Pursaethosides A−E, Triterpene Saponins from *Entada pursaetha*

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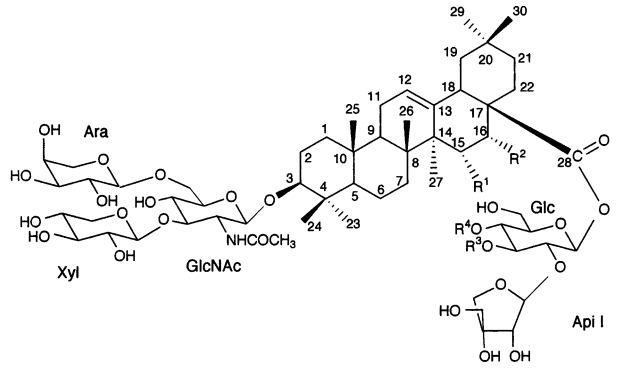
Five new triterpenoid saponins, pursaethosides A−E (**1**−**5**), were isolated from the *n*-BuOH extract of the seed kernels of *Entada pursaetha* along with the known phaseoloidin. The structures of **1**−**5** were elucidated mainly by spectroscopic data interpretation and chemical degradation. Pursaethosides C−E (**3**−**5**) possess as a common structural feature entagenic acid as aglycon, which is rare among triterpene saponins. Compounds **2**−**4** and phaesolidin were found to be not cytotoxic when tested against HCT 116 and HT-29 human colon cancer cells.

Entada pursaetha DC. (Mimosaceae) is a large, woody, climbing vine. The seeds are extremely hard and poisonous. The leaves and stems, however, are an ingredient in the all-purpose medicinal teas of local people in western Cameroon. The kernels are also used by these local people as a common musical instrument for traditional dances. The seeds and roots of several related species, namely, *Entada africana, E. abyssinica*, *E. phaseolides*, and *E. scandens*, are used in indigenous medical systems for various ailments such as liver diseases,[1,2] sleeping sickness, stomachache, and hemorrhoids.[3] Furthermore, they have emetic and febrifuge activities.[4] The occurrence of oleanolic acid, echinocystic acid, and entagenic acid glycosides has been reported from these species.[5−9] Previous phytochemical investigations of *E. pursaetha* have described several natural products structurally related to amino acid glycosides[10] and triterpenoids.[11] Although some authors have reported the presence of hemolytic and toxic saponins from *Entada* species,[12] nothing is known about their structures. In our search for new biologically active glycosides from plants grown in Cameroon,[13−16] we have investigated the seed kernels of *E. pursaetha*. Herein, we report the isolation and structure elucidation of five new triterpene saponins designated as pursaethosides A−E (**1**−**5**) along with the known glycoside phaseoloidin[17] from the seed kernels of this plant.



	R^1	R^2	R^3	R^4
1	H	H	H	H
2	H	OH	H	H
3	OH	OH	H	H
4	OH	OH	H	Api II
5	OH	OH	Api II	Glc II

Results and Discussion

A successive maceration of the powdered seed kernels of *E. pursaetha* in CH_2Cl_2−MeOH (1:1) and MeOH yielded after evaporation of solvent a crude extract that was suspended in water and successively partitioned between hexane, EtOAc, and *n*-BuOH. Partial evaporation of the EtOAc solution afforded the known phaseoloidin[17] upon crystallization, and the *n*-BuOH layer yielded after evaporation of the solvent a crude saponin mixture. This mixture was fractionated by vacuum-liquid chromatography (VLC) over silica gel, and the different fractions, purified by repeated medium-pressure liquid chromatography (MPLC) over silica gel, yielded five saponins, pursaethosides A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**). Their structures were elucidated mainly by 1D and 2D NMR spectroscopy (COSY,

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Table 1. ^{13}C NMR (150 MHz) and ^{1}H NMR (600 MHz) Data for the Aglycon Moieties of Compounds **1**−**5** in Pyridine-d_5[a,b]

	1		**2**		**3**		**4**		**5**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.7	0.93, 1.50	38.7	0.95, 1.55	38.6	0.96, 1.50	37.2	[c]	38.4	0.90, 1.48
2	26.5	1.70, [c]	26.0	1.75, 2.18	26.0	1.75, 2.18	26.7	[c]	25.9	[c]
3	88.9	3.30	89.0	3.23 br d (10.7)	89.1	3.24 br d (10.7)	89.3	3.30	89.5	3.23
4	38.7		38.5		38.4		39.1		38.6	
5	55.2	0.74	55.2	[c]	55.4	[c]	55.8	[c]	55.2	0.78
6	18.5	[c]	18.4	[c]	18.1	[c]	20.9	1.60, 1.75	18.4	[c]
7	33.6	1.30, [c]	33.1	1.50[c]	36.4	1.88, 2.05	36.1	[c]	36.5	1.95, 2.12
8	40.7		39.6		39.5		41.6		40.9	
9	47.0	1.64	46.7	1.68	46.9	1.68	46.8	[c]	46.9	1.67
10	36.7		36.6		36.7		37.3		36.6	
11	23.0	0.80, 1.90	23.5	0.80, 1.95	23.4	0.81, 1.95	24.2	[c], 2.02	23.5	[c], 2.00
12	122.3	5.34 t-like	122.1	5.56 t-like	124.3	5.58 t-like	123.0	5.56 t-like	124.3	5.70 t-like
13	144.2		144.0		144.1		145.1		144.0	
14	42.0		41.6		47.4		48.8		47.1	
15	27.7	1.30, 2.02	35.7	1.85, 2.25	68.0	4.35 d (4.8)	68.2	4.50 d (4.8)	68.0	4.45 d (5.0)
16	23.5	0.92, [c]	73.5	5.24, br s	77.9	4.97 d (4.8)	78.9	5.02 d (4.8)	78.2	5.04
17	46.7		48.8		48.3		48.4		48.3	
18	41.0	3.02	41.0	3.28 br d (11.7)	41.4	3.30 br d (11.7)	41.6	3.30	41.5	3.40
19	46.1	1.20, 2.60	46.3	1.18, 2.60	46.7	1.21, 2.65	47.5	1.40, 2.60	46.3	1.28, 2.70
20	30.0		30.3		30.2		30.9		30.3	
21	35.0	[c]	36.4	1.85, 2.05	35.4	1.15, 2.25	35.4	[c]	35.4	1.26, 2.38
22	31.6	1.64, 1.80	31.2	2.08, 2.28	31.3	2.05, 2.20	31.2	[c]	31.2	2.18, 2.34
23	27.7	1.08 s	27.7	1.03 s	27.7	1.02 s	28.3	1.15 s	27.6	1.14 s
24	16.5	0.84 s	16.6	0.82 s	16.6	0.82 s	17.2	0.98 s	16.6	0.91 s
25	16.8	0.92 s	15.2	0.77 s	15.3	0.75 s	15.9	0.88 s	15.2	0.82 s
26	17.2	1.08 s	17.0	0.94 s	17.5	1.06 s	18.2	1.20 s	17.5	1.16 s
27	25.5	1.21s	26.7	1.71 s	20.3	1.69 s	20.9	1.80 s	20.3	1.76 s
28	175.4		175.9		175.5		174.2		175.7	
29	32.4	0.90 s	32.8	0.86 s	32.8	0.86 s	33.4	0.97 s	32.8	0.98 s
30	25.8	0.98 s	24.2	0.89 s	24.1	0.92 s	24.9	1.05 s	24.1	1.06 s

[a] Assignments are based on HSQC, HMBC, DEPT, TOCSY, and NOESY experiments; δ in ppm; proton coupling constants (J) in Hz are given in parentheses. [b] Overlapping ^{1}H NMR signals are reported without designated multiplicity. [c] Not determined.

TOCSY, NOESY, HMBC, HMQC) and by FABMS and HRESIMS as well as chemical degradation. One additional compound was isolated and identified by analysis of its spectroscopic data (FABMS and 2D NMR) as 2-*O*-β-D-glucopyranosyloxy-5-hydroxyphenylacetic acid (phaseoloidin), a known compound previously characterized from *Entada phaseoloides*.[17]

Pursaethoside A (**1**) was obtained as a white amorphous powder and has the molecular formula $C_{59}H_{95}NO_{25}$, which was determined from its positive-ion HRESIMS ($[M + Na]^+$ *m/z* 1240.6107 calcd 1240.6091) and confirmed by ^{13}C NMR and DEPT analysis. Its negative-ion FABMS showed a quasi-molecular ion peak at *m/z* 1216 $[M - H]^-$, indicating a molecular weight of 1217. Further fragment ion peaks were observed at *m/z* 1084 $[(M - H) - 132]^-$, 922 $[(M - H) - 132 - 162]^-$, 658 $[(M - H) - 132 - 162 - 2 \times 132]^-$, and 455 $[(M - H) - 132 - 162 - 2 \times 132 - 203]^-$, corresponding, respectively, to the successive loss of one pentosyl, one hexosyl, two pentosyls, and one acetylamino-deoxyhexosyl moiety. The fragment ion at *m/z* 455 corresponded to the pseudomolecular ion of the aglycon. Upon acid hydrolysis with 2 N TFA at 120 °C, **1** afforded an aglycon that was identified as oleanolic acid by co-TLC comparison with an authentic sample and from the 2D NMR spectra of **1** (Table 1) compared with literature values.[18,19] The sugars obtained from the saponin hydrolysate were identified as glucose, arabinose, xylose, and apiose by co-TLC comparison with authentic samples. However, the presence of an *N*-acetylamino group [^{1}H NMR δ_H 2.16 (3H, s, MeCO) and δ_H 9.02 (1H, br s, NH); ^{13}C NMR δ_C 23.0 and 171.4], together with the ^{1}H and ^{13}C NMR data of the C-2 of the glucose moiety (δ_H 4.44 and δ_C 57.0), suggested the presence of one 2-(acetylamino)-2-deoxyglucose (GlcNAc) unit from an analysis of previously published data.[20] In addition, the alkaline hydrolysis of **1** with a 5% KOH aqueous solution afforded a prosapogenin that by further acid hydolysis yielded oleanolic acid, two sugars (xylose and arabinose) identified by TLC comparison with authentic samples, and *N*-acetylglucosamine. On the basis of the above evidence, it was determined that compound **1** is a bidesmosidic glycoside of oleanolic acid with three sugars (*N*-acetylglucosamine, xylose, and arabinose) located at the C-3 position of the aglycon, with two others (glucose and apiose) linked at C-28 of the aglycon through an ester bond. This was confirmed by the downfield ^{13}C NMR chemical shifts of C-3 at δ_C 88.9 and the upfield ^{13}C NMR chemical shifts of C-28 at δ_C 175.4 (Tables 1 and 2).

The ^{1}H NMR spectrum of **1** displayed five sugar anomeric protons at δ_H 4.82 (d, J = 6.9 Hz), 4.94 (d, J = 7.2 Hz), 5.00 (d, J = 4.0 Hz), 5.96 (d, J = 7.1 Hz), and 6.22 (d, J = 3.0 Hz), giving correlations with five anomeric carbon atoms at δ_C 105.4, 104.1, 102.0, 93.9, and 110.2, respectively, in the HMQC spectrum (Table 2), confirming that this saponin contains five sugar units. The ring proton atoms of each monosaccharide residue were assigned starting from the readily identifiable anomeric proton using COSY with the aid of TOCSY and NOESY NMR spectra. After assignments of the protons, the ^{13}C NMR resonances of each sugar unit were identified by HMQC and further confirmed by HMBC. Evaluation of spin−spin couplings and chemical shifts then allowed the identification of one terminal α-arabinopyranosyl (T-Ara), one terminal β-xylopyranosyl (T-Xyl), one terminal β-apiofuranosyl (T-Api I), one 2-(acetylamino)-2-deoxy-β-glucopyranosyl (GlcNAc), and one β-glucopyranosyl (Glc I) ester unit. The absolute configuration of sugar residues was determined to be D for Glc, Xyl, and Api and L for Ara, by GC analysis of chiral derivatives of sugars in the acid hydrolysate (see Experi-

Table 2. ^{13}C NMR (150 MHz) and ^{1}H NMR (600 MHz) Data for the Sugar Moieties of Compounds **1**−**5** in Pyridine d_5[a,b]

	1		**2**		**3**		**4**		**5**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
C-3: GlcNAc 1	104.1	4.94 d (7.2)	104.1	4.93 d (6.8)	104.1	4.93 d (6.8)	105.0	5.00 d (6.8)	103.9	5.00 d (7.0)
2	57.0	4.44	57.0	4.42	57.0	4.42	58.1	4.50	56.4	4.48
3	79.7	4.35	79.6	4.32	79.7	4.36	80.4	4.50	82.0	4.42
4	71.8	4.00	71.8	4.02	71.8	4.02	73.1	4.37	72.9	4.18
5	78.0	3.86	78.2	3.86	78.2	3.86	77.7	4.00	77.1	3.96
6	69.0	4.08, 4.48	68.8	4.06, 4.48	68.9	4.06, 4.48	69.6	4.18, 4.62	67.6	4.56, 4.63
Me(CO-NH-)	23.0	2.16 s	23.0	2.14 s	23.0	2.12 s	23.8	2.12 s	23.0	2.21 s
Me(*C*O-NH-)	171.4		171.3		171.3		170.0		171.2	
Me(CO-*NH*-)		9.02 br s		9.05 br s		9.00 br s		8.90 br s		9.18 br s
Xyl 1	105.4	4.82 d (6.9)	105.5	4.84 d (7.3)	105.4	4.84 d (7.3)	106.3	4.98 d (7.7)	106.2	4.78 d (7.1)
2	74.8	3.90	74.7	3.88	74.7	3.88	75.5	4.00	75.6	3.98
3	77.0	3.98	77.0	3.96	77.0	3.96	77.7	4.62	76.9	4.62
4	70.2	4.06	70.2	4.05	70.2	4.05	71.0	4.25	70.2	4.22
5	66.5	3.50, 4.30	66.5	3.48, 4.30	66.5	3.48, 4.30	67.4	3.58, 4.38	66.6	3.57, 3.97
Ara 1	102.0	5.00 d (4.0)	101.8	4.98 d (5.0)	101.8	4.98 d(4.0)	102.0	5.15 d (6.8)	102.7	5.18 d (6.4)
2	71.8	4.28	71.6	4.28	71.6	4.28	72.9	4.42	72.8	4.42
3	74.4	4.38	74.9	4.30	74.9	4.30	73.1	4.45	74.2	4.66
4	67.0	4.32	66.9	4.34	66.9	4.32	71.3	4.25	68.1	4.26
5	63.5	3.66, 4.20	63.8	3.66, 4.20	63.7	3.65, 4.20	64.3	3.72, 4.28	65.6	3.68, 4.20
C-28: GlcI 1	93.9	5.96 d (7.1)	94.0	5.94 d (7.4)	93.9	5.96 d (7.1)	95.3	6.15 d (7.4)	93.9	6.04 d (7.6)
2	78.5	4.20	78.2	4.17	78.3	4.18	78.8	4.30	78.7	4.22
3	77.5	4.18	77.8	4.16	77.9	4.16	76.0	4.70	84.9	4.02
4	70.2	4.08	70.3	4.06	70.3	4.06	80.5	4.53	80.0	4.74
5	77.0	3.98	77.0	3.96	77.0	3.96	78.6	3.92	78.3	3.92
6	61.6	4.16, 4.30	61.4	4.13, 4.28	61.5	4.16, 4.30	62.3	4.28, 4.32	61.6	4.20, 4.26
Api I 1	110.2	6.22 d (3.0)	110.2	6.28 d (3.6)	110.2	6.23 d (3.6)	110.8	6.35 d (3.2)	110.0	6.23 br s
2	77.1	4.70	77.5	4.72	78.2	4.62	78.6	4.75	80.0	4.74
3	78.7		79.7		79.6		79.8		79.8	
4	74.4	4.26, 4.44	74.4	4.22, 4.44	74.4	4.22, 4.44	75.2	4.25, 4.48	74.4	3.96, 4.32
5	64.5	4.05, 4.14	64.4	4.05, 4.14	64.3	4.04, 4.12	65.6	4.10, 4.15	64.4	4.14, 4.20
Api II 1							110.9	5.55 d (3.6)	110.6	6.08 br s
2							78.7	4.62	78.1	4.70
3							79.7		79.7	
4							75.5	4.30, 4.52	74.5	4.50, 4.66
5							65.6	3.90, 4.10	64.5	4.16, 4.20
Glc II 1									103.7	5.36 d (7.3)
2									74.0	3.98
3									77.7	4.28
4									70.8	4.12
5									77.2	4.18
6									61.4	4.34, 4.46

[a] Assignments are based on HMBC, HSQC, and DEPT experiments; δ in ppm; proton coupling constants (J) in Hz are given in parentheses. [b] Overlapping ^{1}H NMR signals are reported without designated multiplicity.

mental Section).[20,21] The sequences of the sugar chains were obtained by HMBC and NOESY experiments. Cross-peak correlations observed in the HMBC spectrum between signals at δ_H 4.94 (d, J = 7.2 Hz) (GlcNAc-1) and δ_C 88.9 (Agly-3) showed that the GlcNAc moiety is linked at C-3 of the aglycon. This was confirmed by the NOESY correlation observed between signals at δ_H 4.94 (d, J = 7.2 Hz) (GlcNAc-1) and δ_H 3.30 (Agly-3). The 3,6-disubstitution of the GlcNAc moiety was shown by downfield chemical shifts at δ_C 79.7 (GlcNAc-2) and 69.0 (GlcNAc-6) in the ^{13}C NMR spectrum by ca. 5 and 7 ppm, respectively (Table 2).[9,22] The correlation observed in the HMBC spectrum between the signals at δ 4.82 (d, J = 6.9 Hz) (Xyl-1) and δ_C 79.7 (GlcNAc-3) suggested the location of the T-Xyl unit at C-3 of GlcNAc. This was confirmed by the reverse correlation observed between H-3 (δ 4.35) of GlcNAc and C-1 (δ 105.4) of Xyl. Further correlations were also depicted between H-1 (δ 5.00 (d, J = 4.0 Hz)) of Ara and C-6 (δ 69.0) of GlcNAc, indicating the location of the T-Ara at C-6 of GlcNAc. These results showed the sequence of the sugar chain at C-3 to be 3-*O*-β-D-xylopyranosyl-(1→3)-*O*-[α-L-arabinopyranosyl-(1→6)]-2-(acetylamino)-2-deoxy-β-D-glucopyranosyl. Further correlations observed in the HMBC spectrum between H-1 (δ 5.96 d, J = 7.1 Hz) of Glc I and the signal of the carbon at δ_C 175.4 (Agly-28) proved a glucosidic ester linkage at the C-28 of the aglycon. A correlation observed in the NOESY spectrum between signals of protons at δ 4.20 (Glc I-2) and δ 6.22 (d, J = 3.0 Hz) (Api-1) suggested that the T-Api unit was linked at the C-2 position of Glc I. This was confirmed by the HMBC correlation observed between H-1 (δ 6.22 (d, J = 3.0 Hz) of Api I and C-2 (δ 78.5) of Glc I. Thus, the structure of **1** was elucidated as 3-*O*-β-D-xylopyranosyl-(1→3)-[α-L-arabinopyranosyl-(1→6)]-2-acetylamino-2-deoxy-β-D-glucopyranosyloleanolic acid 28-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside (pursaethoside A).

Pursaethoside B (**2**) was isolated as a white amorphous powder, and the molecular formula was determined as $C_{59}H_{95}NO_{26}$ from the HRESIMS ($[M + Na]^+$ *m/z* 1256.6095, calcd 1256.6040) and ^{13}C NMR spectroscopic data. The negative-ion FABMS displayed a quasi-molecular ion peak at *m/z* 1232 $[M - H]^-$, 16 mass units higher than that of **1** and indicating a molecular weight of 1233. Further fragment ion peaks were observed at *m/z* 938 $[(M - H) - 132 - 162]^-$, 806 $[(M - H) - 132 - 162 - 132]^-$, and 471 $[(M - H) - 132 - 162 - 2 \times 132 - 203]^-$, corresponding, respectively, to the successive loss of one pentosyl, one hexosyl, two pentosyl, and one acetylamino-deoxyhexosyl moiety, as in compound **1**. The comparison of the NMR data (see Tables 1 and 2) indicated that **2** has a different aglycon but exactly the same sugar chains at both positions C-3 and C-28 as **1**. The only difference was the variation of

chemical shifts of C-15 and C-16 of the two aglycons. In the ^{13}C NMR spectrum, the resonances at δ 27.7 of C-15 and δ 23.5 of C-16 of **1** appeared at δ 35.7 and 73.5, respectively, for **2**. In the ^{1}H NMR spectrum, the resonance of H-16 of **2** appeared at δ 5.24 and indicated its position geminal to a hydroxyl group. Cross-peak correlations were depicted in the $^{1}H-^{1}H$ COSY spectrum between H-16 (δ 5.24, br s) and H_2-15 [δ 1.85 (br d, J = 12.4 Hz), δ 2.25 (m)], thus confirming the location of the hydroxyl group at C-16. The 16α-configuration of the hydroxyl unit was evident from the small J value of H-16 resonating as a broad singlet in the ^{1}H NMR spectrum characteristic of a β equatorial proton. The aglycon part of **2** was then identified as echinocystic acid.[23] This was also confirmed by the fragment ion at *m/z* 471 present on the FABMS and representing the pseudomolecular ion of this aglycon. Sugar analysis was carried out using the same method used for **1**, and after the acid hydrolysis, echinocystic acid was confirmed as the aglycon from co-TLC comparison with an authentic sample. Thus, compound **2** was defined as 3-*O*-β-D-xylopyranosyl-(1→3)-*O*-[α-L-arabinopyranosyl-(1→6)]-2-acetylamino-2-deoxy-β-D-glucopyranosylechinocystic acid 28-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside (pursaethoside B).

Pursaethoside C (**3**) was isolated as a white amorphous powder, and its molecular formula was deduced as $C_{59}H_{95}NO_{27}$ on the basis of HRESIMS ($[M + Na]^+$ *m/z* 1272.5930, calcd 1272.5989) and ^{13}C NMR analysis. The negative-ion FABMS of **3** exhibited a quasi-molecular ion peak at *m/z* 1248 $[M - H]^-$, 16 mass units higher than that of **2** and indicating a molecular weight of 1249. Subsequent peaks were observed at *m/z* 954 $[M - H - 132 - 162]^-$ and 487 $[M - H - 132 - 162 - 2 \times 132 - 203]^-$, corresponding to the successive loss of one pentosyl, one hexosyl, two pentosyls, and one acetylamino-deoxyhexosyl moiety, respectively, as in compounds **1** and **2**. The NMR data of this compound (see Tables 1 and 2) revealed that, like compound **2**, compound **3** differs from **1** only in the aglycon and contains exactly the same sugar chains at both the C-3 and C-28 positions. The only difference was the variation of chemical shifts of C-15 and C-16 in the aglycons. In the ^{13}C NMR spectrum, the carbon resonances at δ 35.7 of C-15 and δ 73.5 of C-16 of **2** changed into δ 68.0 and 77.9, respectively, for **3**. In the ^{1}H NMR spectrum, the resonances of H-15β and H-16β of **3**, deduced from the HMQC spectrum as occurring at δ 4.35 and 4.97, respectively, indicated the geminal position of each to a hydroxyl group, which were confirmed by cross-peak correlations observed in the $^{1}H-^{1}H$ COSY spectrum of **3** between ^{1}H NMR signals at δ 4.35 (H-15) and 4.97 (H-16). In the HMBC spectrum, correlations were observed between the deshielded methyl signal at δ_H 1.69 (CH_3-27) and the carbons at δ_C 39.5 (C-8), 144.1 (C-13), 46.7 (C-14), and 68.0 (C-15). Further correlations observed between the signals at δ_H 4.97 (H-16) and δ_C 68.0 (C-15), 41.4 (C-18) proved the location of two vicinal hydroxyl groups at C-15 and C-16, respectively. The β-axial configuration of H-15 was deduced from the NOESY cross-peak between H-15 at δ 4.35 (d, J = 4.8 Hz) and Me-26 at δ 1.06 (s). Furthermore, the coupling constant between H-15 at δ 4.35 (d, J = 4.8 Hz) and H-16 at δ 4.97 (d, J = 4.8 Hz) and the cross-peak in the NOESY spectrum between H-15 and H-16 are in agreement with the assigned stereochemistry of the hydroxyl groups as 15α and 16α. All of these data were confirmed by the fragment ion at *m/z* 487 present in the FABMS and representing the pseudomolecular ion of this aglycon. Consequently, the aglycon was identified as entagenic acid, for which the structure was revised as 3β,15α,16α-trihydroxyolean-12-en-28-oic acid by Okada et al.,[24] in contrast to the earlier proposition of Barua et al.[25] (3β,21α,22α-trihydroxyolean-12-en-28-oic acid). Compound **3** was thus elucidated as 3-*O*-β-D-xylopyranosyl-(1→3)-*O*-[α-L-arabinopyranosyl-(1→6)]-2-acetylamino-2-deoxy-β-D-glucopyranosylentagenic acid 28-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside (pursaethoside C).

Pursaethoside D (**4**) was obtained as a white amorphous powder, and its molecular formula was deduced as $C_{64}H_{103}NO_{31}$ on the basis of HRESIMS ($[M + Na]^+$ *m/z* 1404.6454, calcd 1404.6412) and ^{13}C NMR analysis. Its negative-ion FABMS displayed a quasi-molecular ion peak at *m/z* 1380 $[M - H]^-$, 132 mass units higher than that of **3** and indicating a molecular weight of 1381. Other fragment ion peaks were observed at *m/z* 1248 $[(M - H) - 132]^-$, 954 $[(M - H) - 132 - 132 - 162]^-$, and 487 $[(M - H) - 132 - 132 - 162 - 132 - 132 - 203]^-$, suggesting the elimination of four pentosyls, one hexosyl, and one *N*-acetyldeoxyhexosyl moiety. This indicated that **4** contains one additional pentosyl moiety compared to **3**. The NMR data of **4** (see Tables 1 and 2) were quite similar to those of **3**. The main difference was the presence of additional signals in **4** that were determined to be from a terminal β-D-apiofuranosyl (T-Api II) moiety[26] (Table 2) [anomeric proton at δ_H 5.55 (d, J = 3.6 Hz), anomeric carbon at δ_C 110.9, one hydroxyl-containing methine carbon at δ_C 78.7, one quaternary carbon atom at δ_C 79.7, and two hydroxyl-containing methylene carbons at δ_H 65.6 and 75.5]. Comparison of the NMR data of **4** and **3** (Tables 1 and 2) indicated that these two compounds have the same aglycon and the same sugar chain at C-3, thus demonstrating that an additional apiofuranosyl moiety (Api II) is located at the C-28 sugar chain of the aglycon. Its location was shown from the HMBC experiment. Cross-peak correlations were observed in the HMBC spectrum between the proton at δ_H 4.53 (Glc I-4) and the anomeric carbon of Api II (δ_C 110.9) and also between the proton at δ_H 5.55 (d, J = 3.6 Hz) (Api II-1) and the deshielded carbon at δ_C 80.5 (Glc I-4), indicating the location of Api II at C-4 of the glucopyranosyl ester (Glc I). Thus, the structure of **4** was established as 3-*O*-β-D-xylopyranosyl-(1→3)-[α-L-arabinopyranosyl-(1→6)]-2-acetylamino-2-deoxy-β-D-glucopyranosylentagenic acid 28-*O*-β-D-apiofuranosyl-(1→2)-[β-D-apiofuranosyl-(1→4)]-β-D-glucopyranoside (pursaethoside D).

Pursaethoside E (**5**) was obtained as a white amorphous powder and had a molecular formula of $C_{70}H_{113}O_{36}N$ ($[M + Na]^+$ *m/z* 1566.6903, calcd 1566.6940), which was determined from its positive-ion HRESIMS and confirmed by ^{13}C NMR and DEPT analysis. Its negative-ion FABMS displayed a quasi-molecular ion peak at *m/z* 1542 $[M - H]^-$, 162 mass units higher than that of **4** and demonstrating a molecular weight of 1543. Other significant ion peaks were observed at *m/z* 1380 $[(M - H) - 162]^-$, 1248 $[(M - H) - 162 - 132]^-$, 1116 $[(M - H) - 162 - 132 - 132]^-$, 954 $[(M - H) - 162 - 132 - 132 - 162]^-$, and 487 $[(M - H) - 162 - 132 - 132 - 162 - 132 - 132 - 203]^-$. Comparison of these ion fragments with those of **4** revealed the presence of one additional hexosyl moiety in **5**. The ^{1}H and ^{13}C NMR signals of **5** determined from the 1D and 2D NMR spectra revealed supplementary signals that were assigned to those of a terminal β-D-glucopyranosyl moiety (Glc II)[26] (Tables 1 and 2) [anomeric proton at δ_H 5.36 (d, J = 7.3 Hz), an anomeric carbon at δ_C 103.7, and hydroxyl-attached methine carbons at δ 74.0, 77.7, 70.8, and 77.2, and a further signal at δ 61.4 (CH_2OH)]. The spectroscopic data were almost superimposable on those of **4** in terms of the

trisaccharide chain at C-3 of the aglycon (Table 2), thus indicating that the additional terminal β-D-glucopyranosyl moiety is located on the C-28 oligosaccharidic chain of the aglycon. In addition to the deshielded value of C-2 (δ 78.7) of the β-D-glucopyranosyl ester (Glc I) unit, as in compounds **1**−**4**, deshielded values were also observed for C-3 (δ 84.9) and C-4 (δ 80.0) of Glc I (Table 2), thus indicating the location of the two remaining sugar residues (one glucopyranosyl and one apiofuranosyl unit) at these positions. The position of substitution of each sugar was shown by HMBC and NOESY experiments. A cross-peak correlation observed in the HMBC spectrum between the proton at δ_H 4.74 (Glc I-4) and the carbon at δ_C 103.7 (Glc II-1) and another between the proton at δ_H 5.36 (Glc II-1) and the carbon at δ_C 80.0 (Glc I-4) enabled the location of the terminal glucopyranosyl moiety (Glc II) to be established at C-4 of the Glc I. The remaining terminal apiofuranosyl moiety (Api II) was then located at position C-3 of the Glc I, and this was confirmed by the cross-peak correlation observed in the NOESY spectrum between the protons at δ_H 4.02 (Glc I-3) and δ_H 6.08 (Api II-1). Therefore, the structure of **5** was established as 3-*O*-β-D-xylopyranosyl-(1→3)-[α-L-arabinopyranosyl-(1→6)]-2-acetylamino-2-deoxy-β-D-glucopyranosylentagenic acid 28-*O*-β-D-apiofuranosyl-(1→2)-[β-D-apiofuranosyl-(1→3)]-[β-D-glucopyranosyl(1→4)]-β-D-glucopyranoside (pursaethoside E).

Saponins having entagenic acid as aglycon are rarely encountered in nature. Entagenic acid was found in *Entada phaseoloides*[5,7] and *E. scandens*[27] and could represent a chemotaxonomic marker for this genus.

Since triterpene or steroid saponins have been reported to possess, to varying degrees, cytotoxic activity against a large variety of cancer cell lines,[28] we have tested compounds **2**−**4** and phaesoloidin for cytotoxicity against HCT 116 and HT-29 human tumor cell lines. However, as determined by the MTT assay,[29] no significant effect could be found in these bioassays (IC_{50} > 100 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotations were measured using an AA-10R automatic polarimeter. IR spectra (KBr) were recorded on a Perkin-Elmer 881-IR spectrophotometer. The 1D and 2D NMR spectra (^1H−^1H COSY, NOESY, HMQC, and HMBC) were performed using a UNITY-600 spectrometer at an operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4L-X computer system (600 MHz for ^1H and 150 MHz for ^{13}C spectra). Conventional pulse sequences were used for COSY, HMBC, and HMQC NMR spectra. The carbon type (CH_3, CH_2, CH) was determined by DEPT experiments. All chemical shifts (δ) are given in ppm units with reference to tetramethylsilane (TMS) as internal standard, and the coupling constants (*J*) are in Hz. Samples were measured in pyridine-d_5. FABMS (negative-ion mode) were obtained on a JEOL-SX-102 spectrometer, using glycerol as matrix, and HRESIMS was carried out on a Q-TOF 1-micromass spectrometer. GC analysis was carried out on a Thermoquest gas chromatograph using a DB-1701 capillary column (30 m × 0.25 mm, i.d.) and He as carrier gas. Vacuum-liquid chromatography (VLC) was carried out using silica gel 60 (Merck, 70−200 μm), and medium-pressure liquid chromatography (MPLC) employed silica gel 60 (Merck, 15−40 μm), a Gilson M 305 pump, and a Buchi column (460 × 25 mm and 460 × 15 mm). TLC and HPTLC were carried out on precoated Kieselgel 60 F_{254} (Merck) plates, with the solvent systems used for monosaccharides and saponins being $CHCl_3$−MeOH−AcOH−H_2O (60:32:12:8), and for aglycons, $CHCl_3$−MeOH (9:1). Spray reagent used for saponins and aglycons was Komarowsky reagent (a 5:1 solution of 2% 4-hydroxybenzaldehyde in MeOH and 50% H_2SO_4 solution), with the diphenylamine/phosphoric acid reagent used for sugars.

Plant Material. The kernels of *Entada pursaetha* collected in the Noun Division of western Cameroon were bought in a Dschang market (Menoua Division, western province of Cameroon) in December 2003. The plant material was identified by Dr. Benoit Satabie at the Cameroonian National Herbarium, Yaoundé, where a voucher specimen (No. 12116/SFR/CAM) has been deposited.

Extraction and Isolation. Dried, powdered seed kernels (3.5 kg) of *E. pursaetha* were extracted with MeOH−CH_2Cl_2 (1:1) (6 L) at room temperature for 24 h. After removal of solvent, the residual powder was re-extracted with MeOH (3 L) for 12 h. Evaporation of solvent from combined extracts in vacuo gave an oily residue (68 g), which was suspended in water (500 mL) and submitted to successive partitions with hexane (3 × 200 mL), ethyl acetate (3 × 150 mL), and H_2O-saturated *n*-BuOH (3 × 200 mL). Partial evaporation of the EtOAc solution produced a precipitate that was separated into EtOAc-insoluble and EtOAc-soluble portions. This precipitate was collected and successively washed with small quantities of Me_2CO and cold EtOAc to furnish the known phaseoloidin (1.25 g).[17] Since the EtOAc-soluble portion and the BuOH extract were similar by co-TLC, they were combined and evaporated to dryness, affording a brown gum (35 g). A part of the above extract (30 g) was submitted to VLC (silica gel 70−200 μm) with $CHCl_3$ containing increasing amounts of MeOH, yielding, after evaporation in vacuo, five subfractions: I (2.3 g), II (2. 2 g), III (3.1 g), IV (1.2 g), and V (2.3 g). Subfractions III and IV were combined and submitted to column chromatography (Sephadex LH-20, MeOH) and thereafter purified by repeated MPLC (silica gel 15−40 μm) with $CHCl_3$−MeOH−H_2O (64:40:8) and $CHCl_3$−MeOH−H_2O (65:35:10, lower phase), to yield **1** (27 mg), **2** (18 mg), and **3** (58 mg). Subfraction V was submitted to column chromatography (Sephadex LH-20, MeOH) for fractionation and purified by repeated MPLC (silica gel 15−40 μm) with $CHCl_3$−MeOH−H_2O (64:40:8), to yield **4** (183 mg) and **5** (18 mg).

Pursaethoside A (1): white amorphous powder; $[\alpha]_D^{22}$ −15° (*c* 0.06, MeOH); IR (KBr) ν_{max} 3500−3390 (OH), 2950 (CH), 1740 (CO of ester group), 1655 (C=C), 1450, 1010 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5), Tables 1 and 2; HRESIMS *m/z* 1240.6107 $[M + Na]^+$ (calcd for 1240.6091, $C_{59}H_{95}NO_{25}Na$); FABMS (negative) *m/z* 1216 $[M - H]^-$, 1084 $[(M - H) - 132]^-$, 922 $[(M - H) - 132 - 162]^-$), 658 $[(M - H) - 132 - 162 - 2 \times 132]^-$), 455 $[(M - H) - 132 - 162 - 2 \times 132 - 203]^-$.

Pursaethoside B (2): white amorphous powder; $[\alpha]_D^{22}$ −27° (*c* 0.06, MeOH); IR (KBr) ν_{max} 3500−3395 (OH), 2820 (CH), 1745 (CO of ester group), 1685 (C=C), 1430, 1110, 1010 cm^{-1}; $_1H$ and ^{13}C NMR (pyridine-d_5), Tables 1 and 2; HRESIMS *m/z* 1256.6095 $[M + Na]^+$ (calcd for 1256.6040, $C_{59}H_{95}NO_{26}Na$); FABMS (negative) *m/z* 1232 $[M - H]^-$, 938 $[M - H - 132 - 162]^-$, 806 $[(M - H) - 132 - 162 - 132]^-$, 471 $[(M - H) - 132 - 162 - 2 \times 132 - 203]^-$.

Pursaethoside C (3): white amorphous powder; $[\alpha]_D^{22}$ −21° (*c* 0.06, MeOH); IR (KBr) ν_{max} 3500−3390 (OH), 2890 (CH), 1650 (C=C), 1480, 1110 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5), Tables 1 and 2; HRESIMS *m/z* 1272.5930 $[M + Na]^+$ (calcd for 1272.5989, $C_{59}H_{95}NO_{27}Na$); FABMS (negative) *m/z* 1248 $[M - H]^-$, 954 $[(M - H) - 132 - 162]^-$), 487 $[(M - H) - 132-162 - 2 \times 132 - 203]^-$.

Pursaethoside D (4): white amorphous powder; $[\alpha]_D^{22}$ −33.3° (*c* 0.066, MeOH); IR (KBr) ν_{max} 3366 (OH), 2956 (C−H), 1720 (C=O of ester), 1655 (C=C), 1448, 1109, 1021 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5), Tables 1 and 2; HRESIMS *m/z* 1404.54 $[M + Na]^+$ (calcd for 1404.6412, $C_{64}H_{103}NO_{31}Na$); FABMS (negative) *m/z* 1380 $[M - H]^-$ ($C_{64}H_{102}NO_{31}$), 1248 $[(M - H) - 132]^-$, 954 $[(M - H) - 132 - 132 - 162]^-$, and 487 $[(M - H) - 132 - 132 - 162 - 132 - 132 - 203]^-$.

Pursaethoside E (5): white amorphous powder; $[\alpha]_D^{22}$ −18.1° (*c* 0.066, MeOH); IR (KBr) ν_{max} 3414 (OH), 2952 (C−H), 1715 (C=O of ester), 1653 (C=C), 1450, 1111, 1016 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5), Tables 1 and 2; HRESIMS *m/z*

1566.6903 $[M + Na]^+$ (calcd for 1566.6940, $C_{70}H_{112}NO_{36}Na$); FABMS (negative) *m/z* 1542 $[M - H]^-$, 1380 $[(M - H) - 162]^-$, 1248 $[(M - H) - 162 - 132]^-$, 1116 $[(M - H) - 162 - 132 - 132]^-$, 954 $[(M - H) - 162 - 132 - 132 - 162]^-$, and 487 $[(M - H) - 162 - 132 - 132 - 162 - 132 - 132 - 203]^-$.

Acid Hydrolysis of 1−5. A solution of each compound (2.5 mg) in H_2O (1 mL) and 2 N aqueous CF_3COOH (10 mL) was refluxed at 100 °C in a water bath for 2 h. The mixture was then diluted in water (10 mL) and extracted with CH_2Cl_2 (3 × 4 mL). The combined CH_2Cl_2 extracts were washed with H_2O and evaporated to afford the aglycon. After repeated evaporations of the aqueous layer by adding MeOH to remove the acid, the crude sugar residue was analyzed by TLC (silica gel) in comparison with standard sugars, and the absolute configuration of sugar residues was determined by GC analysis of their chiral derivatives as described in the previous papers.[18,21]

Alkaline Hydrolysis of 1−5. Each saponin (3 mg) was refluxed at 100 °C on a water bath with 5% aqueous KOH solution (10 mL) for 2 h. The mixture was adjusted to pH 6 with a dilute HCl solution and then extracted with H_2O-saturated BuOH (3 × 4 mL). The combined BuOH extracts were washed with H_2O and evaporated to afford the prosapogenin. The acid hydrolysis of the prosapogenins resulting from **1** and **2** afforded, respectively, oleanolic acid and echinocystic acid, identified by co-TLC comparison with authentic samples. The same aglycon resulted from the acid hydrolysis of **3**−**5** and was identified as entagenic acid by comparison of its ^{13}C NMR data with literature values.[24]

MTT Cytotoxicity Assay. The bioassay was carried out according to the method described in ref 29 with two human colorectal cancer cell lines (HCT 116 and HT-29). Paclitaxel was used as a positive control. The antiproliferative effect of **2**−**4** and phaesoloidin was monitored using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). HCT 116 and HT-29 cells were seeded at an initial density of 5000 or 10 000 cells/well in 96-well plates and treated with medium containing various concentrations of the test compounds. DMSO controls (0.01%) did not affect cell proliferation. After 96 h, 20 μL of MTT solution (5 mg/mL in PBS) was added to the culture medium and the reaction mixture was incubated at 37 °C in a 5% CO_2 atmosphere for 4 h. The MTT solution was aspired, and 200 μL of DMSO was added. The optical density was measured spectrophotometrically at 570 nm. The results are expressed as a percentage of cell survival. The experiments were repeated three times. Paclitaxel exhibited IC_{50} values of 9.9 ± 6.3 and 14.4 ± 8.4 ng/mL against HCT 116 and HT-29, respectively.

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