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Bitter Steroid Glucosides, Vernoniosides A1, A2, and A3, and Related B1 from a Possible Medicinal Plant, *Vernonia amygdalina*, used by Wild Chimpanzees.

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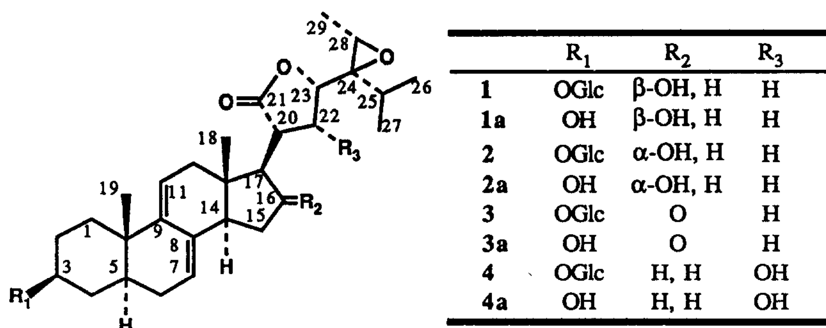
Abstract: *From Vernonia amygdalina, a possible medicinal plant used by wild chimpanzees, three bitter steroid glucosides, vernoniosides A1, A2, and A3 and a nonbitter vernonioside B1, were isolated. The oxygenation patterns of the aglycone parts were new, especially the pattern of the carboxyl group at C21. The oxygen functionalities at C16 were important for the bitter taste.*

Some plants may be consumed for medicinal purposes by wild chimpanzees.[1,2] *Vernonia amygdalina* (Compositae), a tree growing throughout tropical Africa, is one of the most likely examples of such plants. At the Mahale Mountain in Tanzania, Huffman and Seifu [2] observed that shoots of this tree were chewed by an apparently sick adult female chimpanzee. *V. amygdalina* is used in tropical Africa as anthelmintic, antiscorbutic, and a quinine substitute, and it is eaten as a traditional tonic food in west Africa. These facts suggest that *V. amygdalina* contains a variety of physiologically or biologically active compounds. Some sesquiterpene lactones have been isolated as antitumor agents by Kupchan *et al.*[3] and as insect antifeedants by Ganjian *et al.*[4] To search for further physiologically and biologically active compounds, we have investigated the bitter compounds, in particular.[5] The structures of a bitter compound, vernonioside A_1, and a nonbitter related vernonioside B_1 were briefly reported,[6] without detailed physicochemical data or chemical characteristics. We recently isolated two new related bitter compounds, vernoniosides A_2 and A_3, and identified the configuration of the hydroxy group at C16 of vernonioside A_1. Here, we describe in full the isolation, structures, and bitterness of these steroid glucosides.

The bitter *n*-butanol soluble part of a methanolic extract of the dried leaves of *V. amygdalina* contained a group of compounds that appeared as violet or blue spots on TLC with 0.5% vanillin in sulphuric acid-ethanol solution, and was separated by 4 steps of column chromatography and then purified by preparative HPLC on μBondasphere C_{18} to yield the bitter compounds vernonioside A_1 (**1**), A_2 (**2**), and A_3 (**3**) and a related nonbitter compound, vernonioside B_1 (**4**).



	R_1	R_2	R_3
1	OGlc	β-OH, H	H
1a	OH	β-OH, H	H
2	OGlc	α-OH, H	H
2a	OH	α-OH, H	H
3	OGlc	O	H
3a	OH	O	H
4	OGlc	H, H	OH
4a	OH	H, H	OH

Fig. 1. Structures of Vernoniosides and Their Aglycones.

The results of FABMS indicated that the molecular formulae of **1**, **2**, and **4** were $C_{35}H_{52}O_{10}$ and that the molucular formula of **3** was $C_{35}H_{50}O_{10}$. UV spectra of these compounds showed triple maxima at 235, 243, and 250 nm, indicating the presence of a conjugated diene chromophore. An IR absorption band due to a γ-lactone was observed with each compound. The following structural information was common to all of the compounds, as reported previously.[6] They were ß-glucopyranosides of the C_{29} stigmastane-type of steroids, and each contained an isopropyl group at C24, a trisubstituted epoxy ring system at C24-C28, and a γ-lactone cyclized between C21 and C23.

^1H-^1H and ^1H-^{13}C COSY NMR spectra of **4** confirmed the partial connectivities such as C23-C22-C20-C17, C6-C7, and C11-C12. The triple absorption maxima in the UV spectrum of **4** suggested the presence of a $\Delta^{7,9(11)}$ diene, as reported of some triterpenoids and steroids.[7] Hydrolysis of **4** with β-glucosidase gave the natural aglycone (**4a**), the spectral data of which corresponded with those of **4** except for ^{13}C NMR signals arising from C2, C3, and C4. The ^{13}C NMR signals of C2, C3, and C4 shifted from 30.15, 77.07 (or 78.50), and 34.54 ppm in **4** to 32.58, 70.28, and 38.78 ppm in **4a** (Table 1). From these data, the structure of vernonioside B_1 seems to be **4** (Fig. 1).

The final structure of the steroid part of vernonioside B1 was found to be that of **4a** by X-ray diffraction analysis.[6] The perspective view of **4a** is shown in Fig. 2, where the $\Delta^{7,9(11)}$ diene is shown;

Table 1. ^{13}C NMR Assignments of **1**, **2**, **3**, **4**, **3a**, **4a**, and **4b**.

C	1	2	3	4	3a	4a	4b
1	35.01	34.92	34.83	35.01	35.11	35.30	35.75
2	30.14	30.08	30.06	30.15	32.49	32.58	32.63
3	77.05[a]	76.98[a]	76.93[a]	77.07[a]	70.14	70.28	70.28
4	34.52	34.48	34.40	34.54	38.69	38.78	39.32
5	39.30	39.16	39.11	39.25	39.57	39.73	41.31
6	30.26	30.19	30.06	30.27	30.20	30.39	22.38
7	120.63	120.99	122.72	120.68	122.91	120.85	36.07
8	136.13	135.87	133.41	136.53	133.47	136.59	x[b]
9	144.15	144.09	144.77	143.84	145.02	144.08	142.10
10	36.23	36.18	36.42	36.12	36.48	36.17	36.96
11	119.45	118.76	117.76	119.75	117.76	119.76	27.12
12	41.77	41.32	39.93	42.16	40.00	42.20	25.77
13	42.89	43.28	40.94	42.63	40.98	42.67	45.48
14	50.22	49.18	46.13	51.93	46.22	51.03	151.33
15	37.03	36.02	37.64	23.51	37.69	23.55	116.62
16	72.12	74.89	214.51	28.08	214.53	28.10	36.63
17	57.07	60.14	62.80	45.81	62.83	45.84	47.56
18	13.91	13.91	14.24	12.68	14.28	12.70	16.87
19	19.60	19.48	19.50	19.52	19.69	19.70	18.58
20	38.69	40.26	37.36	51.01	37.38	52.03	46.10
21	178.43	177.97	177.70	176.36	177.71	176.38	177.24
22	31.05	29.31	28.50	77.04	28.50	73.05	79.46
23	77.28	77.38	77.24	80.11	77.23	80.11	82.99
24	64.17	64.02	64.38	63.81	64.38	63.82	82.13
25	29.76	29.74	29.66	30.33	29.67	30.33	30.85
26	18.70	18.73	18.75	18.49	18.76	18.49	17.34
27	18.88	18.89	18.75	18.65	18.76	18.65	18.07
28	55.28	55.40	55.32	56.17	55.31	56.18	81.09
29	13.21	13.19	13.22	13.19	13.23	13.19	13.94
1'[c]	102.29	102.25	102.32	102.28			
2'[c]	75.36	75.30	75.37	75.34			
3'[c]	78.65	78.60	78.69	78.64			
4'[c]	71.74	71.71	71.80	71.74			
5'[c]	78.52[a]	78.46[a]	78.56[a]	78.50[a]			
6'[c]	62.89	62.86	62.93	62.88			

[a] The values of C3 and C5' may be reversed.

[b] This olefin carbon signal was not assigned exactly because it was superimposed by the signals arising from pyridine-d_5.

[c] The carbons of glucose were numbered as 1' to 6'.

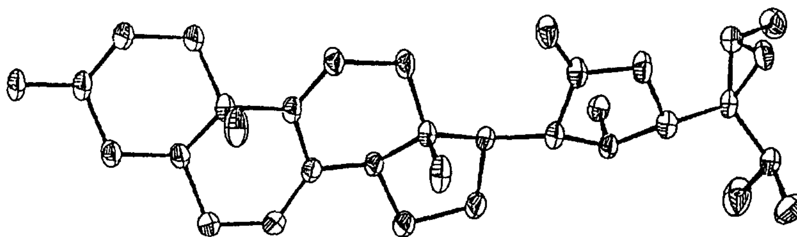


Fig. 2 Perspective View of **4a**.

Table 2. ^{1}H NMR Assignments of **1**,**2**,**3**, and **4**.

H	1	2	3	4
1α	1.30	1.26	1.29	1.28
1β	1.91	1.86	1.87	1.88
2α	2.18	2.16	2 15	ca. 2.2
2β	1.72	1.71	1.75	ca. 1.7
3	3.96	3.95	ca. 4.0	ca. 4.0
4α	2.07	2.02	2.04	2.05
4β	1.48	1.41	1.47	1.47
5	1.37	1.34	1.35	1.38
6α	1.83	1.82	ca. 1.8	ca. 1.8
6β	1.83	1.82	ca. 1.8	ca. 1.8
7	5.44, br s	5.40, br s	5.30, br s	5.38, br s
11	5.56, br d, *J*=6.3 Hz	5.50, br d, *J*=5.9 Hz	ca. 5.5, br d	5.55, br d, *J*=6.29 Hz
12α	2.19	2.34	ca. 2.3	2.37
12β	3.12, dd, *J*=17.8, 6.7 Hz	2.77, dd, *J*=17.4, 6.6 Hz	ca. 2.4	3.25, dd, *J*=18.0, 6.9 Hz
14	2.24	2.82	2.67	2.30
15α	2.53, dt, *J*=12.8, 6.9, 6.9 Hz	2.16	2.44, dd, *J*=18.0, 7.9 Hz	1.82
15β	1.91	2.16	2.17, dd, *J*=18.4, 12.7 Hz	ca. 1.5
16α	4.65, br s	-	-	1.65
16β	-	4.58	-	2.38
17	1.89	2.37	3.04	2.41
18	1.18, s	0.75, s	0.73, s	0.75, s
19	0.89, s	0.85, s	0.83, s	0.87, s
20	3.37, q, *J*=9.7 Hz	3.07, m	ca. 3.08	3.04, dd, *J*=9.6, 4.0 Hz
22α	2.28	2.32	2.32	-
22β	2.73, m	2.52, q, *J*=11.8 Hz	2.32	4.77, br s
23	4.89, dd, *J*=9.9, 5.9 Hz	4.93, dd, *J*=10.6, 5.5 Hz	4.90, t, *J*=8.1 Hz	4.98, d, *J*=2.3 Hz
25	1.89	1.86	1.92	2.01
26	1.07, d, *J*=7.2 Hz	1.08, d, *J*=7.2 Hz	1.04, d, *J*=7.2 Hz	1.25, d, *J*=7.4 Hz
27	1.28, d, *J*=7.1 Hz	1.28, d, *J*=7.0 Hz	1.26, d, *J*=6.7 Hz	1.29, d, *J*=7.6 Hz
28	2.98, q, *J*=5.3 Hz	3.01, q, *J*=5.6 Hz	3.17, q, *J*=5.6 Hz	3.63, q, *J*=5.5 Hz
29	1.23, d, *J*=5.5 Hz	1.19, d, *J*=5.5 Hz	1.24, d, *J*=5.4 Hz	1.32, d, *J*=6.1 Hz
1'	5.07, d, *J*=7.6 Hz	5.01, d, *J*=7.6 Hz	5.05, d, *J*=7.8 Hz	5.02, d, *J*=7.6 Hz
2'	4.07	4.06	4.08	4.06
3'	4.38	4.31	4.31	4.33
4'	4.30	4.27	4.27	4.30
5'	4.00	3.96	4.02	4.03
6'	4.38	4.40, br dd, *J*=11.9, 5.0 Hz	4.42	4.41, dd, *J*=11.9, 5.3 Hz
	4.60	4.57, br d, *J*=10.0 Hz	4.60	4.58, dd, *J*=11.8, 2.1 Hz

this allowed us to assign most of the ^{13}C (Table 1) and ^{1}H (Table 2) NMR signals of **4**, which was helpful for the structure elucidation of other vernoniosides.

Hydrolysis of **4** with HCl gave an aglycone (**4b**) with a molecular ion peak at m/z 470 in its EIMS, indicating that the molecular formula of **4b** was $C_{29}H_{42}O_5$. The UV spectrum of **4b** had a single maximum at 250 nm (ε 14000) arising from a diene chromophore. The ^{1}H NMR spectrum of **4b** showed only an olefinic proton signal at 5.45 ppm, which was coupled with methylene proton signals at 2.44 and 2.88 ppm. The methylene proton signals were coupled with a methine proton signal arising from 17-H (2.54 ppm) in the ^{1}H-^{1}H COSY NMR spectrum at 500 MHz. Thus, double migrations of double bonds from $\Delta^{7,9(11)}$ to $\Delta^{8,14}$ by proton addition and elimination reactions occurred during acid hydrolysis (Fig. 3). Furthermore, the ^{13}C NMR signals arising from C22, C24, and C28 shifted downfield from 73.05, 63.82, and 56.18 ppm in **4a** to 79.46, 82.13, and 81.09 ppm, respectively, in **4b** (Table 1). Additionally, the methine proton signal arising from 28-H shifted downfield from 3.64 ppm in **4a** to

4.31 ppm in **4b**. These shifts of the ^{13}C and ^{1}HNMR signals could be explained by the formation of a five-membered ether ring accompanied with opening of the epoxy ring as shown in Fig. 3.

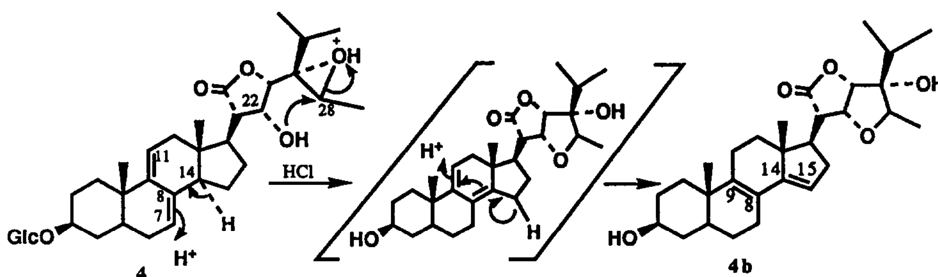


Fig. 3. Reaction of **4** Hydrolyzed with HCl to **4b**.

The structure of vernonioside A_1 was previously found to be that of **1** by comparison of ^{13}C and ^{1}H NMR data (Table 1 and 2, respectively) with those of **4**, except for the stereochemistry at C16.[6] Vernonioside A_2 (**2**) was found to be an isomer of **1** and **4** by its FABMS and by HR-EIMS of its natural aglycone **2a** obtained by enzymatic hydrolysis. ^{1}H-^{1}H and ^{1}H-^{13}C COSY NMR spectra of **2** clearly demonstrated the connectivity of C23-C22-C20-C17-C16-C15-C14, as for **1**. The C22 and C16 of **2** were of methylene (^{1}H NMR; 2.32 and 2.52 ppm) and hydroxymethine (^{1}H NMR; 4.58 ppm), respectively. Thus, **2** was identified as an epimer of **1** at C16.

Vernonioside A_3 had an IR absorption band at 1740 cm^{-1} and a ^{13}C NMR signal at 214.51 ppm (Table 1), indicating the presence of an additional carbonyl group other than that of C21. In the ^{1}H-^{1}H COSY NMR spectrum, both 15α-H and 15β-H appeared as double doublets (2.44 ppm, J=18.0, 7.9 Hz and 2.17 ppm, J=18.4, 12.7 Hz, respectively) coupled with a methine proton signal at 2.67 ppm arising from 14-H. Thus, **3** seemed to be a 16-oxo-derivative.

By $NaBH_4$ reduction of **3**, two products were obtained in the ratio of 5:1. The major product was identified spectroscopically as **1** and the minor one as **2**. Probably, hydride stereoselectively attacked the carbonyl carbon at C16 from the less hindered α-side to yield a β-hydroxy derivative as the major product. The results of $NaBH_4$ reduction of **3**, therefore, indicated not only that **3** is a 16-oxo-derivative, but also that **1** and **2** are 16β-hydroxy and 16α-hydroxy derivatives, respectively. Further evidence identifying the configurations at C16 of both **1** and **2** was their ^{1}H and ^{13}C NMR spectra. The ^{13}C NMR signals of C16 and C17 of **1** resonated at a higher field than those of **2**. Furthermore, the ^{1}H NMR signal due to 18-methyl resonated at a lower field and the 14- and 17-methine signals resonated at a higher field in **1** than **2**. These tendencies were in agreement with the data

reported on 2-alkyl hydroxycyclopentane and certain steroids.[8,9] On irradiation of 16-H, 2% of the NOE of the 18-methyl signal was observed in **2a**, but none was observed in **1a**. Thus, the configurations of the hydroxy group at C16 of **1** and **2** were β and α, respectively (Fig. 1).

The amounts needed for bitter taste of **1**, **2**, and **3** were 7, 5, and 5 μg, respectively (Table 3), which were almost equal to the amount needed of quinine sulphate. Vernonioside B_1 was not bitter even at 200 μg, suggesting that the presence of an oxygen atom at C16 may be important for the bitter taste. The glucosyl moiety was also important for bitter taste because aglycones **1a**, **2a**, and **3a** had small bitterness.

Table 3. Bitterness of Vernoniosides and Their Aglycones.

Compounds	Bitterness (μg)
1	7
1a	130
2	5
2a	150
3	5
3a	>200
4	>200
4a	>200
4b	>200

Acknowledgments

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Experimental

General remarks

All melting points were measured on a Yanagimoto microapparatus and are uncorrected. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200; IR, Shimadzu IR-435 (KBr); ORD, JASCO model J-5; ^1H and ^{13}C NMR, Brucker AC250 (250 MHz for ^1H, ref. TMS) and GE GN-500 (500 MHz for ^1H, ref. TMS); MS, JEOL JMS-DX300 (70 eV, 300 μA); HPLC was done with a μBondasphere C_{18} (ODS) column (Waters Associates Inc.). GC was done with an OV-1 column (FFS capillary column, 50 m x 0.24 mm i.d.). The bitterness was measured as the minimum amount for bitter taste by a filter paper method, in which a filter paper containing a known amount of test sample (1 cm^2, 0.2 mm, thick) was put on the tongue.[5]

Extraction of V. amygdalina *and isolation of 1, 2, 3, and 4*

Dried leaves of *V. amygdalina* (800 g), which had been collected and air-dried in 1987, were extracted with MeOH at room temperature for 7 days. The crude extract (94.9 g) was partitioned with *n*-hexane-MeOH-water (5:9:1) to yield a bitter lower layer. This portion was partitioned with EtOAc-water (1:1), and the bitter lower layer obtained (49.9 g) was further partitioned with water-saturated *n*-BuOH to yield a bitter *n*-BuOH-soluble part (17.6 g). The bitter part was chromatographed on Amberlite XAD-2 eluted stepwise with MeOH-water. The bitter eluate (80% MeOH) was rechromatographed on silica gel eluted stepwise with $CHCl_3$-MeOH to afford 7.5-10% MeOH eluates. The combined eluate was further

separated on silica gel 60H with toluene-acetone (2:3) under pressure to yield a bitter fraction (2.1 g). After being rechromatographed on ODS gel (MeOH-water/3:2), the bitter fraction was purified by HPLC on μBondasphere (acetonitrile-water/36:64, 8 ml/ min) to yield vernonioside A_1 (**1**, 21.2 mg, Rt 21.7 min), A_2 (**2**, 13.7 mg, Rt 19.0 min), A_3 (**3**, 31.2 mg, Rt 26.5 min) and B_1 (**4**, 217 mg, Rt 30.0 min). Vernonioside A_1 (**1**): Colorless needles, mp 244-246°C. $[\alpha]_D^{24}$ +35.2° (c 0.23, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 236 (12000), 243 (14000), 251 (9000). FABMS m/z: 633 (MH^+, $C_{35}H_{52}O_{10}$+H). Vernonioside A_2 (**2**): Colorless plates, mp 254-256°C. $[\alpha]_D^{22}$ +10.7° (c 0.30, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 235 (15000), 243 (17000), 250 (11000). FABMS m/z: 655 {$(M+Na)^+$, $C_{35}H_{52}O_{10}$+Na}. Vernonioside A_3 (**3**): Colorless needles, mp 200-203°C. $[\alpha]_D^{24}$ -30.0° (c 0.25, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1775, 1740. UV λmax (MeOH) nm (ε): 235 (11000), 242 (12000), 250 (8000). FABMS m/z: 631 (MH^+, $C_{35}H_{50}O_{10}$+H). Vernonioside B_1 (**4**): Colorless needles, mp 208-211°C. $[\alpha]_D^{25}$ +31.9° (c 0.94, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1775. UV λmax: 235 nm (ε 13000), 243 nm (ε 15000), 250 nm (ε 10000). FABMS m/z: 633 (MH^+, $C_{35}H_{52}O_{10}$+H).

GLC analysis of sugar moiety of 4

Vernonioside B_1 (**4**) (100 μg) was hydrolyzed with 2N TFA (200 μl) at 125°C in a sealed tube for 1 hr. After the solvent was evaporated, the residue was dissolved in pyridine (20 μl), to which N,O-bis(trimethylsilyl)trifluoroacetamide (10 μl) was added. The mixture was left for 1 hr and the products were analyzed by GLC on OV-1 at 170°C. TMS-glucoses were observed at Rt 8.0 min and Rt 12.8 min.

Enzymatic hydrolysis

Vernonioside B_1 (10 mg) was dissolved in Triton X-100 (0.2 ml), and 50 mM sodium citrate-NaOH buffer (pH 5.0, 20 ml) and β-glucosidase (32 mg) were added. After incubation of the mixture at 35°C for 72 hr, the reaction products were extracted with *n*-BuOH (20 ml) three times. After chromatography of the *n*-BuOH-soluble part on silica gel and then ODS gel, the natural aglycone was recrystallized from EtOAc. **4a**: Colorless plates, mp 205-210°C. $[\alpha]_D^{25}$ +40.8° (c 1.03, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 236 (13000), 242 (14000), 250 (10000). ^1H NMR δ (pyridine-d_5) ppm: 0.79 (3H, s), 1,00 (3H, s), 1.26 (3H, d, *J*=7.4 Hz), 1.30 (3H, d, *J*=7.4 Hz), 1.32 (3H, d, *J*=5.5 Hz), 1.45 (1H), 1.5 (1H), 1.51 (1H), 1.6 (1H), 1.62 (1H), 1.8 (2H), 1.9 (2H), 1.92 (2H), 1.99 (1H), 2.0 (1H), 2.1 (1H), 2.38 (1H), 2.39 (1H), 2.40 (1H), 2.44 (1H), 3.07 (1H, dd, *J*=9.9, 4.1Hz), 3.29 (1H, dd, *J*=17.9, 6.8 Hz), 3.64 (1H, q, *J*=5.6 Hz), 3.80 (1H, m), 4.79 (1H, br s), 5.00 (1H, d, *J*=2.4 Hz), 5.42 (1H, br s), 5.62 (1H, br d, *J*=6.2 Hz). HR-EIMS m/z: 470.3028 (M^+, calcd. for $C_{29}H_{42}O_5$, 470.3032). By the same procedure, three natural aglycones, **1a** (2.6 mg), **2a** (5.2 mg), and **3a** (2.6 mg), were obtained from 10 mg of each vernonioside and recrystallized from MeOH. **1a**: Colorless needles, mp 256-258°C. $[\alpha]_D^{28}$ +51.3° (c 0.08, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 236 (14000), 242 (1600), 251(11000). ^1H NMR δ (pyridine-d_5) ppm: 1.02 (3H, s), 1.07 (3H, d, *J*=7.2 Hz), 1.21 (3H, s), 1.23 (3H, d, *J*=6.7 Hz), 1.29 (3H, d, *J*=7.1 Hz), 1.42 (1H), 1.55 (1H), 1.61 (1H), 1.79 (1H), 1.89(1H), 1.90(1H), 1.91 (1H), 1.93 (2H), 1.96 (1H), 2.02 (1H), 2.16 (1H), 2.22 (1H), 2.26 (1H), 2.27 (1H), 2.55 (1H, dt, *J*=12.6, 7.4, 7.4 Hz), 2.74 (1H, m), 2.99 (1H, q, *J*=5.5 Hz), 3.16 (1H, dd, *J*=17.6, 6.6 Hz), 3.39 (1H, m), 3.86 (1H, m), 4.65 (1H, m), 4.90 (1H, dd, *J*=10.2, 5.9 Hz), 5.48 (1H, br s), 5.63 (1H, br d, *J*=6.1 Hz). HR-EIMS m/z: 470.3014 (M^+, calcd. for $C_{29}H_{42}O_5$, 470.3032). **2a**: Colorless plates, mp 229-231°C. $[\alpha]_D^{19}$ +34.5° (c 0.26, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1760. UV λmax (MeOH) nm (ε): 235 (14000), 242 (17000), 251 (11000). ^1H NMR δ (pyridine-d_5) ppm: 0.79 (3H, s), 0.98 (3H, s), 1.08 (3H, d, *J*=7.2 Hz), 1.19 (3H, d, *J*=5.5 Hz), 1.29 (3H, d, *J*=7.1 Hz), 1.38 (1H), 1.53 (1H), 1.60 (1H), 1.76 (1H), 1.87 (1H), 1.90 (2H), 1.96 (2H), 2.13 (1H), 2.17 (2H), 2.32 (1H), 2.39 (1H), 2.42 (1H), 2.54 (1H, m), 2.81 (1H, dd, *J*=17.3, 6.7 Hz), 2.86 (1H), 3.01 (1H, q, *J*=5.5 Hz), 3.07 (1H, dt, *J*=12.1, 7.6, 7.6 Hz), 3.84 (1H, m), 4.58 (1H, m), 4.93 (1H, dd, *J*=10.7, 5.5 Hz), 5.45 (1H, br s), 5.58 (1H, br d, *J*=5.6 Hz). HR-EIMS m/z: 470.3038 (M^+, calcd. for $C_{29}H_{42}O_5$, 470.3033). **3a**: Colorless needles, mp 228-230°C. $[\alpha]_D^{26}$ -12.5° (c 0.12, MeOH). IR υmax (KBr) cm^{-1}: 3400, 1770, 1740. UV λmax (MeOH) nm (ε): 234 (11000), 242 (12000), 250 (8000). ^1H NMR δ (pyridine-d_5) ppm: 0.76 (3H, s), 0.96 (3H, s), 1.04 (3H, d, *J*=7.2 Hz), 1.24 (3H, d, *J*=5.7 Hz), 1.26 (3H, d, *J*=7.3 Hz), 1.43 (1H), 1.50 (1H), 1.65 (1H), 1.80 (1H), 1.88 (2H), 1.89 (1H), 1.95

(1H), 2.00 (1H), 2.19 (1H, dd, *J*=18.2, 12.8 Hz), 2.20 (1H), 2.34 (2H), 2.40 (2H), 2.47 (1H), 2.70 (1H), 3.06 (2H), 3.17 (1H, q, *J*=5.6 Hz), 3.84 (1H, m), 4.89 (1H, t, *J*=8.1 Hz), 5.35 (1H, br s), 5.62 (1H, br d, *J*=5.4 Hz). HR-EIMS m/z: 468.2859 (M^+; calcd. for $C_{29}H_{40}O_5$, 468.2876).

Hydrolysis of 4 with HCl

To **4** (8.9 mg) in EtOH (0.2 ml), 2.0N HCl (5 ml) and toluene (5 ml) were added. After the solution was refluxed for 6 hr, the products in the upper layer were purified by preparative TLC (silica gel plate, toluene-MeOH/8:3) to yield an aglycone (**4b**, 3.8 mg). **4b**: Colorless needles, mp 259-261°C. $[\alpha]_D^{28}$ +13.3° (c 0.24, MeOH). IR νmax (KBr) cm^{-1}: 3400, 1775. UV λmax (MeOH) nm (ε): 250 (16000). ^1H NMR δ (pyridine-d_5) ppm: 1.02 (3H, s), 1.12 (3H, d, *J*=7.1 Hz), 1.16 (1H), 1.17 (3H, s), 1.25 (3H, d, *J*=6.6 Hz), 1.33 (3H, d, *J*=6.6 Hz), 1.37 (1H), 1.44 (2H), 1.54 (1H), 1.64 (1H), 1.72 (2H), 1.87 (1H), 2.07 (1H), 2.11 (1H), 2.12 (1H), 2.24 (3H), 2.38 (1H), 2.44 (1H), 2.54 (1H), 2.88 (1H), 2.98 (1H, br d, *J*=13.2 Hz), 3.11 (1H, dd, *J*=10.0, 6.5 Hz), 3.85 (1H, m), 4.31 (1H, q, *J*=6.4 Hz), 4.89 (1H, dd, *J*=6.5, 4.7 Hz), 4.96 (1H, d, *J*=4.7 Hz), 5.44 (1H, br s).

Reduction of 3 with $NaBH_4$

To **3** (11.5 mg) in MeOH (2 ml), $NaBH_4$ (2 mg) was added. The mixture was left for 5 min while being stirred, and diluted acetic acid (about 20%, 1 ml) was added. After the solvent was evaporated, the products were extracted with *n*-BuOH. Purification of the *n*-BuOH extract by preparative HPLC on μBondasphere C_{18} (acetonitrile-water/35: 65) yielded vernonioside A_1 (**1**, 3.7 mg, R_t 32.0 min) and A_2 (**2**, 1 mg, R_t 28.4 min).

X-ray diffraction analysis of 4a

Crystal data of the single crystal used for X-ray analysis were as follows: monoclinic, space group $p2_1$, a=17.082(1), b=12.236(1), c=6.230(1) Å, β=92.81°(1), V=1301.0 $Å^3$, Z=2, Dx=1.202 g/cm^3. Intensity data of 2179 diffractions within 2Ø<130° were collected by a Rigaku AFC-5UD four-circle diffractometer with graphite-monochromated CuKα radiation (λ=1.54178 Å), and 2179 unique reflections with Fo>3σ (F) were used for the structure determination by a MULTAN 84 program system[10] (Rint=0.050). The perspective view is shown in Fig. 2.

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