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NEW ANTIFEEDANT TRITERPENE GLYCOSIDES FROM THE CARIBBEAN SPONGE *ERYLUS FORMOSUS*

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Two groups of antifeedant triterpene glycosides were identified from the Caribbean sponge *Erylus formosus*. The structure of formoside B, a novel *N*-acetyl amino derivative of the known penasterol tetrasaccharide formoside, was elucidated using NMR and mass spectral data. Four triterpene hexasaccharides and two triterpene trisaccharides, characterized by a 31-carbon aglycone, proved difficult to isolate and therefore only the structure of their aglycone was determined. Gas chromatographic analysis of derivatized saccharides from these mixtures established the carbohydrate content of these compounds. All of the triterpene glycosides isolated contributed to the chemical defenses of this sponge, although with differing activities.

Keywords: Sponge; chemical defense; formoside; *Erylus formosus*

INTRODUCTION

In a recent survey of Caribbean sponge chemical defenses [1], crude extracts of *Erylus formosus* (Choristida/Astrophorida) were found to deter feeding by the predatory reef fish *Thalassoma bifasciatum* in aquarium assays. In the present study, bioassay-guided fractionation using both aquarium and field assays was employed to isolate the deterrent molecules, a mixture of triterpene glycosides [2]. The major metabolite of *E. formosus* collected in

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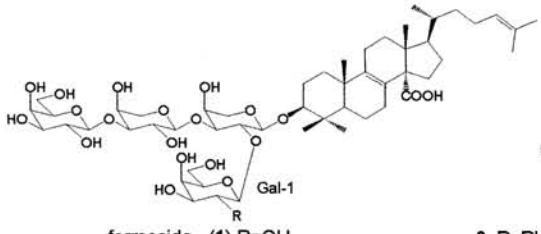
the Bahamas was formoside (**1**), a previously reported penasterol tetrasaccharide [3]. Although this compound represented approximately 90% of the mass of triterpene glycosides in *E. formosus* from the Bahamas, other triterpene glycosides were also found that proved to be more potent antifeedants when concentrations were normalized.

The deterrent metabolites belong to two classes of triterpene glycosides with distinct aglycones: penasterol derived compounds (formoside (**1**) and formoside B (**2**)), and compounds with a novel homologated triterpene aglycone. In this paper, we provide evidence for the structure of formoside B (**2**), a new *N*-acetyl aminoglycoside derivative of formoside. The second group of compounds proved difficult to separate into distinct compounds; thus the terpene alcohol **3** corresponding to the aglycone of this group was characterized, and the carbohydrate composition of each mixture was determined using degradative analysis. Tentative saccharide assignments to the individual compounds in each mixture are offered.

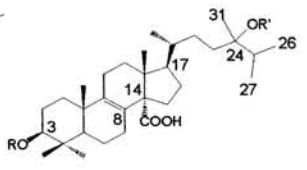
RESULTS AND DISCUSSION

Liquid partitioning, column chromatography, and reversed-phase HPLC were used to purify formoside (**1**) and formoside B (**2**) from *Erylus formosus* collected in the Bahamas, and yielded two other fractions containing very closely related mixtures of triterpene glycosides. *E. formosus* from the Florida Keys did not contain either **1** or **2**, but did contain the same two mixtures of other triterpene glycosides, in addition to a third mixture. These compounds and mixtures deterred fish feeding in field and aquarium assays [2].

Characterization of formoside (**1**) was accomplished by comparison of spectroscopic properties including ^{1}H- and ^{13}C-NMR and FAB and EI mass spectral data with published data [3]. The structure of formoside B (**2**) was elucidated by comparison of spectral data with that of **1**. The



formoside (**1**) R=OH
formoside B (**2**) R=$NHCOCH_3$



3 R, R' = H
HPLC fractions A, B, C: R, R' = saccharides

parent ion observed in the high resolution FAB mass spectrum ($[M+Cs]^+$ $m/z = 1218.4825$) indicated a molecular formula of $C_{54}H_{87}O_{21}N$ for **2**, suggesting that **2** differed from **1** by the substitution of an hydroxyl for an *N*-acetyl amino group. The upfield portion of the ^{1}H-NMR spectrum (δ 0–3) appeared identical to that of **1**, except for the presence of a methyl single at δ 2.03 that showed an HMQC correlation to a methyl carbon signal at δ 23.5 and an HMBC correlation to a carbonyl carbon signal at δ 173.8. ^{13}C-NMR shifts pertaining to the triterpene aglycone of **2** matched those of **1**, indicating that **1** and **2** shared the penasterol aglycone and that the *N*-acetyl amino group was positioned on one of the saccharides. Comparison of the saccharide anomeric ^{1}H chemical shifts for **1** and **2** supported the hypothesis that the *N*-acetyl amino group was at galactose-1 in **2**, making it an *N*-acetyl 2-amino-2-deoxy-D-galactose. In the NMR spectrum of **1**, the anomeric proton of galactose-1 resonated as a doublet at δ 4.71, whereas in **2** this signal was shifted to δ 4.92. Carbon-2 of galactose-1 (δ 73.5 in **1**) was significantly further upfield in the spectrum of **2** (δ 57.7), indicating a nitrogen linkage instead of a carbinol methine. All other carbon resonances of saccharide moieties in **2** were within 1–2 ppm of those of **1**, and most were within 0.5 ppm.

The other antifeedant triterpene glycosides isolated from *Erylus formosus* were characterized as mixtures of closely related compounds, HPLC fractions A, B, and C. Analytical diode array HPLC analysis, and NMR spectral data indicated that these three mixtures each contained two major compounds in an approximate 1 : 1 ratio, as well as a number of other minor constituents. Attempts to purify the compounds in these binary mixtures were not met with success; methods used included normal and reversed-phase flash column chromatography, modified silica (amino, cyano) TLC, size-exclusion column chromatography, reversed-phase HPLC in isocratic and gradient modes, and acetylation followed by purification by normal-phase HPLC. Finally, partial characterization of each mixture was afforded by mass spectral analysis and by hydrolysis, followed with GC carbohydrate analysis and NMR and mass spectral analysis of the aglycone.

For HPLC fraction A, probable molecular ions observed in the FABMS suggested two major hexasaccharides with molecular weights of 1454 and 1484. The exact mass of the higher mass component (A1) of fraction A as determined by negative ion MALDI and FABMS $([M-H]^-$ m/z 1482.7057 and 1482.7167, respectively), supported a molecular formula of $C_{69}H_{113}O_{33}N$. A molecular formula of $C_{68}H_{111}O_{32}N$ was suggested for the lower mass component (A2) of fraction A by negative ion MALDI

TABLE 1 Proposed carbohydrate composition of triterpene glycosides in HPLC fractions A, B and C

Saccharide	*HPLC fraction A*[a]		*HPLC fraction B*		*HPLC fraction C*[a]	
	A1	*A2*	*B1*	*B2*	*C1*	*C2*
Arabinose	2	3	3	2	2	3
Galactose	1	1	1	2		
Glucose	2	1				
Xylose					1	
N-acetyl glucosamine	1	1	1	1		
Unknown amino sugar			1	1		

[a]compounds in these fractions are proposed to contain one *O*-acetyl group on an unknown saccharide.

and FABMS ($[M\text{-}H]^-$ m/z 1452.7069 and 1452.7101, respectively). Gas chromatographic analysis of hydrolyzed and derivatized saccharides indicated that fraction A contained arabinose, galactose, glucose, and *N*-acetyl glucosamine in an approximate molar ratio of 5:2:2:5:2:5, with unknown linkages and configurations at the anomeric carbons. Combining these results with mass spectral data suggested that compound A1 could have contained two arabinose sugars, one galactose, two glucose sugars, one *N*-acetyl glucosamine, and one *O*-acetyl moiety on a saccharide (Table 1). Compound A2 would then have contained three arabinose sugars, one galactose, one glucose, one *N*-acetyl glucosamine, and one *O*-acetyl group. These constitutions were consistent with the NMR spectral data of fraction A: ester and/or amide carbonyl carbons were observed at δ 173.5 and 172.6, that showed HMBC correlations from methyl singlets at δ 2.04 and 1.97, respectively. As expected, *O*-acetyl groups were cleaved during hydrolysis prior to carbohydrate analysis, and thus could not be assigned to specific residues.

For HPLC fraction B, mass spectral analysis generated two sodium adduct parent ions at 1519 and 1549 by positive ion FABMS. The molecular formula of one of the triterpene hexasaccharides, B1, was suggested to be $C_{70}H_{117}O_{31}N_3$ by negative ion MALDI and positive ion FAB ($[M\text{-}H]^-$ m/z 1494.7489 and $[M+Cs]^+$ 1628.6815, respectively). The other molecular ion was too weak for exact mass determination but could be deduced by mass difference from B1, with B2 being 30 mass units heavier than B1, just as A2 and A1 differed by 30 mass units (i.e. by one $-CH_2O-$ group). Thus, it is likely that B2 was represented by the formula $C_{71}H_{119}O_{32}N_3$. These formulae provide for 14 degrees of unsaturation, consistent with B1 and B2 as hexasacharides of the aglycone **3**, with two *N*-acetyl residues on sugars. NMR spectral data, including ^1H methyl singlets at δ 1.95 and 2.02 correlating in the HMBC spectrum to ^{13}C-NMR signals at δ 172.3

and 172.5, matched these partial structures. Gas chromatographic carbohydrate analysis showed that HPLC fraction B contained arabinose, galactose, *N*-acetyl glucosamine, and an unknown amino sugar in an approximate 5:3:2:2 ratio. It is probable that B1 contained three arabinose sugars, one galactose, one *N*-acetyl glucosamine, one unknown amino sugar, and that B2 differed only from B1 by having one more galactose and one less arabinose (Table 1). Analysis of the complex NMR spectral data for HPLC fraction B did not reveal further aspects of the structures.

HPLC fraction C appeared to contain two isomeric components with molecular formula $C_{48}H_{78}O_{17}$, supported by negative ion HRFABMS measurement ($[M\text{-}H]^-$ m/z 925.5183). The carbohydrate content of this binary mixture of trisaccharides was arabinose and xylose, in an approximately 3, 4, or 5:1 ratio, with compound C1 likely containing two arabinose sugars, one xylose, and one *O*-acetyl moiety, and C2 containing three arabinose sugars with one *O*-acetyl group (Table 1). One ester carbonyl (δ 172.5), which showed HMBC correlations from a methyl singlet at δ 1.96 (HMQC to δ 22.4) confirmed the presence of a common *O*-acetyl group in compounds C1 and C2.

Despite extensive overlap in the NMR spectra that prevented the full structural elucidation of the six major triterpene glycosides in fractions A, B and C, it was possible to characterize the identical aglycone, **3**, of these compounds. When examined by EIMS, each of the mixtures gave a base peak at m/z 426, corresponding to loss of water and CO_2 from $C_{31}H_{52}O_4$, and both ^{1}H- and ^{13}C-NMR spectral data appeared identical for the terpene portions of the molecules. The ^{13}C chemicals shifts for C-3 and C-24 in the natural product mixtures, δ 92.2 and 89.6, respectively, were downfield relative to normal alcohols, indicating that saccharides were attached at both of these carbons. ^{13}C chemical shifts comparable to those of formoside (**1**) revealed that the ring systems of these two terpene cores were identical, and that the differences resided in the side chain (Table 2). An extra methyl singlet with ^{1}H-NMR shift δ 1.29 (^{13}C shift δ 20.2, C-31) had HMBC correlations to carbons at δ 89.6 (C-24), 35.4 (C-25), and 33.3 (C-23), established that C-31 was attached to C-24, which indicated that the aglycone of HPLC fractions A, B, and C was methylated and hydroxylated at C-24.

Acid hydrolysis and subsequent NMR and mass spectral characterization of the partially dehydrated alcohol of fractions A and B, confirmed the aglycone structure **3**. The positive ion HRFABMS of the hydrolysis product showed a $[M+H]^+$ ion at m/z 471.3826 (fraction A) and 471.3823 (fraction B), supporting a molecular formula of $C_{31}H_{52}O_4$ for

TABLE 2 NMR spectral data of aglycone side chain of triterpene glycosides in HPLC fractions A, B, and C[a]

Carbon #	$\delta\ ^{13}C$	$\delta\ ^{1}H$	*HMBC correlation to*
21	19.4	0.92 d	C-17, 20, 22
22	30.5	1.40 m	
23	33.3	1.82 m	
24	89.6	–	
25	35.4	2.35 m	C-23, (weak), 24, 26, 27, 31
26	17.5[b]	0.88 d	C-24, 25
27	17.8[b]	0.91 d	C-24, 25
31	20.2	1.29 s	C-23, 24, 25

[a]identical data were acquired for all 3 fractions so are only shown once.
[b]may be interchanged.

both. Elimination of water, as expected for a tertiary alcohol, gave a mixture of olefinic isomers.

Sponge triterpene glycosides possess a wealth of interesting biological activities, including cytotoxicity [5], immunosuppressive activity [6], and thrombin receptor antagonist activity [7]. Other triterpene glycosides isolated from diverse collections of *Erylus formosus*, erylosides E and F, have been shown to facilitate calcium release from cells [8]. In this study, formoside (**1**) showed antiviral activity (3.5 μg/mL vs. HSV-1) and modest antibacterial activity (31.3 μg/mL vs. *Corynebacterium xerosis*). HPLC fractions A, B, and **1** were active against amphotericin B-resistant *Candida albicans* (7.8, < 3.9, < 3.9 μg/mL, respectively) [9].

Each of the compounds discussed in this report contribute to the chemical defenses of the sponge *Erylus formosus*. When taking into account differences in the natural concentration of different compounds, triterpene glycosides in HPLC fractions A and B were more active than formoside (**1**) [9], suggesting that aglycone side chain methylation and hydroxylation, and/or more extensive glycosylation contributes to greater feeding deterrence. In particular, glycosylation at two sites on the triterpene (as in HPLC fractions A, B, C) rather than one (as in **1** and **2**) might be expected to alter the biological characteristics of these compounds. Triterpene and steroidal glycosides in terrestrial plants have been shown to cause digestive irritation in animals by altering surface tension of stomach contents thereby trapping gas produced by bacterial fermentation [10]. The mechanism of feeding deterrence in reef fishes is uncertain and begs attention. This study of sponge chemical defenses shows that predators differentiate between triterpene glycoside structures and respond to increasing concentrations of particular metabolites [2]. With increasing frequency of reports of triterpene glycosides from diverse marine organisms, we suggest that these compounds

likely fulfill important ecological functions in environments such as coral reefs that have strong predatory pressure.

EXPERIMENTAL SECTION

General Chemical Methods

Methanol was distilled before use, analytical solvents were used for HPLC, and all other solvents used were reagent grade. Semi-preparative HPLC was performed using a Waters pump with a Waters R403 refractive index detector. Analytical HPLC was performed using a Hewlett Packard Series II 1090 liquid chromatograph with diode array UV detection (210 to 400 nm). HPLC columns used were Rainin Dynamax 60 Å C_{18} silica. ^{1}H, ^{13}C, DEPT, and two-dimensional inverse-detected NMR experiments (COSY, HMQC, HMBC) were performed on Varian Inova 300 and Gemini 400 MHz spectrometers. NMR spectra were recorded in deuterated methanol or chloroform and referenced to CHD_2OD (^{1}H δ 3.31, ^{13}C δ 49.0) or $CHCl_3$ (^{1}H δ 7.24, ^{13}C δ 77.0). Infrared spectra were recorded on a Perkin Elmer 1600 Series FTIR system. UV measurements were made on a Perkin Elmer Lambda 3B UV/VIS spectrophotometer. Optical rotations were measured in chloroform using a Rudolph Research Autopol III polarimeter with a path length of 10 cm recorded at the sodium D line. Low resolution electron impact mass spectra were obtained on a Hewlett-Packard 5988A mass spectrometer.

Sponge Collection and Identification

Erylus formosus was collected by SCUBA at 5 to 15 m depth from several locations in the Bahamas and from White Banks Dry Rocks, Florida Keys. Identification was made by comparisons of spicule and tissue preparations with published accounts [4]. Sponges were measured by volumetric displacement and then stored at −20°C until extraction.

Extraction and Isolation of Antifeedant Compounds

Sponge material was freeze-dried, extracted with methanol and methanol/dichloromethane (1 : 1) two to three times with each solvent mixture, and the extracts were concentrated *in vacuo* and combined. The crude extracts

and chromatography factions were tested in aquarium assays using the generalist reef fish *Thalassoma bifasciatum*, and in field assays using a natural assemblage of reef fishes. Details of assay design and analysis are found elsewhere [1,2].

Solvent partitioning using *n*-butanol and water separated the deterrent components from most salts and carbohydrates. Further desalting and partial purification was achieved by vacuum liquid chromatography using reversed-phase (C_{18}) silica gel, eluting first with water, and then methanol. Fractionation of the methanol eluate was performed by reversed-phase flash column chromatography, eluting with a gradient of methanol/water (4 : 1) to methanol to ethyl acetate. Deterrent fractions were further purified by semi-preparative reversed-phase HPLC with methanol/water (9 : 1). Repetitive HPLC purification led to the isolation of formoside (**1**, 3.7–7.9 mg/mL) and formoside B (**2**, 0.052 mg/mL) from collections of *Erylus formosus* made in the Bahamas. These collections also yielded two mixtures of closely related novel triterpene glycosides, HPLC fractions A (0.21–0.39 mg/mL) and B (0.23–0.41 mg/mL). A collection made in the Florida Keys yielded no detectable quantities of **1** or **2**, but contained fractions A and B, as well as an addition fraction from HPLC, fraction C (0.036 mg/mL).

Formoside B (**2**) was isolated as a white amorphous solid (3.0 mg from 58 mL of fresh sponge): $[\alpha]_D^{20} - 10.3°$(*c* 0.13, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 211 (3.7); IR (thin film) ν_{max} 3348 (br), 2978, 2355, 1655, 1073 cm^{-1}; ^{1}H-NMR (CD_3OD, 300 MHz) δ 0.80 (3H, s), 0.91 (3H, s), 0.94 (3H, d, $J = 6.3$), 1.06 (3H, s), 1.08 (3H, s), 1.3–2.4 (br m), 1.60 (3H, s), 1.67 (3H, s), 2.03 (3H, s), 3.1–4.2 (br m), 4.36 (1H, d, $J = 7.8$), 4.49 (1H, d, $J = 6.9$), 4.52 (1H, d, $J = 8.0$), 4.92 (1H, d, $J = 7.2$), 5.09 (1H, t, $J = 7.8$); ^{13}C-NMR (CD_3OD, 100 MHz) δ 16.8, 17.8, 18.6, 19.2, 19.5, 20.2, 23.5, 23.5, 25.9, 26.0, 27.9, 28.3, 28.9, 29.3, 30.5, 32.9, 36.6, 37.2, 37.5, 38.6, 40.8, 48.1, 51.7, 52.1, 57.7, 62.8, 62.8, 64.0, 66.8, 67.1, 70.0, 70.5, 70.6, 72.2, 73.0, 73.2, 74.9, 76.1, 77.1, 77.8, 78.7, 84.9, 86.0, 92.1, 102.1, 106.3, 106.5, 106.6, 126.5, 130.0, 132.0, 140.8, 173.8, 180.0; HRFABMS *m/z* $[M + Cs]^+$ 1218.4900 (calcd for $C_{54}H_{87}O_{21}NCs$, 1218.4825).

HPLC fraction A (mixture) was isolated as a white amorphous solid (12.2 mg from 58 mL of fresh sponge): $[\alpha]_D^{20} + 1.0°$(*c* 0.29, CH_3OH); UV (CH_3OH) $\lambda_{max} < 210$; IR (thin film) ν_{max} 3424 (br), 2931, 1631, 1373, 1255, 1073 cm^{-1}; ^{1}H- and ^{13}C-NMR spectra contained overlapping resonances, see below for terpene assignments; HRMS *m/z* $[M\text{-}H]^-$ 1482.7057 (MALDI), 1482.7167 (FAB) (calcd for $C_{69}H_{112}O_{33}N$, 1482.7117), $[M\text{-}H]^-$ *m/z* 1452.7069 (MALDI), 1452.7101 (FAB) (calcd for $C_{68}H_{110}O_{32}N$, 1452.7011); positive FABMS *m/z* 1507, 1477; positive

MALDI and ESIMS *m/z* 1507, 1477; negative FAB and ESIMS *m/z* 1483, 1453; EIMS *m/z* 426, 173, 161, 135, 112, 69, 55.

HPLC fraction B (mixture) was isolated as a white amorphous solid (24.0 mg from 58 mL of fresh sponge): $[\alpha]_D^{20} - 3.6°$ (*c* 0.39, CH_3OH); UV (CH_3OH) $\lambda_{max} < 210$; IR (thin film) ν_{max} 3425 (br), 2955, 1702, 1643, 1367, 1255, 1073; ^1H- and ^{13}C-NMR spectra contained overlapping resonances, see below for terpene assignments; HRMS *m/z* $[M\text{-}H]^-$ 1494.7489 (MALDI) (calcd for $C_{70}H_{116}O_{31}N_3$, 1494.75929) and $[M+Cs]^+$ 1628.6815 (FAB) (calcd for $C_{70}H_{117}O_{31}N_3Cs$, 1628.6725); positive FABMS *m/z* 1549, 1519; negative ESIMS *m/z* 1495, 1525; EIMS *m/z* 426, 409, 285, 239, 173, 161, 135, 69, 55.

HPLC fraction C (mixture) was isolated as a white amorphous solid (7.6 mg from 210 mL of fresh sponge); $[\alpha]_D^{20} - 20°$ (*c* 0.19, CH_3OH); UV (CH_3OH) $\lambda_{max} < 210$; IR (thin film) ν_{max} 3425 (br), 2943, 1731, 1637, 1467, 1373, 1255, 1067, 1002; ^1H- and ^{13}C-NMR spectra contained overlapping resonances, see below for terpene assignments; HRFAMBS *m/z* $[M\text{-}H]^-$ 925.5183 (calcd for $C_{48}H_{77}O_{17}$, 925.51608); positive FABMS *m/z* 949.5.

HPLC fractions A, B and C terpene NMR spectral data: ^1H-NMR (CD_3OD, 400 MHz) δ 0.80 (3H, s), 0.88 (3H, d, $J=6.6$), 0.91 (3H, d, $J=6.6$), 0.92 (3H, s), 0.93 (3H, d), 1.06 (3H, s), 1.09 (3H, s), 1.29 (3H, s), 1.2–2.4 (br m); ^{13}C-NMR (CD_3OD, 100 MHz) δ 16.8 (C-30), 17.5 (C-26), 17.8 (C-27), 18.5 (C-18), 19.3 (C-6), 19.4 (C-21), 20.2 (C-19), 20.2 (C-31), 23.5 (C-11), 27.8 (C-2), 28.3 (C-29), 28.9 (C-12), 29.2 (C-7), 30.5 (C-22), 30.5 (C-16), 32.9 (C-15), 33.3 (C-23), 35.4 (C-25), 36.6 (C-1), 37.8 (C-20), 38.6 (C-10), 40.8 (C-4), 48.0 (C-13), 5.18 (C-5), 52.0 (C-17), 64.3 (C-14), 89.6 (C-24), 92.2 (C-3), 129.4 (C-8), 140.9 (C-9), 180.7 (C-28); additional NMR spectral data see Table 2.

Hydrolysis of HPLC Fractions A and B Yielding Aglycone 3

10 mg of HPLC fractions A and B were separately dissolved in 1 mL of ethanol containing concentrated HCl (2 drops). These solutions were stirred in sealed vials at 65°C for 16 h and, following cooling K_2CO_3 (60 mg) and H_2O (2 drops) were added. Ethanol (5 mL) was added and each suspension was filtered through cotton and dried *in vacuo*. The crude products were separated from more polar constituents by partitioning between ethyl acetate and water and by normal phase HPLC (chloroform/methanol [49 : 1]), yielding the alcohol **3**, isolated as a clear, colorless oil (mixture with Δ-iso-

mers, 3.6 mg from 5.9 mg HPLC fraction A, 1.4 mg from 9.0 mg HPLC fraction B): $[\alpha]_D^{20} - 61.6°$ (*c* 0.13, $CDCl_3$); UV ($CDCl_3$) λ_{max} (log ε) 242 (3.1); IR (thin film) ν_{max} 3389 (br), 2919, 2849, 2355, 1684, 1454, 1372 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.76 (3H, d, $J=1.8$), 0.80 (3H, s), 0.85 (3H, d, $J=6.3$), 0.92 (3H, d, $J=6.2$), 0.96 (3H, d, $J=7.0$), 0.98 (3H, s), 1.02 (3H, s), 1.0–2.3 (br m), 3.23 (1H, dd, $J=11.4$, 4.2), 5.09 (< 1H, br m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 15.5, 17.2, 18.1, 18.4, 18.6, 19.4, 20.2, 20.5, 21.6, 22.3, 27.7, 27.8, 27.9, 29.4, 29.6, 31.2, 31.4, 31.5, 34.2, 34.3, 35.4, 36.1, 36.9, 37.1, 37.6, 38.9, 50.1, 50.4, 50.8, 63.3, 78.6, 120.6, 122.4, 128.2, 129.6, 141.2, 177.0, (C-24 not observed); HRFABMS *m/z* [M-H_2O + H]$^+$ 471.3823, 471.3826 (calcd for $C_{31}H_{50}O_3$, 471.3838).

Carbohydrate Composition Analysis

HPLC fractions A, B and C (1 mg) were each hydrolyzed using freshly prepared 1 M methanolic HCl for 16 h at 80°C. The released sugars were derivatized with Tri-Sil and the samples analyzed by GC using a Supelco column. Myo-inositol was used as internal standard along with common monosaccharide derivatives. Mole percents HPLC fraction A: arabinose (38.3), xylose (2.4), galactose (15.4), glucose (17.8), *N*-acetyl glucosamine (18.2), unknown amino sugar (7.9). Fraction B: arabinose (39.1), xylose (3.5), galactose (23.7), glucose (5.7), *N*-acetyl glucosamine (16.5), unknown amino sugar (same sugar as in fraction A, 11.5). Fraction C: arabinose (73.1), xylose (18.2), galactose (3.4), glucose (2.4), *N*-acetyl glucosamine (2.9).

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