMR spectrometer from Bruker, Karlsruhe.

Spectroscopic data:

2,3,5-trithiahexane **(1)**:
MS: *m/z* (rel. int.) = 140 (11) M^+, 142 (1) $M^+ + 2$, 93 (4), 61 (100), 46 (9).
^{1}H-NMR ($CDCl_3$): δ (ppm) 2.21 (s, 3H), 2.48 (s, 3H) and 3.83 (s, 2H).
^{13}C-NMR ($CDCl_3$): δ (ppm) 15.2, 23.4 and 44.3.

2,3,4,6-tetrathiaheptane **(2)**:
MS: *m/z* (rel. int.) = 172 (4) M^+, 107 (8), 93 (44), 79 (10), 61 (100), 46 (14).
^{1}H-NMR ($CDCl_3$): δ (ppm) 2.23 (s, 3H), 2.57 (s, 3H) and 3.98 (s, 2H).
^{13}C-NMR projected from HSQC (in $CDCl_3$): δ (ppm) 15.5, 22.9 and 44.1.

2,4,5,7-tetrathiaoctane **(3)**:
MS: *m/z* (rel. int.) = 186 (7) M^+, 93 (6), 61 (100), 46 (7).
^{1}H-NMR ($CDCl_3$): δ (ppm) 2.22 (s, 6H) and 3.92 (s, 4H).
^{13}C-NMR ($CDCl_3$): δ (ppm) 15.2 and 45.1

2,4,5,6,8-pentathianonane **(4)**:
MS: *m/z* (rel. int.) = 218 (1) M^+, 154 (11), 139 (55), 93 (30), 61 (100), 46 (9).
^{1}H-NMR ($CDCl_3$): δ (ppm) 2.22 (s, 6H) and 3.99 (s, 4H).
^{13}C-NMR ($CDCl_3$): δ (ppm) 15.5 and 44.5

2,3,4,6,8-pentathianonane **(5)**:
MS: *m/z* (rel. int.) = 218 (0.1) M^+, 108 (24), 106 (43), 93 (24), 61 (100), 46 (11).
^{1}H-NMR ($CDCl_3$): δ (ppm) 2.26 (s, 3H), 2.41 (s, 3H), 4.28 (s, 2H) and 4.34 (s, 2H)
^{13}C-NMR ($CDCl_3$): δ (ppm) 15.6, 17.4, 42.7 and 67.3.

2,4,5,7,9-pentathiadecane **(6)**:
MS: *m/z* (rel. int.) = 232 (1) M^+, 124 (16), 107 (24), 93 (9), 61 (100), 46 (9).
^{1}H-NMR ($CDCl_3$): δ (ppm) 2.14 (s, 3H), 2.22 (s, 3H), 3,80 (s, 2H), 3.92 (s, 2H) and 4.10 (s, 2H).

2,4,5,7,8,10-hexathiaundecane **(7)**:
MS: *m/z* (rel. int.) = 264 (0.5) M^+, 139 (72), 93 (44), 61 (100), 46 (9).
^{1}H-NMR ($CDCl_3$): δ (ppm) 2.22 (s, 6H), 3.94 (s, 4H) and 4.20 (s, 2H).
^{13}C-NMR ($CDCl_3$): δ (ppm) 15.4, 45.5 and 50.2

2,3,5,6,8,10-hexathiaundecane **(8)**:
MS: *m/z* (rel. int.) = 264 (0.4) M^+, 93 (10), 61 (100), 46 (6).
^{1}H-NMR ($CDCl_3$): δ (ppm) 2.22 (s, 3H), 2.40 (s, 3H), 3.99 (s, 2H), 4.03 (s, 2H) and 4.40 (s, 2H).
^{13}C-NMR projection from HSQC ($CDCl_3$): δ (ppm) 15.1, 16.4, 44.9, 52.9 and 56.9.

1,2,4-Trithiolane **(9)**:
MS: *m/z* (rel. int.) = 126 (12) $M^+ + 2$, 124 (100) M^+, 78 (87), 60 (16), 46 (15).

Dimethyltetrasulfide **(10)**:
MS: *m/z* (rel. int.) = 158 (100) M^+, 79 (96), 64 (31), 47 (29).

Tris-methylthiomethane **(11)**:
MS: m/z (rel. int.) = 154 (17) M^+, 106 (71), 61 (100), 46 (8).

2,3,5,7-tetrathiaoctane **(12)**:
MS: m/z (rel. int.) = 186 (4) M^+, 139 (21), 107 (59), 93 (10), 61 (100), 46 (9).

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