

## FURANOSESQUITERPENOIDS OF *COMMIPHORA ERYTHRAEA* AND *C. MYRRH*

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**Key Word Index**—*Commiphora myrrh*; *C. erythraea*; Burseraceae; furanosesquiterpenoids; 2-*O*-acetyl-8,12-epoxygermacra-1(10),4,7,11-tetraene; 2-*O*-methyl-8,12-epoxygermacra-1(10),4,7,11-tetraene; ixodicides.

**Abstract**—The isolation and structure determination of two new furanosesquiterpenoids, 2-*O*-acetyl-8,12-epoxygermacra-1(10),4,7,11-tetraene and 2-*O*-methyl-8,12-epoxygermacra-1(10),4,7,11-tetraene, from gum exudates of *Commiphora myrrh* are reported. These two compounds, together with the known furanodienone isolated from *C. erythraea*, were found to have ixodocidal activity against *Rhipicephalus appendiculatus* tick larvae.

### INTRODUCTION

*Commiphora myrrh* and *C. erythraea* plants grow wild in the north-eastern province of Kenya and adjoining areas of Somalia and Ethiopia. Both plants yield economically important gum exudates which have been collected for centuries as medicinal and perfumery substances. So far there has been no detailed report on the chemical analysis of these gums, commercial samples of which are often adulterated with gums of other *Commiphora* species. There are several uninformative reports [1] on the constituents of *Commiphora* gums claimed to originate from the Red Sea area down to the Horn of Africa. There are also accounts on gums generally referred to as myrrh [2] but the absence of information linking the gums to the locality of collection and the particular species of *Commiphora* has made the available literature unreliable.

Accounts of a chemical analysis of gums referred to as Arabian myrrh have been given [3,4]. From these accounts several sesquiterpenoids of the eudesmane type were implicated as constituents of Arabian myrrh; however, their structures differ from the furanosesquiterpenoids of *C. myrrh* and *C. erythraea* reported here. Judging from our preliminary steam distillation experiments and information from other sources [5,6], there is no doubt that the plants reported here are *C. myrrh* (Nees) Engler, the source of myrrh oil and *C. erythraea* Engler, the source of opopanax oil. Unfortunately chemical analyses of gums from these plants have been limited to analyses of steam-volatile oils [5,7].

### RESULTS AND DISCUSSION

In our search for naturally occurring insecticides among local African traditional medicines we

encountered resinous gums of *C. myrrh* and *C. erythraea*, which are locally known as malmal and habaq-hagar-ad in the Somali vernacular respectively, as materials used on livestock against ticks. Careful screening of the various hexane, benzene, methylene dichloride, ethyl acetate, methanol and water fractions of the two gums resulted in isolation of sesquiterpenoids 1-3 from hexane fractions. These are moderately toxic to larvae of *Rhipicephalus appendiculatus* ticks responsible for transmitting organisms causing East Coast Fever in cattle. In bioassay tests according to Stone and Haydock [8], 1 had LD<sub>50</sub>, 6.0% and both 2 and 3 had LD<sub>50</sub>, 10%. Compound 1 is the well-known furanodienone (1*E*, 4*E*)-8,12-epoxygermacra-1(10)-4,7,11-tetraen-6-one [9]; 2 and 3 are reported here for the first time.

Chromatography of crude hexane extracts of *C. erythraea* gums on silica gel afforded furanodienone (1), mp 90.5°, with IR, UV and NMR data identical to those published [9]. The identification of 2 and 3 was therefore easy since their NMR spectra in CCl<sub>4</sub> were identical in many respects to furanodienone. The presence of a furan moiety in all three compounds was as usual [10] characterized by a one proton NMR signal at *ca.*  $\delta$  7.05 as a broad singlet coupled to a narrow doublet (1.1 Hz) of methyl hydrogens at 1.9 ppm. Furthermore, furan-ring induced retro-Diels-Alder fragmentation in the mass spectrum common to these type of compounds [10-12] resulted in significant mass peaks at *m/z* 122 for 1 and *m/z* 108 for 2 and 3. Absence of oxygenation at C-6 or C-9 in 2 and 3 therefore accounted for the ion at *m/z* 122 in 1 appearing as a peak of medium intensity at *m/z* 108 for the other two.

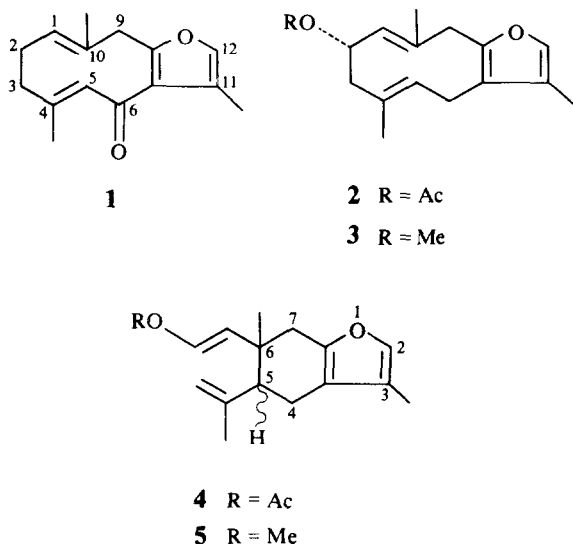
The germacrane ring moiety in 2 and 3 was readily inferred from the presence of two vinyl methyl resonances at *ca.* 1.7 and 1.4 ppm, and the olefinic proton resonances at 5.0-5.2 ppm. From the NMR data (Table 1), it can be seen that all three compounds share a common carbon skeleton. Chemical shift assignments were in all cases aided by spin-spin

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Table I.  $^1\text{H}$  NMR data\* of sesquiterpenoids

Compound	H-1	H-2	H-5	H-6	H-9	H-12	Me-4	Me-10	Me-11	Other
1†	5.2 <i>br dd</i> (10.4)	>2.50	5.80 <i>br s</i>	—	3.70 <i>br s</i>	7.06 <i>br s</i>	1.95 <i>br d</i> (1.2)	1.32 <i>br d</i>	2.14 <i>d</i> (1.5)	—
2‡	5.0 <i>br d</i> (10.4)	5.63 <i>ddd</i> (10.4, 4.5)	5.20 <i>br dd</i>	3.08 <i>br d</i> (8)	3.56 <i>br s</i>	7.22 <i>br s</i>	1.72 <i>br d</i> (ca 1.2)	1.44 <i>br d</i> (ca 1.1)	1.96 <i>d</i> (1.5)	2.13 <i>s</i> (OAc)
3†	5.0 <i>br d</i> (10.4)	4.04 <i>ddd</i> (10.4, 4.5)	5.08 <i>br dd</i>	3.05 <i>br d</i> (8)	3.50 <i>br s</i>	7.08 <i>br s</i>	1.65 <i>br d</i> (ca 1.2)	1.35 <i>br d</i> (ca 1.1)	1.91 <i>d</i> (1.5)	3.33 <i>s</i> (OMe)

\*Spectra were recorded on a 60 MHz instrument; chemical shifts in ppm; assignment aided by  $^1\text{H}$ - $^1\text{H}$  decoupling; coupling constants (Hz) in parentheses.†Recorded in  $\text{CCl}_4$ .‡Recorded in  $\text{CDCl}_3$ .



decoupling experiments. In the NMR spectra of **2** and **3**, resonances of H-1 and H-2 were easily identified; these parameters together with mass spectral information clearly established C-2 as the position of germacranolide ring oxygenation. As expected, H-2 of **2** resonated at a lower field than H-2 of **3**. The resonances of C-3 protons to which H-2 was coupled were clearly resolved in the spectrum of **2** recorded at 90 MHz in  $C_6D_6$ . Accordingly, large couplings (10.4 Hz) between H-2 and H-1 and the same (10.4 Hz) between H-2 and one H-3 were observed. Coupling parameters of this magnitude in the NMR call for an almost *trans*-diaxial orientation between H-1 and H-2 and in turn H-2 to H-3 $\alpha$ . Molecular models indicate that this arrangement is possible if the acetoxyl group in **2** or methoxyl group in **3** attain an  $\alpha$ -orientation. Similarly constituted germacrane at C-2 have been reported to show similar coupling interactions [13, 14].

Pyrolysis of **2** and **3** separately afforded unstable mixtures of elemenes\* **4** and **5**. In both cases the original C-1 and C-2 protons in **2** and **3** showed large couplings (13 Hz) in **4** and **5** indicating that their original *trans*-orientation to each other remained unchanged. This is consistent with the observation that if the Cope rearrangement of **2** and **3** as 1,5-dienes proceeds stereospecifically via a quasi-chair transition state [15, 16], then the orientation of their C-1 and C-2 substituents should remain unaltered in **4** and **5**. A coupling parameter of 13 Hz for *trans*-vinyl protons has been observed in other enol esters [13]. The data therefore favour an  $\alpha$ -orientation assignment for the acetoxyl or methoxyl group at C-2 in **2** and **3** respectively.

\*From the studies of thermal rearrangements of furanodienone and isofuranodienone [17] it is reasonable to suggest that products **4** and **5** occur as mixtures due to the presence of geometrically isomerized intermediates in the transition state involving the ten-membered ring double bonds rather than the substituents at C-1 and C-2 assuming new orientations.

## EXPERIMENTAL

**General procedures.** Proton NMR spectra were recorded at 60 MHz using solvents indicated and with TMS as an internal standard. Double resonance expts were all carried out using a double resonance component attached to the same instrument. One FT-NMR 90 MHz spectrum for **2** was kindly provided by Dr. A. S. Perlin of McGill University, Montreal. UV spectra were recorded in EtOH and IR spectra in  $CCl_4$  and  $CS_2$ . Mps are uncorr. Mass spectral measurements were performed on a Finnigan GC/MS Model 1015D operating at 70 eV. The GC component was equipped with a column of 3% SE-30 on Gas Chrome Q. Samples were introduced in the mass spectrometer by either solid probe or through the GC component. Polarimetric readings were kindly provided by Dr. M. Barbier of Centre National de la Recherche Scientifique, France.

**Collection of gums.** The gums of *C. myrrh* and *C. erythraea* were collected from the surrounding areas of the bore hole at Dambas, 20 km from Tarbaj, a small village town north of Wajir in the north-eastern province of Kenya. Specimens of twigs, fruits and leaves belonging to trees from which the gums were collected were identified by Mr. J. Gillet of the Kenya Herbarium in Nairobi and kept as specimens No. 16240 for *C. myrrh* and No. 16241 for *C. erythraea* of the herbarium.

**Extraction of 1, 2, and 3 from gums.** Crude gums of *C. erythraea* (1 kg) were triturated at room temp. in hexane using a Waring blender. Separation of insoluble materials by filtration and washing afforded, after drying followed by evapn of solvent *in vacuo*, a pale-yellow viscous syrup (256 g, 25.6%). A portion of the extract (10 g) was added to a Si gel column and eluted with petrol (bp 40–60°). The first fraction to be eluted was a fairly sweet scented, colourless oil (1 g, 10%). On changing the solvent to 1%  $CH_2Cl_2$  in petrol (bp 40–60°) **1** (0.5 g, 5%) was eluted preceded by small quantities of unidentified oils. **1** had mp 90.5–91.5°,  $[\alpha]_D^{20} \pm 0$  ( $CHCl_3$ ;  $c$  0.93), lit. [9, 17] 89.5–90.5°,  $[\alpha]_D^{20} \pm 0$  ( $c$  10); MS  $m/z$  (rel. int.): 230 ( $M^+$ , 20), 122 (100).

Crude gums of *C. myrrh* (1 kg) were treated as for **1** above. The hexane fraction amounted to 240 g (24%) of syrup oil. Chromatography of a portion of syrup (10 g) on Si gel as for **1** above gave a pungent viscous oil (5.3 g, 53%) which resinsified on standing in air. Elution with 3%  $CH_2Cl_2$  in hexane gave **2** (0.43 g, 4.3%) followed by **3** (0.2 g, 2%). **2** had mp 82–83°;  $[\alpha]_D^{20} + 75^\circ$  ( $CHCl_3$ ;  $c$  0.93). FT-NMR, 90 MHz ( $C_6D_6$ ):  $\delta$  6.95 (1H, *br s*, H-12), 5.62 (1H, *td*,  $J = 10.4, 4.5$  Hz, H-2), 4.9 (1H, *br d*,  $J = 10.4$  Hz, H-1), 4.86 (1H, *br dd*, H-5), 3.48 (1H, *d*,  $J = 15.2$  Hz, H-9 $\beta$ ), 3.23 (1H, *d*,  $J = 15.2$  Hz, H-9 $\alpha$ ), 2.98 (2H, *m*, H-6 $\alpha, \beta$ ), 2.43 (1H, *dd*,  $J = 11.28, 4.5$  Hz, H-3 $\beta$ ), 1.85 (1H, *dd*,  $J = 10.4, 11.28$  Hz, H-3 $\alpha$ ), 1.66 (3H, *s*, Me-CO), 1.60 (3H, *d*,  $J = 1.1$  Hz, Me-13), 1.35 (3H, *br s*, Me-14), 1.17 (3H, *s*, Me-15). MS analysed calc. for  $C_{17}H_{22}O_3$ , MW 274. Found:  $m/z$  (rel. int.): 274 ( $M^+$ , 0.3), 214 (0.76), 159 (1), 108 (4), 91 (4.7), 43 (100). **3** had mp 84–85°;  $[\alpha]_D^{20} + 68^\circ$  ( $CHCl_3$ ;  $c$  1.106); MS analysed calc. for  $C_{16}H_{22}O_2$  MW 246. Found:  $m/z$  (rel. int.): 246 ( $M^+$ , 3), 159 (11), 108 (10), 105 (11), 91 (29), 85 (17), 77 (17), 45 (100).

**Pyrolysis.** Portions of **2** and **3** weighing 50 mg each were introduced in tubes which were then filled with  $N_2$  and sealed. The sealed tubes were then heated at 200° for 5 min. On cooling the products were spectroscopically examined without separation. The products of **2**, i.e. **4**, had: IR  $\nu_{max}$  ( $CCl_4$ ) 1760, 1235  $cm^{-1}$  (vinyl acetate);  $\delta$  ( $C_6D_6$ ) 7.25 (1H, *d*,  $J = 13$  Hz,  $AcO-CH=C$ ), 7.0 (1H, *br s*, H-2), 5.47 and 5.73 (1H, *d*,  $J = 13$  Hz,  $AcO-CH=CH$ ), 4.55 and 4.9 (2H, *br m*,  $H_2C=C$ ), 2.9–1.9 (4H, *m*,  $CH_2$  groups), 1.8 and 1.5 (each 3H, *br m*, two

$\text{CH}_3\text{-C=}$  groups), 0.97 (3H, s, Me-6). The pyrolysis products of **3**, i.e. **5**, had:  $\delta$  ( $\text{CCl}_4$ ) 6.92 (1H, br s, H-2), 6.18 and 6.12 (1H each, d,  $J = 13$  Hz,  $\text{MeO-CH=C}$ ), ca 5.45 (1H, d,  $J = 13$  Hz,  $\text{MeO-CH=CH-}$ ), 3.42 (3H, s,  $\text{CH}_3\text{-O-C=C}$ ), 1.89 (3H, br d, Me-3), 1.76 (3H, br s,  $\text{CH}_3\text{-C=CH}_2$ ) 1.04 (3H, s, Me-6).

**Bioassay tests.** Stones' packet method was used [8]. 21–28 Day-old larvae of *Rhipicephalus appendiculatus* ticks were used. A sample mixed in olive oil was impregnated on Whatman No. 1 filter paper ( $10 \times 10$  cm) and made into a packet in which active live larvae were introduced. The packets were suspended in an incubator kept at  $37^\circ$  and 80% relative humidity. After 12 hr, the packets were opened and live and dead larvae counted. Data analysis was done by the Probit method.

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