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New C_{26} δ-lactones from the Dufour's gland of the urticating ant *Tetramorium aculeatum*

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Abstract. (6R*)-{(2S*)-2-hydroxyheneicos-12-enyl}-5,6-dihydro-2H-pyran-2-one (**1**)° is the major constituent of the secretion of freshly dissected Dufour's gland of the urticating ant *Tetramorium aculeatum.* In solution, compound **1** is slowly transformed into (1S*, 5R*, 7S*)-7-(nonadec-10-enyl)-2,6-dioxabicyclo[3.3.1]nonan-3-one (**2**)° on standing. The structures of compounds **1** and **2** have been established on the basis of their spectral and chemical properties. Compound **1** could be responsible for the urticating properties of the ant.
° IUPAC numbering.
Key words. Tetramorium aculeatum; ant; Dufour's gland; δ-lactone.

Tetramorium aculeatum nests between the leaves of small trees in tropical Africa. It is notorious for its aggressiveness and for causing severe skin irritation by biting and stinging. When abundant, this ant impairs work in coffee plantations, because labourers refuse to work. In Zaire, this ant is named the 'urticating ant'[1, 2]. Dissection of worker ants revealed that they possess a hypertrophied Dufour's gland which reaches the front of the gaster. We report here on the chemical composition of the Dufour's gland secretion of *T. aculeatum.*

Materials and methods

The ants were collected around Yaounde and dipped in methanol, or sent alive to Brussels. The specimens were identified by Dr B. Bolton (British Museum).

Preliminary TLC analysis on silica gel (Macherey-Nagel Sil G/UV 254, 0.25-mm precoated plates; visualization: ceric sulphate; eluent: hexane/acetone 8:2) demonstrated the presence in the methanol extract of dissected Dufour's glands of one major compound and traces of a second one. Both compounds could also be easily detected in the methanolic extract of whole ants.

About 400 ants stored in methanol were extracted with dichloromethane (3 ×) and methanol (3 ×). The extracts were combined and the solvent evaporated under reduced pressure, yielding a solid residue (20.4 mg) that contained the two sought-after compounds. The latter were isolated by chromatography on neutral alumina (activity 1; eluent: hexane 100% to ethyl acetate 100%). This yielded pure **1** (2.2 mg) and **2** (3.8 mg) as oily derivatives whose structures were mainly deduced from their spectral properties.

^{1}H NMR (250 MHz) and ^{13}C NMR (62.8 MHz) spectra were recorded on a Bruker WM 250 spectrometer and are reported in tables 1 and 2. Infrared spectra were taken with a Bruker IFS 25 instrument using NaCl discs on which the compounds had been deposited as a glassy film. The UV spectra were recorded in methanolic solution with a Philips PU 8720 spectrophotometer and the mass spectra with a VG micromass 7070F spectrometer. The CI mass spectra were recorded with ammonia as the reactant gas. The microozonolyses of **1** and **2** were performed as follows: the compound (0.2 mg) was dissolved in methanol/hexane 1:1 (2 ml) and a stream of air enriched with ozone generated by a commercial microozoniser (Litha), was passed through the solution for 5 min. Then, triphenylphosphine (5 mg) was added to the mixture and the resulting solution was analyzed by GLC using a Varian 3700 gas chromatograph equipped with an OV-1 capillary column (25 m × 0.25 mm i.d.), a splitless injector and a flame ionization detector. Nitrogen was the carrier gas and the temperature was programmed from 60° to 140 °C at a rate of 2 °C min^{-1} following an initial delay of 2 min. In these conditions, only one peak, which had the same retention time as *n*-nonanal (coinjection) was observed. This identification was confirmed by

Table 1. ^1H and ^{13}C NMR data for compound **1** (250 and 62.8 MHz/$CDCl_3$/δ/TMS).

*C	^{13}C (BBD)[a]	^1H[a,b]
1	164.0	-
2	121.3	6.02, 1H, ddd, J = 1.8, 1.8 and 9.8 Hz
3	145.1	6.90, 1H, ddd, J = 4.3, 4.3 and 9.8 Hz
4	31.9*	2.41, 2H, m
5	76.9	4.66, 1H, m
6	41.9*	1.95, 1H, m; 1.80, 1H, m
7	69.2	3.85, 1H, m
8	37.7*	1.50, 2H, m
9	25.4	-
10–15 / 20–24	29.5	1.27, 22H, bs
16	27.2	2.00, 2H, m
17 / 18	129.9 / 129.9	5.35, 2H, t, J = 4.9 Hz
19	27.2	2.00, 2H, m
25	22.7	1.27, 2H, bs
26	14.1	0.88, 3H, t, J = 6.5 Hz

*assignments may be interchanged; [a]assignments were made by analogy with reported values for related compounds; [b]assignments supported by a $^1H/^1H$ correlation spectrum.

Table 2. ^1H and ^{13}C NMR data for compound **2** (250 and 62.8 MHz/$CDCl_3$/δ/TMS).

*C	^{13}C (BBD)[a]	^1H[a,b]
1	169.9	-
2	36.9+	2.87 (1H,bd,J = 19); 2.75 (1H,dd,J = 19, 5.4)°
3	65.7*	4.34, 1H, m
4	36.5+	2.00, 2H, m
5	73.1	4.87, 1H, m
6	35.9+	1.50, 2H, m
7	65.8*	3.73, 1H, m
8	31.9	1.50, 2H, m
9–15 / 20–24	29.7 (11C) and 25.2 (1C)	1.26, 24H, bs
16	27.2	2.00, 2H, m
17 / 18	129.9 / 129.9	5.35, 2H, t, J = 4.9 Hz
19	27.2	2.00, 2H, m
25	22.7	1.26, 2H, bs
26	14.1	0.88, 3H, t, J = 6.6 Hz

*and + assignments may be interchanged; [a]assignments were made by analogy with reported values for related compounds; [b]assignments supported by a $^1H/^1H$ correlation spectrum; °AB part of an ABX system.

GC-MS (Finnigan ITD 800 apparatus) using chemical ionization with ammonia as the reactant gas. The mass spectrum $\{(M+H)^+$ at m/z 143$\}$ was superimposable on that of an authentic sample of *n*-nonanal.

Treatment of **1** (0.2 mg) dissolved in dichloromethane (1 ml) with *p*-toluenesulphonic acid (2 mg) for 48 h at 40 °C gave in quantitative yield a compound whose R_f in TLC and ^1H NMR spectrum were identical to that of compound **2**.

Results and discussion

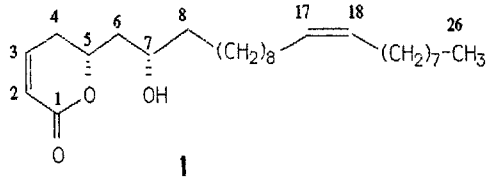
The structures of the two Dufour's gland compounds (**1** and **2**) were established mainly on the basis of their spectral properties.

Compound **1** showed a molecular ion ($M^{+\cdot}$) at m/z 406 daltons in the EI mass spectrum and a quasi molecular ion $(M+NH_4)^+$ at m/z 424 daltons in the CI mass spectrum compatible with the formula $C_{26}H_{46}O_3$. These other spectral properties of **1** indicated the presence of a secondary alcohol (ν_{OH} at 3442 cm^{-1}; 1H multiplet at δ 3.85) and an α,β-unsaturated-δ-lactone substituted at the δ position [$\nu_{C=O}$ at 1720 cm^{-1}; λ_{max} at 205 nm (ε = 12550); three methine signals at δ 6.90, 6.02 and 4.66; ^{13}C signals at δ 164.0, 145.1, 121.3 and 76.9]. These spectral data are entirely compatible with those reported for boronolide[3] and massoilactone[4] which both bear the same δ-lactone moiety. The $^1H/^1H$ COSY spectrum of **1** indicated that the secondary alcohol and the α,β-unsaturated-δ-lactone are linked as indicated in the partial formula **3**.

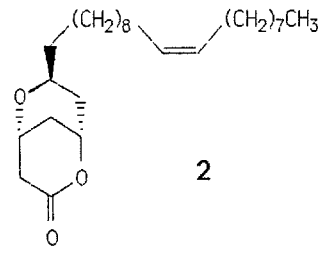
Further prominent features of the ^1H NMR spectrum of **1** were a 3H triplet at δ 0.88, a 26H broad signal at δ 1.27 and a 2H triplet at δ 5.35. These data, together with the presence in the mass spectrum of a series of fragmentation peaks separated by 14 daltons, suggested that the R substituent in partial structure **3** is a long chain of 19 carbon atoms bearing a double bond. The Δ^{17} position of this double bond in the side chain followed from the detection by GC/MS of *n*-nonanal after reductive microozonolysis of **1**.

Selective irradiation of the allylic protons (δ 2.0) collapsed the triplet at δ 27.2 in the ^{13}C NMR spectrum of **1** indicating that this signal may be attributed to the carbon atoms adjacent to the isolated double bond. Such a chemical shift for allylic carbon atoms is characteristic for a Z double bond[5]. This geometry is further confirmed by the lack of any absorption band at about 970 cm^{-1} in the IR spectrum of **1**[6]. All these data, together with the ^{13}C NMR data, reported in table 1, are consistent with structure **1**, the relative configuration of which was deduced by chemical correlation with **2** (see hereunder).

Compound **2** showed a molecular ion at m/z 406 daltons in the EI mass spectrum and a quasi molecular ion at 424 daltons $(M + NH_4)^+$ in the CI mass spectrum, indicating that **1** and **2** are isomers. High resolution mass spectrometry measurements on the molecular ion yielded the molecular formula $C_{26}H_{46}O_3$ (measured: 406.3454; calculated: 406.3449). The other spectral properties of **2** indicated that the δ-lactone is no longer unsaturated ($\nu_{C=O}$ at 1735 cm^{-1}, no absorption above 200 nm in the UV spectrum) and the absence of an hydroxyl group. In contrast, the IR and ^1H NMR spectra of **2** suggested the presence of three methine groups linked to an oxygen atom (ν_{C-O} at 1076 cm^{-1}; three 1H multiplets at δ 4.87, 4.34 and 3.73; ^{13}C NMR signals at δ 73.1, 65.8 and 65.7). All these data led to the hypothesis that **2** is the compound resulting from an intramolecular nucleophilic attack of the hydroxyl group on the α,β-unsaturated-δ-lactone of **1**. This was confirmed by adding *p*-toluenesulphonic acid to a dichloromethane solution of **1** at 40 °C for two days. Under these conditions, **1** was quantitatively transformed into **2**. The ^1H and ^{13}C NMR spectra of **2** reported in table 2 are consistent with the proposed structure. As for **1**, reductive microozonolysis



1



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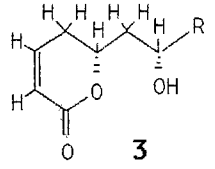

3

of **2** generated *n*-nonanal identified unambiguously by GC/MS.

The relative configurations at C−3, C−5 and C−7 of **2** derive from a careful analysis of its ^{1}H NMR spectrum. Indeed, the equatorial position of the side chain at C−7 is deduced from the chemical shift of H−7 (δ 3.73) which is abnormally shielded. Such a shielding effect can take place only when this proton is in an axial position and thus lies in the shielding cone of the carbonyl group. Moreover, the width at half height of the H−7 signal ($W_{1/2}$ = 23 Hz) is also compatible with an axial position for this proton. Finally, the ^{1}H/^{1}H correlation spectrum of **2** indicated, besides the expected vicinal interproton couplings (^{3}J), long-range couplings (^{4}J) between H−3 and H−5, H−2_{eq} and H−$4_{eq\circ}$, H−$4_{ax\circ}$ and H−6_{eq} (°axial or equatorial relative to the lactone ring) respectively, compatible with W-configurations for these protons[7]. The lack of such long-range couplings between H−7 and H−3 or H−5 is also in agreement with the proposed relative configurations 3R*, 5S*, 7S*.

The conversion of **1** into **2** on acidic treatment implies that they have the same relative configuration at C−5 and C−7. It follows that the alcohol **1** is the 5,7-*syn* diastereoisomer (5R*, 7S*).

Careful dissection of the poison and Dufour's glands of a few specimens of *T. aculeatum*, followed by TLC of the secretion of both types of glands, indicated that the compounds **1** and **2** are present in the Dufour's gland exclusively. Compound **1** is the major one in freshly dissected Dufour's glands. In solution, it is slowly transformed into **2** on standing. This suggests that the alcohol is the compound biosynthesized by the gland, the ether being most probably formed spontaneously. Biogenetically, compound **1** can be viewed as being formed from a diunsaturated fatty acid (26:2;2,17) hydroxylated at C−5 and C−7.

Lactones have already been found in exocrine secretions of various ant species[8]. They usually function as defensive compounds and/or as pheromones. Thus, iridoid lactones were found in the anal glands of several dolichoderines[9], whereas unsaturated δ-lactones have been described in the mandibular glands of males and workers of *Camponotus* spp. (formicine)[4, 10], as well as in the poison gland of fire ant queens (myrmicine)[11]. So far, lactones have been only reported in the Dufour's gland of *Lasius flavus*[12]. In this formicine, they are γ-lactones. In contrast, lactones were not found in the Dufour's secretion of three European *Tetramorium* species[13].

The erratic distribution of lactones in ant taxa and in their various exocrine secretions, illustrates that the chemical composition of ant exocrine secretions can evolve very quickly and much faster than their morphology.

Structurally, the unsaturated δ-lactones of *T. aculeatum* are closely related to massoilactone, isolated from the bark oil of *Cryptocaria massoia* (Lauraceae) and the mandibular gland of *Camponotus* sp.[4]. Massoilactone is known to be a powerful skin irritant and to produce systolic standstill in frog heart muscle[4]. The *T. aculeatum* lactones could be responsible, at least in part, for the irritating properties of these ants. These ants are able to sting, but the sting is not a very powerful structure able to inject deeply the venom in the skin. The lipophilicity of the lactones could facilitate their penetration through the skin or insect exoskeleton. Bites during fighting could also facilitate the penetration of the poison. Besides their defensive and offensive functions, the *T. aculeatum* lactones could serve as pheromones as in other species of ants. Their biological functions are currently being investigated in our laboratories.

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