86. Novel Acetogenins from the Leaves of *Annona purpurea*

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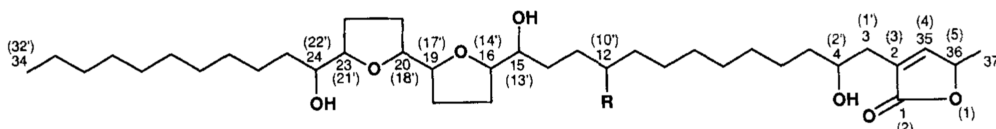
The CH_2Cl_2 extract of the leaves of *Annona purpurea* L. (Annonaceae) showed strong brine shrimp toxicity and pronounced activity against larvae of the yellow-fever mosquito *Aedes aegypti*. Activity-directed fractionation of the extract by a combination of column chromatography on silica gel, gel filtration, and high-pressure liquid chromatography led to the isolation of acetogenins **1–6** as the main active principles. The structures of two novel acetogenins named purpureacin 1 (**5**) and purpureacin 2 (**6**) were elucidated by spectroscopic analysis (UV, EI- and DCI-MS, EI-MS of Me_3Si derivatives, ^1H- and ^{13}C-NMR). The configuration of **5** and **6** was not established due to the limited sample amount. Compounds **3–6** also showed antifungal activity against *Candida albicans*, and **5** was slightly active against *Bacillus subtilis*.

Introduction. – *Annona purpurea* L. (Annonaceae) is a bushy tree native to Middle and South America. Its fruit is edible, and its wood is used for the manufacture of paper. Various parts of the tree are employed in traditional medicine: the fruit juice is a remedy for fever, chills, and jaundice, and a decoction of the inner bark is prescribed for cases of dysentry and edema. In Panama, the leafy branch tips are boiled with those of *Aegyphila martinicensis*, and the resulting decoction is drunk to clear up skin eruptions [1]. The powdered seeds of *A. purpurea* were reported to be insecticidal [2], and several cytotoxic alkaloids [3] were isolated from this plant prior to our own investigation.

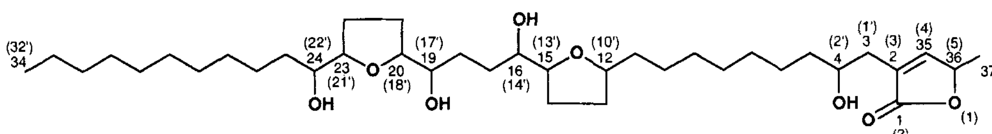
During preliminary screening, the CH_2Cl_2 extract of the leaves of *A. purpurea* exerted strong toxicity towards larvae of the crustacean *Artemia salina* (brine shrimp) and pronounced activity against larvae of the mosquito *Aedes aegypti*, vector of yellow fever. We report here on the activity-guided isolation and structure elucidation of the active constituents, four known and two new γ-lactone acetogenins.

Results. – The air-dried leaves of *A. purpurea* collected in Panama were extracted successively with petroleum ether and CH_2Cl_2. The CH_2Cl_2 extract was submitted to activity-guided fractionation using the brine shrimp toxicity assay [4] and *Aedes aegypti* mosquito larvae toxicity [5]. Compounds **1–6** were isolated by a combination of open column chromatography on silica gel, gel filtration on *Sephadex LH-20*, and low-pressure and semi-preparative high-pressure liquid chromatography (HPLC; see *Exper. Part*).

The structures of the known acetogenins **1–4** were confirmed by UV, MS, ^1H- and ^{13}C-NMR, and $[\alpha]_D$ measurements. Compounds **1** and **2** were identified as rolliniastatin 1 [6] and bullatacin [7], respectively. They belong to the family of the bi(tetrahydrofuran)-



	R	C(15)/C(16)	C(16)/(19)	C(19)/C(20)	C(20)/C(23)	C(23)/C(24)
1 rolliniastatin 1	H	*threo*	*cis*	*threo*	*cis*	*erythro*
2 bullatacin	H	*threo*	*trans*	*threo*	*trans*	*erythro*
6 purpureacin 2	OH					



	C(15)/C(16)	C(19)/C(20)	C(20)/C(23)	C(23)/C(24)
3 cherimoline	*threo*	*threo*	*trans*	*erythro*
4 sylvaticin	*threo*	*erythro*	*trans*	*threo*
5 purpureacin 1				

(bi(THF)) acetogenins (*i.e.* two adjacent THF rings) [8]. Compounds **3** and **4** were identified as the nonadjacent bi(THF) acetogenins cherimoline [8] [9] and sylvaticin [10], respectively (see *Exper. Part*).

A new acetogenin, purpureacin 1 (**5**), was obtained as an amorphous powder. The molecular weight of 638 amu was deduced from DCI-MS recorded in positive- and negative-ion modes. The molecular formula $C_{37}H_{66}O_8$ and the presence of four free OH groups were corroborated by ^{13}C-NMR and EI-MS data of the trimethylsilyl (Me_3Si) derivative of **5**. The UV absorption maximum at 208 nm ($\log \varepsilon = 3.93$) and MS and NMR data (*Tables 1* and *2*) were indicative of an α-substituted α,β-unsaturated γ-methyl-γ-lactone moiety, an OH group at C(4), and two nonadjacent tetrahydrofuran rings flanked by OH groups [8].

Table 1. *^{13}C-NMR Data of Acetogenins* **1–6** *in* $CDCl_3$[a])

C-Atom[b])	**1**	**2**	**6**	**3**	**4**	**5**
C(1)	174.6	174.6	174.6	174.6	174.6	174.6
C(2)	131.2	131.2	131.2	131.2	131.2	131.2
C(3)	33.3	33.3	33.3	33.3	33.3	33.3
C(4)	70.0	70.0	69.9	69.9	69.9	69.9
C(5)	37.4	37.4	37.3	37.4	37.4	37.4
C(6)	26.0	26.0	26.1	26.0	26.0	26.1
C(7) to C(9)	[c])	[d])	[e])	[f])	[g])	[h])
C(10)	[c])	[d])	28.9[i])	[f])	[g])	[h])
C(11)	[c])	[d])	37.5	[f])	[g])	[h])
C(12)	[c])	[d])	71.7[k])	79.3	79.3	79.9
C(13)	25.7	25.6	33.6	[f])	[g])	[h])
C(14)	34.2	33.3	31.9	[f])	[g])	[h])
C(15)	74.0	74.1	74.2	83.3	83.0	82.7[i])
C(16)	83.0	83.0	83.2[l])	74.5	74.5	74.7[k])
C(17)	28.8	28.9	28.9[i])	32.4	32.4	32.1[l])

Table 1 (cont.)

C-Atom[b])	1	2	6	3	4	5
C(18)	27.9	28.4	28.4[i])	35.5	35.4	35.7
C(19)	81.1	82.5	82.5[l])	74.4	72.4	74.4[k])
C(20)	81.0	82.8	82.8[l])	82.2	81.8	82.2[i])
C(21)	25.6	25.5	25.6[m])	[f])	[g])	[h])
C(22)	28.4	28.9	28.4	[f])	[g])	[h])
C(23)	82.9	82.3	82.3[l])	82.0	82.4	81.3[i])
C(24)	71.9	71.2	71.3[k])	71.4	74.2	74.1[k])
C(25)	32.7	32.4	32.4	32.5	33.0	33.3[l])
C(26)	25.6	25.5	25.5[m])	25.5	25.5	25.5
C(27) to C(31)	[c])	[d])	[e])	[f])	[g])	[h])
C(32)	31.9	31.9	31.9	31.9	31.9	31.9
C(33)	22.7	22.7	22.7	22.7	22.7	22.7
C(34)	14.1	14.1	14.1	14.1	14.1	14.1
C(35)	151.8	151.8	151.8	151.8	151.9	151.8
C(36)	78.0	78.0	78.0	78.0	78.0	78.0
C(37)	19.1	19.1	19.1	19.1	19.1	19.1

[a]) Measured at 50.7 MHz; δ in ppm relative to TMS (= 0 ppm). [b]) 'Biogenetic' numbering. [c]) 29.3–29.6 ppm. [d]) 29.3–29.7 ppm. [e]) 29.4–29.7 ppm. [f]) 25.1–29.7 ppm. [g]) 26.1–29.8 ppm. [h]) 27.0–31.7 ppm. [i,k,l,m]) Assignments in vertical column interchangeable.

Table 2. *^{1}H-NMR Data of Compounds* **5** *and* **6** *in $CDCl_3$*[a])

H-Atom[b])	5	6	H-Atom[b])	5	6
H_a–C(3)	2.46 (*dddd*, J = 15.3, 3.6, 1.5, 1.5)	2.47 (*dddd*, J = 15.3, 3.6, 1.4, 1.4)	CH_2(17), CH_2(18)	1.4–1.6	1.7–1.9
H_b–C(3)	2.32 (*dddd*, J = 15.3, 7.7, 1.5, 1.5)	2.32 (*dddd*, J = 15.3, 7.7, 1.4, 1.4)	H–C(19)	3.8–3.9	3.8–3.9
H–C(4)	3.80 (*m*)	3.78 (*m*)	H–C(20)	3.80 (*m*)	3.8–3.9
CH_2(5)	1.4–1.6	1.3–1.5	CH_2(21), CH_2(22)	1.8–2.0	1.7–1.9
CH_2(11)	1.4–1.6	1.3–1.5	H–C(23)	3.8–3.9	3.8–3.9
H–C(12)	3.55 (*m*)	3.53 (*m*)	H–C(24)	3.8–3.9	3.8–3.9
CH_2(13), CH_2(14)	1.8–2.0	1.3–1.5	Me(34)	0.81 (*t*, J = 6.8)	0.80 (*t*, J = 6.6)
H–C(15)	3.85 (*m*)	3.39 (*m*)	H–C(35)	7.12 (*dd*, J = 1.5)	7.12 (*q*, J = 1.4)
H–C(16)	3.42 (*m*)	3.82 (*m*)	H–C(36)	4.99 (*ddq*, J = 1.5, 6.8)	5.00 (*qq*, J = 1.4, 6.8)
			Me(37)	1.37 (*d*, J = 6.8)	1.37 (*d*, J = 6.8)

[a]) Measured at 200.06 MHz; δ values in ppm relative to TMS (= 0 ppm), J in Hz.
[b]) 'Biogenetic' numbering.

The DCI-MS (NH_3, positive-ion mode) of **5** exhibits quasimolecular ions at m/z 639 ($[M + H]^+$) and 656 ($[M + NH_4]^+$), while in the negative DCI-MS, a quasimolecular ion at m/z 638 ($[M - H]^-$) is observed. A series of fragment ions at m/z 621, 603, 585, and 567, arising from sequential losses of H_2O from the quasimolecular ion at m/z 639, are also present in the positive-ion DCI-MS. The EI-MS of the Me_3Si derivative of **5** (see *Fig. 2*, below) lacks the molecular-ion peak, but a fragment ion at m/z 911 resulting from the loss of a Me group is observed. These MS data confirm the presence of 4 OH groups.

With the aid of a DQ-PSCOSY, ^{1}H-signals at δ 7.12 (*ddd*, J = 1.5, 1.5, 1.5 Hz), 5.00 (*dddq*, J = 1.5, 1.5, 1.5, 6.8 Hz), and 1.37 (3H, *d*, J = 6.8 Hz) in the ^{1}H-NMR spectrum are assigned to *H*–C(β) (=H–C(35)), *H*–C(γ)

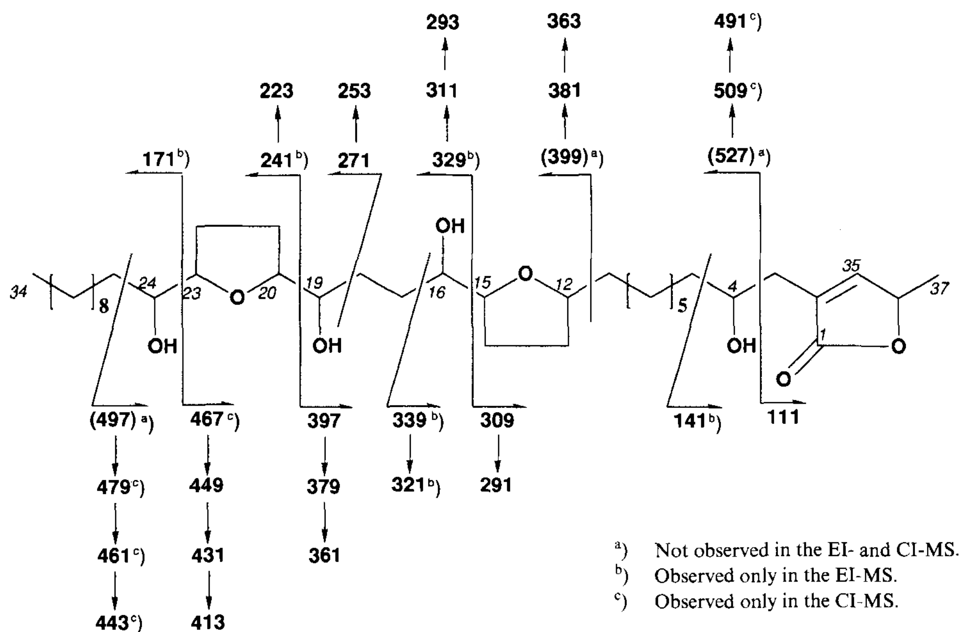
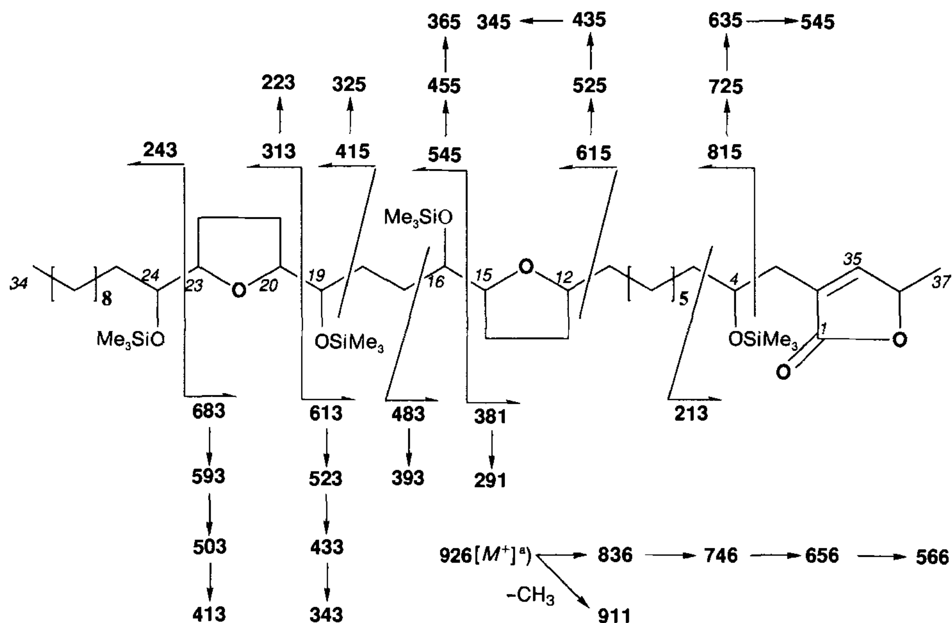


Fig. 1. *Diagnostic fragment ions in the EI- and CI-MS of* **5**



a) The molecular ion was not observed.

Fig. 2. *Diagnostic fragment ions in the EI-MS of the* Me_3Si *derivative of* **5**

(=H–C(36)), and Me–C(γ) (=Me(37)), respectively, and the ^{13}C-NMR signals at δ 176.4 (C), 131.2 (C), 151.8 (CH), 78.0 (CH), and 19.1 (Me) are attributable to C=O (=C(1)), C(α) (=C(2)), C(β) (=C(35)), C(γ) (=C(36)), and Me–C(γ) (=C(37)) of the lactone moiety. ^{1}H-Signals at δ 2.46 (*dddd*, J = 15.3, 3.6, 1.5, 1.5 Hz, H_a–C(3)) and 2.32 (*dddd*, J = 15.3, 7.7, 1.5, 1.5 Hz, H_b–C(3)) and ^{13}C-signals at δ 69.9 (C(4)), 33.3 (C(3)), and 37.3 (C(5)) suggest the presence of an OH group at C(4) [8]. The cleavage between C(3) and C(4), and between C(4) and C(5) yields fragment ions at m/z 111 and 141 in the EI- and DCI-MS of **5**, respectively (*Fig. 1*), and at m/z 213 in the EI-MS of its Me_3Si derivative (*Fig. 2*).

The ^{13}C-NMR spectrum of **5** is similar to those of sylvaticin [10] (**4**) and bullatalicin [11]. The ^{13}C-signals at δ 82.7, 82.2, 81.3, and 79.9 (C(12), C(15), C(20), and C(23)) are readily attributable to the C-atoms of nonadjacent THF rings. The resonances at δ 74.7, 74.4, and 74.2 are characteristic of oxygenated methin C-atoms adjacent to THF rings (C(16), C(19), C(24)). The presence of two nonadjacent THF ring is also confirmed by the EI- and the DCI-MS of **5** (*Fig. 1*) and the EI-MS of the Me_3Si derivative (*Fig. 2*); their fragmentation patterns are similar to those of **3**, **4**, and bullatalicin [11].

Thus, compound **5** has the same C-skeleton as cherimoline (**3**) and sylvaticin (**4**) and, hence is one of their stereoisomers. Due to the limited sample amount (1.2 mg), the configuration could not be established. It should be mentioned here that the relative configurations around the two THF rings (C(15)/(16), C(19)/(20), and C(23)/(24)) in Annonaceous acetogenins are determined with the aid of empirical ^{1}H- and ^{13}C-NMR shift rules established with a series of model compounds [12–14]. This implies unambiguous assignments of the relevant ^{1}H- and ^{13}C-NMR signals through heteronuclear correlation experiments. The configuration of the γ-lactone region also remains open, the NMR and CD spectral data of diastereoisomeric model compounds being virtually indistinguishable [8]. Stereoisomers of purpureacin 1 (**5**) with known relative configuration of the bi(THF) core are cherimoline [9] (**3**), sylvaticin [10] (**4**), bullatalicin [11], and gigantecin [15]. Since their NMR spectral data differ from those of **5**, this acetogenin has to be a new compound.

The other new acetogenin, purpureacin 2 (**6**), was obtained as an amorphous powder. The molecular weight of 638 amu was deduced from the DCI-MS. The MS and NMR data indicated that **6** also has four OH groups and a terminal γ-methyl-γ-lactone moiety as found in compounds **1**–**5**.

In the positive-ion DCI-MS of **6**, quasimolecular ions at m/z 639 ($[M + H]^+$) and 656 ($[M + NH_4]^+$) are observed. A series of fragment ions at m/z 621, 603, 585, and 567 in the positive-ion DCI-MS suggested a successive elimination of 4 molecules of H_2O. The presence of 4 OH groups is corroborated by a molecular ion at m/z 926 and a distinct fragment ion at m/z 911 ($[M - Me]^+$) in the EI-MS of the Me_3Si derivative of **6**. The ^{1}H-signals at δ 7.12 (*ddd*, J = 1.4, 1.4, 1.4 Hz, H–C(35)), 4.99 (*dddq*, J = 1.4, 1.4, 1.4, 6.8 Hz, H–C(36)), and 1.37 (*d*, J = 6.8 Hz, CH_3(37)) and the ^{13}C-signals at δ 174.6 (C(1)), 131.2 (C(2)), 151.8 (C(35)), 78.9 (C(36)), and 19.1 (C(37)), as well as the UV absorption maximum at 207 nm, suggest the presence of an α,β-unsaturated α-substituted γ-methyl-γ-lactone moiety. The ^{1}H-NMR spectrum exhibits nonequivalent protons at δ 2.47 (*dddd*, J = 15.1, 3.6, 1.4, 1.4 Hz, H_a–C(3)) and 2.32 (*dddd*, J = 15.1, 8.1, 1.4, 1.4 Hz, H_b–C(3)). The ^{13}C-signals at δ 33.3, 69.9, and 37.3 attributable to C(3), C(4), and C(5) reveal that an OH group is located at C(4). The fragment ions at m/z 111 and 141 in the EI- and DCI-MS of **6**, respectively (*Fig. 3*), and at m/z 213 in the EI-MS of its Me_3Si derivative (*Fig. 4*) support of this partial structure.

The signals of 6 O-bearing C-atoms appear at δ 71.3, 74.2, 82.3, 82.5, 82.8, and 83.0 (C(15), C(16), C(19), C(20), C(23), C(24)); they are superimposable with those of bullatacin [7] (**2**) and rolliniastatin 2 [16]. The ^{13}C-NMR spectrum shows one additional oxygenated methin C-atom at δ 71.6. The location of this OH group at C(12) was determined by MS analysis as follows: The EI and DCI-MS of **6** shows fragment ions at m/z 269 arising from the cleavage between C(12) and C(13), and at m/z 381 and 363 due to sequential losses of H_2O from the fragment C(12)–C(34) (*Figs. 3* and *4*). The EI-MS of the Me_3Si derivative also displays fragment ions resulting from a cleavage of the C(12)–C(13) (m/z 413) and C(11)–C(12) bonds (m/z 615; *Fig. 4*). A detailed analysis of the MS of **6** and of its Me_3Si derivative is given in *Figs. 3* and *4*.

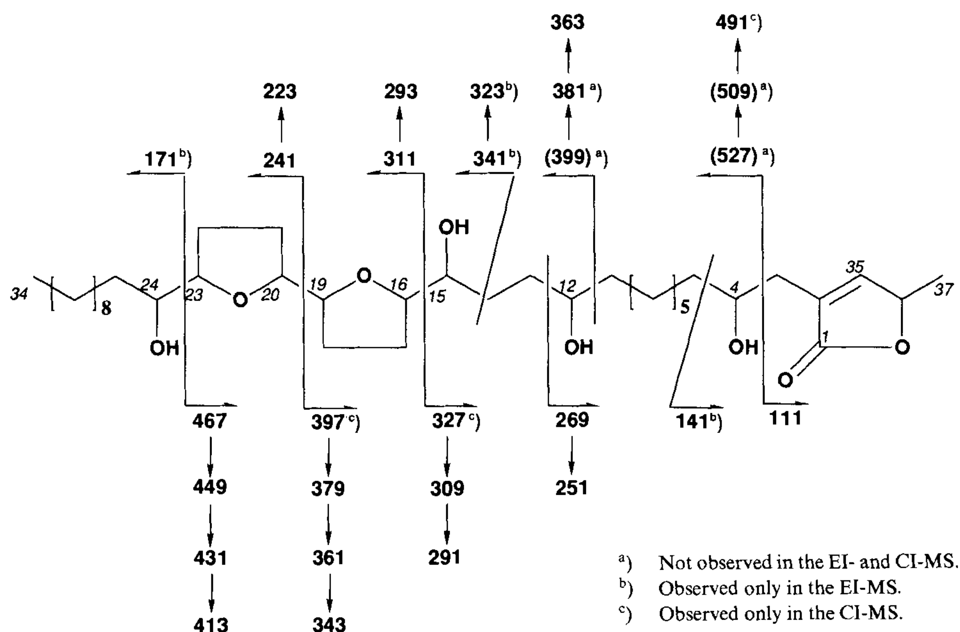


Fig. 3. *Diagnostic fragment ions in the EI- and CI-MS of* **6**

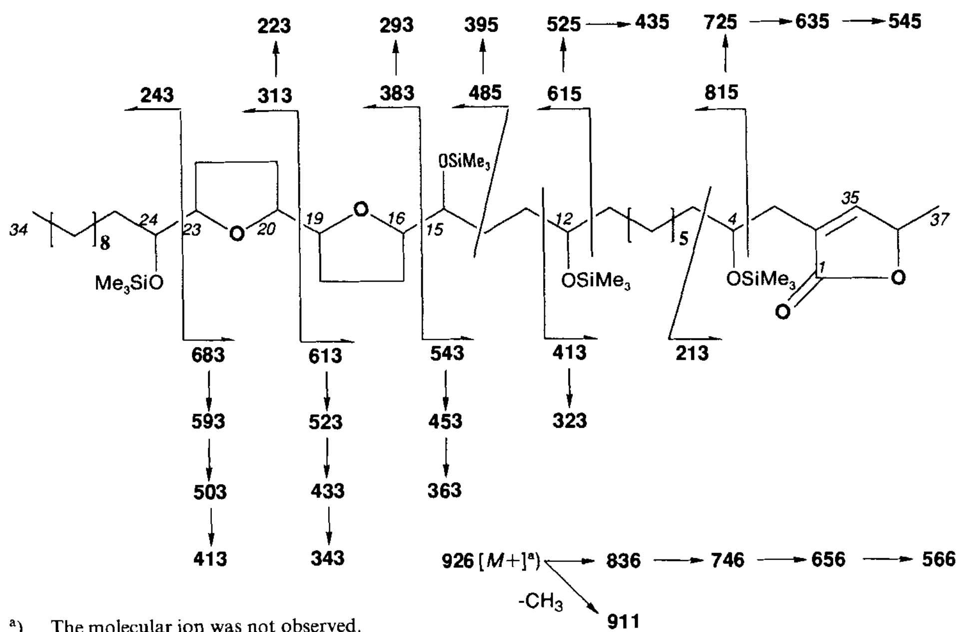


Fig. 4. *Diagnostic fragment ions in the EI-MS of the Me_3Si derivative of* **6**

On the basis of this spectral evidence, structure **6** was assigned to this new acetogenin. Purpureacin 2 (**6**) belongs to the group of bi(THF) acetogenins [8]. As in the case of **5**, the small amount of **6** (1.7 mg) precluded the determination of its configuration.

Compounds **1**–**6** were tested for brine shrimp toxicity (*Artemia salina*) [4], larvicidal activity (*Aedes aegypti*) [5], and antimicrobial activity against *Candida albicans* and *Bacillus subtilis* [17]. The results are summarized in *Table 3*.

Table 3. *Biological Activities of the CH_2Cl_2 Extract and of Compounds* **1**–**6**

	Brine shrimp toxicity[a]) [μg/ml]	Larvicidal activity[b]) [μg/ml]	Antifungal activity[c]) [μg]	Antibacterial activity[d]) [μg]
Extract	3.9 (4.5–3.3)	10	100	not active
1	0.0049 (0.0061–0.0036)	0.2	not active	not active
2	0.091 (0.011–0.0074)	0.3	not active	not active
3	0.40 (0.50–0.32)	2.0	20	not active
4	0.57 (0.68–0.47)	1.0	0.5	not active
5	0.53 (0.65–0.43)	2.0	0.05	20
6	0.38 (0.47–0.30)	1.0	1	not active
References[e])	5.0	0.1	0.001	0.001

[a]) Toxicity towards *Artemia salina*, expressed as LC_{50}/24 h. In parentheses 95% confidence intervals, calculated by probit analysis.
[b]) Activity against larvae of *Aedes aegypti*, expressed as LC_{100}/24 h.
[c]) Antifungal activity against *Candida albicans*: minimal amount inhibiting fungal growth on TLC plate.
[d]) Antibacterial activity against *Bacillus subtilis*: minimal amount inhibiting bacterial growth on TLC plate.
[e]) Reference compounds: taxol for brine-shrimp toxicity; diazion for larvicidal, miconazole for antifungal, and chloramphenicol for antibacterial assays.

Discussion. – From the leaves of *Annona purpurea*, four known and two new Annonaceous acetogenins, purpureacin 1 (**5**) and purpureacin 2 (**6**) were isolated through activity-directed fractionation. The first representative of this unique class of compounds was reported only ten years ago [18], and their occurrence appears at present to be limited to the family Annonaceae. Compound **6** is the first example of a bi(THF) acetogenin bearing an OH group at C(12). A possible biosynthetic pathway leading to Annonaceous acetogenins was discussed by *McLaughlin* and coworkers [8]. They proposed that the THF rings are biosynthesized through the epoxidation of diene, triene, or triene ketone intermediates followed by 'zipper-like' epoxide-ring opening and closure. Thus, purpureacin 2 (**6**) would be formed in a pathway different from other bi(THF) acetogenins such as compounds **1** and **2**. It might originate from the same precursor as acetogenins **3**–**5**, with THF ring closure occurring between C(16) and C(19) instead of C(12) and C(15).

Many of the Annonaceous acetogenins known to date display potent bioactivities, including pesticidal, cytotoxic, antitumour, antimicrobial, and antimalarial activities and brine shrimp toxicity [8] [19]. As shown in *Table 3*, compounds **1**–**6** are strongly toxic to larvae of the yellow-fever mosquito *Aedes aegypti*. Rolliniastatin 1 (**1**) and bullatacin (**2**) are among the most potent larvicidal acetogenins known to date. These results are in accord with preliminary structure-activity relationships established by *McLaughlin* and coworkers [8], who found pesticidal activity and brine shrimp toxicity for acetogenins

bearing adjacent THF rings. All Annonaceous acetogenins were patented as pesticides by *Mikolajczak et al.* [20], and an acetogenin-rich fraction of *Asimina triloba* is in evaluation for licensing as a natural pesticide [21]. In addition, compounds **3–6** are strongly fungicidal, and **5** also exhibits weak activity against *B. subtilis*. The antimicrobial effects of acetogenins **3–6** corroborates the use of *A. purpurea* in traditional medicine for the treatment of skin afflictions.

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Experimental Part

General. TLC: silica gel *60 F_{254}* precoated Al sheets (*Merck*, Darmstadt); detection with tetrazolium blue (0.5% in MeOH/5N NaOH 1:1). Open column chromatography (CC): silica gel (15–40 μm and 63–200 μm, *Merck*); *Sephadex LH-20* (*Pharmacia*). Low-pressure liquid chromatography (LPLC): *Lobar-RP-8* column (40–63 μm; 27 × 2.5 cm (i.d.); *Merck*) equipped with a *Duramat-80* pump (*Chemie und Filter*, Regensdorf). Semi-prep. HPLC of **1–6**: *Shimadzu LC-8A*, *Rheodyne* injector, *LKB-2151* UV detector; *Nucleosil-C18-Varioprep* column (7 μm, 25 × 2.1 cm (i.d.); *Macherey-Nagel*), $MeCN/H_2O$ 85:15 for **1** and **2**, $MeOH/H_2O$ 85:15, flow rate 10 ml/min for **3–6**; *LiChrosorb-Si-60* column (7 μm, 250 × 4.6 mm (i.d.); *Knauer*), hexane/i-PrOH 77:23, flow rate 1 ml/min, for **5** and **6**; detection at 210 nm. Anal. HPLC (purity controls): *Hewlett-Packard 1090*, photodiode array detector; *Nucleosil-C18* column (5 μm, 250 × 4 mm (i.d.); *Macherey-Nagel*), $MeCN/H_2O$ 85:15, flow rate 1 ml/min, for **1–6**; *LiChrosorb-Si-60* column (7 μm, 250 × 4.6 mm (i.d.); *Knauer*), hexane/i-PrOH 77:23, flow rate 1 ml/min, for **5** and **6**; detection at 210 nm. UV: *Varian-DMS-100S* spectrophotometer; $\lambda_{max}(\log\varepsilon)$ in nm. ^1H- and ^{13}C-NMR: *Varian-VXR-200* instrument at 200.07 and 50.2 MHz, resp., in $(D)CHCl_3$; chemical shifts δ in ppm rel. to TMS as internal standard, J in Hz; ^1H-correlations by DQ-PSCOSY experiments; ^{13}C-multiplicities by DEPT pulse sequence; ^1H- and ^{13}C-assignments for the major compounds by 2D heteronuclear one-bond and multiple-bond correlation. MS: *Finnigan-MAT-TSQ-700* instrument; EI at 70 eV; positive- and negative-ion mode DCI with NH_3; trimethylsilyl derivatives: the acetogenin (100 μg) was treated with 100 μl of silylating mixture *Fluka II* according to *Horming* (*Fluka*) at 80° for 30 min, the mixture diluted with H_2O and extracted with hexane, and the org. layer subjected to EI-MS.

Plant Material. Annona purpurea was collected in August 1989 at Cierro Jefe, Province of Panama, Panama, and authenticated by Prof. *Mireya D. Correa*. A voucher specimen is deposited at the Herbarium of the University of Panama, Panama City.

Extraction and Isolation. The air-dried leaves (220 g) were ground and extracted at r.t. successively with petroleum ether and CH_2Cl_2 to afford 4.5 g and 5.5 g of extracts, respectively. The CH_2Cl_2 extract was subjected to CC (silica gel 63–200 μm, column 58 × 5 cm (i.d.), step-gradient $CHCl_3/MeOH$ 49:1→1:1). Six fractions (*I–IV*) were collected. Fraction *IV* was submitted to gel filtration (*Sephadex LH-20*, $CH_2Cl_2/MeOH$ 1:1) to give four fractions (*A–D*). LPLC (*RP-8*, $MeOH/H_2O$ 7:3→9:1) of fraction B yielded four fractions (*1–4*). Gel filtration of fraction *4* (*Sephadex LH-20*, CH_2Cl_2/hexane/MeOH 10:10:1) afforded five fractions (*a–e*). Compounds **1** (3.1 mg) and **2** (9.7 mg) were obtained from fraction *b* by semi-prep. HPLC (*Nucleosil C18*, $MeCN/H_2O$ 85:15). Fraction *V* was resubmitted to CC (silica gel 15–40 μm, column 43 × 3.5 cm (i.d.), step-gradient $CHCl_3$→$CHCl_3/MeOH$ 9:1). Ten fractions (*A′ – J′*) were collected, of which *E′* and *F′* were subjected to gel filtration (*Sephadex LH-20*, CH_2Cl_2/hexane/MeOH 10:10:1). Semi-prep. HPLC (*Nucleosil C18*, $MeOH/H_2O$ 85:15) of the 3rd fraction of *E′* afforded **3** (8.8 mg) and **4** (2.7 mg). Reversed-phase semi-prep. HPLC (*Nucleosil C18*, $MeOH/H_2O$ 85:15) followed by normal phase HPLC (*Lichrosorb Si 60*, hexane/i-PrOH 77:23) of the 3rd fraction of *F′* afforded **5** (1.2 mg) and **6** (1.7 mg). The isolation of **1** and **2** was directed by the brine shrimp toxicity assay and that of **3–6** by the *Aedes aegypti* larvicidal assay.

rel-*(5*R*,2′*S*)*-rel-*(13′*R*,14′*R*,17′*S*,18′*S*,21′*R*,22′*S*)-3-(14′,17′:18′,21′-Diepoxy-2′,13′,22′-trihydroxydotriacontyl)-5-methylfuran-2(5*H*)-one* (= *Rolliniastatin 1* = *3-{2,13-Dihydroxy-13-{octahydro-5′-(1-hydroxyunde-*

*cyl)[2,2'-bifuran]-5-yl}tridecyl}-5-methylfuran-2(5*H*)-one;* **1**). Colorless waxy solid. TLC (silica gel, $CHCl_3$/MeOH 9:1): R_f 0.53. HPLC: k' 3.85 (*Nucleosil C18*). $[\alpha]_D = +20.6$ ($c = 0.31$, MeOH; [6]: $[\alpha]_D = +25.2$, CH_2Cl_2). UV: 207 (3.93). ^{1}H-NMR: 0.87 (*t*, $J = 6.8$, CH_3(34)); 1.42 (*d*, $J = 6.8$, CH_3(37)); 2.38 (*dddd*, $J = 1.6, 1.6, 7.8, 15.3$, H_b–C(3)); 2.53 (*dddd*, $J = 1.5, 1.5, 3.6, 15.3$, H_a–C(3)); 3.39 (*m*, H–C(15)); 3.75–3.95 (*m*, H–C(4), H–C(16), H–C(19), H–C(20), H–C(23), H–C(24)); 5.05 (*ddq*, $J = 1.6, 1.6, 6.8$, H–C(36)); 7.18 (*ddd*, $J = 1.2, 1.6, 1.6$, H–C(35)). ^{13}C-NMR: *Table 1*. DCI-MS (NH_3, pos.-ion mode): 640 ($[M + NH_4]^+$), 623 ($[M + H]^+$), 605 ($[M + H - H_2O]^+$), 587 ($[M + H - 2H_2O]^+$), 579 ($[M + H - 3H_2O]^+$), 451, 433, 415, 381, 363, 345, 312, 311, 293, 241, 223, 141.

rel-*(5*S*,2'*R*)*-rel-*(13'*R*,14'*S*,17'*S*,18'*S*,21'*S*,22'*S*)-3-(14',17':18',21'-Diepoxy-2',13',22'-trihydroxydotriacontyl)-5-methylfuran-2(5*H*)-one* (= *Bullatacin*; **2**). White, amorphous powder. TLC (silica gel, $CHCl_3$/MeOH 9:1): R_f 0.53. HPLC: k' 3.02 (*Nucleosil C18*). $[\alpha]_D = +12.8$ ($c = 0.49$, MeOH; [7]: $[\alpha]_D = +13.0$, $CHCl_3$). UV: 209 (4.04). ^{1}H-NMR: 0.86 (*t*, $J = 6.8$, CH_3(34)); 1.42 (*d*, $J = 6.8$, CH_3(37)); 2.37 (*dddd*, $J = 1.5, 1.5, 8.2, 15.1$, H_b–C(3)); 2.53 (*dddd*, $J = 1.5, 1.5, 3.2, 15.1$, H_a–C(3)); 3.38 (*m*, H–C(15)); 3.75–3.95 (*m*, H–C(4), H–C(16), H–C(19), H–C(20), H–C(23), H–C(24)); 5.05 (*ddq*, $J = 1.5, 1.5, 6.8$, H–C(36)); 7.14 (*ddd*, $J = 1.5, 1.5, 1.5$, H–C(35)). ^{13}C-NMR: *Table 1*. DCI-MS (NH_3, pos.-ion mode): 640 ($[M + NH_4]^+$), 623 ($[M + H]^+$), 605 ($[M + H - H_2O]^+$), 587 ($[M + H - 2H_2O]^+$), 579 ($[M + H - 3H_2O]^+$), 451, 433, 415, 381, 363, 345, 312, 311, 293, 241, 223, 141.

rel-*(5*R*,2'*S*)*-rel-*(10'*R *or* S*,13'*R*,14'*R*)*-rel-*(17'*R*,18'*R*,21'*R*,22'*S*)-3-(10',13':18',21'-Diepoxy-2',14',17',22'-tetrahydroxydotriacontyl)-5-methylfuran-2(5*H*)-one* (= *Cherimoline = 3-{9-{5-{1,4-Dihydroxy-4-[tetrahydro-5-(1-hydroxyundecyl)furan-2-yl]butyl}tetrahydrofuran-2-yl}-2-hydroxynonyl}-5-methylfuran-2(5*H*)-one;* **3**). White, amorphous powder. TLC (silica gel, $CHCl_3$/MeOH 9:1): R_f 0.27. HPLC: k' 1.38 (*Nucleosil C18*). $[\alpha]_D = +8.0$ ($c = 0.88$, MeOH; [9]: $[\alpha]_D = +64$). UV: 209 (3.98). ^{1}H-NMR: 0.88 (*t*, $J = 6.7$, CH_3(34)); 1.43 (*d*, $J = 6.8$, CH_3(37)); 2.38 (*dddd*, $J = 1.4, 1.4, 8.0, 15.1$, H_b–C(3)); 2.52 (*dddd*, $J = 1.4, 1.4, 3.7, 15.1$, H_a–C(3)); 3.35–3.45 (*m*, H–C(16), H–C(24)); 3.70–3.92 (*m*, H–C(4), H–C(12), H–C(15), H–C(19), H–C(20), H–C(23)); 5.05 (*ddq*, $J = 1.5, 1.5, 6.8$, H–C(36)); 7.18 (*ddd*, $J = 1.5, 1.5, 1.5$, H–C(35)). ^{13}C-NMR: *Table 1*. DCI-MS (NH_3, pos.-ion mode): 656 ($[M + NH_4]^+$), 639 ($[M + H]^+$), 621 ($[M + H-H_2O]^+$), 603 ($[M + H-2H_2O]^+$), 595 ($[M + H-3H_2O]^+$), 467, 449, 431, 413, 397, 379, 361, 343, 327, 311, 309, 293, 291, 241.

rel-*(5*R*,2'*S*)*-rel-*(10'*R *or* S*,13'*R*,14'*R*)*-rel-*(17'*R*,18'*S*,21'*S*,22'*R*)-3-(10',13':18',21'-Diepoxy-2',14',17',22'-tetrahydroxydotriacontyl)-5-methylfuran-2(5*H*)-one* (= *Sylvaticin*; **4**). White, amorphous powder. TLC (silica gel, $CHCl_3$/MeOH 9:1): R_f 0.26. HPLC: k' 1.88 (*Nucleosil C18*). $[\alpha]_D = +4.4$ ($c = 0.27$, MeOH). UV: 207 (3.96). ^{1}H-NMR: 0.87 (*t*, $J = 6.8$, CH_3(34)); 1.42 (*d*, $J = 6.8$, CH_3(37)); 2.38 (*dddd*, $J = 1.5, 1.5, 8.0, 15.1$, H_b–C(3)); 2.52 (*dddd*, $J = 1.5, 1.5, 3.6, 15.1$, H_a–C(3)); 3.40 (*m*, H–C(24)); 3.48 (*m*, H–C(16)); 3.73 (*m*, H–C(4)); 3.78–3.93 (*m*, H–C(12), H–C(15), H–C(19), H–C(20), H–C(23)); 5.05 (*ddq*, $J = 1.5, 1.5, 6.8$, H–C(36)); 7.18 (*ddd*, $J = 1.5, 1.5, 1.5$, H–C(35)). ^{13}C-NMR: *Table 1*. DCI-MS (NH_3, pos.-ion mode): 656 ($[M + NH_4]^+$), 639 ($[M + H]^+$), 621 ($[M + H-H_2O]^+$), 603 ($[M + H - 2H_2O]^+$), 595 ($[M + H - 3H_2O]^+$), 467, 449, 431, 413, 397, 379, 361, 343, 327, 311, 309, 293, 291, 241.

*3-(10',13':18',21'-Diepoxy-2',14',17',22'-tetrahydroxydotriacontyl)-5-methylfuran-2(5*H*)-one* (= *Purpureacin 1*; **5**). White, amorphous powder. TLC (silica gel, $CHCl_3$/MeOH 9:1): R_f 0.24. HPLC: k' 0.83 (*Nucleosil C18*), 5.9 (*LiChrosorb Si 60*). $[\alpha]_D = -3.3$ ($c = 0.12$, MeOH). UV: 208 (3.93). ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. EI- and DCI-MS: *Fig. 1*. EI-MS of the Me_3Si derivative: *Fig. 2*.

*3-(14',17':18',21'-Diepoxy-2',10',13',22'-tetrahydroxydotriacontyl)-5-methylfuran-2(5*H*)-one* (= *Purpureacin 2*; **6**). White, amorphous powder. TLC (silica gel, $CHCl_3$/MeOH 85:15): R_f 0.28. HPLC: k' 0.83 (*Nucleosil C18*), 7.78 (*LiChrosorb Si 60*). $[\alpha]_D = +6.5$ ($c = 0.17$, MeOH). UV: 207 (3.90). ^{1}H-NMR: *Table 2*. ^{13}C-NMR: *Table 1*. EI- and DCI-MS: *Fig. 3*. EI-MS of the Me_3Si derivative: *Fig. 4*.

Brine Shrimp Toxicity Assay (*Artemia salina*; [4] modified). *A. salina* kysts are incubated in artificial salt water at r.t. for 24 h. Hatched larvae (first instar) are collected and incubated for a further 24 h, during which they moult into second instar. The assay is performed on this homogeneous population. Samples are solubilized in DMSO (solvent concentration in the test vials, 1%). All samples are tested in triplicate.

Larvicidal Assay (*Aedes aegypti*) [5]. The assay is performed on second-instar larvae. Samples are solubilized in DMSO (solvent concentration in test samples, 1%). All samples are tested in triplicate.

Antimicrobial Assays. Bioautography agar overlay method with *Candida albicans* and *Bacillus subtilis* [17].

Notes Added in Proof. – *1*) While this manuscript was in press, two stereoisomers of purpureacin 1, namely cherimolin-1 (D. Cortes, S.H. Myint, B. Dupont, D. Davoust, *Phytochemistry* **1993,** *32,* 1475) and bullatanocin (Z.-M. Gu, X.-P. Fang, M.J. Rieser, Y.-H. Hui, L.R. Miesbauer, D.L. Smith, K.L. Wood, J.L. McLaughlin, *Tetrahedron* **1993,** *49,* 747) have been reported. Cherimolin-2 and bullatanocin are identical. However, their NMR data do not correspond with those of purpureacin 1 (**5**).

2) Cherimoline (**3**) has been renamed cherimolin-1 (D. Cortes, B. Figadère, A. Cavé, *Phytochemistry* **1993,** *32,* 1467).

3) For bullatacin (**2**), the relative configurations at C(15)/(16) and C(23)/(24) has been revised to *threo* and *erythro*, respectively (X.-P. Fang, M.J. Rieser, Z.M. Gu, G.X. Zhao, J.L. McLaughlin, *Phytochem. Anal.* **1993,** *4,* 27).

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