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New 1-alk(en)yl-1,3,5-trihydroxycyclohexanes from the Dufour gland of the African ant *Crematogaster nigriceps*

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Abstract—The Dufour gland of the African ant *Crematogaster nigriceps* contains a mixture of at least eight 1-heptadec(en)yl- and 1-nonadec(en)yl-1,3,5-trihydroxycyclohexane derivatives, the mono-unsaturated components being the major ones (about 65%). They are accompanied by small amounts of (*Z*,*Z*)-dienic derivatives (<10%). The structures, the relative and absolute configurations, and the preferred conformation of these new compounds have been established by spectroscopic and chemical methods, whereas the positions of the double bond in the alkenyl chains were determined by DMDS derivatisation followed by linked scan EIMS.

Ants of the genus *Crematogaster* are able to raise their abdomen forwards and over the thorax and head. In many *Crematogaster* species, the venom is emitted as a froth that accumulates on the spatulate portion of the sting, and thus can be easily applied to the integument of enemies. In the three European species of *Crematogaster* ants, the venom is produced by both the Dufour and the poison glands. The Dufour gland contains complex mixtures of long chain derivatives bearing a (*E*,*E*)-cross-conjugated dienone linked to a primary acetate function.[1–3] When the venom is emitted, these compounds are transformed into highly electrophilic and toxic 4-oxo-2,5-dienals by an esterase and an oxidase stored in the poison gland.[2] Other types of long chain derivatives have been subsequently found in ants belonging to this genus, such as alkenylfurans in an unidentified species from Papua New Guinea,[4] and (13*E*,15*E*,18*Z*,20*Z*) - 1 - hydroxypentacosa - 13,15,18,20-tetraen-11-yn-4-one acetate in an unidentified Brazilian species.[5] Recently, several furanocembrene diterpenes were also found in two Brazilian species (*C. brevispinosa rochai* and *C. brevispinosa ampla*).[6,7]

To further investigate the defensive mechanisms in this genus and to assess whether the composition of the Dufour gland secretion could be used for taxonomic purposes, we have now studied another *Crematogaster* species, *C. nigriceps*, originating from Africa. The ants were collected from swollen thorns of *Acacia drepanolobium* trees in August, 2001 on the Laikipia plateau of central Kenya (0°15′N, 37°45′ E, elevation: 1800 m). This ant appears to be an obligate acacia ant, and in Laikipia is apparently restricted to this acacia species.[8] When its host tree is disturbed, hundreds of ants swarm out of the swollen thorns and attack.[9] In addition to biting their attackers, they produce a pungent odour easily detected by human 'attackers'. The relationship between the ants and the acacia is evolutionarily parasitic to the tree. The ants prune the tree, which greatly reduces successful reproduction in its host tree.[8,10]

Five hundred ants from a colony maintained in the laboratory were exhaustively extracted with a 1:1 mixture of CH_2Cl_2 and MeOH, affording 165.5 mg of crude extract that was chromatographied on a silica gel column using hexane/acetone 7:3 as eluent. This procedure afforded a fraction (FA, 30.1 mg), homogeneous by TLC on silica gel, containing the Dufour gland constituents. However, the MS and NMR data of FA showed that it was still a mixture of several compounds which all possess a 1,3,5-trihydroxycyclohexane ring, and which differ from each other by the length of the

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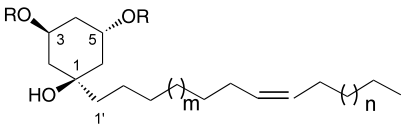
hydrocarbon side chain at C-1, and/or by the position of the double bond(s) in the side chain (vide infra).

The SIMSMS of FA showed three $[M+Na]^+$ ions at m/z 419, 393, and 391, whereas the CIMS(NH_3) displayed $[M+H-H_2O]^+$ peaks at m/z 379, 353, and 351, thus leading to the conclusion that the major constituents of this fraction were compounds having molecular weights of 396, 370 and 368 Da. In EIMS, no molecular ion could be detected, but instead three $[M-H_2O]^{+\bullet}$ peaks at m/z 378 (HRMS: 378.3501, calc. for $C_{25}H_{46}O_2$: 378.3498), 352 (HRMS: 352.3334, calc. for $C_{23}H_{44}O_2$: 352.3341), and 350 (HRMS: 350.3192, calc. for $C_{23}H_{42}O_2$: 350.3185) were present. It turns out that the major compounds of FA possessed the molecular formula $C_{25}H_{48}O_3$ (396 Da), $C_{23}H_{46}O_3$ (370 Da), and $C_{23}H_{44}O_3$ (368 Da), respectively. Based on their molecular formula, the compounds having a molecular weight of 396 and 368 Da possess two degrees of unsaturation which, according to the ^1H and ^{13}C NMR data[11] of FA, correspond to one ring and one carbon–carbon double bond. As will be shown later, each of these molecular formulae corresponds to three isomers of double bond position (**1a**–**c** and **1d**–**f**). These major components are accompanied by the dihydro-analogue **1g** ($C_{23}H_{46}O_3$, MW: 370 Da). The presence of dihydro-analogue **1h** ($C_{25}H_{50}O_3$, MW: 398 Da), although highly probable, could not de ascertained by MS, presumably because of its small concentration. Finally, the ^1H NMR spectrum of FA also showed the presence of small amounts of components exhibiting the characteristic signals[1] of (*Z*,*Z*)-conjugated dienes (**1i** and/or **1j**) at δ_H 5.46, and 6.26. The ratio of mono-unsaturated (**1a**–**f**), saturated (**1g/1h**), and dienic (**1i**/**1j**) components, as determined by ^1H NMR, was 65:27:8. Catalytic hydrogenation of FA afforded a mixture of two compounds, **1g** and **1h** [EIMS: $M^{+\bullet}$ at m/z 398 and 370; $[\alpha]_D$ 11.5 ($c=0.72$, MeOH)], whose ^1H NMR spectrum was nearly identical to that of FA, except for the disappearance of the signals of the vinyl hydrogen atoms at δ_H 5.37 and of the allylic hydrogen atoms at δ_H 2.03.

The ^1H and ^{13}C NMR data of FA[11] showed the presence, in the major compounds (**1a**–**f**), of two secondary (δ_H 4.35, tt, 11.4 and 4.2 Hz, δ_C 64.1 and δ_H 4.31, bs, δ_C 69.3) and one tertiary (δ_C 76.0) hydroxyl groups, and of a mono-unsaturated alkyl chain (δ_C 130.6 and 130.5; δ_H 5.37, m). The connectivity from C-2 to C-6 was deduced from $^1H/^1H$ COSY data and by HMBC correlations, in particular the 1,3 relationship of the two secondary alcohols. The presence of a cyclohexane ring was established by the observation of HMBC correlations between both H_2-2 (δ_H 1.45 and 1.91) and H_2-6 (δ_H 1.32 and 2.11), and C-1 at δ_C 76.0. From the MS and NMR data, an alkyl or alkenyl chain bearing 17 or 19 carbon atoms must also be present in the major components of FA. The only possible location for this chain is at C-1, which was confirmed by HMBC correlations between H_2-1′ at δ_H 1.38 and 1.48, and C-1. Finally, the values of the coupling constants measured for the two carbinol methines at δ_H 4.31, bs and 4.35, tt, $J=11.4$ and 4.2 Hz, clearly showed that the first one is equatorial, and the other one axial. This implied a *trans* relationship between the hydroxyl groups at C-3 and C-5 in all the components of FA, as shown in Fig. 1. It should be noted that in all these compounds, C-1 is chirotopic but not stereogenic.[12] Treatment of FA with acetic anhydride in pyridine led to a mixture of diacetylated compounds **2a**–**j**[13] (EIMS: major $M^{+\bullet}-H_2O$ peaks at m/z 462 and 434; ^1H NMR: 2 CH_3 signals at δ_H 2.03 and 2.08), confirming the presence of two secondary hydroxyl groups in **1a**–**j**. The

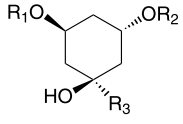


R = H

1a: m = 6; n = 1
1b: m = 4; n = 3
1c: m = 2; n = 5
1d: m = 8; n = 1
1e: m = 6; n = 3
1f: m = 4; n = 5

R = Ac

2a: m = 6; n = 1
2b: m = 4; n = 3
2c: m = 2; n = 5
2d: m = 8; n = 1
2e: m = 6; n = 3
2f: m = 4; n = 5



$R_1 = R_2 = H$

1g: $R_3 = C_{17}H_{35}$
1h: $R_3 = C_{19}H_{39}$

$R_1 = R_2 = Ac$

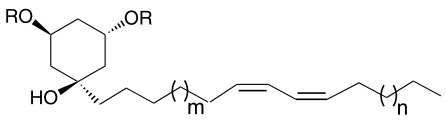
2g: $R_3 = C_{17}H_{35}$
2h: $R_3 = C_{19}H_{39}$

$R_1 = H$; $R_2 = (S)$-MTPA

5a: $R_3 = C_{17}H_{35}$
5b: $R_3 = C_{19}H_{39}$

$R_1 = H$; $R_2 = (R)$-MTPA

6a: $R_3 = C_{17}H_{35}$
6b: $R_3 = C_{19}H_{39}$



R = H

1i: n+m = 6
1j: n+m = 8

R = Ac

2i: n+m = 6
2j: n+m = 8

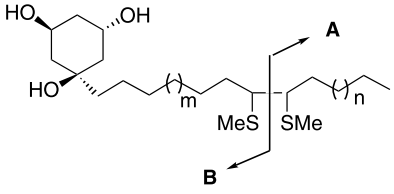
Figure 1.

configuration of the carbon–carbon double bond in the mono-unsaturated chains of **1a**–**f** was *Z* as shown by the chemical shift of the allylic carbon atoms (δ_C 27.6 and 27.8).[14] At this stage, it remained only to determine (i) the position(s) of the double bond in the mono-unsaturated analogues **1a**–**f**; (ii) the preferred conformation of the cyclohexane ring of **1a**–**j**; and (iii) their absolute configuration.

Treatment of FA with DMDS and a catalytic amount of iodine[15] afforded a mixture of DMDS derivatives (**3a**–**f**) that were analyzed by linked scan MS–MS. As in the case of other *Crematogaster* long chain derivatives,[3,4] the results (Fig. 2 and Table 1) showed that for each chain length (C_{17} and C_{19}), there are three positional double bond isomers, namely Δ^5 (**1a**, **1d**), Δ^7 (**1b**, **1e**), and Δ^9 (**1c**, **1f**) (if one starts the numbering of the side chain from the terminal methyl group). In the two series, the Δ^5 isomer was always the major one. The position of the (*Z*,*Z*)-diene in **1i**–**1j** was not determined.

As shown in Fig. 3, these molecules may exist as two interconverting chair conformations. Several arguments point to conformation **A** (OH at C-1 axial and alk(en)yl chain equatorial) being more stable than the inverted chair (**B**). It is known that in *cis*-1-methylcyclohexane-1,3-diol, it is the conformation with the two hydroxyl groups in an axial position which exists predominantly in aprotic solvents.[16] Moreover, conformation **A** is stabilized by an intramolecular hydrogen bond between the OH groups at C-1 and C-3, which is not possible for **B**. Finally, in the ^{13}C NMR spectrum of **1a**–**h**, CH_2-1′ absorbs at a rather low field (δ_C 44.6), which also suggests an equatorial position for the alkyl side chain.[17]

This hypothesis was definitely proved by treatment of the mixture of **1g** and **1h** with phenylboronic acid and



3a: m = 6; n = 1 **3d**: m = 8; n = 1
3b: m = 4; n = 3 **3e**: m = 6; n = 3
3c: m = 2; n = 5 **3f**: m = 4; n = 5

Figure 2.

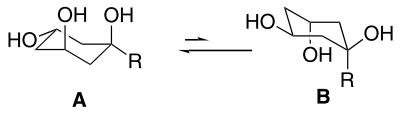


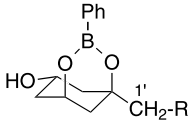
Figure 3.

$MgSO_4$ in benzene. The two-dimensional NMR spectra of the resulting phenylboronate derivatives[18] (**4a**–**b**, Fig. 4) allowed us to assign the signals of C-1 to C-6 and C-1′, and showed that C-1′ experienced only a slight shielding (−1.7 ppm) on going from **1g**–**h** to **4a**–**b**.

Since C-1′ is β to the boronate, this small shielding is in agreement with literature data.[19] This value, together with the other ^{13}C NMR data of **4a**–**b**,[18] indicates that no conformational change (e.g. **A** to **B**) takes place during boronate formation. Indeed, if the preferred conformation of **1g**–**h** were **B**, formation of the boronate ester would have necessitate a change to conformation **A**, resulting in a strong deshielding (about 5 ppm) of C-1′ on going from an axial to an equatorial position.[17] It turns out that compounds **1a**–**h** exist preferentially under conformation **A** (Fig. 3).

In order to determine the absolute configuration of compounds **1g**–**h**, they were treated with (*R*)- and (*S*)-MTPA chlorides and DMAP in dry THF. This led to the selective acylation of the equatorial C-5 OH group, and afforded the corresponding (*S*)- and (*R*)-MTPA mono-esters **5a**–**b** and **6a**–**b** (Fig. 1). Analysis of their ^1H and $^1H/^1H$ COSY spectra allowed us to assign all the signals of the cyclohexane hydrogens and of CH_2-1′ of these esters. Positive $\Delta\delta$[20] ($\Delta\delta = \delta_S - \delta_R$) were observed for OH-1, H_2C-6 and H_2C-1′, whereas negative values were observed for HC-3_{eq}, OH-3 and H_2C-4, which is consistent[21] with a (3*S*,5*S*) absolute configuration.

Compounds **1a**–**j** are new natural products that extend the array of secondary metabolites isolated from the Dufour gland of *Crematogaster* ants. Structurally, they depart from the long chain conjugated dienones[1–3] and



4a: R = $C_{16}H_{33}$
4b: R = $C_{18}H_{37}$

Figure 4.

Table 1. Characteristic fragment ions (*m*/*z*) of DMDS derivatives **3a**–**f** obtained by linked scan MS–MS

No.°	*m*	*n*	A[a]	B	B-H_2O	B-2H_2O	B-3H_2O
3a	6	1	117 (70)	345	327	309	291
3b	4	3	145 (6)	–	–	281	263
3c	2	5	173 (24)	–	–	253	235
3d	8	1	117 (57)	–	355	337	319
3e	6	3	145 (18)	345	327	309	291
3f	4	5	173 (25)	–	–	281	263

[a] Relative intensity (%) for each chain length in parentheses.

furan derivatives[4] already reported by the presence of a trihydroxylated cyclohexane ring. We do not know yet whether these compounds play a role in the chemical defense of these ants. Work is currently under way to test this possibility and will be reported elsewhere.

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11. ^{1}H NMR data (600 MHz, $CDCl_3$, ppm) of **1a**–**1j**: 1.91 (bd, 14.4, H-2_{eq}), 1.45 (m, H-2_{ax}), 4.31 (bs, H-3_{eq}), 2.29 (bd, 12.5, H-4_{eq}), 1.40 (m, H-4_{ax}), 4.35 (tt, 11.4, 4.2, H-5_{ax}), 2.11 (bd, 12.5, H-6_{eq}), 1.32 (m, H-6_{ax}), 1.48 and 1.38 (m, H_2-1'), 2.03 (m, CH_2–C=), 5.37 (m, HC=), 1.50–1.30 (m, $(CH_2)_n$), 0.92 and 0.91 (two t, 6.6, CH_3), 3.10, 2.69 and 1.60 (bs, OH). Small signals pertaining to **1i**–**j** at 5.46 (dt) and 6.26 (bd). ^{13}C NMR data (75.4 MHz, $CDCl_3$, ppm) of **1a**–**j**: 76.0 (C-1), 40.7 (C-2), 69.3 (C-3), 42.7 (C-4), 64.1 (C-5), 46.9 (C-6), 44.6 (C-1′), 130.6 and 130.5 (C=), 27.8 and 27.6 (**C**–C=), 32.6, 32.57, 30.7, 30.4, 30.3 (several superimposed peaks), 30.2, 30.0, 29.96, 29.6, 28.1, 23.6, 23.3, 23.0 (CH_2), 14.8 and 14.7 (CH_3). Small signals pertaining to **1i**–**j** at 132.7 and 124.2.
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13. ^{1}H NMR data (600 MHz, $CDCl_3$, ppm) of **2a**–**j**: 1.88 (bd, 15.0, H-2_{eq}), 1.63 (m, H-2_{ax}), 5.33 (m, H-3_{eq}), 2.20 (bd, 13.5, H-4_{eq}), 1.57 (m, H-4_{ax}), 5.28 (tt, 10.5, 3.6, H-5_{ax}), 2.09 (bd, 12.5, H-6_{eq}), 1.45 (m, H-6_{ax}), 1.48 and 1.36 (H_2-1′), 2.02 (m, CH_2–C=), 5.34 (m, HC=), 1.40–1.30 (m, $(CH_2)_n$), 0.89 and 0.88 (two t, 6.6, CH_3), 2.45 (bs, OH), 2.08 and 2.03 (two s, CH_3CO).
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18. **4a**–**b**: EIMS: $M^{+\cdot}$ at m/z 484 (2%) and 456 (18%); ^{13}C NMR (75.4 MHz, $CDCl_3$, ppm): 136.0 (C-1 Ph), 134.5 (2, *o*-Ph), 131.3 (*p*-Ph), 128.2 (2, *m*-Ph), 74.8 (C-1), 38.5 (C-2), 68.9 (C-3), 41.3 (C-4), 65.5 (C-5), 45.7 (C-6), 42.9 (C-1′), 32.6, 30.7, 30.4, 30.3, 30.0, 23.5, 23.4 (CH_2), 14.8 (CH_3).
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20. $\Delta\delta = \delta_S - \delta_R$: OH-1: +0.06; H-$2_{ax}$: 0; H-$2_{eq}$: −0.01; H-$3_{eq}$: −0.02; OH-3: −0.15; H-$4_{ax}$: -0.11; H-$4_{eq}$: −0.06; H-$5_{ax}$: 0; H-$6_{ax}$: +0.11; H-$6_{eq}$: +0.05; H_2C-1′: +0.01 and +0.02.
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