New Acetogenins from the Seeds of *Annona coriacea*

by **Thayana da C. Alves**[a]), **Mariele R. S. Gonçalves**[a]), **Francyne C. S. Correia**[a]), **Virgínia C. da Silva**[a]), **Paulo T. de Sousa Jr.**[a]), **Mário G. de Carvalho**[b]), **Raimundo Braz-Filho**[b]), and **Evandro L. Dall'Oglio***[a])

[a]) Universidade Federal de Mato Grosso, Departamento de Química, 78060-900, Cuiabá, Mato Grosso, Brazil (e-mail: dalloglio.evandro@gmail.com)

[b]) Universidade Federal Rural do Rio de Janeiro, Departamento de Química, 23800-000, Seropédica, Rio de Janeiro, Brazil

From the hexane and MeOH extracts of *Annona coriacea* MART. (Annonaceae) seeds, two novel acetogenins, coriapentocins A and B (**1** and **2**, resp.) were isolated. The known acetogenin bullacin (**3**) was also isolated from the hexane extract. The structures of compounds **1**–**3** were elucidated by NMR and MS analysis, and relative configurations were established by comparison with literature data.

Introduction. – The family Annonaceae consists of *ca.* 135 genera and 2500 species [1]. In Brazil, 26 genera are recorded, comprising *ca.* 260 species [2]. The acetogenins, which are a series of secondary metabolites isolated exclusively from Annonaceae species, have been identified in various plants parts, mainly in the leaves, stems, and seeds, in only eleven genera [3]. These natural products exhibit a range of important biological features, such as antitumor, pesticidal, antimicrobial, cytotoxic, antiparasitic, vermicidal, immunosuppressive, antihelminthic, abortive, antiprotozoal, appetite-inhibiting, antimalarial, and antiemetic activities [1–3]. Herein, we report the structure elucidation of two novel isomeric mono-THF acetogenins, mainly by using ESI-LC/MS and HR-ESI-MS techniques. The compounds coriapentocin A (**1**) and its isomer coriapentocin B (**2**) were identified in the hexane and MeOH extracts, respectively, from the seeds of *A. coriacea*. The known acetogenin bullacin (**3**) was also identified as a major component of the former extract (*Fig. 1*).

Results and Discussion. – In the fractionation of the hexane extract of the seeds of *A. coriacea*, the MeOH fraction provided two acetogenins which were confirmed by the peaks observed by ESI-LC/MS corresponding to the mono-THF acetogenin coriapentocin A (**1**) and the known 'adjacent' bis-THF acetogenin bullacin (**3**). Compound **3** provided peaks of *quasi*-molecular ions ($[M+Na]^+$ and $[M+H]^+$), at *m*/*z* 645.3 and 623.3, respectively. HR-ESI-MS Experiments afforded a *quasi*-molecular ion ($[M+H]^+$) peak at *m*/*z* 623.4894, compatible with the calculated value for $C_{37}H_{67}O_7^+$ (calc. 623.4887). These results, along with 1D- and 2D-NMR experiments, and comparison with literature data, confirmed the structure of **3** [4]. ESI-MS Experiments for compound **1** afforded peaks of *quasi*-molecular ions at *m*/*z* 635.1 and 613.1, corresponding to the $[M+Na]^+$ and $[M+H]^+$ adducts, respectively. HR-ESI-MS Experiments led to a peak at *m*/*z* 613.4696 corresponding to $[M+H]^+$ ion from **1**, compatible with the calculated value for $C_{35}H_{65}O_8^+$ (calc. 613.4679).

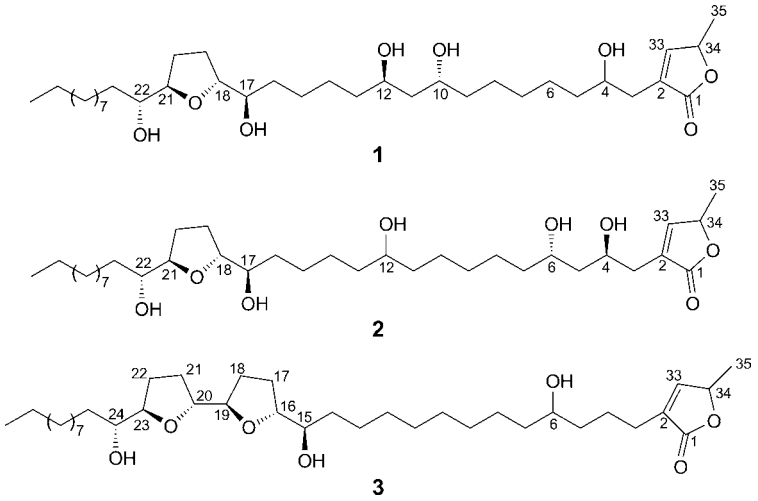

Fig. 1. *Acetogenis from the seeds of A. coriacea.*

The analysis of the MeOH extract by ESI-LC/MS afforded another mono-THF acetogenin, named coriapentocin B (**2**) of which a major peak at *m/z* 613.1, corresponding to $[M+H]^+$, as well as the peak of the Na-adduct ($[M+Na]^+$) at *m/z* 635.1 were detected. The HR-ESI Mass spectrum displayed an $[M+H]^+$ peak at *m/z* 613.4283, matching with the calculated value for $C_{35}H_{65}O_8^+$ (calc. 613.4679), compatible with coriapentocin B (**2**).

The ^{1}H- and ^{13}C-NMR data of **1** and **2** were very similar (*Tables 1* and *2*) as well as the positive tests when submitted to *Kedde* reagent [5]. The structural differences between **1** and **2** were detected by the ESI-MS/MS analysis in positioning of the 1,3-diol moietiy.

Substance **1** showed characteristic IR absorption bands at 3401 (OH), 2924 and 2846 (Me, CH_2), 1751 (C=O), 1656 (α,β-unsaturated C=C), 1460 and 1375 (CH_2 and Me), and 1073 (C–O) cm^{-1}.

The ^{1}H-NMR spectrum of **1** (*Table 1*) exhibited signals at δ(H) 7.38 (*s*) and 5.11–5.14 (*m*) assigned by HSQC to C-atom signals at δ(C) 153.0 (C(33)) and 78.4 (C(34)) of the γ-lactone moiety, respectively. In the ^{13}C-DEPT-Q spectrum, signals of the other corresponding vinylic C-atom at 130.1 (C(2)), the C=O group at 175.1 (C(1)), and the signal in a higher field at δ(C) 17.8 corresponding to the Me(35) were observed. These signals confirmed the presence of an α,β-unsaturated-γ-lactone moiety in **1**. The COSY spectrum evidenced the connection of the Me(35) group (δ(H) 1.42 (*d*, $J=6.8$)) to H–C(34) (δ(H) 5.11–5.14 (*m*)). The HMBCs (*Fig. 2*) between the signals of CH_2(3) group and that at δ(C) 175.1 (C(1)) confirmed the alkyl vicinity and the connection to the C-atom resonating at δ(C) 130.1 (C(2)). HMBCs were also observed for the signals of CH_2(3) group with those of the C-atoms resonating at δ(C) 69.0 (C(4)) and 36.9 (C(5)), which evidenced the presence of the HO–C(4) group (*Fig. 2, a*).

According to the HMBCs, the cross-peaks evidenced the positions of the O-bearing C-atoms by correlations between the *multiplet* at δ(H) 2.00–2.21 with the super-

Table 1. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp., in CD_3OD) *Data of Coriapentocin A* (**1**). Atom numbering as indicated in *Fig. 1.*

Position	**1**	
	δ(H)	δ(C)
1	–	175.1
2	–	130.1
3	2.26 (*dd*, J = 14.5, 8.0, H_α), 2.44 (br. *d*, J = 14.5, H_β)	32.6
4	3.83 – 3.85 (*m*)[a])	69.0
5	1.32 – 1.37 (*m*)[a])	36.9
6 – 9	1.32 – 1.37 (*m*)[a])	22.4 – 37.0
10	3.52 – 3.63 (*m*)[a])	80.0
11	1.51 – 1.55 (*m*)	40.5
12	3.37 – 3.71 (*m*)[a])	70.6
13 – 16	1.32 – 1.37 (*m*)[a])	22.4 – 37.0
17	3.43 – 3.45 (*m*)[a])	73.9
18	3.83 – 3.85 (*m*)[a])	83.0
19 – 20	2.00 – 2.21 (*m*)	28.2 – 28.3
21	3.83 – 3.85 (*m*)[a])	82.6
22	3.43 – 3.45 (*m*)[a])	72.6
23 – 31	1.32 – 1.37 (*m*)[a])	22.4 – 37.0
32	0.93 (*t*, J = 6.5)	13.1
33	7.38 (*s*)	153.0
34	5.11 – 5.14 (*m*)	78.4
35	1.42 (*d*, J = 6.8)	17.8

[a]) Signals overlaped.

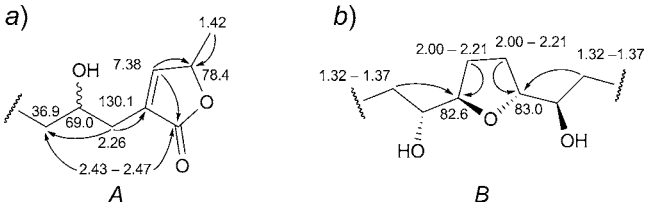

Fig. 2. *HMBCs* (H → C) *of the α,β-unsaturated γ-lactone moiety* (*A*) *and the mono-THF α,α′-dihydroxylated system* (*B*) *of* **1**

imposed signals at δ(C) 28.2 – 28.3 (C(19), C(20)) and the long-range couplings CH_2(23)/C(21), CH_2(20)/C(21), CH_2(16)/C(18), and CH_2(19)/C(18), confirming a α,α'-dihydroxylated mono-THF system connecting two aliphatic chains (*Fig. 2, b*). A comparative analysis of ^{1}H- and ^{13}C-NMR data with those of natural models allowed us to propose a relative configuration to the mono-THF ring as *threo*/*trans*/*threo* [5][6].

The 1,3-diol moiety in compound **1** was evidenced by HMBCs, showing long-range couplings from the signal of H–C(10) (δ(H) 3.52 – 3.63) to that of C(12), and additional analysis of COSY with the coupling signals of H–C(10) to that of H–C(12) (δ(H) 3.37 – 3.71) and the *multiplet* of H–C(11) (δ(H) 1.51 – 1.55) (*Table 1*). For the acetogenin **2**, the positioning of the 1,3-diol group was deduced from HMBC spectra

with the correlations H–C(6) (δ(H) 3.67–3.69)/C(6)/C(5) and the couplings detected in the COSY spectrum between the signals of H–C(6) (δ(H) 3.67–3.69) with those of H–C(4)/H–C(5). The HMBC from H–C(10)/H–C(11) (δ(H) 1.36–1.41) to C(12) (δ(C) 70.5) confirmed the absence of the OH group at C(10) (*Table 2*).

The ESI-MS/MS analysis assisted in locating the mono-THF system and lateral side chain on the basis the fragment-ion peaks at *m*/*z* 371.0, 299.1, 271.1, 255.4, 245.0, 241.3, 171.0, and 199.2, 141.0 for α,β-unsaturated-γ-lactone moiety (*Fig. 3*).

The ESI-MS/MS analysis also allowed positioning of the 1,3-diol group in **1** and **2**. In compound **1**, the OH groups were at C(12) and C(10) evidenced by the fragment-ion peaks at *m*/*z* 255.4 and 237.2 (loss of H_2O), 241.3 (C(10)/C(11) bond cleavage), 199.2 (C(8)/C(9) bond cleavage), and 141.0 (C(4)/C(5) bond cleavage), confirming the presence of only one OH group at C(4) (*Fig. 3*).

In compound **2**, the OH groups were at C(6) and C(4) as indicated by fragment-ion peaks at *m*/*z* 199.3 (C(7)/C(8) bond cleavage), as well as 181.7 and 163.0 (loss of H_2O), respectively. Further evidences were provided by the peaks at *m*/*z* 227.2 (C(9)/C(10) bond cleavage) and 185.2 (C(6)/C(7) bond cleavage), establishing the presence of OH groups at C(4) and C(6) (*Fig. 4*).

A comparative analysis of ^{1}H- and ^{13}C-NMR data of compound **1** and **2** with those of models reported in the literature suggest a *pseudo-erythro* configuration for 1,3-diol group in both structures [7][8]. Compound **3** was previously isolated from the stem bark of *A. bullata* [9] and *A. squamosa* [4]; however, this is the first report of its

Table 2. *^{1}H- and ^{13}C-NMR* (500 and 125 MHz, resp., in CD_3OD) *Data of Coriapentocin B* (**2**). Atom numbering as indicated in *Fig. 1*.

Position	**2**	
	δ(H)	δ(C)
1	–	175.1
2	–	130.1
3	2.36–2.39 (*m*), 2.46–2.47 (*m*)	33.0
4	3.84–3.86 (*m*)[a])	69.0
5	1.55–1.61 (*m*)	40.4
6	3.67–3.69 (*m*)	70.3
7–11	1.36–1.41 (*m*)[a])	22.4–33.0
12	3.57–3.60 (*m*)	70.5
13–16	1.36–1.41 (*m*)[a])	22.4–33.1
17	3.42–3.44 (*m*)[a])	73.8
18	3.81–3.87 (*m*)[a])	82.9
19–20	2.00–2.01 (*m*)[a])	28.3–28.2
21	3.81–3.87 (*m*)[a])	82.6
22	3.42–3.44 (*m*)[a])	73.8
23–31	1.36–1.41 (*m*)	22.4–33.1
32	0.92 (*t*, *J* = 6.5)	13.1
33	7.38 (*s*)	153.0
34	5.12 (*q*, *J* = 13.0)	78.4
35	1.42 (*d*, *J* = 6.5)	17.8

[a]) Signals overlaped.

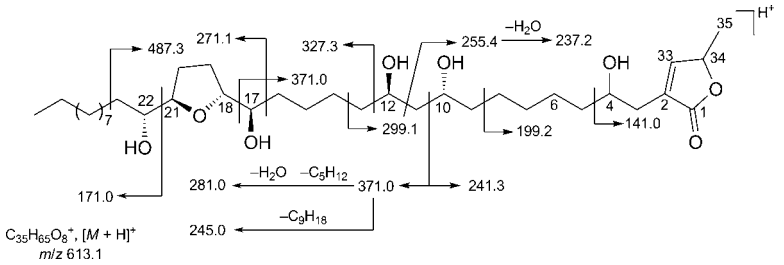

Fig. 3. *Proposed Fragments for the Principal Peaks and Positioning of the 1,3-Diol Group Detected in ESI-MS/MS of* **1**

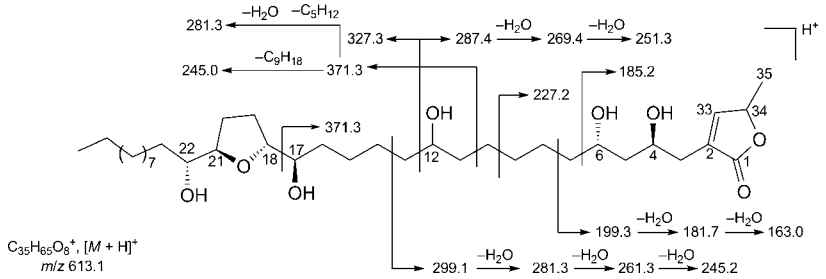

Fig. 4. *Proposed Fragments for Principal Peaks and Positioning of the 1,3-Diol Group Detected in ESI-MS/MS of* **2**

isolation from the *Annona* genus seeds. Compounds **1** and **2** differ by the position of their 1,3-diol moieties and are described herein for the first time.

Experimental Part

General. TLC: Silica-gel plates *60 F254* (SiO_2; *Macherey–Nagel*). Column chromatography (CC): silica gel (SiO_2, 70–230 mesh; *Kieselgel 60*; *Merck*) and *Sephadex LH-20* (*Sigma–Aldrich*), in normal mode and under atmospheric pressure; elution in gradient mode. IR Spectra: *Bomem MB-100* spectrophotometer; KBr discs; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AVANCE-500*; *ca.* 2–5 mg of substance in $CDCl_3$ or CD_3OD (0.5 ml); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-ESI-MS: *Bruker MicrOTOF$_Q$ II*; recorded between *m/z* 50–1500 in positive-ion mode; in *m/z*. LC-ESI- and ESI-MS: LC analyses were conducted with a *Varian ProStar 410* autosampler coupled with a *Varian 500-MS* mass detector ion-trap analyzer and electrospray ionization source (ESI-MS), operating in a positive-ion mode scan in the range of *m/z* 50–2000. The MS spectra were recorded during the HPLC using the following conditions: MS/MS analysis with starting collision-induced dissociation energy was varied in intervals of 5 eV. LC was equipped with a *Gemini C18* 5 μm 110 Å column (250 × 4.6 mm; *Phenomenex*), linear gradient, 0.1% TFA in H_2O (*A*) and MeOH (*B*); column eluted with isocratic flow control of *A* (85%) over 60 min; flow rate, 1 ml/min. The ion-trap MS system is coupled with a LC system including a binary pump (*Varian ProStar 210*) and a UV/VIS detector (*Varian ProStar 325*) at 220 nm. The MSWorkstation *Varian* software, version 6.9.2.

Plant Material. Annona coriacea MART. (Annonaceae) was collected on side roads Tangará da Serra town, Mato Grosso, Brazil. A voucher specimen (No. 269) was deposited with the Laboratory of Botany of Research Center, Studies and Development Agro-Environmental (CPEDA) of Universidade do Estado de Mato Grosso (UNEMAT), Mato Grosso, Brazil.

Extration and Isolation. The seeds of *A. coriacea* were dried at 29°. The dried material was pulverized to afford the powdered seeds (701.0 g) and submitted to successive cold extractions with 8 l of hexane (7×7 d) and 12 l of MeOH (7×7 d) to afford the hexane (110 g) and MeOH (51.9 g) extracts, resp., after solvent evaporation *in vacuo*. The hexane extract (111.0 g) was submitted MeOH/H_2O 9 :1 liquid-liquid partition, obtaining the MeOH fraction (15.3 g). This fraction was submitted to CC ($CHCl_3$ and MeOH) to afford 92 fractions. *Frs. 27–31* (8.63 g) were reunited and submited to CC (hexane, $CHCl_3$, AcOEt, and MeOH) to furnish 47 fractions. *Fr. 30* (144.0 mg) was obtained as a white amorphous precipitate, affording a mixture of two isomeric compounds (sample *A*; 31.2 mg) after MeOH washing. Sample *A* was analyzed by ESI-LC/MS, and at 26.94 min a major peak was observed which corresponded to **1**. *Frs. 32–50* (0.67 g) were reunited and subjected to CC (gradient elution with hexane, $CHCl_3$, AcOEt, and MeOH) to afford 39 fractions. A white precipitate containing a mixture of two isomeric compounds (sample *B*; 28.0 mg) was obtained from *Fr. 22*. Sample *B* was analyzed by LC/MS, and at 55.70 min a major peak corresponding to compound **3** was observed. An aliquot of the MeOH extract (42.5 g) was submitted to CC (gradient elution with $CHCl_3$ and MeOH) to give 159 fractions. *Frs. 39–42* (0.56 g) were reunited by TLC analysis and subjected to CC (gradient elution with hexane, $CHCl_3$, AcOEt, and MeOH) to afford 22 fractions. The subgroup comprising *Frs. 4–6* were reunited (0.41 g) and subjected to CC (*Sephadex LH-20*; MeOH) to give sample *C* as a white waxy material (12.7 mg), from the *Frs. 13–16*. A mixture of four compounds with different LC/MS t_R values was obtained from sample *C*, with a major peak at 29.19 min corresponding to compound **2**.

Coriapentocin A (= *5-Methyl-3-[(8*R*,10*R*,15*R*)-2,8,10,15-tetrahydroxy-15-{(2*R*,5*R*)-tetrahydro-5-[(1*R*)-1-hydroxyundecyl]furan-2-yl}pentadecyl]furan-2(5*H*)-one*; **1**). White powder. IR (KBr): 3401, 2924, 2846, 1751, 1656, 1460, 1375, 1073. ^{1}H- and ^{13}C-DEPT-Q: see *Table 1*. ESI-MS (80 V): 635.1 ($[M+Na]^+$, $C_{35}H_{64}NaO_8^+$), 613.1 ($[M+H]^+$, $C_{35}H_{65}O_8^+$), 487.3, 371.0, 327.3, 299.1, 281.0, 271.1, 255.4, 245.0, 241.3, 237.2, 199.2, 171.0, 141.0. HR-ESI-MS: 613.4696 ($[M+H]^+$, $C_{35}H_{65}O_8^+$; calc. 613.4679).

Coriapentocin B (= *5-Methyl-3-[(2S,4S,15*R*)-2,4,10,15-tetrahydroxy-15-{(2*R*,5*R*)-tetrahydro-5-[(1*R*)-1-hydroxyundecyl]furan-2-yl}pentadecyl]furan-2(5*H*)-one*; **2**). White powder. IR (KBr): 3420, 2960, 2945, 1727, 1649, 1460, 1377, 1071. ^{1}H- and ^{13}C-DEPT-Q: see *Table 2*. ESI-MS (80 V): 635.1 ($[M+Na]^+$, $C_{35}H_{64}NaO_8^+$), 613.1 ($[M+H]^+$, $C_{35}H_{65}O_8^+$), 371.3, 327.3, 299.1, 287.4, 281.3, 269.4, 261.3, 251.3, 245.2, 245.0, 199.3, 185.2, 181.7, 163.0. HR-ESI-MS: 613.4283 ($[M+H]^+$, $C_{35}H_{65}O_8^+$; calc. 613.4679).

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REFERENCES

[1] A. F. Pontes, M. R. de V. Barbosa, P. J. M. Maas, *Acta Bot. Bras.* **2004**, *18*, 281.
[2] L. W. Chatrou, H. Rainer, P. J. M. Maas, in 'Annonaceae (soursop family)', Eds. N. Smith, S. A. Mori, A. Hendersen, D. W. Stevenson, S. V. Heald, Princeton University Press, New Jersey, 2004, p. 18.
[3] N. Li, Z. Shi, Y. Tang, J. Chen, X. Li, *Beilstein J. Org. Chem.* **2008**, *4*, 48.
[4] D. C. Hopp, F. Q. Alali, Z.-M. Gu, J. L. McLaughlin, *Bioorg. Med. Chem.* **1998**, *6*, 569.
[5] F. Q. Alali, X.-X. Liu, J. L. McLaughlin, *J. Nat. Prod*. **1999**, *62*, 504.
[6] A. Bermejo, B. Figadere, M. C. Zafra-Polo, I. Barrachina, E. Estornell, D. Cortes, *Nat. Prod. Rep.* **2005**, *22*, 269.
[7] F.-E Wu, Z.-M. Gu, L. Zeng, G.-X. Zhao, Y. Zhang, J. L. McLaughlin, S. Sastrodihardjo, *J. Nat. Prod.* **1995**, *58*, 830.
[8] F.-E Wu, L. Zeng, Z.-M. Gu, G.-X. Zhao, Y. Zhang, J. T. Schwedler, J. L. McLaughlin, S. Sastrodihardjo, *J. Nat. Prod.* **1995**, *58*, 909.
[9] Z.-M. Gu, X.-P. Fang, L. Zeng, K. V. Wood, J. L. McLaughlin, *Heterocycles* **1993**, *36*, 2221.

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