# Venom Constituents of Three Species of *Crematogaster* Ants from Papua New Guinea[†]

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Fourteen new long-chain derivatives (**1j**−**1l**, **2j**−**2l**, **4a**−**4f**, **6**, and **7**) have been isolated, together with the known 4-oxo-2,5-dienyl acetates **1g**−**1i** and 4-oxo-2,5-dienals **2g**−**2i**, from the defensive secretions of three species of *Crematogaster* ants from Papua−New Guinea. Their structures have been established by HREIMS and [1]H−NMR studies, and by MS−MS analyses of the dimethyl disulfide derivatives **3a**−**3f**, **5a**−**5f**, and **8a**−**8f**. These results, together with those already reported on the three European *Crematogaster* species, suggest that the production of long-chain electrophilic contact poisons is a characteristic feature of this ant genus.

In previous papers, we reported studies aimed at understanding the complex chemical defense mechanisms of the three European species of *Crematogaster* (Formicidae:Myrmicinae) ants.[1−3] These ants do not inject their venom, as do most primitive ant species, but they use their spatulate sting to apply their venom topically on the integument of attacking insects. When workers of *Crematogaster* ants are immobilized with tweezers, a droplet of venom is emitted and accumulates on the spatulate portion of the sting.[1] We have shown that the Dufour's glands of *C. scutellaris*, *C. auberti*, and *C. sordidula* store a mixture of $C_{19}$, $C_{21}$, and/or $C_{23}$ long-chain derivatives (**1a**−**1i**) bearing a (*E,E*) cross-conjugated dienone linked to a primary acetate function.[2] When the venom is emitted, these compounds are transformed into highly electrophilic 4-oxo-2,5-dienals (**2a**−**2i**) by an esterase and an oxygen-dependent oxidase stored in the poison gland.[2] Acetic acid released during this process acts as alarm pheromone.[2,4] In this paper, we report on the results of an investigation of the defensive secretion constituents of three *Crematogaster* species from Papua New Guinea.

## Results and Discussion

The venoms were collected as described previously[1] on bits of filter paper at Laing Island (Papua New Guinea); stored in $CH_3OH$, $CH_2Cl_2$, or hexane; and brought to our laboratory in Brussels for chemical analyses.

TLC of the venom of *Crematogaster* sp. 1 (Si gel $F_{254}$ plates, hexane−EtOAc 8:2, vizualized by UV light at 254 nm) showed the presence of two major spots, one less polar and the other more polar than compounds **1a**−**1i** from *C. scutellaris*. Flash chromatography of the venom



$$H_3C-(CH_2)_n-\underset{H}{C}=\underset{H}{C}-(CH_2)_m-CH=CH-\overset{\overset{O}{\|}}{C}-CH=CH-R$$
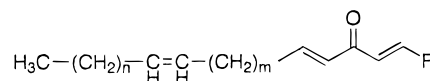
| | n | m | R=CH₂OAc | R=CHO |
|---|---|---|---|---|
| $C_{19}$ | 3 | 7 | **1a** | **2a** |
| | 5 | 5 | **1b** | **2b** |
| | 7 | 3 | **1c** | **2c** |
| $C_{21}$ | 3 | 9 | **1d** | **2d** |
| | 5 | 7 | **1e** | **2e** |
| | 7 | 5 | **1f** | **2f** |
| $C_{23}$ | 3 | 11 | **1g** | **2g** |
| | 5 | 9 | **1h** | **2h** |
| | 7 | 7 | **1i** | **2i** |
| $C_{25}$ | 3 | 13 | **1j** | **2j** |
| | 5 | 11 | **1k** | **2k** |
| | 7 | 9 | **1l** | **2l** |

(12.8 mg) on Si gel afforded two fractions, A (0.7 mg) and B (2.9 mg), homogeneous by TLC. The HREIMS of the less polar fraction A showed the presence of two parent peaks in an approximately 2:1 ratio, at *m/z* 374.3170 (calcd for $C_{25}H_{42}O_2$, 374.3184) and 346.2877 (calcd for $C_{23}H_{38}O_2$, 346.2872), respectively. The ^{1}H-NMR spectrum was nearly identical to those of the 4-oxo-2,5-dienals **2a**−**2i** (majors: **2d**−**2f**) from *C. scutellaris*[1] and **2a**−**2c** from *C. sordidula*.[3] Fraction A was found to be composed of long-chain 4-oxo-2,5-dienals having, respectively, 25 and 23 carbon atoms, and possessing one isolated carbon−carbon double bond in the chain.[1,3] To determine the number of double-bond isomers for each chain length and the position of this double bond in each isomer, fraction A was treated with dimethyl disulfide (DMDS) and a catalytic amount of iodine.[5] The resulting derivatives (**3a**−**3f**) were analyzed by linked scan MS−MS (daughter ions scan), complemented by a fixed neutral loss scan. The results, reported in Table 1, unambiguously showed that for each chain length, there are three positional double-bond isomers, namely $\Delta^5$, $\Delta^7$, and $\Delta^9$ (if one starts the numbering from the terminal $CH_3$ group). Thus, the $C_{23}$ compounds were identified as **2g**−**2i**, already reported from *C. scutellaris*, whereas the $C_{25}$ compounds
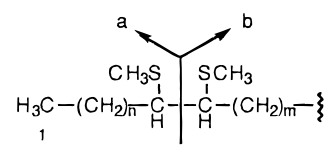
**Table 1.** MS–MS Analyses (Linked Scans: Daughter Ions, and Fixed Neutral Loss*) of Compounds **3a–3f**, **5a–5f**, and **8a–8f**



| | M$^{\cdot+}$ | n | m | C=C$^a$ | a | b | b–AcOH |
|---|---|---|---|---|---|---|---|
| **3a** | 440 | 3 | 11 | $\omega^5$ | 117 | 323 | |
| **3b** | 440 | 5 | 9 | $\omega^7$ | 145 | 295 | |
| **3c** | 440 | 7 | 7 | $\omega^9$ | 173 | 267 | |
| **3d** | 468 | 3 | 13 | $\omega^5$ | 117 | 351 | |
| **3e** | 468 | 5 | 11 | $\omega^7$ | 145 | 323 | |
| **3f** | 468 | 7 | 9 | $\omega^9$ | 173 | 295 | |
| **5a** | 424 | 3 | 11 | $\omega^5$ | 117* | 307 | |
| **5b** | 424 | 5 | 9 | $\omega^7$ | 145* | 279 | |
| **5c** | 424 | 7 | 7 | $\omega^9$ | 173* | 251 | |
| **5d** | 452 | 3 | 13 | $\omega^5$ | 117* | 335 | |
| **5e** | 452 | 5 | 11 | $\omega^7$ | 145 | 307 | |
| **5f** | 452 | 7 | 9 | $\omega^9$ | 173 | 279 | |
| **8a** | 484 | 3 | 11 | $\omega^5$ | 117 | 367 | 307 |
| **8b** | 484 | 5 | 9 | $\omega^7$ | 145 | 339 | 279 |
| **8c** | 484 | 7 | 7 | $\omega^9$ | 173 | 311 | 251 |
| **8d** | 512 | 3 | 13 | $\omega^5$ | 117 | 395 | 335 |
| **8e** | 512 | 5 | 11 | $\omega^7$ | 145 | 367 | 307 |
| **8f** | 512 | 7 | 9 | $\omega^9$ | 173 | 339 | 279 |

$^a$ Carbon atoms are numbered from the terminal CH$_3$.



| n | m | R=CH$_2$OAc | R=CHO |
|---|---|---|---|
| 3 | 11 | **8a** | **3a** |
| 5 | 9 | **8b** | **3b** |
| 7 | 7 | **8c** | **3c** |
| 3 | 13 | **8d** | **3d** |
| 5 | 11 | **8e** | **3e** |
| 7 | 9 | **8f** | **3f** |
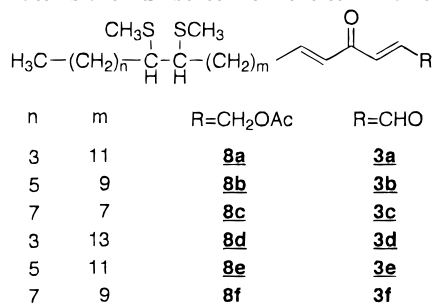
possess structures **2j–2l**. The ratio of C$_{23}$ to C$_{25}$ compounds was estimated to be 29:71 by mass spectrometry.

The GC–CIMS of fraction B showed the presence of two quasi-molecular ions [M + H]$^+$, at $m/z$ 359 and 331, in a 2.2:1 ratio and amounting to more than 85% of the material. HREIMS of this fraction displayed two molecular ions at $m/z$ 358.3227 (major) (calcd for C$_{25}$H$_{42}$O, 358.3235) and 330.2915 (minor) (calcd for C$_{23}$H$_{38}$O, 330.2922). The structures of the components of fraction B were determined to be **4a–4f**, on the basis of a complete spectroscopic study, including 1D and 2D NMR analyses at 600 and 150.87 MHz ($^1$H, $^{13}$C, COSY, HMQC, and HMBC). The assignments of most of the proton and carbon signals are reported in Table 2. Thus, in these compounds, the usual (E,E) cross-conjugated dienone is replaced by a furan ring (H-2 $\delta$ 7.24, d, $J$ = 1.8 Hz; C-2 $\delta$ 141.1; H-3 $\delta$ 6.31, dd, $J$ = 3.1, 1.8 Hz; C-3 $\delta$ 111.0; H-4 $\delta$ 6.09, d, $J$ = 3.2 Hz; C-4 $\delta$ 105.7) conjugated to a (E) double bond attached at C-5 (H-6 $\delta$ 6.12, d, $J$ = 15.8 Hz; C-6 $\delta$ 118.0; H-7 $\delta$ 6.10, dt, $J$ = 15.8, 7.1 Hz; C-7 $\delta$ 130.0), as shown by irradiation of H-4 at $\delta$ 6.09, which simplified the signals of H-6 and H-7. The presence of a furan ring conjugated to a double bond was further established by the fragmentation pattern in HREIMS [diagnostic fragment ions at $m/z$ 94.0421 (calcd for C$_6$H$_6$O, 94.0418) and 107.0493 (calcd for C$_7$H$_7$O, 107.0497)] and by UV ($\lambda_{max}$ 259, 265, and

**Table 2.** NMR Chemical Shift Values ($\delta$) of Compounds **4a–4f**

| | $^{13}$C NMR $(\delta)^{a,b}$ | $^1$H NMR $\delta$(mult, $J$ Hz, int)$^c$ | COSY $(\delta)^c$ |
|---|---|---|---|
| 2 | 141.1 | 7.24 (d, 1.8, 1H) | H3,4 |
| 3 | 111.0 | 6.31 (dd, 3.1, 1.8, 1H) | H2,4 |
| 4 | 105.7 | 6.09 (d, 3.2, 1H) | H2,3 |
| 6 | 118.0$^d$ | 6.12 (d, 15.8, 1H) | H8 |
| 7 | 130.0$^d$ | 6.10 (dt, 15.8, 7.1, 1H) | H8 |
| 8 | 32.7 | 2.14 (q, 7.1, 2H) | H6,7,9 |
| 9 | 29.0 | 1.42 (q, 7.4, 2H) | H8 |
| CH$_2$–C= | 26.9, 27.1 | 2.0 (dt, 6.2, 6.3, 4H) | olefinic H |
| CH=CH | 130.3 | 5.32 (m, 2H) | allylic H |
| (CH$_2$)n | 22–33 | 1.25 (m, $x$H)$^e$ | =C–CH$_2$,CH$_3$ |
| CH$_3$ | 14.1 | 0.87 (bt, 3H) | CH$_2$ |

$^a$ Recorded at 150.87 MHz in CDCl$_3$. $^b$ Assignments based on HMQC and HMBC. $^c$ Recorded at 600 MHz in CDCl$_3$. $^d$ Tentative assignments based on comparison with literature.[9,10] $^e$ C$_{23}$H$_{38}$O: $x$ = 20, C$_{25}$H$_{42}$O: $x$ = 24.

276 nm). The presence of an isolated disubstituted double bond was also proved by NMR ($\delta_H$ 5.32, 2H, m; $\delta_C$ 130.3), its stereochemistry being assigned as $Z$ on the basis of the chemical shift of the allylic carbon atoms[6] ($\delta$ 26.9 and 27.1). To fully establish the structure of fraction B components, it remained only to determine the position of the isolated double bond. This was effected by treatment of 0.2 mg of fraction B with DMDS, and MS analysis of the resulting mixture (**5a–5f**). The EIMS of the latter showed, as expected, two molecular ions at $m/z$ 452 (C$_{25}$, 43%) and 424 (C$_{23}$, 9%). Due to the presence of two different chain lengths and the possibility to have positional double-bond isomers,[1,3] the analysis was again performed by linked scan MS–MS. The results, reported in Table 1, are the same as those obtained for **3a–3f**, thus leading to structures **4a–4f** for the components of fraction B.

TLC (Si gel F$_{254}$, hexane–acetone 8:2, UV$_{254}$) of the defensive secretion extract (5 mg) of *Crematogaster* sp. 2 showed the presence of three major spots that were separated by Si gel flash chromatography, affording, in order of increasing polarity, fraction A (0.74 mg), fraction B (0.85 mg), and fraction C (1.3 mg). In contrast with all the other fractions isolated so far from *Crematogaster* ants, fraction C displayed only one molecular ion in its HREIMS at $m/z$ 318.2564 (C$_{21}$H$_{34}$O$_2$). A detailed NMR study ($^1$H, $^{13}$C, COSY, HMQC, and HMBC) allowed us to establish the presence of partial structure CH$_3$–CH=CH–CO–CH$_2$–CHOH–CH$_2$– at one end of the chain, and of a terminal ethyl group linked to a (E,Z,Z) triene at the other end (Table 3). The combination of the NMR and MS data led to structure **6** for the component of fraction C. Similarly, fraction B contained only one compound, which was quickly identified as a dihydro derivative of **6** (M$^{\cdot+}$ at $m/z$ 320 in EIMS). Its structure was easily established as **7** by comparison of its spectroscopic properties with those of **6**. Indeed, the $^1$H-NMR spectra of both compounds were very similar (Table 3), the most obvious difference being that the spectrum of **7** lacked the vinyl proton signals ($\delta$ 6.82 and 6.05) of the (E)-double bond conjugated to the CO group. Fraction A was still a mixture of compounds that contained the same triene subunit as **6** and **7**, but their complete structure could not be determined.

The venom of *Crematogaster* sp. 3 showed the same major spot in TLC as *C. scutellaris*.[1] Indeed, flash Si gel chromatography of this venom (18 mg) afforded 7.9 mg of a fraction whose $^1$H-NMR spectrum was nearly
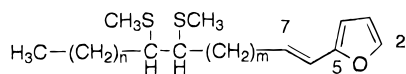
**Table 3.** NMR Chemical Shift Values ($\delta$) of Compounds **6** and **7**

| | 6 | | | 7 |
|---|---|---|---|---|
| | $^{13}$C NMR (d)$^{a,b}$ | $^1$H NMR d(mult, $J$ Hz, int)$^c$ | COSY$^c$ | $^1$H NMR d(mult, $J$ Hz, int)$^d$ |
| 1 | 18.0 | 1.85 (dd, 6.7, 1.5, 3H) | H2,3 | 0.92 (t, 7.4, 3H) |
| 2 | 143.8 | 6.82 (dq, 15.7, 6.8, 1H) | H1,3 | 1.60 (sext, 7.4, 2H) |
| 3 | 132.0 | 6.05 (dq, 15.7, 1.0, 1H) | H1,2 | 2.40 (t, 7.3, 2H) |
| 4 | 201.0 | | | |
| 5 | 45.0 | 2.66$^e$ (17.3, 1.8, 1H) | H6 | 2.59$^e$ (17.4, 3.1, 2H) |
| | | 2.53$^f$ (17.3, 9.1, 1H) | | 2.47$^f$ (17.4, 8.6, 1H) |
| 6 | 67.5 | 3.99 (m, 1H) | OH,H5,7 | 4.02 (m, 1H) |
| OH | | 3.05 (br s, 1H) | H6 | 2.93 (br s, 1H) |
| 7 | 36.0 | 1.35 + 1.45 (m, 2H) | H6 | |
| 8–12 | 29.5 | 1.30 (m, 10H) | | 1.25–1.46 (m, 12H) |
| 13 | 27.0 | 2.10 (m, 2H) | H14,15 | 2.15 (m, 2H) |
| 14 | 132.5 | 5.42 (dt, 10.0, 6.5, 1H) | H13,15 | 5.50 (m, 1H) |
| 15 | 123.0 | 6.36 (dd, 10.0, 10.0, 1H) | H13,14,16 | 6.45 (m, 1H) |
| 16 | 122.5 | 6.06 (g, 1H) | H15,18 | 6.13 (dd, 11.0, 11.0, 1H) |
| 17 | h | 5.90 (dd, 11.0, 11.0, 1H) | H16,18 | 5.96 (dd, 11.0, 11.0, 1H) |
| 18 | 136.5$^i$ | 6.42 (bdd, 15.5, 10.0, 1H) | H16,17,19 | 6.50 (m, 1H) |
| 19 | 125.0$^i$ | 5.70 (dt, 15.5, 6.5, 1H) | H18,20 | 5.74 (dt, 15.7, 6.8, 1H) |
| 20 | 33.0 | 2.05 (m, 2H) | H19,21 | 2.15 (m, 2H) |
| 21 | 13.0 | 0.96 (t, 7.3, 3H) | H20 | 1.02 (t, 7.4, 3H) |

$^a$ Recorded at 150.87 MHz in CDCl$_3$. $^b$ Assignments based on HMQC and HMBC. $^c$ Recorded at 600 MHz in CDCl$_3$. $^d$ Recorded at 250 MHz in CDCl$_3$. $^e$ A part of ABX. $^f$ B part of ABX. $^g$ Superimposed on H3 signal. $^h$ No correlation detected in HMQC. $^i$ Tentative assignment by HMBC.
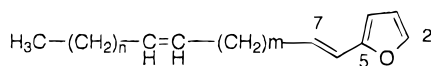


| | n | m |
|---|---|---|
| **4a** | 3 | 11 |
| **4b** | 5 | 9 |
| **4c** | 7 | 7 |
| **4d** | 3 | 13 |
| **4e** | 5 | 11 |
| **4f** | 7 | 9 |



| | n | m |
|---|---|---|
| **5a** | 3 | 11 |
| **5b** | 5 | 9 |
| **5c** | 7 | 7 |
| **5d** | 3 | 13 |
| **5e** | 5 | 11 |
| **5f** | 7 | 9 |



**6**



**7**
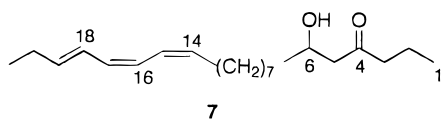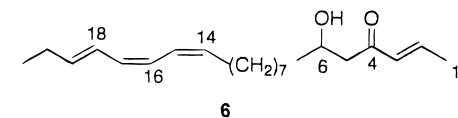
identical to that of the 4-oxo-2,5-dienyl acetates **1a**–**1i** from *C. scutellaris*[1] and **1g**–**1i** from *C. sordidula*.[3] Its HREIMS displayed two molecular ions at $m/z$ 418.3458 (calcd for C$_{27}$H$_{46}$O$_3$, 418.3447) and 390.3129 (calcd for C$_{25}$H$_{42}$O$_3$, 390.3134). Thus, in this species, the lengths of the alkyl chains are the same (C$_{25}$ and C$_{23}$) as those of the compounds of *Crematogaster* sp. 1. Only the positions of the isolated double bond remained to be determined. This was again performed through the formation of the DMDS derivatives and MS–MS analy-

sis of the resulting mixture of compounds (**8a**–**8f**). The results, reported in Table 1, allowed us to assign structures **1j**–**1l** to the C$_{25}$ compounds and to identify the C$_{23}$ compounds with the already known[3] **1g**–**1i**. The ratio of C$_{23}$ to C$_{25}$ compounds was 27:73 by mass spectrometry, similar to that found for *C.* sp. 1.

The three species of *Crematogaster* ants from Papua New Guinea obviously rely on the same type of defense mechanism as their European relatives. This observation strongly suggests that this mechanism is a characteristic feature of the genus, even if only six species have been investigated to date. By analogy with the results obtained on the European species,[1–3] it is highly probable that the compounds reported here originate from the Dufour's gland of these ants. Some of these compounds (**1g**–**1l**) are presumably native Dufour's gland constituents, whereas others (e.g., **2g**–**2l**, **4a**–**4f**) are probably formed, during venom emission or storage, through the action of enzymes from the poison gland on suitable precursors stored in the Dufour's gland.[2] The origin of the furan derivatives **4a**–**4f** from *Crematogaster* sp. 1 is intriguing. Taking into account what is already known of the Dufour's gland chemistry of *Crematogaster* ants, it is highly improbable that they are native compounds. By analogy with the α-angelica lactones **9a**–**9i**, found in aged venoms of *C. scutellaris*, and which are known[2] to be an artifact arising from cyclization of the 4-oxo-2,5-dienals **2a**–**2i**, compounds **4a**–**4f** could arise during storage through a Paal–Knorr[7] reaction on such saturated 1,4-ketoaldehydes as **10** (Scheme 1). Unfortunately, no fresh Dufour's gland extracts of *Crematogaster* sp. 1 were available to check the presence of such compounds.

The new structures reported in this paper are interesting on several accounts. First, in *Crematogaster* sp. 1 and sp. 3, the major derivatives are based on C$_{25}$ chains, instead of C$_{19}$, C$_{21}$, or C$_{23}$ in the European species. This could constitute a useful taxonomic marker. *Crematogaster* sp. 1 and sp. 3 are morphologically very similar, but their venoms are chemically different, even if the furans found in sp. 1 are probably artifacts. The ants were sympatric, but it seems doubtful that these samples represent chemotypes of the same species. This

**Scheme 1.** Proposed pathway for the formation of **4a**−**4f** and **9a**−**9i**.
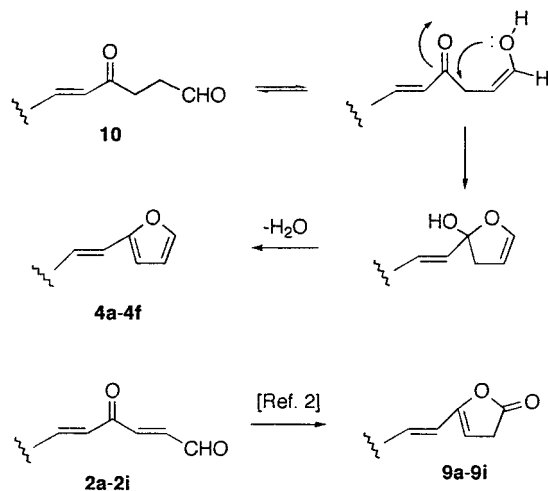


illustrates the difficulty of identifying species of *Crematogaster* from Papua New Guinea. *C. irritabilis* and *C. major* obviously constitute a group of sibling species deserving further studies.

On the other hand, compounds **6** and **7** from *Crematogaster* sp. 2 differ by several features from the pattern found till now in *Crematogaster* ants, namely the presence of a (*E,Z,Z*)-conjugated triene, of a $CH_3$ group at both ends of the carbon chain and a $\beta$-hydroxy ketone moiety. The latter could be a biosynthetic intermediate *en route* to the cross-conjugated dienone normally found in the compounds produced in the Dufour's gland of these ants. Although nothing is known yet concerning the biosynthesis of these compounds, they are presumably derived from fatty acids. In this context, it is worth pointing out that in all *Crematogaster* species examined so far, except for *Crematogaster* sp. 2, there are always three position isomers of the isolated double bond for each chain length ($C_{19}$, $C_{21}$, $C_{23}$, and $C_{25}$). Moreover, the positions of this double bond are always the same with respect to the terminal $CH_3$ group, namely $\omega^5$, $\omega^7$, or $\omega^9$. This suggests either that three different desaturases coexist in these species or, more likely, that there is only one desaturase. Then, once a double bond is introduced into the carbon chain, its position relative to the terminal carbon ($\omega$ value) will be fixed as the process of chain elongation occurs at the functional ($\Delta^1$) carbon.[8] For example, a $\Delta^9$ desaturase acting on a $C_{14}$, a $C_{16}$, or a $C_{18}$ fatty acid would introduce a double bond in the $\omega^5$, $\omega^7$, or $\omega^9$ position, respectively. Subsequent chain elongation of each of these monounsaturated fatty acids by the required number of acetate units (3 to 6) and decarboxylation would afford the characteristic $C_{19}$, $C_{21}$, $C_{23}$, and $C_{25}$ chains. We plan to study the biosynthesis of the defensive compounds of *Crematogaster* ants in the near future.

## Experimental Section

**General Experimental Procedures.** UV spectra were obtained on a Philips PU 8700 Uv-vis spectrophotometer in hexane. IR spectra were recorded on a Bruker IFS 25 instrument as a film on a NaCl disk. EIMS, HREIMS, and MS−MS measurements were performed on a Fisons VG Autospec. The GC−MS were performed on a Finnigan ion trap detector (ITD 800)

coupled to a Tracor gas chromatograph equipped with a 12-m SE 54 capillary column at 60 °C (3 min), programmed at 20 °C/min to 295 °C (hold 30 min). Ion intensities were recorded using chemical ionization (CI) with $NH_3$ as reactant gas. The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 600 and 150.87 MHz (Varian Unity 600 instrument) or, when stated, at 250 MHz (Bruker WM 250 instrument). The chemical shifts ($\delta$) are reported in parts per million, and the coupling constants are reported in Hertz. Flash liquid chromatography was performed over Macherey−Nagel Si gel (0.04−0.063 mm) and TLC analyses on Polygram SilG/ $UV_{254}$ precoated plates (0.25 mm) using hexane−$Me_2$-CO 8:2 as eluent and visualized under $UV_{254}$ light.

**Insect Collections.** Three species, *Crematogaster* sp. 1, sp. 2, and sp. 3, were collected in Papua New Guinea. Workers of arboreal *Crematogaster* sp. 1 were collected from secondary forest near Bogia, while *Crematogaster* sp. 2 workers were found on a dead tree (Hansa Bay, Bogia). *Crematogaster* sp. 3 was collected on a branch in Makarup forest (district of Bogia) and from an arboreal nest in Baitabag forest (district of Madang). Voucher specimens are in the collection of the Musée de Zoologie of Brussels University (reference nos: sp. 1, PNGF 79; sp. 2, PNGF 81; sp. 3, PNGF 19 and 72). Ant workers were seized by tweezers, and the venom that appeared at the tip of the sting was collected on small bits of filter paper. The latter were immediately dipped in $CH_2Cl_2$ (*C.* sp. 1, *C.* sp. 2), hexane (*C.* sp. 3 from Makarup) or $CH_3OH$ (*C.* sp. 3 from Baitabag) to stop or slow down the enzymatic reactions,[2] and the resulting solutions were stored at −18 °C till they were sent to Brussels. In the absence of recent taxonomic revision of the genus, it is presently impossible to identify New Guinean *Crematogaster* with any confidence. Comparison with available types leads to the following conclusions. Species 1 and 3 are close to *C. (Orthocrema) irritabilis* Smith, F. or *C. (Orthocrema) major* Donisthorp. Species 3 is slightly smaller and darker than sp. 1. Species 2 is most probably *C. (Crematogaster) bicolor* Smith, F.

**Extraction and Isolation.** Secretions of 200 *Crematogaster* sp. 1 were exhaustively extracted with $CH_2Cl_2$ affording 12.8 mg of a yellow oil. Flash chromatography of the extract on Si gel (eluent: hexane−$Me_2$CO 9:1) gave 0.7 mg of compounds **2g**−**2l** and 2.9 mg of compounds **4a**−**4f**.

Secretions of 200 *Crematogaster* sp. 2 were extracted 3 times with $CH_2Cl_2$ affording 4.98 mg of a yellow oil. Si gel chromatography of the extract using hexane−$Me_2$-CO 97:3 as eluent afforded 0.74 mg of fraction A, 1.3 mg of compound **6**, and 0.85 mg of compound **7**.

Secretions of 238 *Crematogaster* sp. 3 were exhaustively extracted with $CH_2Cl_2$ affording 18 mg of an yellow oil. The crude extract was processed through a Si gel chromatographic column with hexane−$Me_2$CO 98:2 as eluent to give 7.9 mg of compounds **1g**−**1l**.

**Compounds 1g−1l:** IR (dry film) and ^{1}H-NMR spectra nearly identical to those reported by Daloze *et al.*;[1,3] EIMS (70 eV) *m/z* [M]$^+$ 390 ($C_{25}H_{42}O_3$, <1) and 418 ($C_{27}H_{46}O_3$, 2), 376 (39), 358 (24), 348 (9), 330 (7), 320 (4), 319 (14), 305 (6), 302 (5), 135 (14), 121 (56), 107 (61), 95 (100).

**Compounds 2g−2l:** ^{1}H-NMR data nearly identical to those described by Daloze *et al.*;[1,3] EIMS (70 eV) *m/z*

[M]$^+$ 374 ($C_{25}H_{42}O_2$, 38) and 346 ($C_{23}H_{38}O_2$, 8), 358 (11), 328 (4), 319 (15), 291 (5), 153 (10), 151 (12), 137 (15), 135 (19), 125 (42), 109 (35), 97 (40), 95 (67), 83 (61), 81 (100).

**Compounds 4a−4f:** UV(hexane) $\lambda_{max}$ ($\epsilon$) 259 (4100), 265 (4400), 276 (3200); IR (dry film) $\nu_{max}$ 3006 (=CH), 2926−2854 ($CH_2$), 1464−1446, 1012 cm$^{-1}$; $^1$H NMR, see Table 2; COSY, HMQC, and HMBC fully supported the proposed structure; EIMS (70 eV) $m/z$ [M]$^+$ 330 ($C_{23}H_{38}O$, 6) and 358 ($C_{25}H_{42}O$, 32), 149 (14), 135 (12), 121 (16), 107 (64), 94 (86), 81 (62), 69 (99), 55 (100), 41 (87).

**Compound 6:** IR (dry film) $\nu_{max}$ 3544−3366 (OH), 3025 (=CH), 2962−2856 ($CH_2$), 1674−1630 (CO−C=C), 1192, 1044, 1070 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 600 MHz), see Table 3; COSY, HMQC, and HMBC fully supported the proposed structure; EIMS (70 eV) $m/z$ [M]$^+$ 318 ($C_{21}H_{34}O_2$, 9), 300 (2), 234 (16), 231 (17), 113 (27), 107 (29), 93 (46), 69 (100); HREIMS $m/z$ 318.2564 (calcd for $C_{21}H_{34}O_2$, 318.2559), 234.1984 [M$^{•+}$−$C_5H_8O$ (McLafferty rearrangement), calcd for $C_{16}H_{26}O$, 234.1984], 231.2120 (M$^{•+}$−$C_4H_7O_2$, calcd for $C_{17}H_{27}$, 231.2113) and 216.1877 (M$^{•+}$−$C_5H_8O$−$H_2O$, calcd for $C_{16}H_{24}$, 216.1878).

**Compound 7:** $^1$H NMR (CDCl$_3$, 250 MHz), see Table 3; EIMS (70 eV) $m/z$ [M]$^+$ 320 ($C_{21}H_{36}O_2$, 8), 231 (13), 225 (15), 121 (41), 115 (60), 107 (46), 95 (100).

**Determination of Double Bond Position.** The mixture (0.20 mg) of unsaturated compounds in hexane (500 $\mu$L) were treated with 500 $\mu$L of DMDS and ethereal iodine (3 mg iodine in 200 $\mu$L Et$_2$O) in a sealed microreactor vial. Reaction mixtures were kept overnight at 40−45 °C, cooled, and diluted with hexane (ca. 500 $\mu$L). Iodine was removed by shaking with Na$_2$S$_2$O$_3$ 10$^{-1}$M. The organic phase was evaporated to dryness, yielding the DMDS adduct. The residue was dissolved in hexane and analyzed by mass spectrometry (linked scan MS−MS, daughter ions, and fixed neutral loss scans).

**DMDS derivatives (3a−3f):** EIMS (70 eV) $m/z$ [M]$^+$ 440 ($C_{25}H_{44}S_2O_2$, 3) and 468 ($C_{27}H_{48}S_2O_2$, 6), 351 (3), 323 (13), 295 (19), 267 (7), 173 (82), 145 (51), 117 (23); linked scan (MS−MS analysis) daughter ions of 440: 117, 145, 173, 267, 295, 323; daughter ions of 468: 117, 145, 173, 295, 323, 351.

**Compounds 5a−5f:** EIMS (70 eV) $m/z$ [M]$^+$ 424 ($C_{25}H_{44}OS_2$, 9) and 452 ($C_{27}H_{48}OS_2$, 43), 117 (29), 145 (49), 173 (51), 251 (3), 279 (17), 307 (19), 335 (10); linked scan (MS−MS analysis) daughter ions of 424: 251, 279, 307; daughter ions of 452: 145, 173, 279, 307, 335; neutral loss of 117: from 424 and 452; neutral loss of 145: from 424 and 452; neutral loss of 173: from 424 and 452.

**Compounds 8a−8f:** EIMS (70 eV) $m/z$ [M]$^+$ 484 ($C_{27}H_{48}O_3S_2$, 5) and 512 ($C_{29}H_{52}O_3S_2$, 18), 335 (47), 307 (100), 279 (71), 251 (14), 173 (74), 145 (84), 117 (47); linked scan (MS−MS analysis) daughter ions of 484: 117, 145, 173, 251, 279, 307, 311, 339, 367; daughter ions of 512: 117, 145, 173, 279, 307, 335, 339, 367, 395.

**References and Notes**

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