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A New Triterpenoid Saponin from the Chinese Traditional Medicine *Nothopanax davidii* Harms (Araliaceae)

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Abstract: A new saponin, 3-O-[α-L-arabinopyranosyl-(1-2)-α-L-arabinopyranosyl]-olean-12-ene-28,29-dioic acid-28-[O-α-L-rhamnopyranosyl-(1-4)-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside [1], was isolated from the bark of *Nothopanax davidii* Harms, used to prepare the antiinflammatory Chinese traditional medicine Shu Wu Jia.

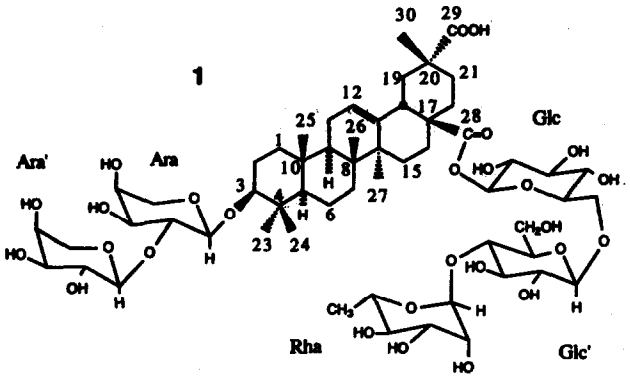
INTRODUCTION

The *Araliaceae* family is one of the most medicinally important plant families. Perhaps the most famous member of this family is ginseng (*Panax ginseng* C. A. Meyer), the tubers of which have been used in traditional Oriental medicines for more than 5000 years.[1,2] Chemical and pharmacological investigations have indicated that triterpenoid saponins are important bioactive components existing in the plants of *Araliaceae* family. For example, more than 50 saponins have been isolated and identified from *Panax* species which show various pharmacological activities including increasing mental efficiency, recovering physical balance, stimulation of metabolic function, and other general health promoting effects.[1,2] Saponins are also abundant in ginseng substitutes,[1,3] such as *Nothopanax delavayi*[4] reported to be antipyretic and antiinflammatory, though unambiguous assays relating the reputed bioactivities to the purified saponins is lacking. Another ginseng substitute of the *Nothopanax* genus commonly employed in Western China, *N. davidii* Harms (known as Yi Ye Liang Wang Cha: 异叶梁王茶), are small trees found in the mountains of Sichuan and neighboring provinces. The bark and leafstalks of this species are used as the antiinflammatory folk medicine Shu Wu Jia (树五加) to treat rheumatism.[5] Five saponins with serratagenic acid as the aglycone have been previously isolated from the bark extracts and their structures reported.[6] An additional, more polar saponin [1] was also isolated, and the structure is reported herein.

RESULTS AND DISCUSSION

Isolation and Preliminary Characterization.

The dried bark of *N. davidii* was extracted by methanol and the saponin fraction isolated according to classic procedures.[6,7] Flash and low pressure chromatography on silica gel gave crude **1**, which was purified by reversed-phase C_{18} HPLC (see Experimental).



Positive ion FAB-MS (LRMS) provided a molecular ion, *m/z* 1220, suggesting a molecular formula of $C_{58}H_{92}O_{27}$, which was consistent with the results of analysis (C, 53.48%; H, 7.752% ; cald. C, 53.49%; H 7.763%; for $C_{58}H_{92}O_{27}\cdot 9/2H_2O$). Efforts to obtain an HRMS analysis of the molecular ion were unsuccessful, nor could water be completely removed from purified **1**.

In order to reduce signal overlap in the ^1H NMR spectrum, the hydroxyl protons were exchanged for deuterium prior to recording the spectra in pyridine-d_5. In addition, 2 drops of D_2O were added to eliminate residual hydroxyl resonances and optimize spectral dispersion. The ^{13}C NMR spectrum showed 58 resonances (Table 1) supporting the molecular formula deduced from the LRMS and analysis.

The presence of five sugars in **1** was apparent from the five anomeric carbon signals (δ 94.9, 101.9, 103.8, 106.5, and 106.7). One of these sugars was rhamnose (δ 101.9), suggested by the distinct methyl proton doublet (Rha.H-6: δ 1.58 ppm, J = 5.9 Hz). Two anomeric protons at δ 6.13 (d, J = 8.1 Hz) and 4.89 (d, J = 7.9 Hz), correlating with carbon signals at δ 94.9 and 103.8, respectively, were tenatively assigned as D-glucose. The significant downfield shift of the anomeric proton at δ 6.13, and the upfield shift of the anomeric carbon at δ 94.9 indicated that this glucose was attached to the aglycone through an ester linkage.[8] The remaining two sugars were both tenatively identified as arabinose based on their anomeric proton and carbon chemical shifts[9] ($^{13}C_1/^1H_1$: δ 106.7/4.70, d, J = 7.1 Hz; 106.5/4.68, d, J = 7.9 Hz; arabinose had also been reported in the saponins previously isolated from *N davidii*).[6] These conclusions were confirmed by acidic hydrolysis of **1**, which afforded L-rhamnose, D-glucose and L-arabinose.

Structure of the Aglycone.

The ^{13}C resonances for the aglycone were easily distinguished, revealing only one oxygenated sp^3 carbon (C_3, δ 88.1), the remaining oxygenated carbon resonances being accounted for by the five sugars. The thirty aglycone carbons were shown by DEPT spectra to be six quaternary carbons (δ 36.3, 38.8, 39.10, 41.4, 41.6, 46.3), four methines (δ 40.1, 47.3, 55.1, 88.1 ppm), ten methylenes (δ 17.7, 22.7, 23.1, 26.0, 27.50, 28.3, 30.8, 32.3, 38.1, 39.13), six methyl groups (δ 14.9, 16.3, 16.7, 19.2, 25.3, 27.46), and four sp^2 carbons (δ 122.6, d; 142.9, s; 175.9, s; 180.9, s). The total of 30 carbons, in combination with the information exhibited in the ^1H NMR spectrum (Experimental): six methyl proton singlets and one broad vinyl proton singlet (δ 5.38

Table 1. NMR Chemical Shift Assignments of 1.

Position	^{13}C Aglycone of 1 (C_5D_5N)[a]	- Sugars -		
		Position	^{1}H (C_5D_5N)[b]	^{13}C (C_5D_5N)[a]
1	38.1 (t)	Glc.1	6.13 (d, 8.1 Hz)	94.9 (d)
2	22.7 (t)	Glc.2	4.60 (o)	72.9 (d)
3	88.1 (d)	Glc.3	4.18 (o)	77.4 (d)
4	38.8 (s)	Glc.4	4.27 (o)	69.6 (d)
5	55.1 (d)	Glc.5	3.99 (o)	77.0 (d)
6	17.7 (t)	Glc.6	4.58, 4.26 (o)	68.2 (t)
7	32.3 (t)	Glc'.1	4.89 (d, 7.9 Hz)	103.8 (d)
8	39.10 (s)	Glc'.2	3.85 (dd, 7.9, 8.1 Hz)	74.3 (d)
9	47.3 (d)	Glc'.3	4.02 (o)	75.5 (d)
10	36.3 (s)	Glc'.4	4.23 (o)	77.6 (d)
11	28.3 (t)	Glc'.5	3.50 (o)	76.2 (d)
12	122.6 (d)	Glc'.6	4.01, 3.97 (o)	60.3 (t)
13	142.9 (s)	Rha.1	5.68 (d, 2.3 Hz)	101.9 (d)
14	41.6 (s)	Rha.2	4.57 (o)	71.7 (d)
15	27.50 (t)	Rha.3	4.46 (dd, 8.7, 2.9 Hz)	71.6 (d)
16	23.1 (t)	Rha.4	4.25 (o)	69.6 (d)
17	46.3 (s)	Rha.5	4.80 (m)	69.6 (d)
18	40.1 (d)	Rha.6	1.58 (d, 5.9 Hz)	17.1 (q)
19	39.13 (t)			
20	41.4 (s)	Ara.1	4.68 (d, 7.9 Hz)	106.5 (d)
21	26.0 (t)	Ara.2	4.35 (dd, 7.4, 6.5 Hz)	74.6 (d)
22	30.8 (t)	Ara.3	4.09 (o)	70.3 (d)
23	27.46 (q)	Ara.4	4.28 (o)	68.6 (d)
24	16.3 (q)	Ara.5	4.22, 3.74 (o)	65.8 (t)
25	14.9 (q)			
26	16.7 (q)	Ara'.1	4.70 (d, 7.1 Hz)	106.7 (d)
27	25.3 (q)	Ara'.2	3.96 (o)	71.5 (d)
28	175.9 (s)	Ara'.3	4.10 (o)	73.6 (d)
29	180.9 (s)	Ara'.4	4.28 (o)	68.6 (d)
30	19.2 (q)	Ara'.5	3.3 - 3.7 (m, 2H)	66.1 (t)

a) Carbon multiplicities from a DEPT experiment; glycosidation sites underlined.
b) "o" = overlapped.

attached to the carbon at δ 122.6), suggested that the aglycone was a pentacyclic triterpene (tetracyclic triterpenes usually have fewer than 5 methyl proton singlets) with one trisubstituted double bond (δ 122.6, d; 142.9, s) and two carbonyl groups (δ 175.9, 180.9). The chemical shifts of the two olefinic carbons and comparison of the remaining chemical shifts with those well-established for pentacyclic triterpenes revealed the characteristic carbons of an olean-12-ene (β-amyrin type) triterpene.[10] Equally revealing was the methine resonance at δ 40.1

ultimately assigned to C_{18}, a diagnostic shift for these triterpenes.[4,6,10] The ^1H NMR spectrum displayed a proton resonance at δ 3.20 bonded to this carbon ($^{13}C/^1H$ heteronuclear COSY spectrum).[11] The absence of a seventh methyl singlet in the ^1H and ^{13}C NMR spectra and the appearance of one more carbonyl carbon in the ^{13}C-NMR spectrum, as well as the relative downfield shift of C_{20} (δ 41.4 ppm) in comparison with methyl oleanolate (δ 30.6)[10] suggested that one methyl group at C_{20} of oleanolic acid had been replaced by a carboxylic acid group. The β-orientation of this methyl group (H_{30}) was established by an NOE with $H_{18\beta}$ observed in the NOESY spectrum; the 20-position carboxylic acid group must therefore be α-oriented. Thus, the aglycone was assigned as 3β-olean-12-ene-28,29-dioic acid (serratagenic acid), originally isolated from *Clerodendron serratum*[12] and the same aglycone as found in the other saponins reported from *N. davidii*[6] and *N. delavayi*,[4] confirmed by comparing the proton and carbon NMR with those reported for the related saponins[4,6] as well as the parent aglycone.[4,12c,13] The five sugars were suggested to be present in two saccharide units, one attached to C_{28} by an ester linkage and the other at C_3. Aglycone carbon assignments were completed by extensive homonuclear and heteronuclear COSY, as well as selective INEPT experiments.

Structure of the Oligosaccharides

The individual proton spin systems for each sugar residue were delineated by homonuclear correlations as detected from the COSY and multistep homonuclear relayed coherence transfer (RCT) experiments.[14] In this way, triple relayed correlations were detected from anomeric protons (H_1) to H_5 via the intervening H_2 (direct coupling), H_3 (single relayed coherence transfer) and H_4 (double relayed coherence transfer). Thus, beginning from the anomeric protons (δ 6.13, Glc.H_1; 5.68, Rha.H_1; 4.89, Glc'.H_1; 4.70, Ara'.H_1; 4.68, Ara.H_1), all 2-position protons of the sugar moieties were easily located in the COSY spectrum (Figure 1), including the correlation between the weakly coupled Rha.H_1 with Rha.H_2 ($^3J_{H1/H2}$ = 2.3 Hz). The remaining proton spins of the rhamnose residue (H_3 through H_6) were also delineated from the COSY spectrum enabling the assignment of Rha.H_6 through Rha.H_3. These rhamnose proton assignments were subsequently confirmed by the double and triple RCT spectra. The two arabinose anomeric protons were overlapped, but distinguished from cross-sections of the COSY spectrum at the frequencies of Ara.H_2 and Ara'.H_2, respectively.

The single RCT spectrum (Figure 2a) showed the relayed coherence transfer from the sugar H_1 to H_3 protons via the intervening H_2.[15] In such a way, most 3-position protons of the sugar residues could be assigned. One ambiguity existed: the cross peaks between the two arabinose anomeric protons with their respective 3-position protons were overlapped due to the near magnetic equivalence of Ara.H_3 and Ara.'H_3. 1,3-Diaxial intraresidue NOE's between the anomeric protons of the two arabinoses with the 3-position protons appeared at exactly the same position as the arabinose H_1/H_3 relayed coherence transfers in the single RCT spectrum, also suggesting that the two arabinose H_3's were near-coincident. This was confirmed by the $^{13}C/^1H$-COSY experiment: the overlapped proton signals of Ara.H_3 and Ara'.H_3 showed correlations with two distinct carbons (δ 73.6, Ara'.C_3 and 70.3, Ara.C_3, ultimately distinguished from their relative ^{13}C T_1 relaxation times and chemical shift analysis, vide infra). Cross-sections from the $^{13}C/^1H$-COSY spectrum at the frequencies of Ara.C3 and Ara'.C3 provided the chemical shifts of these two protons (δ 4.09, Ara.H_3; 4.10, Ara'.H_3).

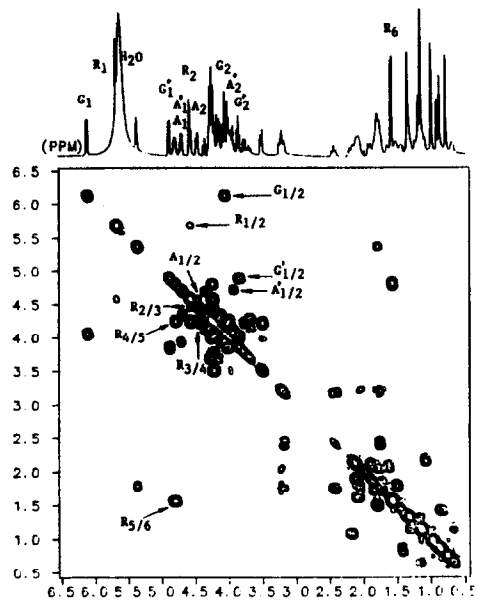


Figure 1. $^1H/^1HCOSY$ spectrum of **1**; sugar anomeric to 2-position proton correlations labelled above diagonal, rhamnose H2 - H6 system labelled below diagonal.

With the assignments of the 3-position protons of individual sugar units, mapping of the sugar spin systems continued to the respective 4-positions, and subsequently to the 5-position protons with double and triple RCT experiments, respectively (Figures 2b and 3). The correlation signals from the anomeric to the 4-position protons of the two glucose residues through two-step relayed coherence transfers were clear in the double RCT spectrum, but only one correlation between the anomeric and the 4-position protons of the two arabinose residues was detectable, which implied that the two 4-position proton signals of arabinoses were also overlapped. The triple relay RCT spectrum (Figure 3) also displayed all correlations between the rhamnose anomeric proton with other rhamnose protons (H_2 through H_5), which was consistent with the assignment of these protons based upon the COSY experiment.[16]

With the COSY and RCT experiments completed, most sugar protons were assigned. The assignment of the 3- and 5-position protons of the glucose residues as well as the 3-position protons of the arabinose units were confirmed by intraresidue NOE's between these protons with the respective anomeric protons due to 1,3-diaxial dipolar interactions observed in the NOESY experiment (vide infra, Figures 4 and 6).

The C_6 methylene protons of the two glucose units were most easily located through their respective $^{13}C/^1H$ correlations in the fixed evolution $^{13}C/^1H$-COSY spectrum[17] after the C_6 resonances had been assigned. (These methylene $^{13}C/^1H$ correlations did not appear in the normal $^{13}C/^1H$-COSY spectrum, as is often a problem for methylene carbons bearing magnetically nonequivalent protons).[18] Since C_6 of glucose and C_5 of arabinose were the only methylene carbons in the region of sugar carbon resonances, they were easily discerned by their multiplicity in the DEPT experiment (δ 68.2, 66.1, 65.8, 60.3). Arabinopyranose C_5 resonances normally appear between 65 - 66 ppm,[19] and glucose C_6 in the range of 60 - 62 ppm except when glycosidated (66 - 69 ppm).[20] Therefore, the most downfield methylene carbon (δ 68.2) was assigned to a glycosidated glucose (Glc.C_6). The remaining methylene resonances were assigned as Ara.C_5 (δ 65.8), Ara.'C_5 (δ 66.1), and Glc'.C_6 (δ 60.3), respectively, according to their chemical shifts. (Distinction of Ara.C_5 and Ara'.C_5 was ultimately based on T_1 relaxation times). The assignments of the methylene protons to each glucose residue were then completed by the vicinal and geminal coupling correlations in the COSY spectrum (Figure 5). With all the sugar protons assigned, a normal $^{13}C/^1H$-COSY spectrum then allowed assignments of the sugar carbon resonances to each individual sugar. The sole exceptions were distinction of carbons attached to the overlapped arabinose protons (H_1's, H_3's, and H_4's). The assignment of these carbons relied upon their relative ^{13}C-T_1 relaxation times and chemical shift analysis after the saccharide sequences were assigned.

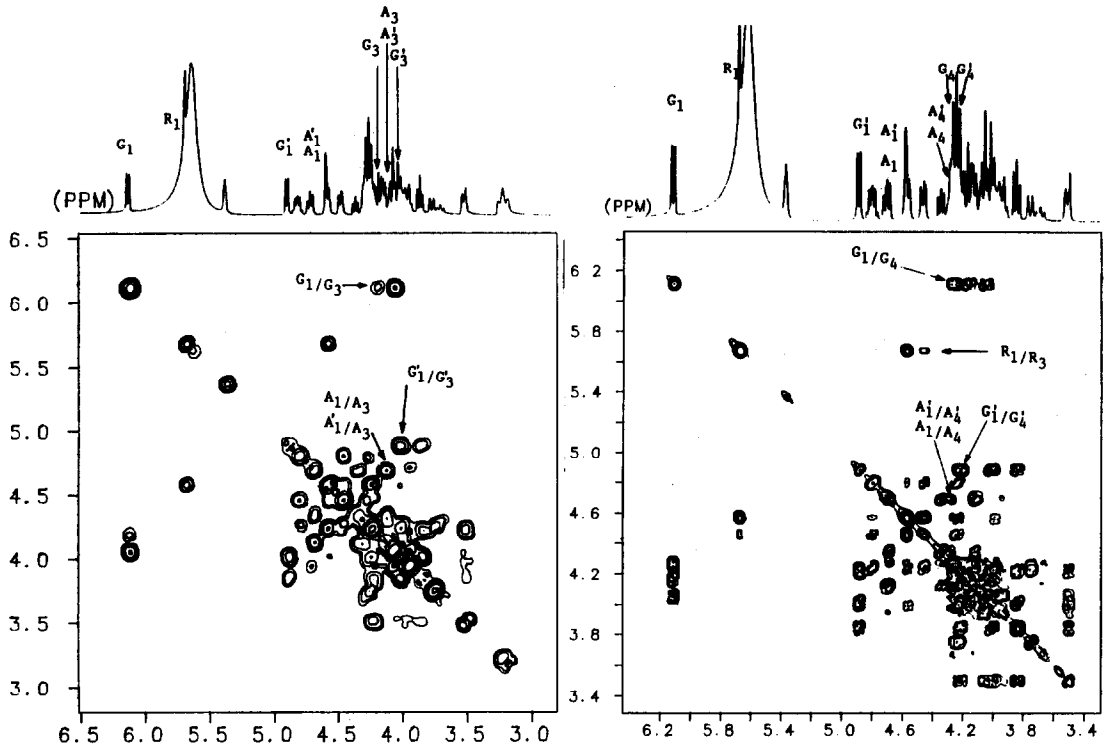


Figure 2 (a) Single RCT spectrum of **1**. Labelled peaks indicate the relayed coherence transfers between anomeric protons of sugar residues with their respective 3-position protons. (b) Double RCT spectrum of **1**. Labelled peaks indicate the relayed coherence transfers between anomeric protons with their respective 4-position protons, and the coherence transfer between Rha.H_1 with Rha.H_3.

Determination of the sugar sequences and linkage sites was accomplished by analysis of the carbon chemical shifts, observation of interresidue NOE's, and the fragmentation patterns in the mass spectrum. Comparing the ^{13}C NMR of **1** with reference data,[21] C_3 of the aglycone, Glc.C_6, Glc'.C_4 and Ara.C_2 displayed downfield shifts of 3 - 10 ppm, indicative of glycosidation. As previously mentioned, one very downfield glucose anomeric proton (Glc.H_1, δ 6.13), which correlated with a relatively high field anomeric carbon (δ 94.9) reflected an ester glycosidic linkage. This was further supported by the appearance of an ester carbonyl carbon (δ 175.9, compared with the carboxylic acid carbonyl resonance: δ 180.9). Since there were two possible ester linkage sites, C_{28} or C_{29}, selective INEPT experiments were performed to determine this connection. Polarization transfer from the H_{30} methyl proton singlet to the carboxylic acid carbonyl carbon (δ 180.9) established that the free carboxylic acid must be C_{29}, and the ester glycosidic bond should be to C_{28}.

The assignment of the sugar sequences was initially suggested by the fragmentation patterns observed in the FAB-MS spectrum. In addition to the molecular ion [M^+, *m/z* 1220], the FAB-MS spectrum of **1** gave ions of *m/z* 1088 and 1074, from loss of the terminal arabinose [M-132] and rhamnose [M-147] sugars, respectively. Additional ions: *m/z* 956 and 750 due to the loss of an [Ara.'-Ara.] disaccharide and [Rha.- Glc.'-Glc.] trisaccharide, were also observed in the FAB-MS spectrum (Figure 4).

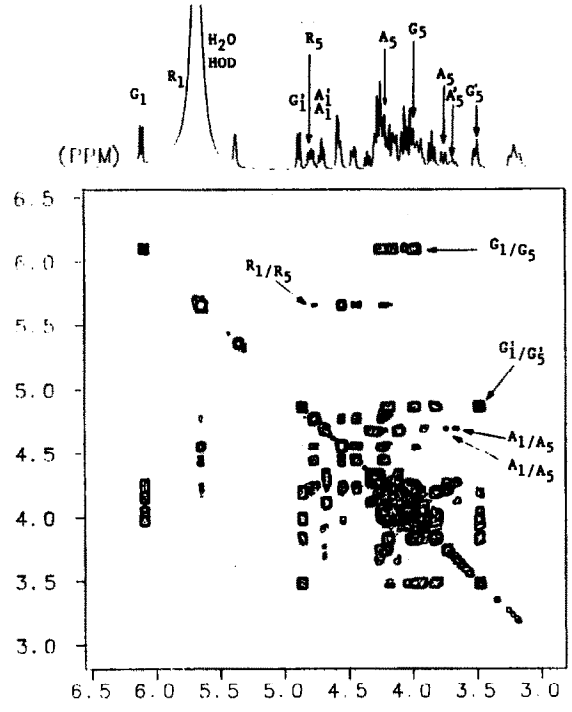


Figure 3. Triple RCT spectrum of **1**. Labelled peaks indicate correlations between anomeric protons of sugars with respective 5-position protons.[16]

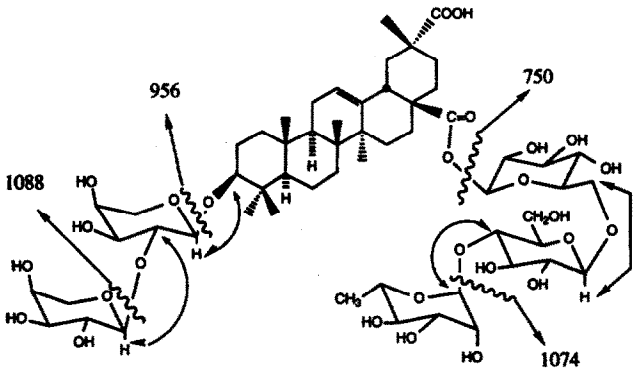


Figure 4. Mass spectral fragmentations and interglycosidic NOE's of **1** for sugar sequence and linkage site determination.

The sequence and the linkage sites of both sugar chains were subsequently confirmed by the observation of interresidue NOE's between the linkage position protons across the glycosidic bonds to the anomeric protons of the next sugar unit or the aglycone (Figures 4 and 6). The NOE's between Rha.H_1 with Glc'.H_4, and Glc'.H_1 with both Glc.H_{6a} and Glc.H_{6b} were observed in the NOESY experiment, suggesting that one saccharide chain had the structure: Rha$^{1\rightarrow4}$Glc'$^{1\rightarrow6}$Glc-, and this chain was attached to the C_{28} carboxylate group through an ester linkage. The NOESY spectrum also displayed a dipolar interaction between Ara.H_1 with the aglycone H_3, connecting this arabinose to the aglycone 3-position. No interresidue NOE's between the second arabinose (Ara'.H_1) with the protons of any other sugar could be detected at ambient temperature due to an unfavorable correlation time.[22] When the temperature was lowered to 2 °C, the correlation times (τ_c) for this terminal arabinose

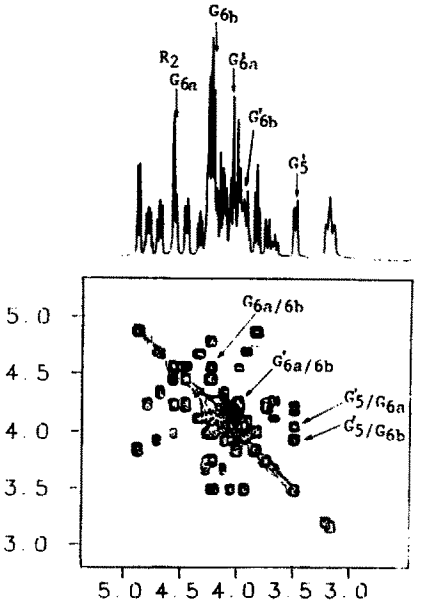


Figure 5. $^1H/^1H$-COSY spectrum of **1**. Labelled peaks indicate geminal and vicinal couplings of glucose C_6 methylene protons.

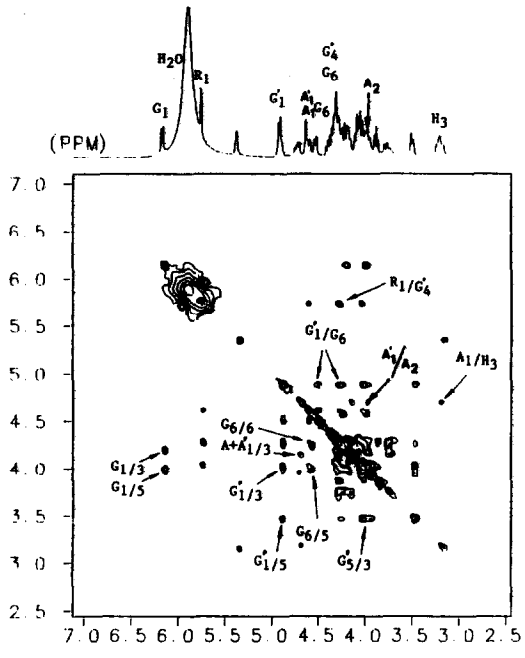


Figure 6 NOESY spectrum of **1** at 2 °C; intraresidue NOE's of sugars are labelled under diagonal and interresidue NOE's are above diagonal. At rt, the NOE between Ara'.H1 and Ara.H2 was not observed.

(Ara') increased sufficiently and a negative NOE between Ara'.H_1 and Ara. H_2 was observed along with the NOE's of all the other sugar residues, establishing the Ara'$^{1\rightarrow2}$Ara disaccharide at the aglycone C_3 (Figure 6). Thus, the sugar sequence and linkage sites deduced from the NOESY experiments were in agreement with that from the analysis of the carbon chemical shifts supported by the mass spectral fragmentations.

With the saccharide chains assigned, distinction of the resonances of the two arabinose sugars was accomplished. For saponins like **1**, molecular mobility of the individual sugar residues increases (τ_c decreases) as the residues are located away from the aglycone, increasing the T_1 relaxation times.[23] Such measurements can be particularly valuable for distinguishing terminal and internal sugar residues in oligosaccharide chains,[18] which was the problem at hand to distinguish the arabinose units. Since the correlation times are viscosity dependent, the measurements were recorded at both ambient temperature and at 40°C. The results (Table 2) indicated that the order of T_1 relaxation times of the sugar residues obtained from the higher temperature experiment was identical as that determined at room temperature, so an additional measurement at higher temperature was not undertaken.

Table 2 ^{13}C-T_1 Relaxation Time of Sugar Units and Anomeric Carbons of **1**.[a]

Sugar	T_1 (sec, 23 °C)		T_1 (sec, 40 °C)	
	Ave. NT_1[b]	Anomeric Carbon	Ave. NT_1[b]	Anomeric Carbon
Glc	0.232	0.232	0.235	0.232
Glc'	0.242	0.240	0.245	0.242
Rha	0.261	0.259	0.266	0.261
Ara	0.314	0.312	0.319	0.314
Ara'	0.342	0.341	0.347	0.343

a) See text for specific T_1's of other arabinose carbons.
b) N = number of attached protons.

The two anomeric carbons of the arabinose units showed distinctive T_1 relaxation times: one (δ 106.7) with the longer relaxation time (0.343 s, 40°C), was assigned to the terminal arabinose C_1, and the other

anomeric carbon (δ 106.5, T_1 = 0.314 s, 40°C) was assigned to the internal arabinose. Similarly, distinguishing the 5-position carbons of the two arabinose residues was achieved by the difference of their T_1 relaxation times (Ara.C_5, T_1= 0.453 s; Ara'.C_5, T_1= 0.469 s). The assignments of the 2-position carbons of the arabinose units were routine based on their chemical shifts: the carbon signal at δ 71.5 with its bonded proton at δ 3.96 was assigned as Ara'.C_2, while the downfield resonance (δ 74.6) with its attached proton at δ 4.35 was assigned as Ara.C_2, the relative downfield chemical shift indicating a glycosidic linkage. The two overlapped arabinose 3-position protons showed correlations with carbons at δ 73.6 and 70.2, which were assigned as Ara'.C_3 and Ara.C_3, respectively, based on a chemical shift analysis. Usually arabinose C_3 carbons appear at δ 72 - 73, and the relatively high field carbon resonance of Ara.C_3 results from a β-glycosidation. The two C_4 resonances of the arabinoses were not resolved (δ 68.6, integration to 2 X C, inverse gated decoupling).

The stereochemistry of each anomeric center was assigned from $^1J_{C1/H1}$ heteronuclear coupling constants and confirmed by the $^3J_{H1/H2}$ coupling constants. The heteronuclear coupling constant ($^1J_{C1/H1}$ =174.6 Hz) of the rhamnose unit indicated that this rhamnose had the usual α-configuration ($^3J_{H1/H2}$= 2.3 Hz).[24] The two glucose residues showed one bond heteronuclear coupling constants between anomeric carbons and protons (Glc, ^1J =158.7 Hz and Glc', 162.0 Hz) typical for β-glucose.[25] In agreement with this, both glucose anomeric protons showed homonuclear couplings with the respective glucose H_2 protons as expected for trans diaxial sugar protons (Glc $^3J_{1/2}$ = 8.1 Hz; Glc' $^3J_{1/2}$ = 7.9 Hz).

The presence of both arabinoses in the $^4C_1$ pyranose forms was apparent from the carbon chemical shifts, the small $^1J_{C1/H1}$ (Ara, J =158.7 Hz; Ara', J = 158.7 Hz) and the large $^3J_{H1/H2}$ (Ara, J = 7.9 Hz; Ara', J = 7.1 Hz) coupling constants. These heteronuclear and homonuclear coupling constants indicated that the anomeric protons were axially oriented, the α-configurations.[26] With the assignments of the anomeric configurations of all sugar residues, the structure of **1** was established as 3-O-[α-L-arabinopyranosyl-(1-2)-α-L-arabinopyranosyl]-olean-12-ene-28,29-dioic acid-28-[O-α-L-rhamnopyranosyl-(1-4)-β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside. The sugars were assumed to be the L-arabinose, L-rhamnose, and D-glucose enantiomers.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were recorded in pyridine-d_5 (0.5 mL with 2 drops D_2O) after exchanging hydroxyl protons for deuterons by 5 X D_2O wash/lyophilization cycles, on a Varian XL-400. The δ 7.55 resonance of residual [4-^1H]pyridine-d_4 and the δ 135.5 resonance of [2-^{13}C]pyridine-d_5 were used as internal references for ^1H and ^{13}C, respectively; ^1H NMR spectra were recorded at 400 MHz and ^{13}C spectra were recorded at 100 MHz. All NMR pulse sequences were run using standard Varian software, version 6.1c, except the fixed evolution heteronuclear COSY spectrum which was added to the sequence library according to Reynolds' program using an evolution period fixed to 20 ms.[17] Selective INEPT experiments were performed with excitation and refocusing delays optimized for $^3J_{CH}$ = 6.5 Hz according to the formulae Δ1 = 1/2J (0.077 s) and Δ2 = 1/3J (0.051 s).[27]

Isolation of 1. Nothopanax davidii Harms were collected in the Ei-mei mountain region of Sichuan Province, China.[28] The dried bark (5 kg) was milled to a powder and extracted with refluxing methanol (3 X 1.5 L, 3 hours per reflux). After filtration, the solvent was removed in vacuo and the residues dissolved/suspended in water (1 L), and then partitioned with petroleum ether (bp 35 - 60 °C), ethyl acetate, and

n-butanol. The residue from the n-butanol soluble fraction was dissolved in methanol and anhydrous acetone was added until precipitation was completed. The precipitate was collected (150 g), and a crude saponin fraction (20 g) obtained by flash chromatography on silica gel, eluting with the lower layer of $CHCl_3/CH_3OH/H_2O$ (7/3/1, then 6.5/3.5/1), producing four fractions. The most polar fraction (2 g) was further separated by low pressure silica gel chromatography ($CHCl_3/CH_3OH/H_2O$, 6.5/3.5/1, lower layer) to provide crude **1** (200 mg). Final purification by reversed phase C_{18} HPLC [Microsorb (5 μm), Rainin, 10 X 250 mm; UV detection, 208 nm; aqueous methanol, 1/1 v/v, flow rate = 1.26 mL/min] gave pure **1** (15 mg) as a white amorphous powder: mp 219 - 224 °C (decompose); $[\alpha]_D^{25}$ -6.89° (*c* = 0.1 MeOH); IR (KBr, cm^{-1}) 3400 (OH), 1720 (COOR) 1642 (-C=C-); ^1H NMR (selected aglycone resonances) δ 5.38 (bs, H_{12}), 3.21 (overlapped m, H_3), 3.20 (overlapped m, H_{18}), 1.33 (s, H_{30}), 1.15 (s, 6H, H_{23} & H_{27}), 0.98 (s, H_{26}), 0.86 (s, H_{24}), 0.77 (s, H_{25}), for sugar resonances, and ^{13}C NMR data, see Table 1; LRMS (FAB, *p*-nitrobenzyl alcohol matrix, *m/z*) 1200 ($[M]^+$, 6%), 1088 (15), 1074 (8), 956 (8), 750 (18); Anal. Calcd for $C_{58}H_{92}O_{27}.9/2H_2O$: C 53.49%, H 7.76; Found: C 53.48%, H 7.75%.

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