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Furanocembranoid Diterpenes as Defensive Compounds in the Dufour Gland of the Ant *Crematogaster brevispinosa rochai*

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Abstract—Two new furanocembranoid diterpenes, crematofuran [(1*R**,11*R**,12*R**)-6,19:11,12-bisepoxycembra-3,6,8(19),15-tetraene] (**1**) and isocrematofuran [(1*R**,3*S**,4*S**)-3,4:6,19-bisepoxycembra-6,8(19),11,15-tetraene] (**2**), have been isolated from the Dufour gland secretion of the Brazilian ant *Crematogaster brevispinosa rochai.* Their structures, including the relative configuration, have been determined by a combination of NMR and molecular mechanics methods. The toxicity of **1** towards other ants is on the same level as that of nicotine. This is the first report of cembranoid diterpenes as defensive compounds in an ant. © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

In ants, two major glands are associated with the sting, the poison and the Dufour glands. Generally, the poison gland secretes the venom which accumulates in the poison sac before being injected into the prey or enemy.[1] In many instances, the main enemies of ants are other competing ants, which are more difficult to fight by stinging than by spraying deterrents, applying contact poisons or emitting sticky secretions. In these cases either the poison gland secretes non-proteinous venoms, e.g. formic acid in formicines or alkaloids in some myrmicines, or chemical defense is taken over by other exocrine glands.[2] In some non-stinging ants, as in the genus *Crematogaster*, it is the enlarged Dufour gland instead of the poison gland which secretes the venom. The three European species of *Crematogaster* ants possess a peculiar defensive mechanism which requires a co-operation between the poison and the Dufour glands. The latter contains complex mixtures of long-chain derivatives bearing an (*E*,*E*)-cross-conjugated dienone linked to a primary acetate function.[3–5] 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.[4] The Dufour gland of New Guinean *Crematogaster* species contains, besides the known 4-oxo-2,5-dienol acetates and 4-oxo-2,5-dienals, long chain furan derivatives.[6] We have now initiated a comparative study of *Crematogaster* ants from the New World in an effort to see whether there are variants to the defensive mechanism already evidenced in European and New Guinean species belonging to this genus. This led recently to the isolation of (13*E*,15*E*, 18*Z*,20*Z*)-1-hydroxypentacosa-13,15,18,20-tetraen-11-yn-4-one 1-acetate, a new acetylenic tetraene derivative from an as yet undetermined Brazilian *Crematogaster* species.[7]

We now report the isolation of two new furanocembranoid diterpenes, crematofuran [(1*R**,11*R**,12*R**)-6,19:11,12-bisepoxycembra-3,6,8(19),15-tetraene] (**1**) and isocrematofuran [(1*R**,3*S**,4*S**)-3,4:6,19-bisepoxycembra-6,8(19),11,15-tetraene] (**2**), from the Dufour gland secretion of the Brazilian ant *Crematogaster brevispinosa rochai* Forel.

Results and Discussion

For the present study, two nests of *C. brevispinosa rochai* were collected at Paraipaba and at Pentecoste, in the Ceará state (Brazil). The venom of four hundred workers was obtained by 'milking' the ants,[5] and stored in methanol. TLC and GC–MS analyses showed that the secretion

Keywords: furanocembranoid diterpenes; *Crematogaster*; Dufour gland.

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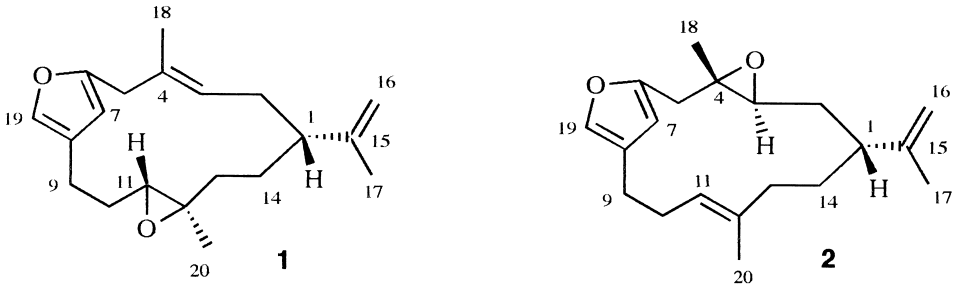

Figure 1. Structures of crematofuran (**1**) and isocrematofuran (**2**).

contained essentially two compounds, **1** and **2**, the first being the major one. The secretion was evaporated under reduced pressure and submitted to a flash chromatography on silica gel (eluent: hexane-acetone, 98:2). This procedure yielded 5.0 mg of the major compound **1** (Fig. 1).

Subsequently, the two compounds were isolated from whole ant extracts: 1,000 workers yielded 52.1 mg of **1** and 34.0 mg of **2**. High-resolution mass measurements showed the molecular formula of **1** to be $C_{20}H_{28}O_2$ (meas., 300.2095; calc., 300.2089), indicating the presence of seven degrees of unsaturation and two oxygen atoms. The planar structure of **1** was determined by an analysis of 1D and 2D NMR spectra (COSY, HMQC and HMBC). This allowed us to detect 20 carbon atoms, which included 8 sp^2 carbon atoms (4 quaternary, 3 methines and 1 methylene) and 12 sp^3 carbon atoms (1 quaternary, 2 methines, 6 methylenes, and 3 methyls). Eight sp^2 carbon atoms imply four double bonds and thus, on the basis of its molecular formula, compound **1** has to be tricyclic. The ^1H and ^{13}C NMR spectra, which were completely assigned (see Table 1), indicated the presence of an epoxide functionality (HC-11: δ_H 2.54; δ_C 64.4; C-12: δ_C 62.4) and a furan ring (see δ of C-6 to C-8 and C-19 in Table 1), thus identifying the nature of four unsaturations and of the two oxygen atoms of the molecule. Two other unsaturations were attributable to a trisubstituted double bond (δ_H 5.04, δ_C 126.5 and 136.4; CH_3 at δ_H 1.53) and an isopropenyl group (δ_H 4.77 and 4.78, δ_C 111.7 and 148.3; CH_3 at δ_H 1.52) and thus, the remaining unsaturation must be a carbocycle. The connectivities C-9/C-10/C-11 and C-3/C-2/C-1/C-14/C-13 were afforded by the COSY spectrum. These partial structures could be connected on the basis of HMBC experiments at 5 and 10 Hz, the results of which are reported in Table 1. Most relevant were the cross peaks observed between H_2-5 and the carbon atoms C-4, C-6, C-7, and C-8, and between H_2-9 and the carbon atoms C-7, C-8, and C-19, which allowed us to connect H_2C-5 and H_2C-9 to C-6 and C-8 of the furan ring, respectively. These results, together with the lack of coupling between the two hydrogen atoms of the furan at δ_H 5.63 and 6.79, demonstrated that the furan ring is 2,4-disubstituted. The configuration of the Δ^3 double bond was

Table 1. NMR data of crematofuran (**1**) (C_6D_6, 600 and 150.87 MHz, δ, J in Hz)

Position	^1H	^{13}C	HMBC[a]	NOESY[b]
CH-1	1.82, tt, 11.4, 3.0	50.6	C-2, C-14, C-15, C-16, C-17	H-2a, **H-2b**, H-3, H-13a, H-14a, H-14b, **H-16a**
CH-2a	1.91, m	33.9	C-1, C-3, C-4, C-14	H-1, **H-2b**, **H-3**
CH-2b	1.98, bq, 12.0		C-1, C-3, C-4, C-14	H-1, **H-2a**, **H-3**, CH_3-17 and/or CH_3-18
CH-3	5.04, dd, 10.8, 4.2	126.5	C-2, C-5, C-18	H-1, **H-2a**, **H-2b**, **H-5b**, H-14b
C-4	–	136.4	–	–
CH-5a	3.02, d, 14.4	39.2	C-4, C-6, C-7, C-8	**H-5b**, CH_3-18
CH-5b	3.19, d, 14.4		C-4, C-6, C-7, C-8	H-3, **H-5a**, H-7, CH_3-18
C-6	–	157.2	–	–
CH-7	5.63, s	108.6	C-6, C-8, C-9, C-19	H-5b, H-9a, H-11, **H-14b**
C-8	–	126.0	–	–
CH-9a	2.16, td, 14.0, 3.6	23.0	C-7, C-8, C-10, C-11, C-19	H-5, **H-6a**, **H-6b**, **H-7**, **H-9b**
CH-9b	2.35, dt, 14.4, 3.6		C-7, C-8, C-10, C-11, C-19	**H-9a**, **H-10a**, H-10b
CH-10a	1.36, m,	30.2	C-9, C-11, C-12	**H-9a**, **H-9b**, **H-10b**, H-11, CH_3-20
CH-10b	1.92, m		C-11, C-12	**H-9a**, H-9b, **H-10a**, H-11
CH-11	2.54, dd, 8.4, 0.6	64.4	C-9, C-10, C-12, C-13	H-7, **H-9a**, **H-10a**, **H-10b**, **H-13a**
C-12	–	62.4	–	–
CH-13a	0.79, td, 12.5, 1.8	38.9	C-1, C-11, C-12, C-14, C-20	H-1, H-11, **H-13b**, **H-14a**, **H-14b**, H-16a
CH-13b	1.96, td, 13.0, 6.6		C-11, C-12, C-14	**H-13a**, **H-14a**, **H-14b**, H-16a, CH_3-17, CH_3-20
CH-14a	0.88, qd, 12.5, 6.6	26.9	C-1, C-13, C-15	H-1, **H-13a**, **H-13b**, **H-14b**, H-16a
CH-14b	1.18, tdd, 12.5, 3.0, 1.8		C-1, C-12, C-13, C-15	H-1, H-3, H-7, **H-13a**, **H-14a**
C-15	–	148.3	–	–
CH-16a	4.77, bs	111.7	C-1, C-15, C-17	**H-1**, H-13b, H-14a
CH-16b	4.78, bs	111.7	C-1, C-15, C-17	**CH_3-17**
CH_3-17	1.52, s	19.0	C-2, C-15, C-16	H-13b, **H-16b**
CH_3-18	1.53, s	17.9	C-1, C-4, C-5, C-6	H-5a, H-5b
CH-19	6.79, s	138.1	C-6, C-7, C-8	H-9b
CH_3-20	0.94, s	17.7	C-11, C-12, C-13	H-10a, H-13b

[a] Experiments optimized for values of J=5 and 10 Hz.
[b] most intense correlations are shown in bold.

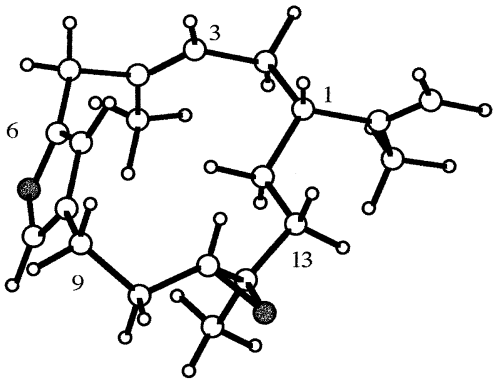

Figure 2. Lowest energy conformer of **1** as determined by NOESY and MM3 methods.

assigned as *E*, on the basis of the NOESY spectrum, showing a cross peak between H-3 and H-5b, but no cross peak between H-3 and the C-18 methyl group, nor between H-5a and the H_2-2. At this stage, it remained only to establish the relative configuration of the three stereogenic carbon atoms of the molecule (C-1, C-11, and C-12). This could again be performed by analysis of the NOESY spectrum, which showed that the epoxide moiety was *trans*. This left only two diastereoisomers to consider, having respectively the H_3C-20 and the isopropenyl groups on the same face or on opposite faces of the cembrane ring. The NOESY correlations listed in Table 1 allowed us to deduce the relative configuration shown in **1**. A preferred solution conformation was tentatively deduced for **1** on the basis of the coupling constants and NOESY correlations reported in Table 1.

The MM3[8] minimum energy conformation (shown in Fig. 2) was in excellent agreement with that deduced from the NMR data. Moreover, the nOe correlations and coupling constants calculated using the MacMimic[9] program were in good agreement with those determined experimentally and reported in Table 1. Compound **1** is thus [(1R^*,11R^*,12R^*)-6,19:11,12-bisepoxycembra-3,6,8(19),15-tetraene], for which we propose the name crematofuran.

Compound **2** had the molecular formula $C_{20}H_{28}O_2$ and is thus an isomer of **1**. Its planar structure was solved by the 1D and 2D NMR methods already described in detail for the structure determination of **1**. This led us to propose that **1** and **2** only differ by the relative positions of the ring double bond and of the epoxide function. The relative configuration of **2** was again deduced by a combination of NMR data (coupling constants and NOESY experiments, see Table 2) and MM3 calculations. The lack of NOESY correlation between H-3 and CH_3-18, as well as between H-11 and CH_3-20 showed that both the epoxide and the Δ^{11} double bond were again *trans*. Thus, only two relative configurations for **2,** either 1R^*,3S^*,4S^* or 1R^*,3R^*,4R^*, remained to be considered. A strong argument in favour of the former came from MM3 calculations on both diastereoisomers. For the 1R^*,3R^*,4R^* stereoisomer, all the low energy conformers displayed an *anti* ($\pm$180°) arrangement of H-2a versus both H-1 and H-3. This geometry should lead to a quartet-type signal for H-2a, as it must have two large ^3J and one large *J*gem. In fact, in the ^{1}H NMR spectrum of **2**, both H-2a (J=13.2, 10.8 and 2.0 Hz) and H-2b (J=13.2, 10.2 and 3.6 Hz) appeared as ddd, which implies that H-2a and H-3, as well as H-2b and H-1 should be *anti* to each other, whereas H-2b and H-3 as well as H-2a and H-1 should be *gauche*. This stereochemical arrangement was realized in the four conformers of lowest energy of the 1R^*,3S^*,4S^* diastereoisomer obtained by MM3 calculations. The coupling constants calculated for the lowest energy

Table 2. NMR data of isocrematofuran (**2**) (C_6D_6, 600 and 150.87 MHz, δ, J in Hz)

Position	^{1}H	^{13}C	HMBC[a]	NOESY[b]
CH-1	1.98, m	43.7	C-2, C-3, C-13, C-14, C-15, C-16	H-2a, H-2b, H-3, H-7, **H-14**, H-16a, H-18
CH-2a	1.26, ddd, 13.2, 10.8, 2.0	35.1	C-1, C-3, C-4, C-14, C-15	H-1, **H-2b**, H-3, H-17
CH-2b	1.75, ddd, 13.2, 10.2, 3.6		C-1, C-3, C-4, C-14	H-1, **H-2a**, H-3, H-17
CH-3	2.69, dd, 10.8, 3.6	61.8	C-2	H-1, H-2a, **H-2b**, H-5a, H-14
C-4	–	60.9	–	–
CH-5a	2.31, AB, 15.0	39.8	C-3, C-4, C-6, C-7, C-18	H-3, **H-5b**, H-7
CH-5b	3.12, AB, 15.0		C-3, C-4, C-6, C-7, C-18	**H-5a**, H-18
C-6	–	153.0	–	–
CH-7	5.80,s	110.1	C-6, C-8, C-19	H-1, H-5a, H-9, H-11, H-14
C-8	–	126.5	–	–
CH_2-9	2.30, m	24.7	C-7, C-8, C-19	H-7, **H-10a**, **H-10b**, H-11, H-19
CH-10a	2.06, m	29.9	C-9, C-11, C-12	**H-9**, **H-10b**, H-11, H-20
CH-10b	2.16, m		C-9, C-11, C-12	**H-9**, **H-10a**, H-11, H-20
CH-11	5.02, t, 7.5	126.4	C-9, C-10, C-13, C-20	H-7, H-9, H-10a, **H-10b**, H-13a, H-13b
C-12	–	136.7	–	–
CH-13a	1.86, m	37.1	C-1, C-11, C-12, C-14	H-11, **H-13b**, H-14, H-16a
CH-13b	1.96, m		C-1, C-11, C-12, C-14	**H-13a**, H-14, H-16a, H-20
CH_2-14	1.34, m	31.1	C-1, C-12, C-13, C-15, C-17	**H-1**, H-3, H-7, H-13a, H-13b, H-16a, H-17
C-15	–	148.3	–	–
CH-16a	4.66, bs	112.5	C-1, C-15, C-17	H-1, H-13a, H-13b, H-14, **H-16b**
CH-16b	4.75, bs		C-1, C-15, C-17	**H-16a**, **H-17**
CH_3-17	1.46, s	19.2	C-1, C-15, C-16	H-2a, H-2b, H-14, **H-16b**
CH_3-18	1.30, s	18.1	C-3, C-4, C-5	H-1, H-5b
CH-19	6.90, s	138.5	C-6, C-7, C-8	H-9
CH_3-20	1.37, s	17.6	C-11, C-12, C-13	H-10a, H-10b, H-13b

[a] Experiments optimized for values of J=5 and 10 Hz.
[b] most intense correlations are shown in bold.

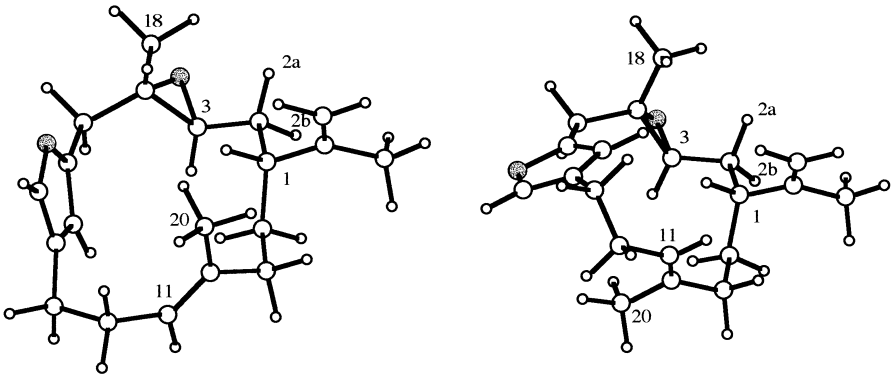

Figure 3. Low energy conformers of **2** as determined by NMR and MM3(92) methods.

conformer by MacMimic[9] were: ($J_{2a,1}$=2.75 Hz; $J_{2a,3}$= 12.5 Hz; $J_{2b,1}$=12.9 Hz; $J_{2b,3}$=4.9 Hz). Accordingly, we propose that compound **2** (isocrematofuran) is (1*R**,3*S**, 4*S**)-3,4:6,19-bisepoxycembra-6,8(19),11,15-tetraene. The MM3 calculations performed on this structure indicated the existence of several low energy conformers (two of them are represented in Fig. 3). This flexibility could be responsible for the observation that several H atoms are less diastereotopic than in crematofuran, or not diastereotopic at all (e. g. H_2-9, H_2-14).

The structures of the two compounds were also supported by mass spectral data. Indeed, whereas in the case of **1** the base peak appears at *m/z* 97 Daltons (C_7H_7O in HREIMS), probably corresponding to the fragment C-9–C-10–furan, it appears in **2** at *m/z* 94 Daltons (C_6H_6O in HREIMS), corresponding to the fragment C-5–furan–C-9, arising from cleavages between C-4 and C-7, and between the two allylic positions, C-9 and C-10.

A comparison of the methanolic extracts of the venom obtained by milking the ants and of dissected Dufour glands was also made by GC–MS. The results clearly showed that the two compounds originate from the Dufour gland of the ant. The presence of **1** and **2** as major component of the defensive secretion of *C. brevispinosa rochai* suggests that they must have repellent and/or toxic properties. The toxicity of **1** was tested by topical application on the ant *Myrmica sabuleti*, and the mortality was recorded after 2, 24, 48 and 72 h. The results reported in Table 3 show that crematofuran is a slow-acting toxin, the maximum toxicity being observed after 72 h. The LD_{50} is estimated to be 11 μg/ant, or 5 μg/ant mg, which is about the same order of magnitude as observed for nicotine.[10] In view of the insecticidal activities of alkylfurans reported recently,[11] the furan moiety could be responsible, at least in part, for the toxicity of crematofuran. It is worth mentioning, however, that the mechanism of action of this class of compounds is not yet understood.[11]

Conclusion

We have demonstrated for the first time the presence in the Dufour gland of an ant of furanocembranoid diterpenes which act as defensive compounds. To our knowledge, the presence of cembrane derivatives in ants has been reported only once, in *Monomorium pharaonis*, where cembrene-A (neocembrene) serves as queen-recognition pheromone.[12] In termites, however, cembrane and cyclized cembrane derivatives are much more common.[13] Thus, a priori, it can not be excluded that **1** and **2** are not produced by the ant itself, but are acquired from the sympatric nasute termite (*Nasutitermes* sp.) whose nests are sometimes colonized by *C. brevispinosa rochai.* To check this hypothesis, we have analysized the defensive secretion of soldiers of *Nasutitermes* sp., collected in the same biotope as *C. brevispinosa rochai.* This led only to the isolation and identification of the already known[14–17] trinervitane diterpenes, **3**–**6** (Fig. 4). A GC–MS analysis of the termite secretion also excluded the presence of **1** and **2**, or of any immediate precursor thereof. On the other hand, we have recently found other cembrane derivatives in the Dufour gland of another Brazilian *Crematogaster* ant.[18] All these results point to a de novo production of cembrane diterpenes by these ants, a conclusion that will be submitted to experimental testing in the near future.

Experimental

General

UV spectra were taken on a Philips PU 8700 uv-vis

Table 3. Percentage of dead workers upon topical application of defensive secretion or crematofuran (**1**), on *Myrmica sabuleti* (20 workers for each experiment)

Time after application	Hexane (blank)	Defensive secretion[a]	3.75 μg of **1**	7.50 μg of **1**	15 μg of **1**	30 μg of **1**
2 h	0	0	0	0	0	0
24 h	2	10	0	15	5	35
48 h	3	90	10	40	30	90
72 h	4	90	10	45	55	100

[a] Amount secreted by one *C. brevispinosa rochai* worker.

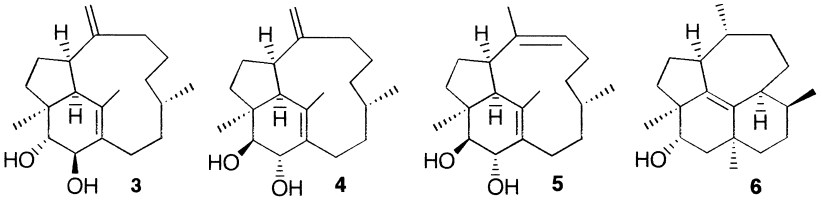

Figure 4. Trinervitane diterpenes isolated from soldiers of the *Nasutitermes* sp. sympatric with *C. brevispinosa rochai*.

spectrophotometer in CH_3OH. IR spectra were recorded on a Bruker IFS 25 instrument as a film on a NaCl disk. EIMS, HREIMS and FABMS measurements were performed on a Fisons VG Autospec. The NMR spectra were recorded in CD_3OD at 600 and 150.87 MHz (Varian Unity 600 instrument). The chemical shifts (δ) are reported in ppm from the solvent, and the coupling constants are given in Hz. The optical rotations were measured on a Perkin Elmer 141 polarimeter (Na-vapor lamp) in a 10 cm cell at room temperature. Thin layer chromatography analyses (TLC) were performed on Polygram SilG/UV_{254} precoated plates (0.25 mm). The compounds were visualized under UV_{254} light and/or by spraying with a 2% ethanolic solution of phosphomolybdic acid, followed by a 3% ceric sulphate solution in 2N H_2SO_4, and heated at 120°C for 5 min. The GC–MS analyses were performed on a Fisons VG Autospec mass spectrometer coupled to a Fisons GC 8065 gas chromatograph equipped with a split injector, and with a 25 m×0.25 mm OV1 fused silica column (Rescom). The conditions were: 1 min at 150°C, then increased 10°C/min to 200°C, then 7°C/min to 300°C. Carrier gas was helium.

Isolation of 1 from the Dufour gland secretion

Nests of arboreal *C. brevispinosa rochai* were collected at Paraipaba and at Pentecoste, in 'caatinga' areas (savanna-like formations) of the Ceará state (Brazil). This species was identified by one of us (J.-C. de B.) by direct comparison with the holotype. Voucher specimens are deposited in the collection of the Musée de Zoologie (ULB). *C. brevispinosa rochai* ants were maintained at a constant temperature of 23°C and were fed with freshly killed cockroaches and a solution of brown sugar. 400 Workers of *C. brevispinosa rochai* were seized by tweezers and the defensive secretion that appears at the tip of the sting was collected on small bits of filter paper and stored in MeOH at −30°C. Secretions were successively extracted with MeOH, acetone and hexane, affording 13.64 mg of a yellow oil. Flash chromatography of the extract on silica gel (eluent: hexane-acetone 98:2) gave 5.0 mg of **1** as a colourless oil.

Table 4. Torsional constants, where 1=C(sp^3), 2=C(sp^2), 49=O(epoxide), 22=C(epoxide), 41=O(furan)

Angle	V1, kcal/mol	V2, kcal/mol	V3, kcal/mol
2-1-2-41	0.0	0.0	0.0
2-1-1-22	0.225	0.41	1.15
22-1-2-41	0.0	0.0	0.0
2-1-22-1	0.225	0.41	1.15
2-1-22-49	0.0	0.0	0.18

Isolation of 1 and 2 from whole ants

1,000 workers of *C. brevispinosa rochai* were successively extracted with MeOH, acetone and hexane to give 239 mg of crude material. The latter was submitted to a flash chromatography on silica gel (eluent: hexane-ether 98:2, 96:4, 90:10) affording 52.1 mg of **1** as a colorless oil and 34.0 mg of **2** as a white solid.

Biological testing

Toxicity tests were performed on *Myrmica sabuleti* workers by topically applying either the defensive secretion of one *C. brevispinosa rochai* worker or 0.1 μl of hexane solutions of varying concentrations (see Table 3) of compound **1**. Each experiment was performed on 20 *M. sabuleti* workers, and the number of dead workers counted after 2, 24, 48 and 72 h. Pure hexane (0.1 μl) was used as a blank.

Computational methods

The calculations of conformational energies and energy-minimized geometries was performed with the MM3(92) molecular mechanics program.[8] In addition to the standard force field parameters of MM3(92), the parameters in Table 4 were used to make it possible to include epoxides in the calculations. The missing torsional parameters were copied from similar MM3 parameters. The geometries of the calculated low energy conformers and the relative energies between these conformers were found not to be critically sensitive to the magnitudes of the estimated torsional parameters. Martin Saunder's stochastic conformational search program, included in MM3(92), was used to find the low energy conformers of $1R^*,11R^*,12R^*$ and $1R^*,11S^*,12S^*$ crematofuran as well as those of $1R^*,3R^*,4R^*$ and $1R^*,3S^*,4S^*$ isocrematofuran. The construction of input structures for the MM3 program and the calculation of coupling constants and nOe correlations was performed with the molecular modeling program MacMimic.[9]

Crematofuran (**1**). oil; $[\alpha]_D^{20}=-14.5$ (c=0.48, hexane); UV (hexane): λ_{max} 215 nm (ϵ 940); IR (NaCl, film): 3056, 2926, 3857, 1643, 1540, 1457–1420, 1380, 1350, 1117, 1143, 888 cm^{-1}; MS: m/z 300 (M^+, $C_{20}H_{28}O_2$, 67), 285 ($C_{19}H_{25}O_2$, 4), 282 ($C_{20}H_{26}O$, 8), 257 (6), 217 ($C_{14}H_{17}O_2$, 17), 191 ($C_{12}H_{15}O_2$, 33), 164 ($C_{10}H_{12}O_2$, 25), 148 ($C_{10}H_{12}O$, 32), 119 (C_9H_{11}, 32), 109 (C_7H_9O, 30), 107 (C_7H_7O, 100), 91 (C_7H_7, 54); ^1H and ^{13}C NMR: Table 1.

Isocrematofuran (**2**). white solid; $[\alpha]_D^{20}=+41.3$ (c=0.46, hexane); UV (hexane): λ_{max} 215 nm (ϵ 5110); IR (NaCl, film): 3078, 2999–2860, 1743, 1645, 1616, 1549, 1456, 1387, 1243, 1119, 893 cm^{-1}; MS: m/z 300 (M^+,

$C_{20}H_{28}O_2$, 13), 285 ($C_{19}H_{25}O_2$, 4), 257 (5), 175 ($C_{12}H_{15}O$, 16), 161 ($C_{11}H_{13}O$, 11), 149 ($C_{10}H_{13}O$, 20), 133 (8), 122 ($C_8H_{10}O$, 17), 107 (C_7H_7O, 19), 94 (C_6H_6O, 100); ^1H and ^{13}C NMR: Table 2.

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