Pergamon

Tetrahedron Letters 41 (2000) 633–637

TETRAHEDRON LETTERS

Five new furanocembrenoids from the venom of the ant *Crematogaster brevispinosa ampla* from Brazil

Sabine Leclercq,[a] Jean Christophe de Biseau,[b] Désiré Daloze,[a,*] Jean-Claude Braekman,[a,*] Yves Quinet [c] and Jacques M. Pasteels [b]

[a]*Laboratory of Bio-organic Chemistry CP 160/07, Department of Organic Chemistry, University of Brussels, Av. F. D. Roosevelt, 50-1050 Brussels, Belgium*

[b]*Laboratory of Animal and Cellular Biology CP 160/12, University of Brussels, Av. F. D. Roosevelt, 50-1050 Brussels, Belgium*

[c]*Departamento de Biologia, Universidade Estadual do Ceará, Av. Paranjana 1700, Campus do Itaperi, 60.740-000 Fortaleza-CE Brazil*

Received 29 October 1999; accepted 10 November 1999

Abstract

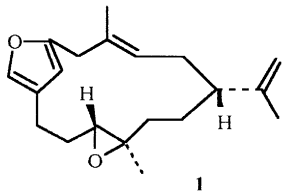
Five new furanocembrenoids (**3**–**7**) were isolated from the Dufour gland secretion of the Brazilian ant *Crematogaster brevispinosa ampla*. The structure of the major component was established by a detailed high-field 1D and 2D NMR study as the dibutanoate **3**. Compounds **4** and **5** were shown to be the two isomeric monoacetate monobutanoates corresponding to **3**, whereas **6** and **7** are the two isomeric monohydroxy monobutanoates.

Keywords: insects; NMR; terpenoids; natural products.

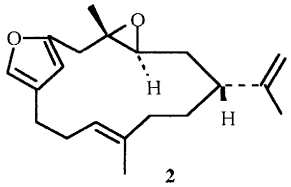
Crematogaster ants are characterized by a peculiar defense strategy. Instead of injecting their venom as most primitive ant species do, they use their spatulate sting to apply their venom topically to the integument of attacking insects. In European[1–3] and New Guinean[4] species, the Dufour gland secretion contains complex mixtures of polyfunctionalized long chain derivatives bearing a (*E,E*)-cross-conjugated dienone linked to a primary acetate function. 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. Recently, two new furanocembrenoid diterpenes, crematofuran (**1**) and isocrematofuran (**2**), have been isolated from the Dufour gland secretion of the Brazilian ant *Crematogaster brevispinosa rochai*.[5] This was the first report of cembrane diterpenes as defensive compounds in ants. In this paper, we report on the results of an investigation of another closely related Brazilian *Crematogaster* taxon, *Crematogaster brevispinosa ampla* Forel[6], leading to the isolation and structure determination of five new furanocembrenoids (**3**–**7**).

* Corresponding author. Fax: 32.2.650.27.98; e-mail: ddaloze@ulb.ac.be and braekman@ulb.ac.be

0040-4039/00/$ - see front matter
PII: S0040-4039(99)02127-9

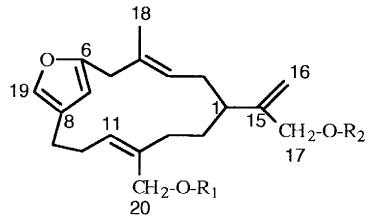


1



2

For the present study, the venom of 200 workers of *C. brevispinosa ampla* (collected at Serra de Baturite, Ceará) was obtained in the usual manner[1] and stored in MeOH. A GC–MS analysis (25 m CP-Sil 5 CB capillary column, programmed from 150°C to 200°C at 5°C/min, then to 300°C at 7°C/min) showed the presence of five peaks displaying molecular ions at *m/z* 386, 386, 428, 428, and 456 Da, the last being the major one. Successive extraction of this material with MeOH, CH_2Cl_2 and hexane gave 19.0 mg of an extract which was submitted to a flash chromatography on silica gel (hexane:acetone, 98:2) affording, in order of increasing polarity, 3.22 mg of **3**, 0.8 mg of a 1:1 mixture of **4** and **5**, and 2.7 mg of a 4:6 mixture of **6** and **7**. A TLC and GC–MS comparison of the methanolic extracts of the venom, obtained by milking the ants, and of dissected Dufour glands showed that compounds **3**–**7** originate from the Dufour gland of the ant.



3: R_1=R_2=CO-*n*-C_3H_7
4: R_1= CO-*n*-C_3H_7, R_2= $COCH_3$
5: R_1=$COCH_3$, R_2= CO-*n*-C_3H_7
6: R_1=CO-*n*-C_3H_7, R_2=H
7: R_1=H, R_2=CO-*n*-C_3H_7

HR-EIMS of **3** displayed an $M^{+\cdot}$ at *m/z* 456.2924 (calcd for $C_{28}H_{40}O_5$: 456.2876) indicating the presence in the molecule of nine degrees of unsaturation and five oxygen atoms. The structure of **3** (without absolute configuration) was solved by a combination of 1D and 2D NMR methods (^1H, ^{13}C, ^1H–^1H COSY, HMQC, HMBC, NOESY) (Table 1). The ^{13}C NMR spectrum, as well as HMQC and HMBC experiments in C_6D_6 allowed us to detect 28 carbon atoms, which included two ester carbonyls, four olefinic methines, five olefinic quaternary carbons, one sp^3 methine, thirteen methylenes [of which two were oxygenated (δ_C 65.9, 62.9) and one was an exomethylene (δ_C 112.7, δ_H 5.0 and 5.15)], and three methyls, one of which was vinylic (δ_H 1.52). Two carbonyl groups and five double bonds accounted for the presence of seven degrees of unsaturation, and thus, on the basis of its molecular formula, **3** must be bicyclic. The ^1H NMR spectrum of **3** (Table 1) exhibited two singlets at δ_H 6.87 and 5.77, attributable to a furan ring, which was further confirmed by the UV spectrum (λ_{max} 217 nm, ε 4100, hexane), and by comparison with the ^1H NMR spectra of **1** and **2**.[5] In the HMBC experiments at 5 and 10 Hz, the cross peaks observed between H_2-5 and C-6, and between H_2-9 and C-8 allowed us to connect H_2C-5 to C-6 and H_2C-9 to C-8 of the furan ring, respectively. These results, together with the lack of coupling between the two hydrogen atoms of the furan and the δ_C values of C-6, C-7, C-8, and C-19, demonstrated that the furan ring is 2,4-disubstituted. The connectivity between C-9/C-10/C-11 and C-13/C-14/C-1/C-2/C-3 was afforded by the ^1H–^1H COSY spectrum. These partial structures could be connected on the basis of HMBC experiments. In particular, the protons H_2-13 at δ_H 1.87 and 2.26 and H_2-10 at δ_H 2.15 gave cross-peaks with the quaternary carbon at δ_C 137.5, allowing us to assign the latter to C-12. The connectivity between partial structures C-6/C-5 and C-3/C-2/C-1/C-14/C-13 could also be deduced from HMBC experiments, leading to a 14-membered carbocycle. The exomethylene H_2-16 gave cross peaks with the carbon atoms C-15, C-1 and C-17. These data allowed us to connect C-15 to C-16 through a double bond, as well as to HC-1 and H_2C-17. The two superimposed AB spin systems, H_2C-17 (δ_H

Table 1
NMR data of **3**, **4**, **5**, **6** and **7** (600 and 150.85 MHz, C_6D_6, δ, J in Hz)

	3					**4, 5**[d]		**6, 7**[d]	
Position	δ_C	δ_H	HMBC (H to C)[a]	COSY (H to H)	NOESY	δ_C[e]	δ_H	δ_C[e]	δ_H
1	45.9	2.02, 1H, tt, 11.2; 3.0	C-2	H-2a, H-14	H-17, H-14	45.5	2.01, m	44.5 (45.0)	2.03 (2.12), tt, 11.4
2a	34.1	2.10, 1H, m	C-1, C-3, C-4, C-14	H-3, H-1	H-1, H-2b, H-3, H-18	34.0	2.08, m	34.0	2.05, m
2b		1.90, 1H, m	C-1, C-3, C-4, C-14	H-1, H-2a, H-3	H-1, H-2a, H-3, H-18		1.89, m		1.84, m
3	126.2	4.97, 2H, m	C-1, C-2, C-5, C-18	H-2a-b, H-18	H-2a, H-5	126.0	5.00, t, 7.8	126.0	5.04, m
4	136.8	-	-	-	-	137.0	- 137.0	-	
5	38.7	3.07&3.15, 2H, AB, 15.0	C-3, C-4, C-6, C-18	H-7, H-18	H-3, H-18	38.5	3.07 & 3.15,AB,9.0	39.0	3.08 & 3.16,AB,14.4
6	156.6	-	-	-	-	156.0	-	157.0	-
7	110.5	5.77, 1H, s	C-6, C-8, C-19	H-5, H-19	H-3, H-5, H-9, H-11	110.0	5.77 (5.76), s	110.0	5.80 (5.79), s
8	126.0	-	-	-	-	126.0	-	126.0	-
9	24.7	2.26, 2H, m	C-8, C-10, C-11, C-12	H-10, H-11	H-7, H-11	24.0	2.28, t, 7.2	24.5	2.27, t, 6.0
10	29.6	2.15, 2H, t, 6.3	C-8, C-9, C-11, C-12	H-9, H-11	H-9, H-11, H-20	29.5	2.16, m	29.0	2.15 (2.05), m
11	131.2	5.19, 1H, t, 7.2	C-9, C-10, C-13, C-20	H-9, H-10	H-7, H-10, H-13a-b	131.0	5.19, t, 7.2	131.5 (129.0)	5.20 (5.08), t, 7.2
12	137.5	-	-	-	-	138.0	-	138.0	-
13a	33.7	1.87, 1H, m	C-1, C-11, C-12, C-14, C-20	H-10, H-13b, H-14	H-3, H-11, H-13b, H-20	33.5	1.85, m	33.5	1.90, m
13b		2.26, 1H, m	C-1, C-11, C-12, C-14, C-20	H-10, H-13a, H-14	H-11, H-13a, H-14		2.22, bt, 7.2		2.25, m
14	31.9	1.46, 2H, m	C-1, C-12, C-13, C-15	H-1, H-13a-b	H-1, H-3, H-11, H-13a-b	31.5	1.45, m	32.0	1.46, m
15	147.9	-	-	-	-	148.0	-	153.0	-
16a	112.7	5.15, 1H, bd, 1.2	C-1, C-15, C-17	H-16b, H-17	H-14, H-16b, H-17	112.0	5.10 (5.12),d,1.2	109.0 (112.0)	5.14, s
16b		5.00, 1H, bs	C-1, C-15, C-17	H-16a, H-17	H-2a-b, H-14, H-16a		4.97, s		4.92 (5.00), s
17	65.9	4.62 & 4.64, 2H, AB,13.8	C-1, C-15, C-16, C-1'	H-16a-b	H-2a, H-16a	65.5	4.62&4.64,AB,13.8	65.0[f](66.0)[g]	3.92[f] (4.64)[g], s
18	17.8	1.52, 3H, s	C-3, C-4, C-5, C-6	H-2a-b, H-3, H-5	H-2a-b, H-5	17.5	1.53, s	17.0	1.55, s
19	138.0	6.87, 1H, s	C-6, C-8	H-7, H-9	H-9	138.0	6.87 (6.88), s	138.0	6.88, s
20	62.9	4.55 & 4.63, 2H, AB,12.6	C-11, C-12, C-13, C-1"	H-11	H-10, H-13a-b	62.5	4.50&4.57,AB,12.6	63.0[f]	4.59&4.62[f],AB,12.0
							(4.53&4.60,AB,12.6)	(61.0)[g]	(3.87[g], AB, 12.0)
1'	173.6[b]	-	-	-	-	173.0	-	174.0	-
2'	36.77	2.10, 2H, t, 7.4	C-1', C-3', C-4'	H-3', H-4'	H-17, H-3', H-4'	36.0	2.10 (2.13), t, 7.2	36.5	2.06 (2.11), t, 6.7
3'	19.4[c]	1.56, quint, 7.4	C-1', C-2', C-4'	H-2', H-4'	H-2', H-4'	19.0	1.55, sext, 7.2	19.0	1.52, m
4'	14.45	0.78, 3H, t, 7.4	C-2', C-3'	H-2', H-3'	H-3'	14.0	0.785 (0.78), t, 7.2	14.0	0.78 (0.76), t, 7.2
1"	173.2[b]	-	-	-	-	171.0	-		
2"	36.8	2.12, 2H, t, 7.4	C-1", C-3", C-4"	H-3', H-4'	H-20, H-3", H-4"	20.5	1.69 (1.70), 3H, s		
3"	19.36[c]	1.56, quint, 7.4	C-1", C-2", C-4"	H-2', H-4'	H-2", H-4"				
4"	14.45	0.78, 3H, t, 7.4	C-2", C-3"	H-2', H-3'	H-3"				

[a] Optimized for $^nJ_{CH}$ = 5 and 10 Hz
[b,c] Assignments may be interchanged
[d] Most of the signals are identical for the two compounds, except in a few cases indicated in brackets
[e] Assignments by HMQC and/or HMBC
[f] These signals correspond to **6**
[g] These signals correspond to **7**

4.62 and 4.64, *J*=13.8 Hz) and H_2C-20 (δ_H 4.55 and 4.63, *J*=12.6 Hz), were deduced from their ^1H and ^{13}C chemical shifts to be vicinal to an oxygen atom. These methylene groups gave HMBC cross peaks, respectively, with the carbonyls at δ_C 173.6 and 173.2, both of which belonged to a butanoic acid moiety, as shown by ^1H–^1H COSY and HMBC. The presence of two butanoate ester functions in **3** was confirmed by diagnostic fragment ions in MS at *m/z* 368 and *m/z* 280, corresponding, respectively, to the loss of one and two butanoic acid moieties from the molecular ion at *m/z* 456. Finally, the configuration of the Δ^3 and Δ^{11} carbon–carbon double bonds was assigned as *E*, on the basis of the NOESY spectrum (see Table 1). It follows from all these data that **3** is (3*E*,11*E*)-6,19-epoxy-17,20-dihydroxycembra-3,6,8(19),11,15-pentaene 17,20-dibutanoate.

A GC–MS analysis of the mixture of compounds **4** and **5** (0.8 mg) showed that they possess the same molecular weight of 428 Da and HR-EIMS established their molecular formula as $C_{26}H_{36}O_5$. The ^1H and ^{13}C NMR spectra revealed that these two compounds are two isomeric furanocembrenoids closely related to compound **3**, in a ratio of approximately 1:1. The only differences with **3** were the presence of a 3H singlet at δ_H 1.7 attributable to an acetate methyl, and of only one triplet at δ_H 0.80 corresponding to the methyl group of a butanoate moiety. These hypotheses were confirmed in HR-EIMS by the presence of fragment ions at *m/z* 368, 340 and 280 Da, corresponding, respectively, to the loss of ethanoic acid, butanoic acid and both. Therefore, we propose that **4** [(3*E*,11*E*)-6,19-epoxy-17,20-dihydroxycembra-3,6,8(19),11,15-pentaene 17-acetate, 20-butanoate] and **5** [(3*E*,11*E*)-6,19-epoxy-17,20-dihydroxycembra-3,6,8(19),11,15-pentaene 17-butanoate, 20-acetate] are the two isomeric monoacetate monobutanoates corresponding to **3**.

Compounds **6** and **7** were also unseparable by flash chromatography on silica gel and were characterized as a mixture. They both had a molecular formula of $C_{24}H_{34}O_4$ ($M^{+\cdot}$ at *m/z* 386.2454 by HR-EIMS; calcd: 386.2457) and thus possess four carbon atoms less than **3**. The ^1H and ^{13}C NMR spectra of the mixture were very similar to those of **3**. However, in the ^1H NMR of **6**, the H_2C-17 appeared at δ_H 3.92 instead of 4.62 and 4.64 in **3**, whereas the signals corresponding to the C-17 butanoic acid moiety were absent. Moreover, HMQC correlations were observed between the signal at δ_H 3.92 and the carbon at δ_C 65.0 confirming that, in **6**, H_2C-17 bears an hydroxyl group. In a similar way, the ^1H and ^{13}C NMR spectra of **7** exibited a singlet at δ_H 3.87 (δ_C 61.0) assigned to H_2C-20 (instead of δ_H 4.55 and 4.63 in **3**), which was thus also vicinal to an hydroxyl group. The presence of hydroxyl and ester functionalities in compounds **6** and **7** was also evident from their IR spectrum, which displayed intense absorption bands at 3577 and 1740 cm^{-1}. Thus, compounds **6** and **7** were, respectively, assigned the structures of (3*E*,11*E*)-6,19-epoxy-17,20-dihydroxycembra-3,6,8(19),11,15-pentaene 20-butanoate and (3*E*,11*E*)-6,19-epoxy-17,20-dihydroxycembra-3,6,8(19),11,15-pentaene 17-butanoate.

This is the second report of furanocembrenoids in the defensive secretion of an ant,[5] confirming that the defensive chemistry of the genus *Crematogaster* is more diversified than had been assumed. Cembrenoids, however, have been found in only two *C. brevispinosa* subspecies, confirming that Dufour defensive compounds constitute a useful chemotaxonomic marker. On the other hand, the allylic ester functions of compounds **3**–**7** recall the allylic acetate of the long chain derivatives present in other species of this genus.[1–4] It was, therefore, interesting to check if *C. brevispinosa ampla* possessed the esterase and oxidase required to transform the ester functions of the furanocembrenes into conjugated and toxic aldehydes as is the case for European *Crematogaster* species.[2,3] A GC–MS analysis of the defensive secretion of *C. brevispinosa ampla*, exposed to air for 30 min before being dipped in MeOH, showed no trace of aldehydes, thus excluding any enzymatic transformation. The toxicity of these new derivatives will be investigated in the near future.

Acknowledgements

This work was supported by grants from the Belgian Fund for Basic Research (Grant 2.4513.90-96) and the French Community of Belgium (ARC 93/98-137). One of us (S.L.) thanks the National Fund for Scientific Research for their financial support. We thank Mr. C. Moulard for the HR-EIMS and Dr. M. Luhmer for the NMR spectra. We also thank Prof. José Jackson Coelho Sampaio, Prof. Crisanto Medeiros de Lima Ferreira and Luiz Gonzaga Sales Jr. for technical support.

References

1. Daloze, D.; Braekman, J. C.; Vanhecke, P.; Boeve, J. L.; Pasteels, J. M. *Can. J. Chem*. **1987**, *65*, 432–436.
2. Pasteels, J. M.; Daloze, D.; Boeve, J. L. *J. Chem. Ecol*. **1989**, *15*, 1501–1511.
3. Daloze, D.; Kaisin, M.; Detrain, C.; Pasteels, J. M. *Experientia* **1991**, *47*, 1082–1089.
4. Leclercq, S.; Braekman, J. C.; Kaisin, M.; Daloze, D.; Detrain, C.; de Biseau, J. C.; Pasteels, J. M. *J. Nat. Prod*. **1997**, *60*, 1143–1147.
5. Leclercq, S.; de Biseau, J. C.; Braekman, J. C.; Daloze, D.; Quinet, Y.; Luhmer, M.; Sundin, A.; Pasteels, J. M. *Tetrahedron* submitted.
6. The species have been identified by comparison with Forel's types. Voucher specimens are in the collection of the 'Musée de Zoologie' of the University of Brussels (Ref. no. Br 18)