A novel D:A-friedooleanane triterpenoid and other constituents of the stem bark of *Dichapetalum barteri Engl.*

Ivan Addae-Mensah,[a]* Sam Adu-Kumi[a], Reiner Waibel[b], and Isaac V. Oppong[a]

[a]*Department of Chemistry, University of Ghana, Legon, Ghana*
[b]*Institute of Pharmacy and Food Chemistry, University of Erlangen, Germany*
E-mail: a-mensah@ug.edu.gh

Dedicated to Professor Berhanu M. Abegaz on his 60th Birthday

Abstract

A novel pentacyclic triterpenoid, 2β-hydroxy-3-oxo-D:A-friedoolenanan-29-oic acid has been isolated from the methanol extract of the stem bark of *Dichapetalum barteri,* and characterised by spectroscopic methods. Also reported for the first time from the plant are two lupane-type triterpenoids betulinic acid and betulonic acid, the friedelane triterpenoids *syn*:canophyllal, *syn*:canophyllol, friedelan-3-one and friedelan-3α-ol (epifriedelinol), the more common triterpenoids β-sitosterol and stigmasterol, as well as seven long chain esters of ferulic acid.

Keyword: *Dichapetalum barteri, Dichapetalaceae,* pentacyclic triterpenoids, 2β-hydroxy-3-oxo-D:A-friedooleanan-29-oic acid, betulinic acid, betulonic acid; canophyllol, canophyllal, ferulic acid esters

Introduction

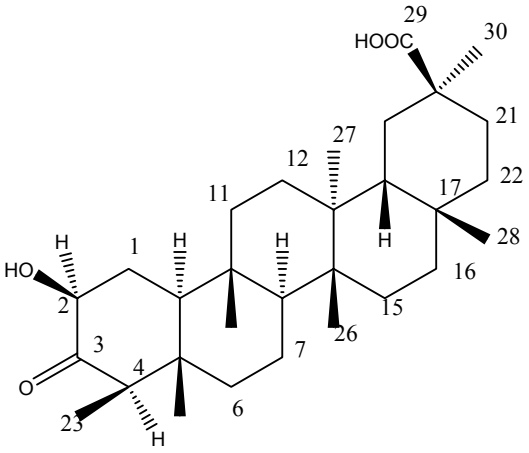
Dichapetalum barteri belongs to the small family Dichapetalaceae (syn. Chailletiaceae), which is distributed throughout the world's tropical and subtropical regions.[1] Some members of the family are known to be highly toxic, particularly to livestock, due to the presence of fluorinated carboxylic acids.[2] The fluorinated acids have been extensively studied. However, very little is known of the non-fluorinated constituents of this plant genus. *N*-methyl alanine and *N*-methyl serine have been reported from *Dichapetalum cymosun,*[3] while friedelin has been found in *D. gelonoides*.[4]

As part of our systematic investigation of the family *Dichapetalaceae* occurring in Ghana, we recently reported a new class of triterpenoids never reported from any other plant family. Eight of these have so far been reported, which we named Dichapetalins A to H.[5,6]

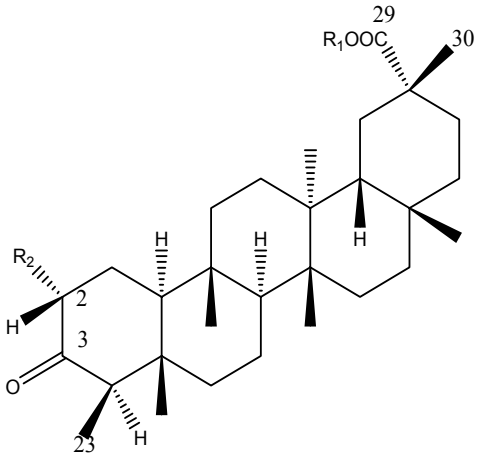
 ©ARKAT USA, Inc.

Biogenetically, their basic structures are characterised by the addition of a C_6-C_2-unit to a cyclodammarane skeleton. These compounds exhibit strong and selective *in-vitro* cytotoxicity.

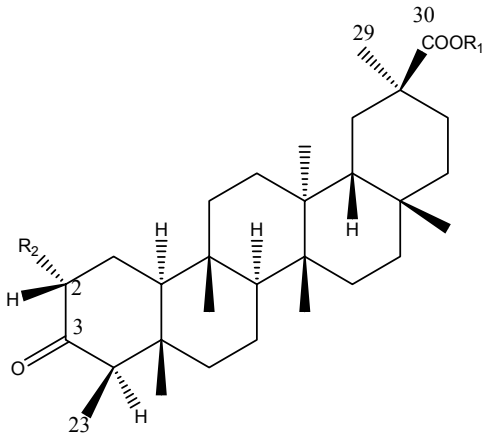
In the present paper, we report the results of studies on the relatively rare species, *D. barteri,* which is said to have strong rodenticidal activity[7] and is also toxic to goats.[8] Fractionation of the methanol extract of the stem bark of the plant yielded a novel pentacyclic triterpenoid identified as 2β-hydroxy-3-oxo-D:A-friedooleanan-29-oic acid **1**, which is isomeric with 2α-hydroxy populnonic acid (2α –hydroxy-octandronic acid) **2b** and 2α-hydroxy-3-oxo-D:A-friedooleanan-30-oic acid **3a**.[9,10]



1



2a. R_1 = R_2 = H
2b R_1 = H; R_2 = OH
2c.R_1 = Me; R_2 = OH
2d. R_1 = Me; R_2 = OAc



3a. R_1 = H; R_2 = OH
3b. R_1 = Me; R_2 =OH
3c. R_1 = Me; R_2 = OAc

Also obtained from the petrol and acetone extracts were several known triterpenoids including the two lupane-type triterpenoids betulinc acid and betulonic acid, as well as seven long-chain esters of E-ferulic acid with the straight chain C_{23} to C_{29} alkan-1-ols.

Results and Discussion

Column chromatography of the methanol extract of the stem bark of the plant gave five main fractions. Further purification of fraction 2 gave compound **1** as off-white needle-like crystals, mp 199-201^{o}C, brown with anisaldehyde reagent. MS gave a molecular ion peak at 472, $C_{30}H_{48}O_4$. ^{1}H-NMR showed signals for six angular methyl singlets at δ 0.71, 0.86, 0.99, 1.05, 1.07 and 1.31ppm as well as a methyl doublet (J = 7.8Hz) at δ 0.96 ppm, which was assigned to methyl protons at position 23. A signal at δ 2.28 ppm (1H, q, J = 7.8Hz), was assigned to a proton at C-4 vicinal to the C-3 carbonyl carbon. The proton geminal to the OH group at C-2 appears as a multiplet at δ 4.12, compared to the C-2 proton in the α-isomer **2b** which occurs at 3.96 and that of compound **3a** (δ 4.00). The remaining 23 protons were assigned as methylene and methine protons occurring between δ 2.52 and 1.15 ppm. A broad signal at δ 3.7, removed by H-D exchange, confirmed the presence of an OH group. The assignment of the ^{1}H-NMR signals is summarised in Table 1.

Comparison of the melting point, ^{1}H and ^{13}C-NMR spectra (Tables 1 and 2) of compound **1** with those of populnonic acid (3-oxo-D:A friedooleanan-29-oic acid) **2a**, 2α-hydroxy populnonic acid methyl ester (*syn*.2α-hydroxy-3-oxo-D:A-friedooleanan-29-oic acid methyl ester) **2c**, 3-oxo-D:A-friedooleanan-30-oic acid methyl ester **3b** and the acetate of 2α-hydroxy-3-oxo D:A-friedooleanan-30-oic acid methyl ester **3c** suggests that compound **1** is different from all these related compounds with which it has the same basic skeleton, and confirms the presence and orientation of the carboxyl and the hydroxyl groups at C-20 and C-2 respectively, and the carbonyl group at C-3.

^{13}C-NMR of the compound showed a singlet at δ 212.4 ppm, assigned to a C-3 ketone, and a doublet at δ 75.2 ppm due to carbon 2. A signal at δ 183.3 ppm was attributed to a carboxyl carbon at C-29. The remaining 27 signals in the ^{13}C-NMR are sp^3- hybridised signals occurring between 75 and 6.5 ppm. DEPT measurements indicated the presence of 8 quaternary, 5 tertiary (CH), 10 secondary (CH_2) and 7 primary (CH_3) carbons. The assignment of the ^{13}C-signals using DEPT measurements are as summarised in Table 1. Comparison of the ^{13}C-signals of **1** with those of **2a, 2b, 3b** and **3c** is as summarised in table 3.

Table 1. ^{1}H and ^{13}C-NMR data for compound **1**

Carbons	DEPT	δ_H (ppm)	δ_C (ppm)	HMBC long-range ^{1}H-^{13}C couplings. Carbons to which protons are coupled
1	CH_2	2.43 (2H, m)	32.6	2,3,5,10
2	CH	4.12 (1H, m); 3.7 (OH br, s)	75.0	1
3	-	-	212.4	-
4	CH	2.28 (1H,q, J = 7.8Hz)	55.6	3,5,10,23,24
5	-	-	43.1	-
6	CH_2	1.75, 1.8 (2H, not well resolved even in expanded spectrum)	41.2	5,7,8,10
7	CH_2	**	18.1	-
8	CH	1.38 (IH, m)	53.2	5,6,7,9,10,13,14,26
9	-	-	37.4	-
10	CH	1.54 (1H, m)	56.5	1,2,3,5,9,11,14,24
11	CH_2	1.50 (2H, m)	35.3	1,10.12.14.25
12	CH_2	1.45 (2H m)	30.2	10,11,13,14,25,27
13	-	-	39.7* s	-
14	-	-	38.1* s	-
15	CH_2	**	32.8	
16	CH_2	**	35.4	-
17	-	-	29.6 s	-
18	CH	1.60 (1H m)	42.5	11,12,13,17,19,20,27
19	CH_2	1.98 (2H, dd J_1 =12.5; J_2 = 5.5 Hz)	31.3	-
20	-	-	40.3 s	-
21	CH_2	dd, J_1 = 13.5Hz, J_2 = 5.0 Hz	28.2	-
22	CH_2	**	38.2	-
23	CH_3	0.96 (3H,d, J=7.8Hz)	6.5	3,4,5
24	CH_3	0.71 (3H, s)	14.7	4,5,6,10
25	CH_3	0.86 (3H, s)	17.8	8,9,10,11
26	CH_3	0.99 (3H s)	20.9	8,13,14,15
27	CH_3	1.05 (3H s)	17.7	12,13,14,18
28	CH_3	1.07 (3H s)	31.8	16,17,18,22
29	-	-	183.3	-
30	CH_3	1.31 (3H, s)	32.0	19,20,21

*Interchangeable values **Signals not well resolved

Table 2. Comparison of ^{1}H-NMR spectral data for compounds 1, 2c, 2d, 3a and 3c [9, 10] δ_H (ppm) (in $CDCl_3$)

Position	**1**	**2c**	**2d**	**3a**	**3c**
2	*4.12 (1H, m); 3.7 (OH br, s)	3.96 q	4.89 br. s	4.00 dd	
3	-	-	-	-	
4	*2.28 (1H,q, J = 7.8Hz)	2.92 q. J = 7	2.62 q	2.90 q	2.64 q
19	1.98 (1H, dd, J_1 =12.5Hz, J_2 = 5.5Hz)				
22	-	0.97 dd, J_1 =13.5, J_2 = 4	0.95 br		
23	*0.96 (3H,d, J 7.8Hz)	0.84 d	0.86 d	0.76 d J 6.7	0.88 d J 5.3
24	0.71 (3H, s)	0.64 s	0.67 s		0.69 s
25	0.86 (3H, s)	0.86 s	0.82 s		0.85 s
26	0.99 (3H s)	0.86 s	0.82 s		0.86 s
27	*1.05 (3H s)	0.86 s	0.84 s		0.87 s
28	1.07 (3H s)	1.07 s	1.06 s		1.08 s
29	-				
30	*1.31 (3H, s)	1.17 s	1.16 s	1.19 s	1.19 s

*Significantly different chemical shifts

The HMBC measurements (Fig. 1) showed that compound **1** exhibited two-bond couplings between H-4 and the C-3 carbonyl carbon, between H-4 and C-5, as well as between H-1 and C-10. There is also a three-bond coupling between H-1 and C-3 and H-1 and C-5, confirming the structure of ring A. The protons of the angular methyl H-30 couple with the carboxyl carbon, locating the carboxyl carbon to be attached to C-20. The proton at C-2 is coupled to C-1 only, unlike **2b** and its derivatives **2c** and **2d** in which this proton is coupled to C-3, C-4and C-10.

The mass spectrum gave the molecular ion at m/z 472, with other significant peaks at 426 (M+ - HCOOH), 411 (426-CH_3), 290 and 289, representing a fraction resulting from cleavage of ring D followed by loss of H, 207, 189, 177, 175. The base peak appeared at m/z 155, due to a fraction resulting from loss of CH_3 and H_2O from M^+ followed by cleavage of ring D.

In addition to compound **1** a number of other known triterpenoids including the cytotoxic agent betulinic acid, betulonic acid, canophyllal, canophyllol, friedelin and a mixture of β-sitosterol and stigmasterol were also isolated for the first time from the plant.

Table 3. Comparison of ^{13}C-NMR spectra of **1, 2a, 2b, 3b** and **3c**, Signals in $CDCl_3$, δ ppm

Carbons	Compounds				
	1	**2a**	**2b**	**3b**	**3c**
1	32.6	22.2	29.4	22.3	28.2
2	75.0	41.5	73.1	44.5	76.5
3	212.4	213.3	213.3	212.6	208.1
4	55.6	58.2	51.9	58.3	54.3
5	43.1	42.1	42.4	42.1	43.1
6	41.2	41.3	40.4	41.3	41.0
7	18.1	18.2	17.7	18.2	18.2
8	53.2	50.7	49.7	53.2	50.4
9	37.4	37.4	36.3	37.5	36.6
10	56.5	59.8	51.6	59.5	53.4
11	35.3	35.4	34.4	35.5	35.1
12	30.2	30.2	29.0	30.3	30.1
13	39.7*	39.1	38.8	39.7	39.2
14	38.1*	39.2	38.6	38.1	39.4
15	32.8	29.7	28.5	32.8	30.4
16	35.4	36.1	35.7	35.5	36.2
17	29.6	30.1	29.8	29.5	29.1
18	42.5	44.2	44.0	42.5	44.5
19	31.3	29.5	29.8	31.4	36.9
20	40.3	40.4	40.0	40.5	40.6
21	28.2	29.5	29.5	28.6	29.9
22	38.2	36.6	36.0	38.3	29.4
23	6.5	6.2	6.0	6.8	6.5
24	14.7	14.6	13.5	14.6	14.1
25	17.8	18.0	17.0	17.6	18.4
26	20.9	18.4	15.4	20.9	16.1
27	17.7	16.3	17.9	17.6	17.5
28	31.8	31.8	31.3	31.9	31.8
29	183.3	184.5	178.6	31.8	31.9
30	32.0	31.6	31.5	179.4	179.4

*Signals interchangeable.

 ©ARKAT USA, Inc.

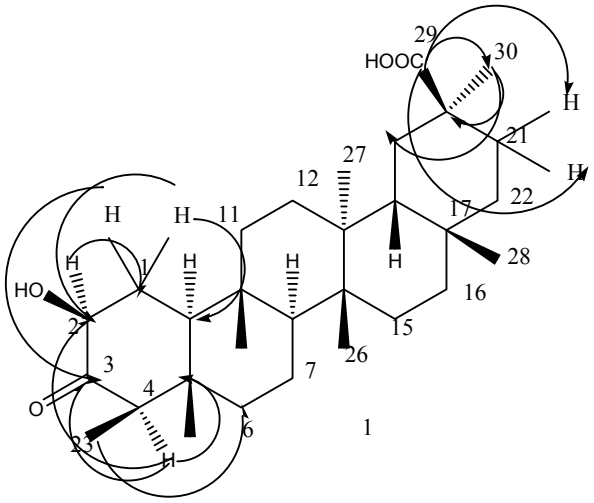
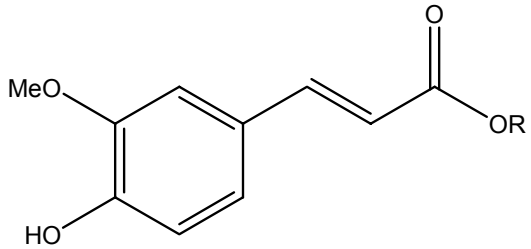


Figure 1

HPLC separation of a white crystalline solid obtained from repeated column chromatography of one of the fractions of the acetone extract gave seven homologous long chain esters of E-ferulic acid (**4a**-**4g**), with molecular ions M^+ at m/z 516, 530, 544, 558, 572, 586 and 600. The most abundant of these was E-ferulic acid hexacosyl ester (**4d)**, the others **(4a**, **4b**, **4c**, **4e**, **4f**, and **4g)** being the tricosyl, tetracosyl, pentacosyl, heptacosyl, octacosyl and nonacosyl esters respectively. These compounds were characterised by spectroscopic methods (UV, including shift behaviour on addition of NaOH, ^{1}H NMR, ^{13}C NMR, MS) and comparison of the resulting data with those reported in the literature.[11,12] All these compounds are reported for the first time in *Dichapetalum barteri.*



4a, R = $CH_3(CH_2)_{22}$; 4b, R = $CH_3(CH_2)_{23}$; 4c, R = $CH_3(CH_2)_{24}$; 4d, R = $CH_3(CH_2)_{25}$
4e, R = $CH_3(CH_2)_{26}$; 4f, R = $CH_3(CH_2)_{27}$; 4g, R = $CH_3(CH_2)_{28}$

Experimental Section

General Procedures. Mps uncorr; Column chromatography, silica gel 60 (Fluka), TLC:0.25 mm silica gel N/UV_{254} (Macherey-Nagel), detection by UV_{254} and by anisaldehyde reagent No 15 according to Stahl [13] UV in MeOH, ^{1}H-NMR at 360 MHz and ^{13}C-NMR at 90 MHz on a Brűker AM 360 instrument, in $CDCl_3$ unless otherwise stated, δ in ppm, TMS as internal standard. EI-MS on a Finnigan TSQ 700 instrument 70 eV, m/z (rel. int > 10% unless key ions.

Plant materials. The stem bark of *Dichapetalum barteri* was collected from a forest grove at Pokuase near Accra. Identification was by (the late) Mr. A. A. Enti, former Curator of the National Herbarium and, Department of Botany, University of Ghana Legon CEO of Forestry Enterprises Limited. Voucher specimens are deposited at the National Herbarium at the University of Ghana, Legon.

Extraction and isolation

2β-hydroxy-3-oxo-D:A-friedooleanan-29-oic acid 1. The air-dried and pulverised stem bark (1.4 kg) was first defatted by Soxhlet extraction with petrol (60-80^{o}). From this extract was obtained mainly fredelan-3-one, D:A friedo-oelanan-3β-ol (epifriedelinol) and β-sitosterol. The plant material was then further extracted for 48 hours with methanol to yield a brown sticky mass (7.88 g). This was chromatographed on a silica gel column with petrol:$CHCl_3$ of increasing polarity to give five main fractions. Trituration of the solid from the second fraction with acetone and subsequent recrystallisation from methanol gave pale yellow to off-white needle-like crystals of **1** (14.5 mg), mp 199-201, UV inactive, brown with anisaldehyde reagent. ^{1}H and ^{13}C Spectroscopic data see tables. MS (rel. int. %) m/z 472 (M^{+}, 8), 426 (14), 411 (7), 373 (7), 318 (14), 284 (30), 264 (22), 207 (15), 191 (44), 163 (81), 155 (100), 109 (44), 69 (45), 55 (58).

Other constituents obtained from the methanol extract were a homologous series of long chain fatty acids (C-16 to C-30), characterised as the methyl esters by GC-MS.

Ferulic acid Esters (4a - 4h). Dried and pulverised stem bark (2.8 Kg) was percolated with cold acetone for 48 hours to give a viscous syrup (55 g). Repeated fractionation of 9 g of this syrup by column chromatography using petrol:$CHCl_3$ of increasing polarity yielded six main fractions. Further purification of fraction 1 gave mainly friedelan-3-one and friedelan 3β-ol. The second and third fractions were combined and rechromatographed with petrol:EtOAc (15:1) to yield a white solid (106 mg), recrystallised from methanol, violet with anisaldehyde reagent, fluorescing intense blue in UV mp 77-78^{o}. Further purification by preparative HPLC yielded seven long-chain esters of ferulic acid with C_{23} to C_{29} moieties, with the hexacosyl ester (20 mg) being the most abundant. IR ν_{max} 3525, 2850, 1725, 1640, 1605, 1520, 1450, 1370, 1320, 1290, 1275, 1220, 1175, 1150, 1125, 1030, 930, 915, 885, 825, 805 and 730 cm^{-1}. UV λ_{max} 216, 234 (sh), 297, 324 nm; + NaOH 212, 250, 300, 308, 377 nm. Other spectroscopic characteristics in agreement with the literature [11, 12]. The yields of the other esters from the HPLC separation were tricosyl ester (0.2 mg), tetracosyl ester (5.2 mg), pentacosyl ester (3.8 mg), heptacosyl ester (2.0 mg), octacosyl ester (2.2 mg) and nonacosly ester (2.6 mg).

Other compounds obtained from the acetone extract are betulonic acid, betulinic acid, syn-canophyllol and syn-canophyllal.

Acknowledgements

A study leave with pay granted to one of us (S A-K) by the Ministry of Education of Ghana through the Ghana Education Service is gratefully acknowledged. Thanks are also due to the Deutsche Forschungsgemeinschaft and the Fonds the Chemischen Industrie in Germany for financial support.

References

1. Dalziel, J. M.; Hutchinson, J. *Useful Plants of West Tropical Africa.* Crown Agents: London, 1955; p 171.
2. Peters, R. *Endeavour* **1954***, 13*, 147.
3. Eloff, J. N.; Grobbelaar, N. *Phytochemistry* **1969**, *8,* 2201.
4. Desai, H. K.; Gawad, D. H.; Govindachari, T. R.; Joshi, B. S.; Kamat, V. N.; Modi, J. D.; Mohamed, P. A.; Parthasarathy, P. C; Patankar, S. J.; Sidhaye, A. R.; Viswanathan, N. *Indian J. Chem.***1970**, *8*, 851.
5. Achenbach, H.; Asunka, S. A.; Waibel, R.; Addae-Mensah, I.; Oppong, I. V. *Nat. Prod. Letters* **1995**, *7,* 93.
6. Addae-Mensah, I.; Waibel, R.; Asunka, S. A.; Oppong, I. V.; Achembach, H. *Phytochemistry***1996**, *43*, 649
7. Hall, R. J. *New Phytol.* **1972**, *71,* 855.
8. Nwude, N.; Parsons, L. E.; Aduadi, O. A. *Toxicology* **1977**, *7*, 23.
9. Estrada, R.; Cardenas, J.; Baldomero, E.; Rodrigues-Hahn, L. *Phytochemistry* **1994***, 36,* 747.
10. Gonzalez, N. L.; Alvarenga, A. G.; Ravelo, I. A.; Bazzochi, I. L. *J. Natural Products* **1995**, *58,* 570.
11. Achenbach, H.; Stőcker, M.; Constenla, M. A. *Z. Naturforsch.***1986**, *41c,* 164.
12. Addae-Mensah, I.; Achenbach, H.; Thoithi, G. N.; Waibel, R.; Mwangi, J. W. *Phytochemistry* **1992**, *31,* 2055.
13. Stahl, E. *Dunnschichtchromatographie* 2nd Edn., Springerl-Verlag: Berlin, **1962**.