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Two cyclohexenone glycosides from the North American fern *Woodwardia virginica* (L.) Smith

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Abstract

New glycosides having multisubstituted cyclohex-2-enones as aglycones and saccharide moieties consisting of three and four glucoses, respectively were isolated from the ethanol extract of the American fern *Woodwardia virginica*. The structures were elucidated using extensive spectroscopic analysis (1D and 2D NMR, MS, IR, CD and UV) including determination of absolute stereochemistry by chemical methods.

Keywords: *Woodwardia virginica*; Blechnaceae; Cyclohexenone glucosides; North American fern

1. Introduction

An ancient family of plants belonging to the divison Pteridophyta are ferns, their early fossils belonging to the Mesozoic era, near 400 million years ago (Hallowell and Hallowell, 2001). Different natural bioactive compounds, including glycosyl derivatives, have been discovered from different fern species. From some fern species sesquiterpene glycosides have been isolated: *Dendrobium nobile* (Ye et al., 2002), *Alangium premnifolium* (Kijima et al., 1998). The protoilludane sesquiterpene glycoside, pteridanoside, was discovered from fern *Pteridium aquilinum* var. *caudatum* (Castillio et al., 1999). Terpene and lignan glycosides have been identified in *Pluchea indica* (Uchiyama et al., 1991), terpenoid glycosides were found in *Picris hieracioides* (Uchiyama et al., 1990), and diterpenes and sesquiterpenes were isolated from *Osteospermum* species (Bohlmann et al., 1983).

Present study is a continuation of our previous investigation of North American ferns (Hanuš et al., 2003) and in this report we describe the isolation and identification of two novel cyclohexenone glycosides from the fern *Woodwardia virginica*.

2. Results and discussion

The leaves of the fern *W. virginica* were extracted by ethanol as was previously described (Hanuš et al., 2003) and the extract was separated on Sephadex LH-20 column. The appropriate fraction was further purified by RP-HPLC to give glycosides (**1** and **2**) (see Fig. 1), which were identified by IR, UV, MS, CD, ^{1}H and ^{13}C NMR spectral data and chemical degradation.

The glucoside **1** (white powder) gave in positive HRFABMS m/z 1047.5366 $[M+H]^+$, corresponding to $C_{51}H_{82}O_{22}$, requiring eleven double bond equivalents. In negative LR-FABMS, ions at m/z 1045 $[M-H]^-$, 883 $[M-H-162]^-$, 721 $[M-H\text{-}2\times162]^-$ and 559 $[M-H\text{-}3\times162]^-$ were corresponding to the loss of one, two and three hexosyl units.

NMR analysis of **1** revealed resonances for two pairs nearly magnetically equivalent isopropyl methyls, 10 olefinic methines, 16 oxymethines, three oxymethylenes and one ketone group (δ_C 201.7 ppm) (see Table 1). The latter observation together with the presence of 12 additional sp^2 hybridized carbons associated with four

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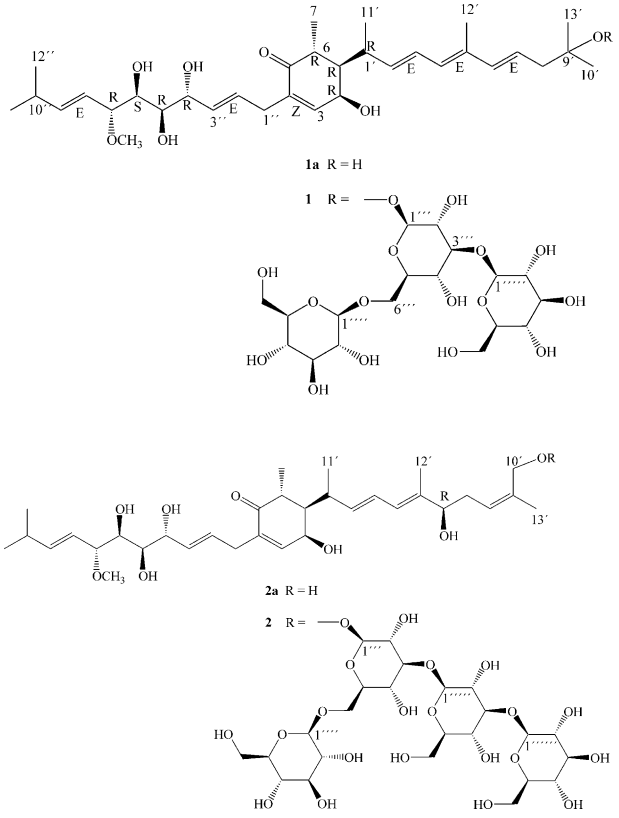

Fig. 1. Structure of woodwardinoside B (**1**) and woodwardinoside C (**2**).

Table 1
^{1}H and ^{13}C NMR spectral data of saccharide parts of the glucosides (**1**, **2**)

C	1	2	1	2
1‴	5.18 (1H, *d*, *J* = 7.6)	4.93 (1H, *d*, *J* = 7.6),	98.8	100.2
2‴	4.30 (1H, *m*)	4.28 (1H, *m*)	73.4	72.8
3‴	4.25 (1H, *m*)	4.23 (1H, *m*)	86.9	87.8
4‴	4.33 (1H, *m*)	4.30 (1H, *m*)	69.3	71.3
5‴	4.10 (1H, *m*)	4.08 (1H, *m*)	76.7	75.5
6‴	4.28 (1H, *m*)	4.31 (1H, *m*)	69.4	68.4
6‴	4.51 (1H, *m*)	4.55 (1H, *m*)		
1⁗	5.35 (1H, *d*, *J* = 7.5)	5.58 (1H, *d*, *J* = 7.7)	102.8	104.4
2⁗	4.08 (1H, *m*)	4.09 (1H, *m*)	75.8	75.3
3⁗	4.17 (1H, *m*)	4.14 (1H, *m*)	78.0	87.6
4⁗	4.12 (1H, *m*)	4.10 (1H, *m*)	71.4	71.6
5⁗	3.98 (1H, *m*)	4.01 (1H, *m*)	78.8	77.3
6⁗	4.20 (1H, *m*)	4.23 (1H, *m*)	62.4	63.0
6⁗	4.38 (1H, *m*)	4.36 (1H, *m*)		
1⁗′	5.12 (1H, *d*, *J* = 7.6)	5.27 (1H, *d*, *J* = 7.8)	104.2	104.8
2⁗′	4.05 (1H, *m*)	4.12 (1H, *m*)	74.9	75.9
3⁗′	4.23 (1H, *m*)	4.18 (1H, *m*)	77.8	77.6
4⁗′	4.14 (1H, *m*)	4.23 (1H, *m*)	71.0	71.2
5⁗′	3.92 (1H, *m*)	4.03 (1H, *m*)	78.3	78.5
6⁗′	4.01 (1H, *m*)	4.21 (1H, *m*)	62.1	62.4
6⁗′	4.35 (1H, *m*)	4.41 (1H, *m*)		
1⁗″	–	5.14 (1H, *d*, *J* = 7.8)	–	104.1
2⁗″	–	4.04 (1H, *m*)	–	76.9
3⁗″	–	4.29 (1H, *m*)	–	78.1
4⁗″	–	4.12 (1H, *m*)	–	70.9
5⁗″	–	3.96 (1H, *m*)	–	78.0
6⁗″	–	4.08 (1H, *m*)	–	62.7
6⁗″	–	4.30 (1H, *m*)		

disubstituted and two trisubstituted double bonds accounted for six double bond equivalents. These observations required for the glucoside **1** named woodwardinoside B to be tetracyclic. In the ^{1}H NMR spectrum of **1**, the signals of 15 oxymethine protons in diaxial conformations ($J = \sim 9.0$ Hz) and three oxymethylenes indicated the presence of the three β-glucopyranosyl groups (Breitmaier and Voelter, 1989). Three anomeric proton signals appeared at δ 5.18, 5.35, and 5.12, in the ^{1}H NMR spectrum, indicating that each glucose has a β-configuration. The corresponding three-anomeric carbons were observed at δ 98.8, 102.8, and 104.2. Also, the downfield shifted ^{13}C NMR resonances among the sugar units were observed at δ 86.9 and 69.4, indicating the probable points of glycosidic linkage in the oligosaccharide to be at Glc-3‴ and Glc-6″. ^{1}H–^{1}H COSY and HMQC experiments revealed the glycosidic attachments at Glc-3‴ (δ 86.9) and Glc-6‴ (δ 69.4) for glucoses (Glc⁗ and Glc⁗′). Further, the HMBC spectrum showed connectivities between the Glc-1‴ proton and C-9′ of the aglycone, the Glc-1⁗ proton and Glc-6‴ carbon, and the Glc-1⁗′ proton and Glc-3‴ carbon. The important HMBC connectivities essential to structure determination of both glucosides are showed on Fig. 1. The name woodwardine B (**1a**) has to be introduced and a formula should be presented. The differences of ^{1}H NMR and ^{13}C NMR values between **1a** (and aglycone part of compound **1**) are mentioned in the Experimental. These changes were produced by glycosidation shift (Tori et al., 1977). The differences were observed only for hydrogens and carbons from H-8′(C-8′) to H-10′(C-10′), including H-13′(C-13′). Thus, **1** was formulated as 9′-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside of woodwardine B (Fig. 2).

By acid (HCl) and also by enzymatic hydrolysis with β-D-glucosidase (EC 3.2.1.21 from almonds), **1** was cleaved to give the aglycone (**1a**) and β-D-glucose, which was confirmed by specific rotation $[\alpha]_D^{23}$ −45.5.

Analysis of the 2D NMR COSY and TOCSY data for **1a** revealed three diagnostic correlation sequences; the first from H-3 to H-4′, incorporating H_3-11′ and H_3-7; the second from H-6′ to H-8′, and H_2-8 and third containing the following sequence, from H-1″ to H-11″, including H-12″. The deshielded chemical shifts of C-4 (δ 67.0), C-9′ (δ 77.8), and from C-4″ to C-7″ (δ 81.5, 72.7, 73.9, 74.4) confirmed that all were substituted by oxygen, which given the considerations outlined above was best accommodated by alcohol functionalities. Furthermore, HMBC correlations from H-3 to C-7 and C-1′, permitted closure of the cyclohexenone ring system as shown and defined the three substructural units as indicated on Fig. 1. Connection of these substructural units as shown was achieved by observation of HMBC correlations from (a) H-4 to C-7 and C-1′, (b) from H-7 to C-4′ and C-5′, (c) from H-6′ to C-8′ and C-9′, and (d)

from H_2-1″ to C-11′, and C-12″. Thus the gross structure for woodwardine B (**1a**) is as shown in Fig. 1.

The geometries of double bonds $\Delta^{2',3'}$, $\Delta^{6',7'}$, $\Delta^{2'',3''}$ and $\Delta^{8'',9''}$ were confirmed to be all *E* by the coupling constants of ($J_{2',3'} = 14.8$, $J_{6'.7'}$ 15.4, $J_{2'',3''} = 14.8$, and $J_{8'',9''} = 15.2$ Hz). The geometry of the trisubstituted double bond ($\Delta^{4',5'}$) was assigned as *E* because the presence of an NOE between H-4′ and H-6′ was observed in the NOESY spectrum and they are according to the NOE and the chemical shift of C-12′ at δ 13.2 (Bax and Summers, 1986).

The relative stereochemistry about C-4, C-5, and C-6 was successfully assigned based on $J_{4,5}$ and $J_{5,6}$. Molecular modeling of all relative stereoisomers about C-4, C-5, and C-6 provided theoretical measures of the H-4–H-5, and H-5–H-6, dihedral angles. These calculations were used to compare theoretical with experimentally measured values for $J_{4,5}$ and $J_{5,6}$ with the best fit being for the relative stereochemistry as shown ($J_{4,5}$ theory 2.8 Hz, experimental 3.2 Hz, $J_{5,6}$ theory 9.8 Hz, experimental 12.2 Hz). This stereochemical assignment was supported by spectroscopic comparison to the known terrestrial plant natural product carvotacetone (Ahmed and Mahmoud, 1997; Jakupovic et al., 1990), which are examples of a large family of related metabolites (Zdero et al., 1991; Sekiguchi and Gaucher, 1979) all possessing a cyclohexenone substructure in common with woodwardine B (**1a**).

^1H–^1H revealed the following correlations: from δ 3.98 to δ 3.54, from δ 3.54 to δ 3.65 and from δ 3.65 to δ 3.80. The connectivity of C-1″ to C-11″ was accomplished also by ^1H–^{13}C correlations. The ^{13}C chemical shifts at C-4″, C-5″, C-6″ and C-7″ were in good agreement with those of polyols in literature (Schnarr et al., 1979). Compound **1** was thus concluded to be an α,ω-substituted tetritol.

It was not possible to directly resolve the problem of the relative stereochemistry across C-4′- C-7′ in **1** or **2,** but comparisons of our experimental J_{H-H} with those from *allo* to *talo* carbohydrate alcohol models and literature data supported our assignment as *manno*. The *J* constants of **1a** can be compared with the dihedral angles of vicinal protons H-4′/H-5′, H-5′/H-6′, H-6′/H-7′ of model compounds and literature data, respectively. Likewise, the J_{H-H} (see Table 1) of **1a** can be compared with J_{H-H} of the D-mannitol (Osawa et al., 1991; Masamune et al., 1986). Accordingly, the very close agreement between the *manno* chiral models and our compounds supports the assignment of the relative stereochemistry of the carbons 4″, 5″, 6″, 7″ as 4*R**, 5*R**, 6*S**, 7*R**.

Because the determination of the relative configuration, see above, of the left tetraoxy side chain is very difficult and also erroneous, we used chemical degradation for determination of the absolute stereochemistry of this part of molecule **1**. After ozonolysis, reduction by $NaBH_3CN$ and peracetylation of products, the 2-*O*-methyl-1,3,4,5,6-penta-*O*-acetyl-D-mannitol (Conchie and Strachan, 1978) was isolated from the reaction mixture. The structure was determined first of all on basis of its MS. The methyl acetate derivative was analyzed by GC–EI-MS and GC–CI-MS. The EI spectrum show the same characteristic fragments as was previously observed (Björndal et al., 1967). The base peak at *m/z* 117 is attributed to the loss of the $C_5H_9O_3$ head group and is obtained when C-1 is acetylated and C-2 methylated. The molecular ion was not observed. Further, the presence of the ions at *m/z* 43 and *m/z* 139 is in accordance with previously published data. Using methane as the reagent gas in CI, the methyl acetate derivative displayed a large molecular ion at *m/z* 407 (M + 1) and two more abundant ions at *m/z* 376 $[M+1–32]^+$ and base peak at *m/z* 348 $[M+1–60]^+$, again this result correspond with previously described mass spectra (McNeil and Albersheim, 1977). Another confirmation of structure was carried by measurement of optical rotation. The value, which was obtained ($[\alpha]_D^{24}$ +29°) was in good agreement with published data (Bonner and Saville, 1960).

High-resolution FABMS analysis of **2** suggested a molecular formula $C_{57}H_{92}O_{28}$ corresponding to $[M+H]^+$ at 1223.5701. The negative FABMS gave $[M-H]^-$ ion at *m/z* 1221 and with prominent fragments at *m/z* 1059 $[M-H-162]^-$, *m/z* 897 $[M-H-2\times162]^-$, *m/z* 735 $[M-H-3\times162]^-$ and *m/z* 573 $[M-H-4\times162]^-$ (cleavage one to four hexose units, respectively). The molecular formula was supported by 57 signals in the ^{13}C NMR spectrum (7×CH_3, 7×CH_2, 39×CH, and 4×quaternary carbon). The high level of oxygenation in the molecular formula pointed to the presence of sugars in the molecule. The glycoside **2** was enzymatically hydrolyzed analogously as compound **1** and the spectra of aglycone (**2a**) that is, woodwardine C, were practically identical, with one exception - right side chain of aglycone **2a**. All of the NMR data were very similar to those mentioned for the above compound **1** except for the signals belonging chain from C-5′ to C-13′. The absence of two signals at δ 6.17 and 5.78 (H-6′ and H-7′, respectively) in the ^1H NMR spectrum of **2** and the presence of a new signals at δ 3.94 and 5.16 suggested that an oxygenated methine and trisubstituted double bond were still present at C-6′ in **2**.

The substitution at C-6′ was confirmed by the COSY correlation between H-6′ and the C-7′ methylene protons at δ 2.29 and 2.43 and by the HMBC correlation between H-6′ and C-5′. The absolute configuration at C-6′ was determined to be *R* by Mosher's method (Ohtani et al., 1991a; 1991b) (Fig. 3). The reaction with MTPACl (α-methoxy-α-trifluormethyphenylacetyl chloride) also esterified further hydroxyl groups (C-4, C-4′, C-5′ and C-6′), but this did not interfere with the determination of stereochemistry of the appropriate secondary alcohol (C-6′).

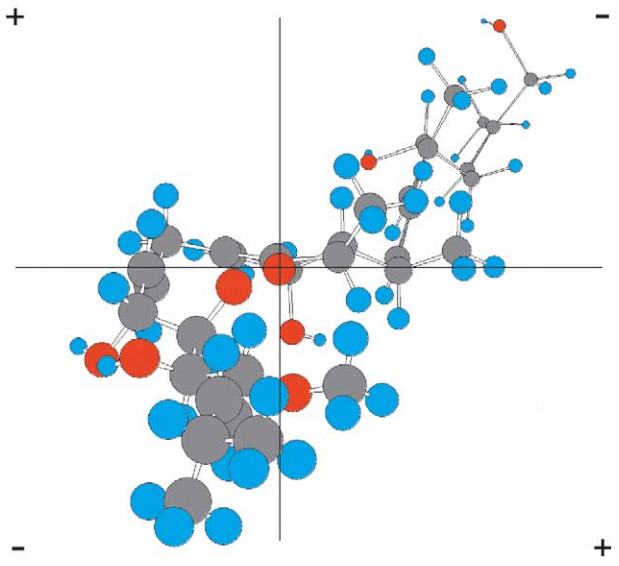

Fig. 2. Projection of woodwardine B into positive and negative contributing quadrants.

Saccharide composition of **2** was identified by GC and the sugars were identified as glucose. In the ^{1}H NMR spectrum of **2**, four anomeric proton signals at δ 4.93, 5.58, 5.27, and 5.14 were observed, corresponding to signals at δ 100.2, 104.4, 104.8, 104.1, respectively, and indicating that **2** possesses four sugar units. All four anomeric protons showed β-glycosidic linkages according to the coupling constants of their anomeric protons (J = 7.6–7.8 Hz). From the ^{1}H–^{1}H COSY and TOCSY spectra, all proton signals belonging to each sugar moiety in **2** were identified, starting from the anomeric protons. All sugar connectivities were established using NOESY and HMBC experiments. In the NOESY spectrum cross-peak signals were observed between H-1‴″ and H-6‴, and between H-1⁗ and H-3‴. The HMBC experiment showed long range correlations between H-1‴ and C-10′, H-1‴″ and C-6‴, H-1⁗ and C-3‴, H-1⁗ and C-3⁗. Thus, the structure of **2** was assigned as the 10′-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside of woodwardine C, that is, woodwardinoside C.

With the relative configuration of the ring substituents of woodwardine C (**2a**) and its co-metabolite (**1**) established, attention was directed to the absolute configuration of these compounds. Woodwardine C (**2a**) has a single chromophore absorbing in the accessible UV region, which gives rise to successive negative CD extreme at 342 nm and positive at 252 nm associated with n–π* and π–π* transitions of the enone system (Fig. 1). These Cotton effects define respectively the 4*R*, 5*R* and 6*R* configurations of the 4-hydroxy and 5,6-dialkyl groups as depicted in structure (**2**). Thus the CD curve of woodwardine C (**2a**) parallels those of the (+)-*epi*-cycloabscisic acid gives rise to successive negative and positive extreme at 341 and 245 nm, respectively, arising from 5*R*, 6*R* and 4*R* configurations. In contrast, 4*R*, 5*S*, 6*S* system of substituted carvotacetone (Jakupovic et al., 1990) and (+)-cycloabscisic acid (Todoroki et al., 1996) and is the mirror image of that of (−)-cycloabscisic acid (Todoroki et al., 1996). This 4*R*, 5*R*, 6*R* absolute configuration of woodwardine C (**2a**) is in agreement with the corresponding relative configuration deduced from the 1.4 Hz long range coupling between 4-H and 6-H (Table 1) and discussed above for woodwardine C (**2a**) itself.

Analysis of the CD spectra of the triene-containing cyclohexenone (**1a**) is potentially complicated by the presence of overlapping chromophores and associated Cotton effects in the 250–300 nm region. This CD spectrum, however, resembles markedly that of woodwardine C (**2a**), with strong negative extreme near 340 nm and positive at 250 nm (see Experimental). This spectrum is clearly dominated by the enone chromophore, with weak additional structure between 250 and 300 nm reflecting the asymmetric environment of the triene. The long wavelength extreme originate solely from the n–π* transition of the enone systems and as with woodwardine C (**2a**) define the chirality. The short wavelength extreme are associated primarily with the π–π* transition of the enone systems, and define the configuration of the 4-hydroxy group. The resulting 4*R*, 5*R*, 6*R* absolute configurations depicted for the woodwardine B (**1a**) again accord with the corresponding relative configurations deduced from ring proton coupling constants (Table 1), as discussed above in the case of woodwardine B (**2a**).

It is known that secondary metabolites such as terpenoids (Murakami and Saiki, 1989) and/or sterols (Chiu et al., 1988) have their taxonomical significance. It will be very interesting to compare this fern from different locations. If it will have consistent glycosidic profile, these glycosides can serve as taxonomic markers.

The structure of the newly identified metabolite is not common. Based upon the previously published works we hypothesize, that the end of the left part of the side chain is derived from a leucin residue, to which acetate units are connected (for example see bengamines in Adamczeski et al., 1989). The central part could be built by acetate units and the right chain by isoprene units (Ahmed and Mahmoud, 1997; Lamnaouer et al., 1991). Only further research can prove or reject this hypothesis.

3. Experimental

3.1. General experimental procedures

UV spectra were measured in heptane within the range of 200–350 nm on a Cary 118 (Varian) apparatus. Optical rotatory dispersion (ORD) measurement was carried out under dry N_2 on a Jasco-500A spectropolarimeter at

24 °C. A Perkin-Elmer Model 1310 (Perkin-Elmer, Norwalk, CT, USA) IR spectrophotometer was used for scanning IR spectroscopy of acids and glycosides as neat films. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H), 125.7 MHz (^{13}C) in mixture of deuterated pyridine and CD_3OD (v/v 1/1). High- and also low-resolution MS were recorded using a VG 7070E-HF spectrometer (70 eV). HRFABMS (positive and/or negative ion mode) were obtained with a PEG-400 matrix. RP-HPLC was carried out using Shimadzu gradient LC system (Shimadzu, Kyoto, Japan). Gas chromatography analysis was made on a Hewlett Packard HP 5980 gas chromatograph (Hewlett Packard Ltd., Czech Republic).

3.2. Plant material, extraction and isolation

The specimens of *W. virginica* (L.) Sm. (Blechnaceae), Virginia chain fern, were collected in Montgomery County, Maryland (USA) in August 2002. Fresh fern leaves were extracted with ethanol (on the spot immediately after collecting) and after that with ethanol/water (70/30). Both extracts were combined and evaporated under reduced pressure to a small volume. The remained plant material was extracted with mixture methanol/dichloromethane (50/50, v/v) with boiling for 25 min. Ethanol–water extract was separated on a Sephadex LH-20 column eluting with MeOH/H_2O (9/1) yielding three fractions. Fraction B was further fractionated by RP-HPLC on a C18-Bondapak column (30 cm×7.8 mm, flow rate 2.0 ml/min) with ACN/H_2O (1/2) to yield compound **1.**

3.3. Acidic hydrolysis and determination of the glycosides

The glycoside (**1**) was refluxed in 2 N HCl (0.5 ml) for 2 h. The aglycone was extracted three times with EtOAc (10 ml). After separation of the organic layer, the aqueous phase was neutralized with $NaHCO_3$, lyophilized and the residue was chromatographed on a column of silica gel (10 g), using CH_2Cl_2/MeOH/H_2O (90/10/1) to

Table 2
The ^{13}C NMR and ^{1}H NMR data as spectral data of aglycones (**1a**–**2a**)

No.	**1a**	**2a**	**1a**	**2a**
1	–	–	201.7	200.7
2	–	–	136.1	138.1
3	6.46 (1H, *dd*, *J*=9.4, 1.7)	6.46 (1H, *d*, *J*=9.4)	142.9	137.9
4	3.79 (1H, *dd*, *J*=9.4, 3.2)	3.79 (1H, *dd*, *J*=9.4, 3.2)	67.0	68.0
5	1.84 (1H, *ddd*, *J*=12.2, 4.0, 3.2)	1.84 (1H, *ddd*, *J*=12.2, 4.0, 3.2)	42.3	42.6
6	2.51 (1H, *dd*, *J*=12.2, 6.4)	2.51 (1H, *dd*, *J*=12.2, 6.4)	40.1	40.5
7	1.21 (3H, *d*, *J*=6.4)	1.21 (3H, *d*, *J*=6.4)	12.2	12.8
1′	2.33 (1H, *ddd*, *J*=4.0, 7.2, 7.6)	2.33 (1H, *ddd*, *J*=4.0, 7.2, 7.6)	28.4	27.8
2′	5.72 (1H, *dd*, *J*=7.2, 14.8)	5.72 (1H, *dd*, *J*=7.2, 14.8)	133.6	132.9
3′	6.67 (1H, *dd*, *J*=14.8, 11.6)	6.03 (1H, *dd*, *J*=14.8, 11.6)	125.7	131.0
4′	6.13 (1H, *d*, *J*=11.6)	6.01 (1H, *d*, *J*=11.6)	131.5	125.3
5′	–	–	136.3	144.1
6′	6.17 (1H, *d*, *J*=15.4)	3.94 (1H, *dd*, *J*=6.5, 7.0)	138.1	76.9
7′	5.78 (1H, *dt*, *J*=15.4, 7.4)	2.29 (1H, *ddd*, *J*=15.0, 7.0, 6.5); 2.43 (1H, *ddd*, *J*=15.0, 7.0, 6.5)	126.0	34.7
8′	2.42 (2H, *d*, *J*=7.4) [+0.03]	5.16 (2H, *dd*, J=6.5, 7.0) [+0.02]	46.4 [−3.2]	127.7 [−0.7]
9′	–	–	77.8 [+7.4]	135.7 [−1.8]
10′	1.26 (3H, *s*) [+0.01]	4.11 (2H, *s*) [+0.07]	27.2 [−1.7]	61.9 [+8.6]
11′	1.16 (3H, *d*, *J*=7.6)	1.16 (3H, *d*, *J*=7.6)	18.4	18.4
12′	1.91 (3H, *s*)	1.71 (3H, *s*)	13.2	13.1
13′	1.26 (3H, *s*) [+0.01]	1.73 (3H, *d*, *J*=0.8) [+0.02]	27.2 [−0.6]	21.1 [−0.4]
1″	2.63 (2H, *dd*, *J*=6.6, 1.7)	2.63 (2H, *dd*, *J*=6.6, 1.7)	35.2	35.2
2″	5.70 (1H, *dt*, *J*=6.6, 14.8)	5.70 (1H, *dt*, *J*=6.6, 14.8)	123.8	123.8
3″	5.59 (1H, *dd*, *J*=14.8, 2.3)	5.59 (1H, *dd*, *J*=14.8, 2.3)	133.2	133.2
4″	3.98 (1H, *dd*, *J*=8.2, 2.3)	3.98 (1H, *dd*, *J*=8.2, 2.3)	81.5	74.5
5″	3.54 (1H, *dd*, *J*=2.9, 8.2)	3.54 (1H, *dd*, *J*=2.9, 8.2)	72.7	76.7
6″	3.65 (1H, *dd*, *J*=2.9, 8.3)	3.65 (1H, *dd*, *J*=2.9, 8.3)	73.9	73.9
7′	3.80 (1H, *dd*, *J*=2.5, 8.3)	3.80 (1H, *dd*, *J*=2.5, 8.3)	74.4	80.4
8″	5.44 (1H, *dd*, *J*=2.5, 15.2)	5.44 (1H, *dd*, *J*=2.5, 15.2)	125.6	128.6
9″	5.79 (1H, *dd*, *J*=6.4, 15.2)	5.79 (1H, *dd*, *J*=6.4, 15.2)	140.1	133.1
10″	2.25 (1H, *ddd*, *J*=6.7, 6.6, 6.4)	2.25 (1H, *ddd*, *J*=6.7, 6.6, 6.4)	30.8	31.8
11″	0.99 (3H, *d*, *J*=6.7)	0.99 (3H, *d*, *J*=6.7)	21.3	22.3
12″	1.00 (3H, *d*, *J*=6.6)	1.00 (3H, *d*, *J*=6.6)	21.3	22.3
OMe	3.52 (3H, *s*)	3.52 (3H, *s*)	52.1	52.1

The numbers in square brackets are values affected by glycosylation shifts.

provide acid for ^{1}H NMR analysis. The identification and the D or L configuration of sugars were determined using gas chromatography (a glass-capillary column Supelco SPB-1). The acetylated (+)-2-butyl derivatives were eluted as peaks with retention times, which were identical with standards of the apropriate tetraacetyl (+)-2-butyl-saccharides according to the method of Gerwig et al. (1978), with some modifications as previously described (Řezanka and Guschina, 2000).

3.3.1. (R)-MTPA and (S)-MTPA esters.

To a CH_2Cl_2 solution (100 μl) of aglycone (0.3 mg), 4-dimethylaminopyridine (1.0 mg), and Et_3N (2 μl) were added at room temperature 2.0 mg of (*R*)-MTPACl [and/or (*S*)-MTPACl], and stirring was continued for 3 h. After evaporation of the solvent, the residue was purified by Si gel TLC (hexane/AcOEt, 2/1) to provide the (*S*)-MTPA and/or ((*R*)-MTPA) ester, respectively as colorless oils (Ohtani et al., 1991a; 1991b).

*3.3.2. 9-O-β-D-Glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-Glucopyranoside of woodwardine B, woodwardinoside B (**1**)*

Colorless powder, $[\alpha]_D^{23}$ −85.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 234 (3.31), 279 (4.24); IR (film) ν_{max} 3450 (OH), 1685 (C=C–C=O); HR-FABMS *m/z* 1047.5369 $[M+H]^+$, calculated for $[C_{51}H_{82}O_{22}+H]^+$ 1047.5375; negative LR-FABMS *m/z* 1045 $[M-H]^-$, 883 $[M-H-162]^-$, 721 $[M-H-2\times162]^-$ and 559 $[M-H-3\times162]^-$; ^{1}H and ^{13}C NMR data, see Table 1.

*3.3.3. (1′R, 2E, 2′E, 2″E, 4R, 4′E, 4″R, 5R, 5″R, 6R, 6′E, 6″S, 7″R, 8″E)-4-Hydroxy-5-(9′-hydroxy-1′, 5′, 9′-trimethyl-deca-2′, 4′, 6′-trienyl)-6-methyl-2-(4″, 5″, 6″-trihydroxy-7″-methoxy-10″-methyl-undeca-2″, 8″-dienyl)-cyclohex-2-enone, woodwardine B (**1a**).*

White crystals, mp 107–108 °C; $[\alpha]_D^{23}$ −45.5 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 234 (3.31), 279 (4.24); IR (film) ν_{max} 3450 (OH), 1685 (C=C–C=O); CD (MeOH) λ_{ext} nm (Δε) 219 (−5.8), 252 (+3.9), 342 (−0.7); HREIMS *m/z* 561.3785 $C_{33}H_{52}O_7$ $[M]^+$, calculated for $[C_{33}H_{54}O_7]^+$ 561.3791; ^{1}H and ^{13}C NMR data, see Table 2.

*3.3.4. 10′-O-[β-D-Glucopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside of woodwardine C, woodwardinoside C (**2**).*

Colorless powder, $[\alpha]_D^{23}$ −142.0 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 234 (3.31), 279 (4.24); IR (film) ν_{max} 3450 (OH), 1685 (C=C–C=O); HRFABMS *m/z* 1223.5701 $[M+H]^+$, calculated for $[C_{57}H_{92}O_{28}+H]^+$ 1223.5696; negative LRFABMS *m/z* 1221 $[M-H]^-$, *m/z* 1059 $[M-H-162]^-$, *m/z* 897 $[M-H-2\times162]^-$, *m/z* 735 $[M-H-3\times162]^-$ and *m/z* 573 $[M-H-4\times162]^-$; ^{1}H and ^{13}C NMR data, see Table 1.

*3.35. (1′R, 2E, 2′E, 2″E, 4R, 4′E, 4″R, 5R, 5″R, 6R, 6′R, 6″S, 7″R, 8′Z, 8″E)-5-(6′, 10′-Dihydroxy-1′, 5′, 9′-trimethyl-deca-2′, 4′, 8′-trienyl)-4-hydroxy-6-methyl-2-(4″, 5″, 6″-trihydroxy-7″-methoxy-10″-methyl-undeca-2″, 8″-dienyl)-cyclohex-2-enone, woodwardine C (**2a**).*

White crystals, mp 98–99 °C; $[\alpha]_D^{23}$ −52.7 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 234 (3.31), 279 (4.24); IR (film) ν_{max} 3450 (OH), 1685 (C=C–C=O); CD (MeOH) λ_{ext} nm (Δε) 219 (−5.8), 252 (+3.9), 342 (−0.7); HREIMS *m/z* 561.3785 $C_{33}H_{52}O_7$ $[M]^+$, calculated for $[C_{33}H_{54}O_7]^+$ 561.3791; ^{1}H and ^{13}C NMR data, see Table 2.

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