Friedelanes from *Crossopetalum lobatum*. A New Example of a Triterpene Anhydride

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Three new triterpenoids, lobatanhydride (**1**), 3α,25-dihydroxyfriedelan-2-one (**2**) and 1β,25-dihydroxyfriedelan-3-one (**3**), were isolated from *Crossopetalum lobatum* LUNDELL. In addition, four known friedelane triterpenes, friedelin (**4**), 2α-hydroxy-3-oxofriedelan-30-oic acid (**5**), 28-hydroxyfriedelane-1,3-dione (**6**), and 29-hydroxyfriedelane-1,3-dione (**7**) were isolated. Lobatanhydride represents the first example of a triterpene anhydride with the *A* ring expanded to a seven-membered ring. The structures were established on the basis of extensive spectral investigation, and by comparison with the respective literature values.

Introduction. – The Celastraceae species have a long history in traditional medicine [1]. They produce an extraordinary variety of bioactive metabolites, including dihydro-β-agarofuran sesquiterpenes [2] and quinone-methide triterpenes [3]. Triterpenes are frequent among the components of species of this family and invariably belong to the friedo-oleane, lupane, oleane, glutinane, taraxerane, ursane, dammarane, and *D*:*B*-friedobaccharane series [3]. Friedelane triterpenoids are reported to have interesting biological, pharmacological, or medicinal activities, including antineoplastic [4] and anti-AIDS activities [5]. Furthermore, reports on triterpene anhydrides are scant in the field of natural products. Up to now, celastranhydride, biogenetically related to the quinone-methides [6], represents the only triterpene anhydride reported from Celastraceae.

Within the context of our investigations of the Celastraceae species, the screening of four species to determine the antimicrobial, cytotoxic [7], and xanthine oxidase inhibitory [8] effects was undertaken. Among these, *Crossopetalum lobatum* LUNDELL was one of the most promising in this study. Further investigations of the chemical constituents from the leaves of *C. lobatum* led to the isolation of three new triterpenes, lobatanhydride (**1**), 3α,25-dihydroxyfriedelan-2-one (**2**), and 1β,25-dihydroxyfriedelan-3-one (**3**). To the best of our knowledge, lobatanhydride represents the first example of a triterpene anhydride with the *A* ring expanded to a seven-membered ring. Their structures were determined by application of 1D- and 2D-NMR techniques. In addition, four friedelane triterpenes were isolated and their structures were identified as friedelin (**4**), 2α-hydroxy-3-oxofriedelan-30-oic acid (**5**), 28-hydroxyfriedelane-1,3-dione (**6**) and 29-hydroxyfriedelane-1,3-dione (**7**) (*Fig. 1*) by comparison of their spectral data with values reported in the literature.

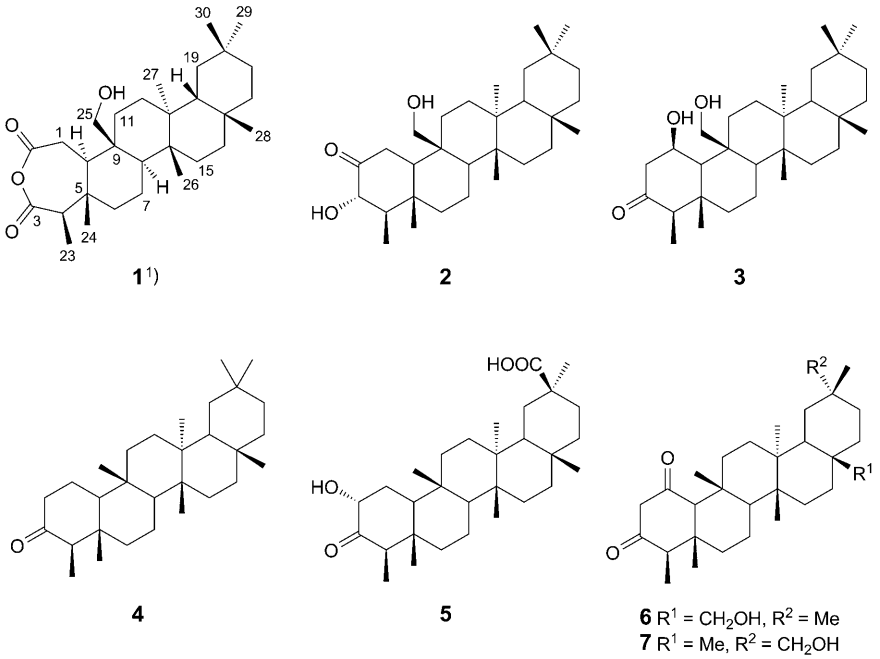

Fig. 1. *Structures of* **1**–**7**

Results and Discussion. – Compound **1**, named lobatanhydride, was isolated as an amorphous solid, and the HR-EI-MS exhibited a molecular-ion peak at *m*/*z* 472.3564 (M^+, calc. 472.3553), indicating the molecular formula $C_{30}H_{48}O_4$. Its IR spectrum displayed absorption bands characteristic of OH (3446 cm^{-1}) and CO (1775 cm^{-1}) groups. The ^{1}H-NMR spectrum (*Table 1*) showed signals for seven Me groups, one of them as *d* at δ(H) 1.09 (J = 7.1) and a HO–CH_2 signal at δ(H) 4.59 and 4.64 (*d* each, J = 7.6, 2 H). A *dd* at δ(H) 2.63 (J = 7.5, 18.9, 1 H), a *q* at δ(H) 2.55 (J = 7.1, 1 H), and a *d* at δ(H) 2.39 (J = 18.9, 1 H), were also observed.

The ^{13}C-NMR spectrum of **1** (*Table 1*) indicated the presence of 30 C-atoms, including seven Me, eleven CH_2, and four CH groups, and eight quaternary C-atoms. The characteristic downfield signal at δ(C) 72.6 was assigned to an OH-substituted C-atom. Moreover, the presence of two C=O groups at δ(C) 170.4 and 177.0, and the lack of absorption bands for COOH groups in the IR spectrum, suggested the presence of an anhydride moiety in the molecule. These data indicated that **1** is a friedelane triterpene with an anhydride and a OH group.

The full assignments of the^{1}H- and ^{13}C-NMR signals were performed by ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments. Therefore, the HMBC (*Table 1*) showed as most relevant correlations those of C(3)[1]) with H–C(4) and Me(23), and correlations of C(2), C(5), and C(9) with CH_2(1). Correlations of CH_2(25) with H–C(8), H–C(10),

[1]) Arbitrary numbering. For systematic names, see *Exper. Part*.

Table 1. ^1H- (400 MHz) *and* ^{13}C-*NMR*[a]) (100 MHz, DEPT) *Data of* **1** *in* $CDCl_3$[1]). δ in ppm, J in Hz.

	δ(H)	δ(C)	HMBC (C → H)
CH_2(1)	2.39 (d, J = 18.9; H_α)	27.8	H–C(10)
	2.63 (dd, J = 7.5, 18.9; H_β)		
C(2)		170.4	CH_2(1), H–C(10)
C(3)		177.0	H–C(4), Me(23)
H–C(4)	2.55 (q, J = 7.1)	47.3	H–C(10), Me(23), Me(24)
C(5)		38.9	CH_2(1), H–C(4), Me(24)
CH_2(6)	1.68–1.70 (m)	34.5	H–C(4), Me(24)
CH_2(7)	1.52[b])	17.1	
H–C(8)	1.48–1.51 (m)	52.0	H–C(10), Me(26)
C(9)		35.3	CH_2(1), CH_2(7), H–C(10)
H–C(10)	1.66 – 1.69 (m)	46.1	Me(24), Me(25)
CH_2(11)	1.84 (br. d, J = 12.8)	32.0	
CH_2(12)	1.41–1.44 (m)	30.1	Me(27)
C(13)		39.6	Me(26), Me(27)
C(14)		38.0	Me(26), Me(27)
CH_2(15)	1.27[b]), 1.48[b])	32.3	
CH_2(16)	1.55–1.58 (m)	35.7	Me(28)
C(17)		30.0	Me(28)
H–C(18)	1.52[b])	42.6	Me(27), Me(28)
CH_2(19)	1.36–1.40 (m)	35.2	Me(30)
C(20)		28.1	Me(29), Me(30)
CH_2(21)	1.27[b]), 1.48[b])	32.6	Me(29), Me(30)
CH_2(22)	0.95–0.98, 1.48–1.51 ($2m$)	39.1	Me(28)
Me(23)	1.09 (d, J = 7.1)	12.5	H–C(4)
Me(24)	1.13 (s)	20.6	H–C(10)
CH_2(25)	4.59, 4.64 ($2d$, J = 7.6)	72.6	H–C(8), H–C(10), CH_2(11)
Me(26)	0.91 (s)	20.2	H–C(8), CH_2(15)
Me(27)	1.06 (s)	18.5	H–C(18)
Me(28)	1.18 (s)	32.1	H–C(18)
Me(29)	0.95 (s)	35.0	Me(30)
Me(30)	1.00 (s)	31.6	Me(29)

[a]) Data are based on DEPT and HSQC experiments. [b]) Overlapping signals.

and CH_2(11) were also observed. The relative configuration of **1** was determined on the basis of a ROESY experiment and biogenetic considerations. Thus, the correlations observed between the H-atom signal at δ(H) 2.39 (d, J = 18.9) and the H-atom signal at δ(H) 1.66–1.69 (m, H–C(10)), and of the H-atom signals at δ(H) 2.63 (dd, J = 7.5, 18.9) and the H-atom signal at δ(H) 1.13 (s, Me(24)), allowed the assignment of the α- and β-CH_2(1) H-atoms. Moreover, correlations of the H-atom signals at δ(H) 4.59, 4.64 (2 d, J = 7.6, CH_2(25)), and the H-atom signals at δ(H) 1.18 (s, Me(28)) and δ(H) 1.00 (s, Me(30)), were also observed. In accord with all these data, the structure of lobatanhydride (**1**) was established as 2,3-*seco*-2,3-epoxy-25-hydroxy-2,3-friedelanedione[1]).

Although celastranhydride [6], a triterpene anhydride, has been reported from species of the Celastraceae family, compound **1** represents the first example of a triterpene anhydride with the *A* ring expanded to a seven-membered ring.

Compound **2** was isolated as an amorphous solid. Its HR-EI-MS revealed a molecular weight at *m/z* 458.3772, consistent with the molecular formula $C_{30}H_{50}O_3$ and six degrees of unsaturation. The IR spectrum displayed absorption bands for OH (3479 cm^{-1}) and C=O (1713 cm^{-1}) groups. The ^{1}H-NMR spectrum (*Table 2*) showed signals for seven Me groups, one of them as a *d* at δ(H) 1.09 ($J = 6.6$). The signals at δ(H) 3.83 (*dd*, $J = 2.3$, 11.7, 1 H) and δ(H) 4.00 and 4.05 (*d* each, $J = 11.5$, 2 H) suggested the presence of a secondary and a primary OH group in **2**, which was supported by the signals at δ(C) 63.7 and 77.2 in the ^{13}C-NMR spectrum (*Table 2*). The signals at δ(H) 3.05 (*dt*, $J = 1.2$, 14.1, 14.3, 1 H) and δ(H) 2.70 (*dd*, $J = 2.8$, 14.1, 1 H) were assigned to a CH_2 group attached to a C=O group. The ^{13}C-NMR and DEPT spectra of **2** showed resonances for 30 C-atoms, including seven Me, eleven CH_2, and

Table 2. *^{1}H-NMR* (400 MHz) *and ^{13}C-NMR*[a]) (100 MHz, DEPT) *Data of* **2** *and* **3** *in $CDCl_3$*[1]). δ in ppm, *J* in Hz.

	2		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)
CH_2(1) or CH(1)	3.05 (*dt*, $J = 1.2$, 14.1, 14.3; H_β) 2.70 (*dd*, $J = 2.8$, 14.1; H_α)	39.0	4.00[b])	75.8
C(2) or CH_2(2)		211.4	2.54–2.56 (*m*; H_α), 1.98[b]) (H_β)	35.0
H–C(3) or C(3)	3.83 (*dd*, $J = 2.3$, 11.7)	77.2		212.3
H–C(4)	1.30–1.34 (*m*)	54.8	2.26 (*q*, $J = 6.7$)	55.9
C(5)		38.0		43.3
CH_2(6)	1.91–1.96 (*m*)	41.1	1.85–1.88 (*m*)	41.8
CH_2(7)	1.47–1.51 (*m*)	17.3	1.46–1.49 (*m*)	17.9
H–C(8)	1.39–1.44 (*m*)	53.8	1.52[b])	53.8
C(9)		41.8		42.0
H–C(10)	1.37[b])	60.8	1.62 (*d*, $J = 11.2$)	57.4
CH_2(11)	1.86–1.91 (*m*)	29.5	1.98[b])	30.2
CH_2(12)	1.37[b])	30.5	1.38–1.41 (*m*)	31.0
C(13)		39.7		39.8
C(14)		38.2		37.5
CH_2(15)	1.29[b]), 1.47[b])	32.7	1.30[b]), 1.50[b])	32.7[b])
CH_2(16)	1.38–1.41 (*m*), 1.56[b])	35.9	1.52[b])	36.0
C(17)		30.0		29.9
H–C(18)	1.56[b])	42.6	1.52[b])	42.7
CH_2(19)	1.18–1.20 (*m*)	35.3	1.20–1.25 (*m*)	35.3
C(20)		28.1		28.1
CH_2(21)	1.29[b]), 1.47[b])	32.7	1.30[b]), 1.50[b])	32.7[b])
CH_2(22)	0.95–0.98, 1.48–1.51 (2*m*)	39.2	0.94–0.96 (*m*), 1.50[b])	39.3
Me(23)	1.09 (*d*, $J = 6.6$)	10.9	0.97 (*d*, $J = 6.8$)	6.7
Me(24)	1.21 (*s*)	14.0	0.82 (*s*)	14.7
CH_2(25)	4.00, 4.05 (2*d*, $J = 11.5$)	63.7	3.85 (*d*, $J = 11.7$), 4.00[b])	63.0
Me(26)	1.00 (*s*)	20.3	1.07 (*s*)	20.1
Me(27)	0.96 (*s*)	18.5	0.99 (*s*)	18.6
Me(28)	1.17 (*s*)	32.2	1.17 (*s*)	32.2
Me(29)	0.95 (*s*)	35.0	0.95 (*s*)	35.0
Me(30)	0.98 (*s*)	31.7	0.99 (*s*)	31.7

[a]) Data are based on DEPT and HSQC experiments. [b]) Overlapping signals.

five CH groups, and seven quaternary C-atoms. A notable feature was the appearance of a downfield quaternary-C-atom signal at δ(C) 211.4, which was due to a C=O group. These data suggested that **2** is a friedelane triterpene with one ketone and two OH groups.

In the ^{1}H,^{13}C-HMBC experiment (*Fig. 2*) of **2**, correlations were observed between CH_2(1)[1]) and C(2), C(3), C(5), and C(10), between H–C(3) and C(4) and C(23), and between CH_2(25) and C(9), C(10), and C(11). Thus, the C=O group was located at C(2), and the primary and secondary OH groups were situated at C(25) and C(3), respectively. The relative configuration of **2** was established on the basis of the ROESY experiment and PC Model [9]. Thus, ROE correlations were observed between the H-atom signal at δ(H) 3.05 (*dt*, J = 1.2, 14.1, 14.3, CH_2(1)) and the H-atom signal at δ(H) 1.21 (*s*, Me(24)), between the H-atom signal at δ(H) 3.83 (*dd*, J = 2.3, 11.7) and the H-atom signals at δ(H) 3.05 and 1.21, and between the H-atom signals at δ(H) 4.00 and 4.05 (2*d*, J = 11.5) and the H-atom signals at δ(H) 1.21 (*s*, Me(24)) and 1.00 (*s*, Me(26)). These correlations allowed the assignments of the α- and β-H-atoms at C(1) and revealed that the secondary OH group at C(3) was in a α-configuration and the primary OH group was at C(25) in a β-configuration as shown in *Fig. 1*.

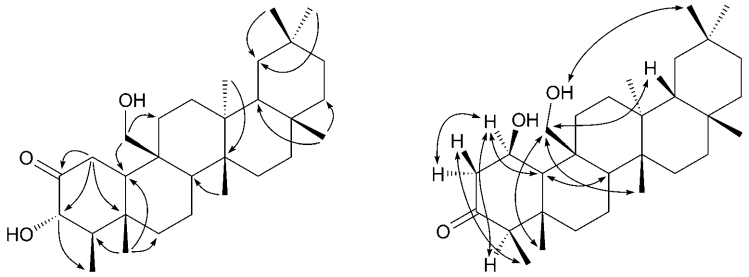

Fig. 2. *Significant HMBC* (H → C) *data for* **2** (left) *and selected ROESY correlations for* **3** (right)

All of these data and a comparison with those found in the literature for 3α-hydroxyfriedelan-2-one [10] established the structure of **2** as 3α,25-dihydroxy-friedelan-2-one[1]).

Compound **3** had the same molecular weight as **2**, and interpretation of the ^{1}H- and ^{13}C-NMR data (*Table 2*) showed it to be a friedelane triterpene with one ketone (δ(C) 212.3), a $HOCH_2$ group (δ(H) 3.85, 4.00, δ(C) 63.0) and a HO–CH group (δ(H) 4.00, partially overlapping, δ(C) 75.8). The presence of absorption bands at 3454 and 1712 cm^{-1}, and 273 nm, in the IR and UV spectra, respectively, supported the presence of these groups. This was confirmed by 2D-NMR experiments (HMBC, ROESY). Thus, the correlations observed in the HMBC experiment between the C-atom signal at δ(C) 212.3 and the H-atom signals at δ(H) 0.97 (*d*, J = 6.8, Me(23)), 2.26 (*q*, J = 6.7, H–C(4)), 2.54–2.56 (CH_2(2)), and 4.00 (partially overlapping, H–C(1)) located the C=O group at C(3)[1]). Moreover, correlation of the H-atom signal at δ(H) 4.00 with the C-atom signals at δ(C) 212.3 (*s*, C(3)), 42.0 (*s*, C(9)), and 57.4 (*d*, C(10)) and correlation of the H-atom signals at δ(H) 3.85, 4.00 with C(9) and C(10), located the secondary and primary OH groups at C(1) and C(25), respectively.

The relative configuration of the OH groups were determined as β by analysis of a ROESY experiment (*Fig. 2*), showing ROE correlations between H–C(1) and H–C(4) and H–C(10), and between CH_2(25) and Me(24) and Me(26). All these data established the structure of **3** as 1β,25-dihydroxyfriedelan-3-one[1]).

Compounds **4**–**7** were elucidated as friedelin (**4**) [11], 2α-hydroxy-3-oxofriedelan-30-oic acid (**5**) [12], 28-hydroxyfriedelan-1,3-dione (**6**) [13], and 29-hydroxyfriedelan-1,3-dione (**7**) [14], respectively, with the help of spectroscopic techniques. Their ^{1}H- and ^{13}C-NMR data were in agreement with the ones reported in the literature.

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Experimental Part

General. TLC: *Polygram® Sil G/UV$_{254}$*, *Macherey–Nagel* foils. Purification was performed using silica gel (SiO_2; 40–63 µm, *Merck*, and *HPTLC-Platten-Sil 20 UV$_{254}$*, *Macherey–Nagel*) and *Sephadex LH-20* (*Pharmacia*). Optical rotations: *Perkin-Elmer 241* automatic polarimeter; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. UV Spectra: *Jasco V-560* instrument, in abs. EtOH. IR Spectra: *Bruker IFS 55* spectrophotometer, in $CHCl_3$. ^{1}H- and ^{13}C-NMR Spectra: *Bruker Avance* at 400 MHz and 100 MHz, resp. EI-MS and HR-EI-MS: *Micromass Autospec* spectrometer.

Plant Material. Crossopetalum lobatum LUNDELL was collected in Margarita, Colon, Panama, in February 2002. A voucher specimen (FLORPAN 1051) is deposited with the Herbarium of the University of Panama.

Extraction and Isolation. The dried leaves of *C. lobatum* (0.5 kg) were exhaustively extracted by refluxing with EtOH, giving 112 g of dried extract. The EtOH soluble extract was taken to vacuum dryness and submitted to a SiO_2 flash chromatography (FC), using hexane/AcOEt mixtures of increasing polarity as eluent to afford 60 fractions, which were grouped after TLC. *Fr. 30–42* were combined, rechromatographed on SiO_2, and eluted with hexane/AcOEt (1:1) to give **1** (3.2 mg), **2** (6.0 mg), **3** (5.4 mg), **4** (160.0 mg), **5** (5.1 mg), **6** (4.0 mg), and **7** (14.5 mg).

Lobatanhydride (=*(5*R*,5a*S*,7a*R*,7b*R*,9a*R*,13a*R*,13b*S*,15a*R*,15b*R*)-Icosahydro-15a-(hydroxymethyl)-5,5a,7b,9a,12,12,13b-heptamethylchryseno[1,2-*d*]oxepine-2,4-dione*; **1**). White amorphous solid. $[\alpha]_D^{20} = -4.0$ ($c = 0.20$, $CHCl_3$). UV (EtOH): 221 (3.03), 219 (3.03). IR (film): 3446, 2952, 2860, 1775, 1730, 1462, 1414, 1382, 1200, 1056, 757. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 472 (3, M^+), 457 (13, [M – Me]$^+$), 410 (6, [457–46, HCOO]$^-$), 398 (13, [M – $MeCH_2COOH$]$^+$), 348 (6), 330 (7), 319 (7), 275 (13), 261 (18), 245 (19), 221 (16), 205 (100), 191 (32), 147 (36), 123 (61), 109 (74), 95 (99). HR-EI-MS: 472.3564 (M^+, $C_{30}H_{48}O_4^+$; calc. 472.3553), 398.3270 ([M – $C_3H_6O_2$]$^+$, $C_{27}H_{42}O_2^+$; calc. 398.3184).

3α,25-Dihydroxyfriedelan-2-one (=*(3*S*,4*R*,4a*S*,6b*R*,8a*R*,12b*S*,14a*R*)-Icosahydro-3-hydroxy-14a-(hydroxymethyl)-4,4a,6b,8a,11,11,12b-heptamethylpicen-2(1*H*)-one*; **2**). White amorphous solid. $[\alpha]_D^{20} = +3.0$ ($c = 0.09$, $CHCl_3$). UV (EtOH): 274 (2.56). IR (film): 3479, 2926, 2858, 1713, 1464, 1386, 1057. ^{1}H-NMR: 3.52 (*d*, $J = 3.5$, HO–C(3), 1 H); for other signals, see *Table 2*. ^{13}C-NMR: *Table 2*. EI-MS: 458 (3, M^+), 443 (5, [M – Me]$^+$), 427 (18, [M – CH_2OH]$^+$), 409 (7, [427 – H_2O]$^+$), 369 (10), 305 (53), 205 (88), 123 (61), 109 (73), 95 (94), 69 (100). HR-EI-MS: 458.3772 (M^+, $C_{30}H_{50}O_3^+$; calc. 458.3760).

1β,25-Dihydroxyfriedelan-3-one (=*(1*R*,4*R*,4a*S*,6b*R*,8a*R*,12b*S*,14a*S*)-Icosahydro-1-hydroxy-14a-(hydroxymethyl)-4,4a,6b,8a,11,11,12b-heptamethylpicen-3(2*H*)-one*; **3**). White amorphous solid. $[\alpha]_D^{20} = -17.0$ ($c = 0.20$, $CHCl_3$). UV (EtOH): 273 (2.51), 262 (2.51). IR (film): 3454, 2946, 2867, 1712, 1457, 1386, 1049, 979. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 458 (4, M^+), 427 (22, [M – CH_2OH]$^+$), 409 (5, [427 – H_2O]$^+$), 369 (8), 305 (61), 275 (14), 263 (17), 247 (19), 233 (11), 205 (100), 191 (28), 123 (44), 109 (56), 95 (70). EI-MS: 458.3789 (M^+, $C_{30}H_{50}O_3^+$; 458.3760).

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