Chimia 43 (1989) 353–354

Surface Lipids Isolated from the Leaves of *Boscia salicifolia* Oliv.**

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Abstract: The surface lipids of the leaves of *Boscia salicifolia* Oliv. were investigated by means of gas chromatography/mass spectrometry. The hydrocarbon fraction contained *n*-alkanes (*n*-$C_{20}H_{42}$ to *n*-$C_{33}H_{68}$) with heptacosane as the main component. Octacosanol proved to be the major constituent of the fraction of the primary *n*-alcohols; the aldehyde fraction consisted of saturated *n*-aldehydes, octacosanal being the main compound here. Furthermore, the phytosterols lupeol, taraxasterol, stigmasterol, and β-sitosterol were isolated.

Boscia salicifolia Oliv., a tropical tree belonging to the Capparidaceae, is quite common in the Ulanga district of Tanzania[1]. Its mashed leaves or the juice obtained from the leaves are used in traditional medicine to aid wound healing processes. So far only few genera of the family Capparidaceae have been investigated with respect to their constituents. The chemotaxonomic knowledge concerning the genus *Boscia* is therefore still rather limited. Since surface lipids are excellent taxonomical markers[2], an investigation of these plant constituents should lead to a better chemotaxonomical description of this genus.

The leaves of *Boscia salicifolia* were extracted exhaustively in succession with petrol ether, chloroform, methanol, and methanol/water. The total surface lipids obtained from the petrol ether extract contained 2.4% hydrocarbons, 44.6% aldehydes, and 51.4% primary alcohols, other lipid classes amounting together to less than 2%. The hydrocarbon fraction contained *n*-alkanes (*n*-$C_{20}H_{42}$ to *n*-$C_{33}H_{68}$) with heptacosane as the main component. Octacosanol proved to be the major constituent of the fraction of the primary saturated unbranched-chain alcohols; the aldehyde fraction consisted of saturated unbranched-chain compounds, octacosanal being the main component here.

The composition of the various classes of compounds is shown in Table 1. The GC signal intensities of the *n*-alkanes are compiled in Table 2. The compounds isolated were in general identified with GC and GC/MS. In the gas chromatograms of the homologous series of unbranched alkanes the separate peaks were assigned using reference compounds[3], as were the main peaks of the alcohol and aldehyde fractions. Use was also made of the fact that, when isothermal chromatograms are run, a linear relationship exists between the retention time and the number of carbon atoms[4].

The sterols lupeol and taraxasterol could be separated from each other by preparative HPLC[5]. They were identified by comparison of their spectral data (^{1}H- and ^{13}C-NMR, MS) with the corresponding values in the literature[6]. Stigmasterol and β-sitosterol could not be separated and were identified by GC/MS with authentic samples.

Experimental

General remarks: HPLC: Spectra-Physics SP 8700 pump and gradient mixer with SP 8400 UV/VIS detec-

Table 1. Composition of surface lipid classes from the leaves of *Boscia salicifolia*.

Chain length	*n*-Alkanes [%][a]	*n*-Alkanols [%]	*n*-Alkanals [%]
20	0.23		
21	0.40		
22	0.63		
23	2.84	5.64	
24	2.64	3.64	1.47
25	6.24	11.82	0.83
26	3.25	2.60	11.05
27	33.27	28.75	3.44
28	4.60	39.68	78.45
29	31.82	4.10	3.05
30	0.88	2.92	
31	3.66		
32	0.33		
33	0.41		
34	0.11		
35	0.06		

[a] Branched-chain alkanes are not mentioned.

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** *Acknowledgement:* The authors wish to thank S. Kaliliamoyo and Dr. M. Tanner, Ifakara, for collecting and drying the leaves of *Boscia salicifolia*. Financial support of this work was provided by the Swiss National Science Foundation (grant No 20-5098.86) and the Ciba-Stiftung, Basel.

Table 2. GC signal intensities of the homologous *n*-alkanes.

Retention time [min]	Signal intensity [%]	Assigned chain length C_nH_{2n+2} (n)
2.35	0.23	20
3.12	0.40	21
4.04	0.63	22
5.11	2.84	23
6.27	2.64	24
7.53	6.24	25
8.79	3.25	26
10.26	33.27	27
11.40	4.60	28
12.86	31.82	29
13.92	0.88	30
15.20	3.66	31
16.37	0.33	32
17.56	0.41	33

tor; preparative Lichrosorb RP 18 column (25 cm × 16 mm, 7 µm, Merck); solvent system: hexane saturated with a mixture of water/diisopropyl ether/2-propanol 97.75:2.00:0.25; detection at $\lambda = 205$ nm; flow 4 mL/min. ^{1}H- and ^{13}C-NMR: Bruker WH 90 at 90 and 22.63 MHz, respectively. GC: HP 5790A capillary gas chromatograph with FID and integrator HP 3390A (Hewlett-Packard); 12 m cross-linked dimethylsilicone fused silica column or 25 m cross-linked phenylmethylsilicone fused silica column (Hewlett-Packard); temperature program 1: 200°C (3 min) → +5°C/min → 300°C (3 min); temperature programm 2: 250°C, isothermal. GC/MS: HP 5790A capillary gas chromatograph with HP 5970A mass-selective detector (Hewlett-Packard); columns and temperature programs as above. Column chromatography: Silica gel 60 (40–63 µm, Chemische Fabrik Uetikon). TLC: Alugram SilG/UV_{254} sheets; detection with vanillin/sulfuric acid[7].

Plant material: Boscia salicifolia Oliv. was collected in spring 1984 4.5 km northwest of Ifakara (Ulanga district, Tanzania). The plant material was identified by Dr. *F. Haerdi* (F. Hoffmann-La Roche AG, Basel and Pharmazeutisches Institut der Universität Basel); a voucher specimen was deposited with the Botanisches Institut der Universität Basel.

Extraction and chromatography: The dried leaves of the plant were ground to a fine powder (100 g) and extracted exhaustively in succession with petrol ether, $CHCl_3$, MeOH, and MeOH/H_2O 1:1. During concentration of the petrol ether extract a mixture of aldehydes and alcohols precipitated as a white solid (0.15 g). Separation was achieved on a silica gel column (10 g) with petrol ether containing increasing amounts of diethyl ether as the eluent. The remainder of the petrol ether extract (0.49 g) was fractionated with flash chromatography on 50 g of silica gel using again petrol ether containing increasing amounts of diethyl ether as the eluent. Fractions 1–4 contained the mixture of homologous *n*-alkanes (14.6 mg), fractions 20–24 consisted of a mixture of lupeol and taraxasterol, whereas fractions 29–31 contained stigmasterol and β-sitosterol.

Alkanes: For gas chromatography 1 µL of a ca. 5% solution of the sample in cyclohexane was injected. The reference mixture was obtained by rinsing a small piece of parafilm M (American Can Company, Greenwich, CT 06830) with cyclohexane. The chromatogram of this solution was calibrated with tetracosane ($C_{24}H_{50}$, purum, Fluka AG) and hexatriacontane ($C_{36}H_{74}$, purum, Fluka AG). Samples of this reference mixture and of the alkane fraction from *B. salicifolia* were gas-chromatographed under identical conditions.

Chromatograms (dimethylsilicone column, temperature program 1; retention time [min] (intensity [%], C_nH_{2n+2})); *B. salicifolia alkane fraction:* 2.35 (0.23, $C_{20}H_{42}$), 3.12 (0.40, $C_{21}H_{44}$). 4.04 (0.63, $C_{22}H_{46}$). 5.11 (2.84, $C_{23}H_{48}$), 6.27 (2.64, $C_{24}H_{50}$), 7.53 (6.24, $C_{25}H_{52}$), 8.79 (3.25, $C_{26}H_{54}$), 10.26 (33.27, $C_{27}H_{56}$), 11.40 (4.60, $C_{28}H_{58}$), 12.86 (31.82, $C_{29}H_{60}$), 13.92 (0.88, $C_{30}H_{62}$), 15.20 (3.66, $C_{31}H_{64}$), 16.37 (0.33, $C_{32}H_{66}$), 17.56 (0.41, $C_{33}H_{68}$); *reference mixture + tetracosane + hexatriacontane:* 2.34 ($C_{20}H_{42}$), 3.11 ($C_{21}H_{44}$), 4.03 ($C_{22}H_{46}$), 5.08 ($C_{23}H_{48}$), 6.24 ($C_{24}H_{50}$), 7.47 ($C_{25}H_{52}$), 8.76 ($C_{26}H_{54}$), 10.05 ($C_{27}H_{56}$), 11.35 ($C_{28}H_{58}$), 12.63 ($C_{29}H_{60}$), 13.90 ($C_{30}H_{62}$), 15.14 ($C_{31}H_{64}$), 16.36 ($C_{32}H_{66}$), 17.55 ($C_{33}H_{68}$), 18.70 ($C_{34}H_{70}$), 19.83 ($C_{35}H_{72}$), 20.92 ($C_{36}H_{74}$).

Aldehydes: ^{1}H-NMR ($CDCl_3$): 9.76 (t, $J = 2$ Hz, 1 H, CHO), 2.43 (t × d, $J = 8$, $J = 2$, 2H, –CH_2–CH_2–CHO), 1.25 (m, R–CH_2–R), 0.88 (t, $J = 6$, 3H, CH_2–CH_3). – GC (dimethylsilicone column, temperature program 1; retention time [min] (intensity [%], $C_nH_{2n}O$)): 11.34 (8.08, $C_{26}H_{52}O$), 12.65 (2.56, $C_{27}H_{54}O$), 13.96 (69.35, $C_{28}H_{56}O$); 15.18 (2.90, $C_{29}H_{58}O$), 16.42 (12.19, $C_{30}H_{60}O$); (phenylmenthylsilicone columne, temperature program 2; retention time [min] (intensity [%], $C_nH_{2n}O$)): 24.74 (11.05, $C_{26}H_{52}O$), 32.00 (3.44, $C_{27}H_{54}O$), 42.04 (78.45, $C_{28}H_{56}O$), 53.87 (3.05, $C_{29}H_{58}O$). – GC/MS (dimethylsilicone column, temperature program 1): 14.28 min, $C_{26}H_{52}O$: M^+ at m/z 380 not visible, 362 (0.2, $[M-18]^+$, H_2O); 15.62 min, $C_{27}H_{54}O$: M^+ at 394 not visible, 376 (1.7, $[M-18]^+$, H_2O); 17.32 min, $C_{28}H_{56}O$: 408 (0.2, M^+); 390 (2.9, $[M-18]^+$, H_2O); 18.34 min, $C_{29}H_{58}O$: 422 (0.2, M^+); 404 (1.8, $[M-18]^+$, H_2O); 19.74 min, $C_{30}H_{60}O$: 436 (0.2, M^+); 418 (2.3, $[M-18]^+$, H_2O): furthermore, each spectrum contained the typical signals of the series $[C_nH_{2n+1}]^+$, $[C_nH_{2n}]^+$, and $[C_nH_{2n-1}]^+$ with a maximum of intensity around n = 3 and 4[8]: 41, 42, 43 (55.3, 11.3, 110.0 [C_3H_5, C_3H_6, C_3H_7]$^+$); 55, 56, 57 (55.3, 19.4, 90.1, [C_4H_7, C_4H_8, C_4H_9]$^+$); 69, 70, 71 (32.1, 12.1, 36.8, [C_5H_9, C_5H_{10}, C_5H_{11}]$^+$). – Reference sample of *octacosanal:* octacosanol (see below) was oxidized with dimethyl sulfoxide and oxalyl chloride in CH_2Cl_2.

Alcohols: ^{1}H-NMR ($CDCl_3$): 3.64 (t, $J = 6$ Hz, 2H, R–CH_2–CH_2–OH), 1.55 (s, br, 1H, OH), 1.26 (m, R–CH_2–R), 0.88 (t, 3H, R–CH_2–CH_3). – GC (phenylmethylsilicone column, temperature program 2; retention time [min] (intensity [%], $C_nH_{2n+2}O$)): 17.38 (5.64, $C_{23}H_{48}O$), 24.84 (3.64, $C_{24}H_{50}O$), 28.91 (11.82, $C_{25}H_{52}O$), 37.56 (2.60, $C_{26}H_{54}O$), 41.74 (28.75, $C_{27}H_{56}O$), 49.09 (39.68, $C_{28}H_{58}O$), 70.24 (4.10, $C_{29}H_{60}O$). – GC/MS (dimethylsilicone column, temperature program 1): 18.20 min, $C_{28}H_{58}O$: 392 (2.1, $[M-18]^+$, H_2O) and the typical signals of the series $(C_nH_{2n+1}]^+$, $[C_nH_{2n}]^+$, and $[C_nH_{2n-1}]^+$ with a maximum of intensity around n = 3 and 4. – Reference sample of *octacosanol:* octacosanoic acid (Fluka AG) was methylated with diazomethane in tetrahydrofuran. The resulting methyl octacosanoate was then reduced with diisobutylaluminum hydride in hexane to octacosanol.

Received: September 22, 1989 [FC 175]

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