References

[1] Zunino, M. P., Novillo Newton, M., Maestri, D. M., Zygadlo, J. A. (1998) Planta Med. 64, 86–87.
[2] Zygadlo, J. A., Rotman, A. D., Perez Alonso, M. J., Velasco-Negueruela, A. (1997) J. Essent. Oil Res. 9, 237–239.
[3] Zygadlo, J. A., Maestri, D. M., Lamarque, A. L., Guzmán, C. A., Velasco-Negueruela, A., Perez Alonso, M. J., Vallejos, M. C., Grosso, N. R. (1996) Biochem. Syst. Ecol. 24, 319–324.
[4] Zygadlo, J. A., Grosso, N. R., Abburrá, R. E., Guzmán, C. A. (1990) Biochem. Syst. Ecol. 18, 405–407.
[5] Cabrera, A. L., Zardini, E. M. (1993) Manual de la flora de los alrededores de Buenos Aires, pp. 627–628, Acme, Buenos Aires.
[6] Burkart, A. (1974) Flora Ilustrada de Entre Ríos, Colección Científica del INTA, pp. 214–217, Buenos Aires.
[7] Toursarkissian, M. (1980) Plantas Medicinales de la Argentina, pp. 33–35, Hemisferio Sur, Buenos Aires.
[8] Van den Dool, H., Kratz, P. (1963) J. Chromatogr. 11, 463–471.
[9] Garneau, F. X., Collin, G., Gagnon, H., Laseve, F. L. J., Chalchat, J. C. (1997) J. Essent. Oil Res. 9, 491–493.
[10] Adams, R. P. (1995) Identification of Essential Oil Components by Gas Chromatography Mass Spectrometry. Allured Publ. Corp., Illinois.
[11] Chisowa, E. H. (1997) J. Essent. Oil Res. 9, 469–470.
[12] de Pascual, J., Bellido, I. S., Torres, C., Perez, M. A. (1980) Rivista Ital. EPPOS 62, 123–126.
[13] Duke, J. A. (1985) CRC Handbook of Medicinal Herbs, pp. 160, CRC press, Florida.
[14] Kaltenbach, G., Schafer, M., Schimmer, I. (1993) J. Essent. Oil Res. 5, 107–108.
[15] Kaltenbach, G., Schimmer, O. (1991) Planta Med. 57, Suppl. Issue 2, A82.

Dr. J. A. Zygadlo

Cátedra de Química Orgánica
Facultad de Ciencias Exactas
Físicas y Naturales
Universidad Nacional de Córdoba
Avda. Velez Sarsfield 299
5000 Córdoba
Argentina
Fax: +54-51-332097

Terpenoid Composition of the Wound-Induced Bark Exudate of *Commiphora tenuis* from Ethiopia

Kaleab Asres[1,2], Andreas Tei[2], Girma Moges[1,2], Frank Sporer[2], and Michael Wink[2,*]

[1] School of Pharmacy, Addis Ababa University, Addis Ababa, Ethiopia
[2] Institut für Pharmazeutische Biologie, Universität Heidelberg, Heidelberg, Germany

Received: September 22, 1997; Revision accepted: January 3, 1997

Abstract: **The bark of *Commiphora tenuis* Vollensen exudes a translucent, free-flowing odoriferous liquid upon wounding which was analysed by capillary GLC and GLC-MS. 42 mono- and sesquiterpenes were detected and 37 identified. The main components of the monoterpenoid fraction were α-pinene (60.8 %), β-pinene (8.8 %), sabinene (6.3 %), α-thujene (8.9 %), limonene (5.5 %), 3-carene (3.7 %), β-myrcene (1.8 %), and β-elemene (1.1 %) constituting 97 % of the oil. Identified sesquiterpenoid components constituted approximately 1.6 % of the oil. Oleanolic acid acetate was isolated and identified as the main triterpene from the resin by ^{1}H- and ^{13}C-NMR. Three other triterpenes of the olean-12-ene group were also detected using GC-MS. The essential oil exhibited antibacterial activities against *Staphylococcus aureus*, *Proteus mirabilis* and *E. coli* with MIC between 0.5 and 1 %.**

Commiphora tenuis Vollesen is one of the 52 species of the genus *Commiphora* (Burseraceae) occurring in Ethiopia. It grows as a multi-stemmed spiny shrub which attains a height of 2 m (1). The plant occurs wild on rocky limestone slopes or black cotton soil at an altitude of 1000 to 1800 m above sea level. It is indigenous to Bale and Sidamo regions of Ethiopia and the adjoining areas of north-eastern part of Kenya (1). When injured, the bark exudes a translucent, free-flowing odoriferous liquid which is widely used by the Somali nomads in south-east Ethiopia for the treatment of animal wounds, particularly of camels. As no scientific investigation has been carried out (to our knowledge) on the exudate, the terpenoid composition of the essential oil was studied by capillary GLC and GLC-MS; furthermore, the triterpene fraction was separated and analyzed by NMR and GLC-MS.

The volatile oil fraction obtained from the bark exudate of *C. tenuis* was subjected to GLC and GLC-MS analyses. About 42 components were recorded and 37 were identified on account of their mass spectra (NBS library) and RI values (2) (Table **1**). Three minor components could not be identified because of their scarcity. Main components (above 1 % of total volatile terpenoids) were α-pinene (60.8 %), α-thujene (8.9 %), β-pinene (8.8 %), sabinene (6.3 %), limonene (5.5 %), 3-carene (3.7 %), β-myrcene (1.8 %), and β-elemene. The other mono- and sesquiterpenes were present in trace amounts. From a related species, *Commiphora guidottii* α-santalene, α-bisabolene, and furanodiene were identified as major and β-santalene, β-bergamotene, β-farnesene, β-bisabolene as minor components (3).

Table 1 Composition of the essential oil in the oleo-resin of *Commiphora tenuis*.

No.	RI	Compound	Area %
1	920	α-Thujene	8.94
2	928	α-Pinene	60.84
3	936	Camphene	0.26
4	961	Sabinene	6.29
5	964	β-Pinene	8.79
6	983	β-Myrcene	1.80
7	1000	3-Carene	3.66
8	1008	*p*-Cymene	0.90
9	1015	β-Thujene	0.91
10	1019	Limonene	5.52
11	1030	*cis*-β-Ocimene	0.15
12	1041	*trans*-β-Ocimene	<0.1
13	1048	γ-Terpinen*	tr[a].
14	1057	unknown	tr[a].
15	1071	α-Pinene-epoxide	tr[a].
16	1077	unknown	tr[a].
17	1090	unknown	tr[a].
18	1095	α-Thujone	tr[a].
19	1104	α-Campholenal	tr[a].
20	1122	*trans*-Verbenol	tr[a].
21	1130	Verbenol	tr[a].
22	1136	Pinocarvone	tr[a].
23	1143	*p*-Mentha-1,5-dien-8-ol*	tr[a].
24	1149	*p*-Mentha-1(7)-dien-8-ol*	tr[a].
25	1162	Terpinen-4-ol	tr[a].
26	1165	*p*-Cymen-8-ol	tr[a].
27	1168	Myrtenal	tr[a].
28	1147	α-Terpineol	tr[a].
29	1179	Myrtenol	tr[a].
30	1275	Bornylacetate	tr[a].
31	1285	Eucarvone	tr[a].
32	1373	Copaene	0.26
33	1378	β-Bourbonene	tr.
34	1385	β-Elemene	1.07
35	1410	β-Caryophyllene	<0.1
36	1447	humulene derivative	tr[a].
37	1454	Alloaromadendrene	tr[a].
38	1470	β-Gurjunene	tr[a].
39	1482	β-Selinene	tr[a].
40	1490	α-Selinene	tr.
41	1494	α-Muurolen	tr[a].
42	1518	δ-Cadinene	tr[a].

[a] tr = traces * = tentative identification RI = Kovats Retention Index

After removal of the gum from the gum-resin by the addition of Me_2CO, the resin obtained was repeatedly subjected to CC using silica gel finally yielding five fractions containing free triterpenes. Saponins were not encountered. Four of these were further purified using silica gel PLC. Analysis of these four fractions led to the detection of triterpenes belonging to the olean-12-ene and/or urs-12-ene group. The main triterpene was characterized as 3β-*O*-acetoxyolean-12-en-28-oic acid (compound **1**). Literature survey indicates that this is the first report with respect to triterpenes in *C. tenuis*.

Compound **1**, (yield: 0.09%), m.p. 265–267 °C. The ^{1}H-NMR spectrum indicated the presence of one OAc (δ = 2.01, s, 3H). The presence of OAc was further confirmed by the ^{13}C data (δ = 170.95). The compound also has one COOH group (δ_c = 183.5). The ^{1}H-NMR spectrum showed, in addition, five signals at δ = 0.86–1.3 due to a total of seven methyl groups. The MS of the TMSi derivative gave M^+ at *m/z* = 570 ($C_{35}H_{56}O_4Si$) with strong peaks at *m/z* = 555 (M^+ – Me), 510 (M^+ – HOAc), 452 (M^+ – HCOOTMS), and 393 [M^+ – (HOAc + COOTMS)]. The characteristic retro-Diels-Alder fragmentation in ring C of oleanene and ursene derived triterpenes having a Δ^{12}-unsaturation was evidenced by the peak at *m/z* = 320. This was also supported by ^{1}H-NMR for one olefinic proton at δ = 5.07 (1H, t-like, H-12). The characteristic fragment corresponding to the loss of the C_{28} group (COOTMS) from the *m/z* = 320 fragment appeared at *m/z* = 203. This is peculiar to olean-12-ene or urs-12-en-28-oic acid derivatives bearing no substituent on the C, D and E rings (4). The acetoxy group, thus, must be on the left hand side of the molecule on either ring A or B. Its position as 3β was strengthened by the δ_H = 4.6 (1H, d, *J* = 8.4 Hz, 3α-H) signal.

Here the oleanene skeleton is favoured to the ursene since NMR signals due to secondary methyl groups are not found. Further evidence is obtained by ^{13}C-NMR chemical shifts of the olefinic carbons (δ = 122.7 and δ = 143.6) and the presence of quartet near δ 33.0 (δ = 32.5, 32.7, 33.1, 33.8) (5, 6). All of the spectroscopic data cited above lead to the structure 3β-acetoxy-olean-12-en-28-oic acid as the structure for this triterpene. Comparison of the results obtained with those in the literature supports this structure (7–10).

The antibacterial activity of the essential oil was tested against *Escherichia coli*, *Enterococcus faecalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and four *Staphylococcus aureus* strains. As can be seen from Table **2** MICs of the 0.5% were obtained against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and the methicilin resistant strain ATCC 1309. Other strains were inhibited at 1% dilutions whereas even 4% dilutions were inactive for *E. coli* ATCC 11229, *P. aeruginosus* ATCC 15442 and ATCC 27853. These results confirm preliminary studies on antimicrobial properties of *C. tenuis* oil. Since many mono- and sesquiterpens exhibit antibacterial activities this

Table 2 Antibacterial activity of the essential oil of *C. tenuis*. Results were obtained from 2 independent sets of experiments which produced identical results.

Concentrations	Bacterial strains 1	2	3	4	5	6	7	8	9	10
4%	+	+	–	–	–	–	–	–	+	–
2%	+	+	–	–	–	–	–	–	+	–
1%	+	+	+	–	–	–	–	–	+	–
0.5%	+	+	+	–	+	–	+	+	+	+
0.25%	+	+	+	+	+	+	+	+	+	+
0.12%	+	+	+	+	+	+	+	+	+	+
0.06%	+	+	+	+	+	+	+	+	+	+
Control	+	+	+	+	+	+	+	+	+	+

+ = bacterial growth – = inhibition

1 = *Pseudomonas aeruginosa* ATCC 27853
2 = *Pseudomonas aeruginosa* ATCC 15442
3 = *Enterococcus faecalis* ATCC 29212
4 = *Staphylococcus aureus* ATCC 25923
5 = *Staphylococcus aureus* ATCC 29213
6 = *Staphylococcus aureus* ATCC 1309 (methicillin resistant)
7 = *Staphylococcus aureus* ATCC 6538
8 = *Proteus mirabilis* ATCC 14153
9 = *Escherichia coli* ATCC 11229
10 = *Escherichia coli* ATCC 25922

result was expected and can explain the utilisation of the exudate by nomads to treat wounds of animals.

Materials and Methods

Plant material: A wound exudate from *C. tenuis* bark was collected in August 1995 from the surrounding areas of Filtu, a small village in Sidamo region about 720 km south-east of Addis Ababa, Ethiopia. Voucher specimens are kept at the School of Pharmacy of Addis Ababa University.

Essential oil isolation: The exudate of *C. tenuis* (46 g) was subjected to steam distillation for 5 h. The distilled oil was taken up in *n*-pentane and the solution dried over anhydrous Na_2SO_4, filtered, and concentrated at low temperature (in vacuo) yielding a colourless odoriferous oil (25.1 g; 54.6 %) with the following properties: n_D^{20} = 1.4566, $[\alpha]_D$ = (+)9.495°; d^{25} = 0.81. For GLC and GC-MS analyses the distillate was dissolved in *n*-pentane.

Essential oil analysis: The oil was subjected to high resolution gas-liquid chromatography under the following conditions: OV1 (Ohio Valley) fused silica capillary column (30 m × 0.25 mm i.d.); carrier gas He; FID detector, det. temp. 300 °C, inj. temp. 250 °C; split 1 : 10; oven temperature program: initial temp. 50 °C 4 min isothermal, 50 – 90 °C at 4 °C min^{-1}, 90 – 300 °C at 10 °C min^{-1}, then 10 min isothermal.

Capillary GLC-MS was performed on OV 1 (30 m × 0.25 mm i.d.) column coupled directly to a quadrupole Finnigan MAT 4500 mass spectrometer. EI-MS were recorded at 45 eV. GLC-Conditions: carrier gas He; split 1 : 10; inj. temp. 250 °C; oven temp. prog.: initial temp. 46 °C 4 min isothermal, 46 – 100 °C at 4 °C min^{-1}, 100 – 300 °C at 8 °C min^{-1}, then 10 min isothermal. Kovats retention indices (RI) were calculated using co-chromatographed standard hydrocarbons. The substances were identified by their RI values (determined in previous analyses in our laboratory) and by comparison of their mass spectra with reference mass spectra in the NBS-library.

Triterpene analysis: M.p.s. were taken on a Reichert (Austria) hot stage apparatus and are uncorrected. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75.5 MHz) spectra were recorded on a Bruker-AC 300 spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard. EI-MS (70 eV) were taken with a Jeol JMS-700 MS station using the following conditions: column OV-1701, 30 m; 200.7 °C isotherm. CC was performed using Merck silica gel (0.063 – 0.2 mm, 150 g) with $CHCl_3$-EtOAc-HOAc (22 : 4 : 1) (System 1) and benzene-Me_2CO (12 : 1) (System 2). PLC plates used were 1 mm Merck silica gel 60 F_{254}. Toluene-EtOAc (4 : 1) and light petroleum ether-EtOAc (9 : 1) were used as developing solvent systems for all fractions. Both Liebermann-Burchard and Carbazole (11) reagents were used interchangeably for detection on TLC plates. For GC-MS, samples (ca. 2 mg) were dissolved with 100 µl MSTFA [*N*-methyl-*N*-(trimethylsilyl)-2,2,2-trifluoroacetamide] (Merck) and left at room temperature for a few minutes to form TMS ether and/or ester derivatives.

The gum-resin of *C. tenuis* (30 g) was taken up in Et_2O and the gum precipitated by the addition of Me_2CO. Repeated chromatography of the resin on a column yielded fractions I and II with System 1 and III, IV and V with System II to give a total of five fractions containing triterpenes. However, only compound **1** was obtained in sufficient quantities to enable both MS and NMR experiments after purification.

Antibacterial activity of the essential oil: The following test organisms were employed: *Escherichia coli* ATCC 11229 and ATCC 25922; *Enterococcus faecalis* ATCC 29212; *Proteus mirabilis* ATCC 14153; *Pseudomonas aeruginosa* ATCC 27853 and ATCC 15442; *Staphylococcus aureus* ATCC 6538, ATCC 25923, ATCC 29213 and ATCC 1309 (methicillin-resistant). All strains were cultured on blood agar overnight and incubated aerobically at 37 °C for 24 h. A broth microdilution method was used to determine minimum inhibitory concentrations (MIC) of the essential oil against the test organisms. The test was performed in Iso-Sensitest broth (ISB; Oxid) supplemented with Tween 80 detergent (Merck) at a final concentration of 0.5 % (v/v) to emulsify the essential oil. Serial dilutions of the oil in ISB were prepared in a 96-microtiterwell tray over the range from 0.06 % to 4.0 % (v/v). Trays were incubated at 37 °C for 24 h. Bacterial growth was evidenced by the presence of a white coloured "pellet" on the well bottom. The MIC's were defined as the lowest concentration which prevents visible growth of the test organisms within 24 hours.

Acknowledgements

We are grateful to Dr. Gross and Dr. Schilling, Organisch-Chemisches Institut (Universität Heidelberg) for running the GC-MS and NMR spectra of triterpenes and to M. Harkenthal (Institut für Pharmazeutische Biologie, Universität Heidelberg) for carrying out the antimicrobial tests. Thanks also go to KAAD (G. M.) and DAAD (K. A.) for financial assistance.

References

[1] Vollesen, K. (1989) in: Flora of Ethiopia, (Heidelberg, I., Edwards, S., eds.), Vol. 3, pp. 442 – 478, The National Herbarium, Addis Ababa University, Addis Ababa.
[2] De Pooter, H. L., Schamp, N. M. (1986) Prog. Essential Oil Res. 139 – 149.
[3] Craveiro, A., Corsano, S., Proietti, G., Strappaghetti, G. (1983) Planta Med. 48, 97 – 98.
[4] Budzikiewicz, H., Wilson, J. M., Djerassi, C. (1963) J. Am. Chem. Soc. 85, 3688 – 3699.
[5] Doddrell, D. M., Khong, P. W., Lewis, K. G. (1974) Tetrahedron Lett. 27, 2381 – 2384.
[6] Mahato, S. B., Kundu, A. P. (1994) Phytochemistry 37, 1517 – 1575.
[7] Bin-Zakaria, M., Jeffreys, J. A. D., Waterman, P. G., Zhong, S-M. (1984) Phytochemistry 23, 1481 – 1484.
[8] Chakravarty, A. K., Mukhopadhyay, S., Das, B. (1991) Phytochemistry 30, 4087 – 4092.
[9] Dictionary of Organic Compounds (1982) 5th edn., p. 1033. Chapman & Hall, London.
[10] Joshi, K. C., Bansal, R. K., Patni, R. (1978) Planta Med. 34, 211 – 214.
[11] Ghosh, P., Thakur, S. (1982) J. Chromatogr. 240, 515 – 517.

Prof. Dr. M. Wink

Universität Heidelberg
Institut Pharmazeutische Biologie
Im Neuenheimer Feld 364
D-69120 Heidelberg
Germany
E-mail: Michael.Wink@urz.uni-heidelberg.de
Fax: +49/(0)6221-544884