Phytochemistry 69 (2008) 1710–1715

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Antiectoparasitic activity of the gum resin, gum haggar, from the East African plant, *Commiphora holtziana*

Michael A. Birkett [a,*], Sate Al Abassi [b], Thomas Kröber [c], Keith Chamberlain [a], Antony M. Hooper [a], Patrick M. Guerin [c], Jan Pettersson [b], John A. Pickett [a], Robin Slade [d], Lester J. Wadhams [a]

[a] *Centre for Sustainable Pest and Disease Management, Biological Chemistry Department, Rothamsted Research, Harpenden, Herts., AL5 2JQ, United Kingdom*
[b] *Department of Entomology, Swedish University of Agricultural Sciences, P.O. Box 7044, SE-750 07 Uppsala, Sweden*
[c] *Institute of Zoology, University of Neuchâtel, Rue Emile-Argand 11, 2007 Neuchâtel, Switzerland*
[d] *Arid Land Resources Ltd., P.O. Box 810, Nanyuki, Kenya*

ARTICLE INFO

Article history:
Received 2 November 2007
Received in revised form 18 February 2008
Available online 9 April 2008

Keywords:
Gum haggar
Commiphora holtziana
Repellent
Tick
Mite
(+)-Germacrene-D

ABSTRACT

The mechanism of ixodid tick (Acari: Ixodidae) repellency by gum haggar, a resin produced by *Commiphora holtziana* (Burseraceae), was investigated by evaluating activity against the cattle tick, *Boophilus microplus*. In an arena bioassay, a hexane extract of the resin of *C. holtziana* exhibited a repellent effect lasting up to 5 h. The hydrocarbon fraction of the resin extract was shown to account for the repellent activity, and was analysed by coupled gas chromatography–mass spectrometry (GC–MS). Major sesquiterpene hydrocarbons were tentatively identified as germacrene-D, δ-elemene and β-bourbonene. The identity and stereochemistry of the former compound was confirmed as the (+)-isomer by peak enhancement using enantioselective GC, whereas the latter 2 compounds, which are most likely degradation products of germacrene-type precursors, were identified through isolation by preparative gas chromatography followed by microprobe-NMR spectroscopy. GC comparison of gum haggar with another resin, *C. myrrha*, which was inactive in the tick bioassay, showed that the latter contained much lower levels of these hydrocarbons. To assess the suitability of the gum haggar resin as a general acarine repellent, further tests were made on a major acarine pest of European and US animal husbandry systems, the red poultry mite, *Dermanyssus gallinae* (Acari: Dermanyssidae). Gum haggar extract, and the isolated hydrocarbon fraction, showed strong repellent effects in an olfactometer assay, and again gum myrrh showed no effect. These findings provide a scientific basis for the observed anti-tick properties of gum haggar, and demonstrate the potential for its development as a general acarine repellent for use in animal husbandry systems.

1. Introduction

Commiphora spp. shrubs (Burseraceae) are native East African species, found in Kenya, Uganda, Tanzania, Ethiopia and Somalia (Beentje, 1994; Gillett, 1991). One of these species, *C. holtziana*, is a thornless tree with hairy trifoliate leaves, yellow flowers and almost round slightly hairy fruits. The trunk and branches are blue and covered with a thin white papery bark peeling in sheets. Another species, *C. myrrha*, is a thorny tree which has hairless toothed leaves with a large terminal leaflet and two tiny lateral leaflets. Male flowers are about 5 mm long and come out just before the rains. Fruits are about 1.2 cm long including the pronounced beak. The bark has a silvery sheen and peels in pieces. Both species are also known to secrete gum resins, the products from *C. holtziana* and *C. myrrha* being known as "gum haggar" and "gum myrrh", respectively.

The resins from *C. holtziana* and *C. myrrha* have been collected for centuries and found wide use in a number of folk traditional practices. These include use as ointments to heal wounds, oral medicines and perfumery substances (Kokwaro, 1976; El Ashry et al., 2003; Lemenih et al., 2003). More recently, the antitumour activity of these resins has been studied (Zhu et al., 2001). Farmers also use traditional formulations of gum resins, in particular the resin from *C. holtziana*, to kill and repel tick pest populations on camels and cattle, and for mange control (Chikamai and Gachathi, 1994).

The chemical composition of *C. holtziana* and *C. myrrha* gum resins has been reported elsewhere (Provan et al., 1987; Cavanagh et al., 1993; Zhu et al., 2001, 2003; Dekebo et al., 2002; Morteza-Semnani and Saeedi, 2003; Marongiu et al., 2005; Hanus et al., 2005; Ahmed et al., 2006). These studies show that the resins

* Corresponding author. Tel.: +44 1582 763133; fax: +44 1582 762595.
E-mail address: mike.birkett@bbsrc.ac.uk (M.A. Birkett).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.02.017

comprise mainly sesquiterpene hydrocarbons and furanosesquiterpenes, some of which are known from unrelated studies to be effective arthropod repellents, e.g. germacrene-D has been shown to be an effective aphid repellent (Bruce et al., 2005). Other hydrocarbons found in *C. holtziana* include β-selinene, β-elemene, δ-elemene, γ-elemene, α-cubebene and β-bourbonene (Provan et al., 1987).

Despite the wide use of gum haggar in tick control by East African farmers, the scientific basis of tick repellency has not been fully elucidated. A previous study has investigated the acaricidal activity of chemical components from *Commiphora* spp. resins, including *C. erythraea* and *C. myrrha* (Marudufu, 1982). Although the tick repellency of *C. erythraea* resin has also been demonstrated (Carroll et al., 1989), the components which are responsible for the activity were not identified.

The aim of this study was to confirm the repellency of gum haggar using the cattle tick, *Boophilus microplus* (Acari: Ixodidae) as a model species, and to identify the active chemical components using coupled GC–mass spectrometry (GC–MS) and NMR spectroscopy. The chemical components were compared with gum myrrh, collected from *C. myrrha*, which is known locally to be only weakly active or inactive in this context. Identification of the active components would provide the underpinning science required for local commercial production of the resins as tick repellents, and therefore control of tick-borne diseases. Furthermore, it would provide a means of assessing the quality of the material produced, i.e. the robustness of future commercial production. The study also investigated the repellent activity of the resins towards another major acarine pest, the red poultry mite, *Dermanyssus gallinae* (Acari: Dermanyssidae) which is found in chicken production systems, in order to determine the potential for developing the resins as more general acarine repellents.

2. Results

2.1. Tick behaviour

In control experiments, a high number of *B. microplus* larvae walked onto the hexane treated surfaces (Fig. 1A). The hexane extract of gum haggar was repellent at the concentration tested (1% v:v, Fig. 1B). Ticks approaching the treated surface were noticed to mostly turn back (not recorded numerically) before entering the 16 mm ring surrounding the treated surface, as demonstrated by the low number of ticks counted at defined time periods in this area. The repellent action was already lost in the second ring, i.e. at 16–26 mm from the treated surface (Fig. 1B). After 2–3 h the repellent effect was reduced as some ticks walked onto the treated surface and the percentage of ticks in the surrounding rings increased. No ticks were counted on the treated surface after 4–5 h, but this effect was not accompanied by a reduction in the number of ticks on the surrounding rings. After 7 h, the number of larvae walking onto the treated surface increased, and after 24 h, the repellent effect had disappeared (data not shown). The hydrocarbon fraction of gum haggar extract, prepared by Florisil® column chromatography, was less active than the crude extract, as revealed by the earlier entry of a higher number of ticks to the 16 mm ring surrounding the treated zone in the first hour (Fig. 1C). The hexane extract of gum myrrh was not repellent at the concentration tested (1% v:v), i.e. the distribution of ticks between the different zones was similar to the control experiments (Fig. 1D).

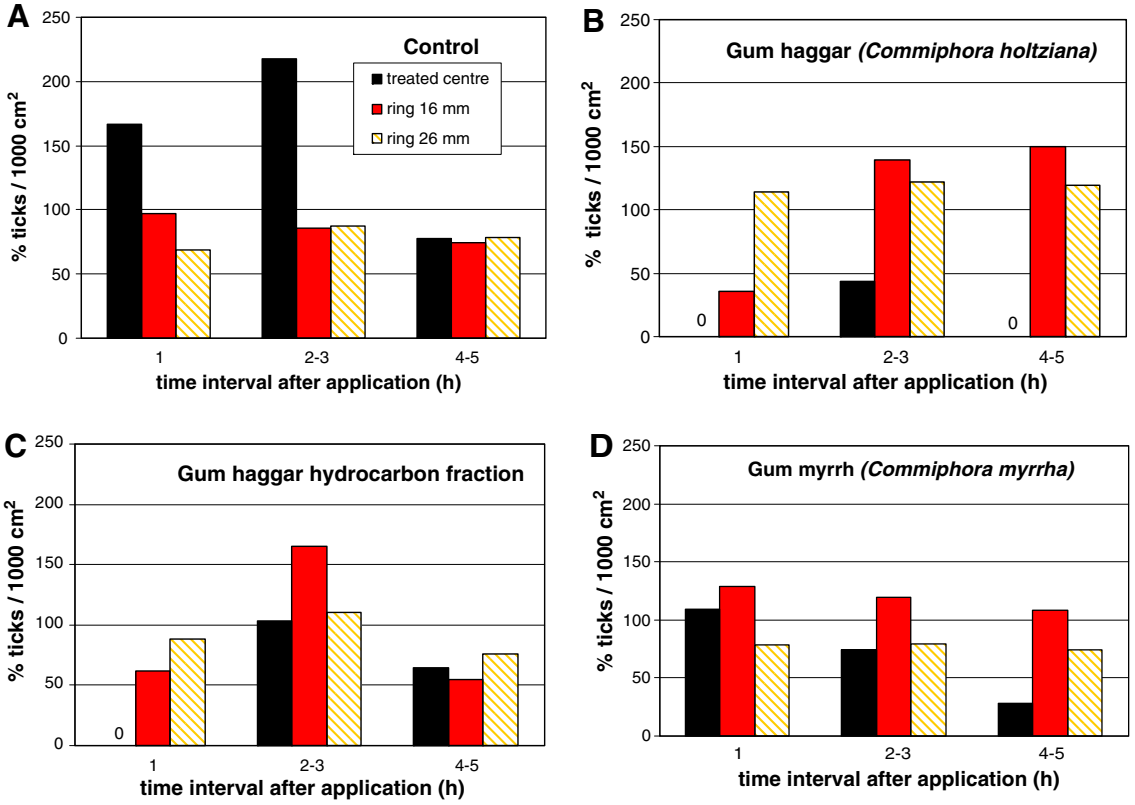

Fig. 1. Effect of gum haggar, *Commiphora holtziana*, hexane extract, a gum haggar hydrocarbon fraction obtained by small-scale liquid chromatography of the hexane extract over Florisil®, and gum myrrh, *C. myrrha*, hexane extract on *Boophilus microplus* larval behaviour. The median number of ticks was calculated for each time interval (1 h, 2–3 h and 4–5 h). The median counts on the central patch and on the inner three rings were then expressed as the number per unit area and percentages calculated relative to the number of ticks on the outer ring (26–36 mm) for each time interval and treatment.

Table 1
Behavioural responses of fed and starved adult red poultry mites, *Dermanyssus gallinae*, in a four-way olfactometer, to gum haggar, *Commiphora holtziana*, hexane extract, a gum haggar hydrocarbon fraction obtained by small-scale liquid chromatography of the hexane extract over Florisil®, and gum myrrh, *C. myrrha*, hexane extract

	Observations[a] (mean ± SE)			
	Treated	Control[b]	*P*	*N*
Tests with fed mites				
10% (v:v) *C. holtziana* extract	3.00 ± 0.68	5.83 ± 0.68	0.03	20
10% (v:v) *C. holtziana* hydrocarbon fraction	1.70 ± 0.63	6.10 ± 0.78	0.003	10
10% (v:v) *C. myrrha* extract	4.25 ± 1.30	4.40 ± 1.24	NS[c]	10
Tests with starved mites				
10% (v:v) *C. holtziana* extract	1.70 ± 0.65	2.20 ± 0.82	0.003	10
0.5% (v:v) *C. holtziana* extract	2.55 ± 0.21	2.15 ± 0.63	NS[c]	20

Figures refer to the mean number of visits in the treatment arm and the mean number of visits in three control arms during a 20 min period.
[a] Cumulative counts over 20 min.
[b] Control: paraffin oil.
[c] NS: not significantly different (paired *t*-test).

2.2. Mite behaviour

Olfactometer assays showed that the hexane extract of gum haggar, presented as a formulation in paraffin oil was repellent towards starved and fed adult *D. gallinae* at the concentration tested (10% v:v), but not at a lower concentration (0.5% v:v) for starved mites (Table 1). The hydrocarbon fraction of gum haggar extract, prepared by liquid chromatography and also formulated in paraffin oil (10% v:v), was also repellent for fed mites, suggesting that the activity of the crude extract was retained in this fraction. In contrast, the hexane extract of gum myrrh (10% v:v) showed no significant activity compared to the control.

2.3. Chemical composition of gum haggar hydrocarbon fraction

GC–MS analysis of the behaviourally active hydrocarbon fraction of gum haggar extract revealed the presence of over 15 sesquiterpene hydrocarbons (Fig. 2; Table 2). The most abundant compounds were tentatively identified as δ-elemene (16.69%), β-bourbonene (20.83%) and germacrene-D (11.64%). Using authentic samples of (+) and (−) isomers, the identity of germacrene-D was confirmed by peak enhancement on GC, and the stereochemistry determined to be the (+)-isomer by peak enhancement using enantioselective GC. ^{1}H NMR spectroscopy of pure materials prepared by preparative-scale GC confirmed the identity of the other 2 compounds. The stereochemistry of the other 2 compounds was not determined due to unavailability of authentic samples of stereoisomers for enantioselective GC.

Table 2
Compounds identified from coupled GC–MS analysis of gum haggar, *Commiphora holtziana*, hydrocarbon fraction obtained by small-scale liquid chromatography of hexane extract over Florisil®

Peak no. (see Fig. 2)	Compound[a]	% Peak area by GC
1	δ-Elemene[b,c]	16.69
2	α-Copaene	2.67
3	β-Bourbonene[b,c]	20.84
4	α-Bergamotene	2.43
5	β-Ylangene	4.56
6	β-Caryophyllene	2.10
7	Calarene	5.72
8	γ-Muurolene	3.53
9	γ-Elemene	2.26
10	Humulene	2.63
11	Bicyclogermacrene	2.07
12	(+)-Germacrene-D[d]	11.64
13	β-Selinene	3.55
14	α-Selinene	2.56
15	δ-Cadinene	2.79

[a] Tentative identification unless otherwise stated.
[b] Identification confirmed by ^{1}H NMR.
[c] Stereochemistry not assigned.
[d] Stereochemistry determined by peak enhancement using enantioselective GC.

3. Discussion

The results of this study confirm that gum haggar, obtained from the plant *C. holtziana*, is significantly repellent towards ticks,

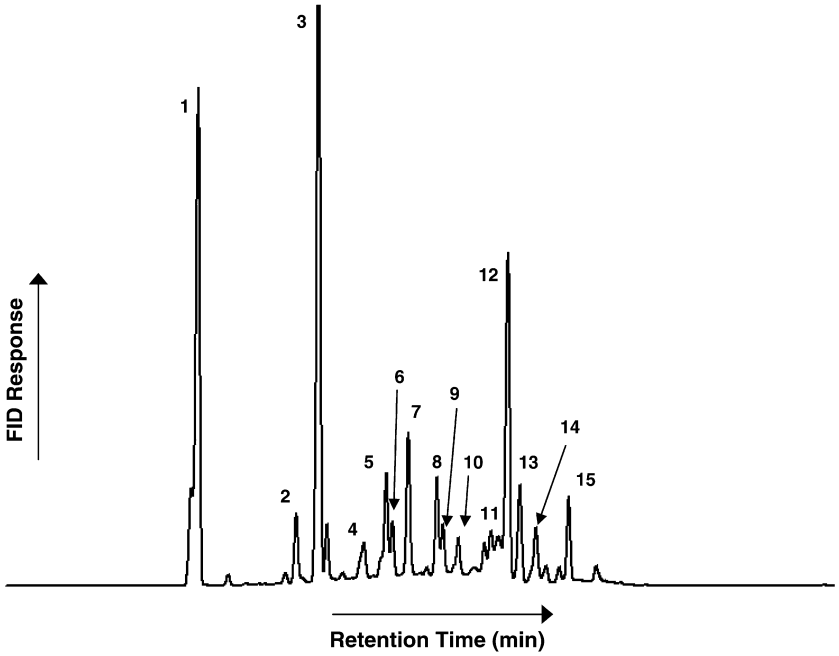

Fig. 2. Total ion chromatogram (TIC) obtained by coupled GC–MS analysis of gum haggar, *Commiphora holtziana*, hydrocarbon fraction obtained by small-scale liquid chromatography of the hexane extract over Florisil®. Analysis was obtained from a non-polar HP-1 column. Peak numbers correlate to compounds listed in Table 2 identified by coupled GC–MS.

and that, in contrast, gum myrrh, obtained from *C. myrrha*, possesses no, or at least little, activity. These data are in line with ethnobotanical use by farmers in East Africa of gum haggar to repel ticks, when applied as a traditional formulation to the skin of cattle. The data suggest that one or more of the sesquiterpene hydrocarbons, present in greater amounts in gum haggar compared with gum myrrh, are responsible for the repellent activity of the resin, as indicated by the activity of the hydrocarbon fraction isolated by column chromatography. Some of the hydrocarbons have been previously reported as components of *C. holtziana* (Provan et al., 1987). Both resins contain large amounts of furanosesquiterpenoids, but the higher level of sesquiterpene hydrocarbons in gum haggar is due to a lower level of oxidation which is needed for formation of the furanosesquiterpenoids (Provan et al., 1987). Thus, the repellent activity is accounted for by germacrene-type compounds, predominantly (+)-germacrene-D, which is known from other studies to be an effective arthropod repellent (Bruce et al., 2005). The presence of other sesquiterpene hydrocarbons, including δ-elemene and β-bourbonene, in the hydrocarbon fraction is likely to be accounted for by either rearrangement or isomerisation of germacrene-type precursors upon extraction/isolation (Newman, 1972; Yoshihara et al., 1969). The disappearance of the activity of the fraction after only one hour in the laboratory assay can be rationalised by the chemical nature of (+)-germacrene-D, which is volatile and thus likely to evaporate, and which as mentioned above, is prone to rapid aerial oxidation and rearrangement. It is likely that the remainder of the resin provides both antioxidant protection of this compound, and acts as a natural slow-release formulation. Evidence for these suggestions is provided by the crude resin being biologically active against the cattle tick for up to 5 h after application, i.e. much longer than that for the hydrocarbon fraction alone. This longevity of activity could be valuable in exploiting gum haggar further for local protection of livestock.

The use of African plant oils and resins to control tick pests through repellency, immobilization or acaricidal activity has been studied elsewhere. The essential oil of molasses grass, *Melinis minutiflora*, has been shown to repel the brown-ear tick, *Rhipicephalus appendiculatus* (Mwangi et al., 1995), as has the essential oil of the shrub, *Cleome monophylla* (Ndungu et al., 1995), *Ce. hirta* (Ndungu et al., 1999), and the East African shrub, *Gynandropsis gynandra* (Lwande et al., 1999). The gum resin from *C. erythraea* has been shown to repel the lone star tick, *Amblyomma americanum* and the American dog tick, *Dermacentor variabilis* (Carroll et al., 1989). The results of this study confirm the reported anti-tick properties of gum haggar from *C. holtziana*. Furthermore, this study is the first report of the repellency of a plant-derived material for the red poultry mite, *D. gallinae*, and demonstrates the potential for using gum haggar as a general purpose acarine repellent. Further studies are underway to determine whether the repellent effect can be extended to other invertebrate pest species affecting animal health and welfare, and even to the control of phytophagous pests.

4. Materials and methods

4.1. Collection of gum resins

The gum resins of *C. holtziana* ssp. *holtziana* Engl. (gum haggar) and *C. myrrha* var. molmol Engl. (gum myrrh) were collected from branches and trunks of shrubs (5–7 years old) located in the Kaisuit desert, in the Marsabit district of Northern Kenya, and sent to Rothamsted Research for preparation of extracts required for behavioural bioassay studies and chemical analysis. Voucher specimens of these plants were deposited at the National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia: *C. holtziana* (072793) and *C. myrrha* (072825).

4.2. Gum resin extraction

For tick behavioural bioassays, extracts of gum haggar and gum myrrh were prepared by sonicating material (200 mg of each) in hexane (2 ml) for 10 min, and leaving at ambient temperature overnight. The stock solutions (10% v:v in hexane) were diluted to 1% (v:v) with hexane prior to use in bioassays. Additionally, a gum haggar hydrocarbon fraction was also used in bioassays. This was prepared by grinding the resin (5 g) into a powder and extracting the powder with hexane (100 ml) for 24 h at ambient temperature. The extract was dried ($MgSO_4$) and filtered. A portion of this extract (5% v:v, 3 ml) was subjected to liquid chromatography through Florisil® (100–200 mesh), using hexane as the eluant, with the eluant (5% v:v) being used in bioassays.

For mite behavioural bioassays, 5% (v:v) hexane extracts of gum haggar and gum myrrh were prepared as described above. A portion of each stock solution (20 ml) was concentrated to half the volume, i.e. 10% v:v, and mixed with paraffin oil (10 ml). The hexane was evaporated under a gentle stream of nitrogen to give 10% (v:v) solutions in paraffin oil. Additionally, a portion of the gum haggar stock solution (5%, 1 ml) was diluted × 10, i.e. 0.5% v:v, and paraffin oil (10 ml) added. The hexane was evaporated under a gentle stream of nitrogen to give a 0.5% (v:v) solution in paraffin oil. A portion of the gum haggar stock solution (5%, 10 ml) was concentrated to half the volume, i.e. 10% v:v, and was passed through a short column of Florisil® using hexane as the eluant. This was concentrated to 5 ml and paraffin oil (5 ml) added. The hexane was evaporated under a gentle stream of nitrogen to give a 10% (v:v) solution in paraffin oil.

4.3. Gas chromatography (GC)

GC analysis was performed on a HP6890 gas chromatograph equipped with a cool on-column injector, flame ionization detector (FID), and fitted with a HP-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature was set at 30 °C for 1 min, then programmed at 5 °C/min to 150 °C, held at this temperature for 0.1 min, then programmed at 10 °C/min to 250 °C. The carrier gas was hydrogen.

Enantioselective GC was performed on a 5890A gas chromatograph equipped with a β-cyclodextrin (Supelco beta-DEX™120; 30 m × 0.25 mm i.d., film thickness 0.25 μm) capillary column. The oven temperature was maintained at 40 °C for 1 min, then programmed to increase at 3 °C/min to 150 °C, then at 5 °C/min to 180 °C. The temperature was maintained at 180 °C for 15 min. The carrier gas was hydrogen.

4.4. Coupled GC–mass spectrometry (GC–MS)

GC–MS analyses were carried out using a fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm, DB-5), fitted with an on-column injector, which was directly coupled to a magnetic sector mass spectrometer (Thermo-Finnigan MAT95 XP, Bremen, Germany). Ionization was by electron impact (70 eV, source temperature 250 °C). The oven temperature was maintained at 30 °C for 5 min, and then programmed at 5 °/min to 250 °C. The carrier gas was helium. Tentative identifications were made by comparison of spectra with mass spectral databases (NIST, 2005), and confirmed by peak enhancement on GC using authentic samples of chemicals.

4.5. Preparative-scale GC–nuclear magnetic resonance (NMR) spectroscopy

Preparative GC was performed on a HP5890 gas chromatograph equipped with a cool on-column injector, flame ionization detector

(FID), and fitted with a megabore HP-1 capillary column (30 m × 0.53 mm i.d., film thickness 5 μm). The oven temperature was maintained at 40 °C for 1 min, then programmed to increase at 5 °C/min to 150 °C, then maintained at this temperature for 20 min. Compounds were collected by cold trapping (solid CO_2) in a short length of silica tubing (40 cm, 0.53 mm i.d.), and eluted with deuterated benzene (C_6D_6, 100 μl) directly into a microprobe-NMR tube. ^{1}H NMR spectroscopy was performed using a Bruker Avance 500 MHz NMR spectrometer equipped with a 2.5 mm microprobe.

4.5.1. δ-Elemene

12 μg of a compound tentatively identified as an elemene isomer by GC–MS was isolated and determined to be δ-elemene, due to features including the presence of 5 double bond protons and a pendant isopropyl group. δ_H (500 MHz, C_6D_6) 6.00 (1H, *dd*, *J* 10.8, 17.5), 5.47 (1H, *br s*), 5.13 (1H, *dd*, *J* 1.2, 17.6), 5.09 (1H, *br s*), 5.08 (1H, *dd*, *J* 1.2, 10.9), 4.95 (1H, *s*), 2.84 (1H, *br s*), 2.26 (1H, quintet, *J* 6.8), 2.00 (1H, *br q*, *J* 6.4), 1.86 (3H, *s*), 1.5–1.7 (2H, *m*), 1.19 (1H, *dd*, *J* 6.8, 6.9), 1.11 (3H, *d*, *J* 6.9), 1.11 (3H, *d*, *J* 6.9), 1.09 (3H, *s*).

4.5.2. β-Bourbonene

3.6 μg of a compound tentatively identified as a bourbonene isomer by GC–MS was isolated and determined to be β-bourbonene due to features including an exomethylene moiety. δ_H (500 MHz, C_6D_6) 5.01 (2H, *s*), 2.64 (1H, *br m*), 2.55 (1H, *m*), 2.33–2.40 (2H, *m*), 1.97 (1H, *m*), 1.84 (1H, *dd*, *J* 7.4, 12.8), 1.77 (1H, *br d*, *J* 12.7), 1.5–1.7 (5H, *m*), 1.30 (1H, *m*), 1.04 (3H, *s*), 1.00 (3H, *d*, *J* 6.6), 0.98 (3H, *d*, *J* 6.6).

4.6. Chemicals

Samples of β-bourbonene (78% pure by GC) and δ-elemene (98% pure by GC) were obtained using preparative-scale GC as described above. Authentic samples of (+) and (−)-germacrene-D were obtained by incubation of farnesyl pyrophosphate with purified, expressed (+) or (−)-germacrene-D synthase and subsequent hexane extraction and purification through a short column of silica gel (BDH, 40–63 μm)/$MgSO_4$ (10:1) (Prosser et al., 2004).

4.7. Ticks and mites

Boophilus microplus (Canestrini), the cattle tick, was obtained from Novartis Animal Research SA, St. Aubin, FR, Switzerland, where they were reared on cattle. Tick larvae were held in an environmental cabinet under long-day conditions of 10:10 h, 28 °C, 85% relative humidity separated by 2 h ramps representing dawn and dusk. Larvae of *B. microplus* used in experiments were 2–10 weeks old.

Poultry red mites, *Dermanyssus gallinae*, used in olfactometer experiments, were collected from poultry sheds at Lövsta Research Station, Swedish University of Agricultural Sciences, Uppsala, in traps made of pieces of corrugated cardboard (7 × 14 cm). This method is based on positive thigmo-kinesis behaviour of the mites. Collected mites were either used directly for experiments, or stored in a refrigerator at 5 °C and used in bioassays within two weeks.

4.8. Tick behaviour

A glass crystallizing dish (90 mm diameter, 50 mm high) was used to study the behaviour of *B. microplus* larvae. A 10 mm circular patch and concentric rings of 16, 26 and 36 mm diameter were marked on the underside of the dish. The stimuli (10 μl) or the solvent hexane (10 μl) were applied to the central surface inside the dish, resulting in a concentration of 125 μg extract/cm^2. After allowing to dry for 5 min, the dish was placed upside down into a large Petri dish over 1000–2000 *B. microplus* larvae. When sufficient ticks had reached the roof of the dish (10–30 min), the number of ticks in one video frame at different time intervals (at three intervals in the first hour, and at two intervals each in 2–3 h and 4–5 h intervals) was counted on the central treated circle and in the three concentric rings. The median number of ticks was calculated for each time interval (1 h, 2–3 h and 4–5 h). The median counts on the central patch and on the inner three rings were then expressed as the number per unit area and percentages calculated relative to the number of ticks on the outer ring (26–36 mm) for each time interval and treatment.

4.9. Mite behaviour

Behavioural assays using adult *D. gallinae* were carried out in a four-way olfactometer as described previously (Pettersson, 1970). The apparatus consisted of a star-shaped arena with four extended arms to which stimuli could be introduced. Air was drawn towards the centre of the olfactometer. One test mite was placed in the centre of the olfactometer and its position noted every 2 min for 20 min. Each experiment was replicated 10–20 times and the results analysed by paired *t*-test; the mean number of visits into the treatment arm was compared with the mean number of visits to the three control arms. If the mite did not move between two consecutive observations the experiment was terminated and the data discarded. Stimuli tested were applied in 0.5 μl microcaps. Control stimuli comprised of paraffin oil. Mites were not sexed prior to use, and were regarded as starved if they were used more than 12 h after capture in poultry sheds.

Acknowledgements

Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council (BBSRC) of the United Kingdom. Mite bioassays were made possible by a grant from The Swedish Board of Agriculture. Arid Land Resources Ltd. acknowledges the role of partners in the development of off-take of gums and resins in Northern Kenya. These include the Kenya Forestry Research Institute (KEFRI), World Agroforestry Centre (ICRAF), Kenya Forestry Department, Kenya Agricultural Research Institute (KARI), Semi Arid Lands Training and Improvement Centre (SALTLIC), and Gum and Resins Associates (GARA). The authors thank Novartis Animal Health SA, St. Aubin, Switzerland, for supplying the cattle ticks.

References

Ahmed, F., Ali, M., Singh, O., 2006. New compounds from *Commiphora myrrha* (Nees) Engl. Pharmazie 61, 728–731.

Beentje, H.J., 1994. Kenya Trees, Shrubs and Lianas. The National Museum of Kenya, Nairobi, Kenya.

Bruce, T.J.A., Birkett, M.A., Blande, J., Hooper, A.M., Martin, J.L., Khambay, B.P.S., Prosser, I., Smart, L.E., Wadhams, L.J., 2005. Response of economically important aphids to components of *Hemizygia petiolata* essential oil. Pest Management Science 61, 1115–1121.

Carroll, J.F., Maradufu, A., Warthen, J.D., 1989. An extract of *Commiphora erythraea*: a repellent and toxicant against ticks. Entomologia Experimentalis et Applicata 53, 111–116.

Cavanagh, I.S., Cole, M.D., Gibbons, S., Gray, A.I., Provan, G.J., Waterman, P.G., 1993. A novel sesquiterpene, 1,2-epoxyfurano-10(15)-germacren-6-one, from the resin of *Commiphora holtziana* Engl. Flavour and Fragrance Journal 8, 39–41.

Chikamai, B., Gachathi, N., 1994. Gum and resin resources in Isiolo District, Kenya: ethnobotanical and reconnaissance survey. East African Agriculture Forest Journal 59 (4), 345–351.

Dekebo, A., Dagne, E., Sterner, O., 2002. Furanosesquiterpenes from *Commiphora sphaerocarpa* and related adulterants of true myrrh. Fitoterapia 73, 48–55.

El Ashry, E.S.H., Rashed, R.N., Salama, O.M., Saleh, A., 2003. Components, therapeutic value and uses of myrrh. Pharmazie 58, 163–168.

Gillett, J.B., 1991. Burseraceae. In: Flora of Tropical East Africa. A.A. Balkema, Rotterdam and Brookfield.

Hanus, L.O., Rezanka, T., Dembitsky, V.M., Moussaieff, A., 2005. Myrrh – *Commiphora* chemistry. Biomedical Papers 149, 3–28.

Kokwaro, J.O., 1976. Medicinal Plants of East Africa. General Printers Ltd., Nairobi, Kenya.

Lemenih, M., Abebe, T., Olsson, M., 2003. Gum and resin resources from some *Acacia*, *Boswellia* and *Commiphora* species and their economic contributions in Liban, south-east Ethiopia. Journal of Arid Environments 55, 465–482.

Lwande, W., Ndakala, A.J., Hassanali, A., Moreka, L., Nyandat, E., Ndungu, M., Amiani, H., Gitu, P.M., Malonza, M.M., Punyua, D.K., 1999. *Gynandropsis gynandra* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. Phytochemistry 50, 401–405.

Marongiu, B., Piras, A., Porcedda, S., Scorciapino, A., 2005. Chemical composition of the essential oil and supercritical CO_2 extract of *Commiphora myrrha* (Nees) Engl. and of *Acorus calamus* L. Journal of Agricultural and Food Chemistry 53, 7939–7943.

Marudufu, A., 1982. Furanosesquiterpenoids of *Commiphora erythraea* and *C. myrrh*. Phytochemistry 21, 677–680.

Morteza-Semnani, K., Saeedi, M., 2003. Constituents of the essential oil of *Commiphora myrrha* (Nees) Engl Var. molmol. Journal of Essential Oil Research 15, 50–51.

Mwangi, E.N., Essuman, S., Kaaya, G.P., Nyandat, E., Munyinyi, D., Kimondo, M.G., 1995. Repellence of the tick *Rhipicephalus appendiculatus* by the grass *Melinis minutiflora*. Tropical Animal Health and Production 27, 211–216.

Ndungu, M., Lawande, W., Hassanali, A., Moreka, L., Chhabra, S.C., 1995. *Cleome monophylla* essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilus zeamis*) repellents. Entomologia Experimentalis et Applicata 76, 217–222.

Ndungu, M.W., Chabra, S.C., Lwande, W., 1999. *Cleome hirta* essential oil as livestock tick (*Rhipicephalus appendiculatus*) and maize weevil (*Sitophilis zeamais*) repellent. Fitoterepia 70, 514–516.

Newman, A.A., 1972. Chemistry of Terpenes and Terpenoids. Academic Press, London and New York.

NIST, 2005. NIST mass spectral search for the NIST/EPA/NIH mass spectral library version 2.0. Office of the Standard Reference Data Base, National Institute of Standards and Technology, Gaithersburg, Maryland.

Pettersson, J., 1970. An aphid sex attractant I biological studies. Entomologica Scandinavica 1, 63–73.

Prosser, I., Altug, I.G., Phillips, A.L., Konig, W.A., Bouwmeester, H.J., Beale, M.H., 2004. Enantiospecific (+) and (−)-germacrene-D synthases, cloned from goldenrod, reveal a functionally active variant of the universal isoprenoid-biosynthesis aspartate-rich motif. Archives of Biochemistry and Biophysics 432, 136–144.

Provan, G.J., Gray, A.I., Waterman, P.G., 1987. Sesquiterpenes from the Myrrh-type resins of some Kenyan *Commiphora* species. Flavour and Fragrance Journal 2, 109–113.

Yoshihara, K., Ohta, Y., Sakai, T., Hirose, Y., 1969. Germacrene-D, a key intermediate of cadinene group compounds and bourbonenes. Tetrahedron Letters 27, 2263–2264.

Zhu, N., Kikuzaki, H., Sheng, S., Sang, S., Rafi, M.M., Wang, M., Nakatini, N., DiPaola, R.S., Rosen, R.T., Ho, C.T., 2001. Furanosesquiterpenoids of *Commiphora myrrha*. Journal of Natural Products 64, 1460–1462.

Zhu, N.Q., Sheng, S.Q., Sang, S.M., Rosen, R.T., Ho, C.T., 2003. Isolation and characterization of several aromatic sesquiterpenes from *Commiphora myrrha*. Flavour and Fragrance Journal 18, 282–285.