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Glycosides of benzodioxole-indole alkaloids from *Narcissus* having axial chirality

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ABSTRACT

Glycosides of benzodioxole-indole alkaloid 6-hydroxy-galanthindole (7-(6′-(hydroxymethyl)benzo[*d*][1′,3′]dioxol-5′-yl)-1-methyl-1*H*-indol-6-ol) having axial chirality were isolated from *Narcissus* cultivar 'Dutch Master'. The structure, including absolute configuration, was determined by means of extensive spectroscopic data such as UV, IR, CD, MS, 1D and 2D NMR spectra, and computational chiroptical methods. The aglycone has a structure containing two aromatic moieties with substituents hindering rotation about the biaryl axis and is connected to a saccharide moiety linked at C-6 and made up of one, two, or three sugars (glucose, α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranose, and trisaccharide ([β-D-xylopyranosyl(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranose).

1. Introduction

Daffodil is a common English name of *Narcissus*, a genus of monocotyledonous plants from the Amaryllidaceae family. They are perennial terrestrial plants with bulbs. All *Narcissus* species have a central trumpet-, bowl-, or disc-shaped corona surrounded by a ring of six floral leaves called the perianth, which is united into a tube at the forward edge of the 3-locular ovary. The traditional daffodil of folklore, poetry, and field may have a yellow to golden-yellow color. The American Daffodil Society (ADS) cites between 50 and 100 excluding species variants and wild hybrids, which are widespread in South Europe, Asia, and North Africa; in other regions they are rather rare (Bastida et al., 2006).

Most of the species of *Narcissus* will hybridize but, significantly, there is great variation in the fertility of the offspring, depending upon the degree of relationship between the parents. Hybridization has become very popular, resulting in thousands of commercial *Narcissus* cultivars that are in most cases larger and more robust than their wild parents. ADS divides all *Narcissus* into 13 horticultural divisions, based partly upon flower form and partly upon genetic background. More than 27,000 cultivar names have accumulated (Kington, 2008).

The Amaryllidaceae alkaloids represent a large and still expanding group of predominantly isoquinoline alkaloids, the majority of which are not known to occur in any other family of plants. Since the isolation of the first alkaloid, lycorine (named also narcissine), from *Narcissus pseudonarcissus* in 1910 (Ewins, 1910), substantial progress has been made in examining the Amaryllidaceae plants, although they still remain a relatively untapped phytochemical source (Bastida and Viladomat, 2002). At present, over 300 alkaloids have been isolated as a result of the continuing search for novel alkaloids with pharmacological activity in the Amaryllidaceae family. Although the structures of these alkaloids vary considerably, they are considered to be biogenetically related (Jin, 2009). The large numbers of structurally diverse Amaryllidaceae alkaloids are classified into nine main skeleton types (Bastida et al., 2006).

Approximately a third of the total number of alkaloids isolated from Amaryllidaceae have been found in the genus *Narcissus* (Bastida and Viladomat, 2002). Up to now, about 40 wild species and around 100 cultivars have been studied in relation to the presence of alkaloids, which means that more than half of the *Narcissus* species or varieties have still to be explored in this aspect (Bastida et al., 2006).

A number of glycosyl derivatives of alkaloids were isolated from different Amaryllidaceae species. The pancratiside was isolated from *Haemanthus kalbreyeri* (Ghosal et al., 1989), and kalbreclasine and 4-*O*-β-D-glucosylnarciclasine were isolated, respectively, from *Zephyranthes flava* (Ghosal et al., 1986), *H. kalbreyeri* (Ghosal et al., 1985), and *Pancratium maritimum* (Abou-Donia et al., 1991). In addition, two others derivatives of pancratistatin, telastaside, and 1-*O*-(3-*O*-β-D-glucopyranosylbutyryl)-pancratistatin, were isolated from the *Polytela gloriosa* (Ghosal et al., 1990) and from *Zephyranthes carinata* (Kojima et al., 1998), respectively. Further, two novel Amaryllidaceae alkaloids, having substituted glucose and named cripowellin A and B were isolated from bulbs of *Crinum powellii* (Velten et al., 1998).

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This report is part of our investigation of compounds having axial chirality (Rezanka and Dembitsky, 2006; Rezanka and Sigler, 2007; Rezanka et al., 2008, 2009) within the framework of a comprehensive program on the chemistry and biotoxicity of natural compounds. We now report the identification of 6-hydroxy-galanthindole and of its glycosides having axial chirality.

2. Results and discussion

Minced bulbs of *Narcissus* (11.2 kg), cultivar 'Dutch Master' were exhaustively extracted with 95% EtOH by cold percolation. The combined extracts were concentrated, then defatted, acidified, filtered and alkalinized to ca. pH 10 and extracted successively with *n*-BuOH. The *n*-BuOH extract was separated on Sephadex LH-20 and the fractions were further purified by preparative RP-HPLC to give four compounds: **1** 13.2 mg (1.18×10^{-4}%), **2** 6.5 mg (5.8×10^{-5}%), **3** 14.2 mg (1.27×10^{-4}%) and **4** 7.2 mg (6.42×10^{-5}%). The compounds were identified by their IR, UV, CD, MS, and ^{1}H and ^{13}C NMR spectroscopic data.

Compound (**1**) was isolated as a colorless amorphous solid. The molecular formula of **1** was assigned to be $C_{17}H_{15}NO_4$ from its pseudomolecular ion peak $[M+H]^+$ at *m/z* 298.1082 in the high-resolution fast-atom bombardment mass spectrum (HR-FAB-MS). The UV spectrum showing absorption bands at 222, 288 and 296 nm indicated the presence of a conjugated system. The ^{1}H NMR and ^{13}C NMR data of **1** were similar to those for a previously described compound, i.e. galanthindole (Unver et al., 2003), except for the presence of a hydroxy group in **1** (Table 1). Information for all of the functional groups and their locations in the molecule was obtained from the HMBC, ^{1}H–^{1}H COSY and NOESY spectra (Fig. 1).

The ^{1}H NMR data of **1** (Table 1) revealed six aromatic H-atoms, two of which were *para*-oriented, appearing at δ 6.82 (H-4′, *s*) and 7.04 (H-7′, *s*) on benzene ring. The remaining protons appeared as two *ortho*-oriented two pairs of hydrogens, as further confirmed by a ^{1}H–^{1}H-COSY spectrum, see Fig. 1.

The ^{1}H NMR spectrum of **1** also displayed signals at δ 3.29, 6.03 and 6.04, characteristic of one MeN and one OCH_2O group, respectively. Further, a CH_2OH group was identified from the signals at δ 4.20 and δ 64.5 in the ^{1}H and ^{13}C NMR spectra, respectively.

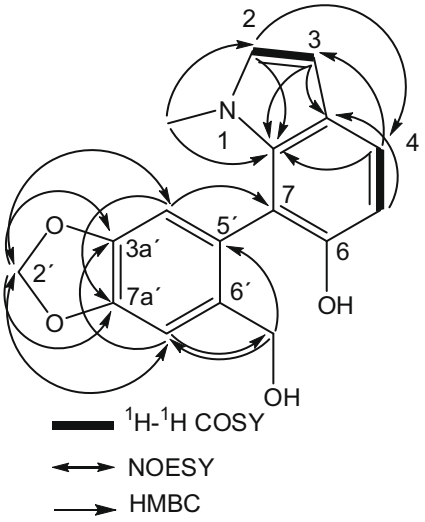

Fig. 1. Structure of the new compound (**1**); only important HMBC, COSY, and NOESY correlations are shown.

The N-atom of the MeN group was connected to both C-2 at δ 130.9 and C-7a at 131.0, which was supported by HMBC correlations between C-2, C-7a, and MeN. Indole ring was joined to benzene ring through C-5′ at δ 131.8 and C-7 at 104.8, in accord with HMBC correlations between H-4′ and C-7. The CH_2OH group was attached to benzene ring at C-6′ 132.0, as confirmed by HMBC correlations between CH_2 and C-5′ and C-7′, respectively. The OCH_2O group was placed in 3a′ and 7a′ positions of the benzene ring, as deduced from HMBC correlations between OCH_2O and C-3a′ and C-7a′ and also by NOESY (see Fig. 1). The information obtained from 2D NMR and ^{13}C NMR data, and a comparison with previously reported data for galanthindole (Unver et al., 2003) supported the structure of **1**. Based on the above results, compound **1** could be identified as 7-(6′-(hydroxymethyl)benzo[*d*][1′,3′]dioxol-5′-yl)-1-methyl-1*H*-indol-6-ol, a new benzodioxole-indol alkaloid, named 6-hydroxy-galanthindole (**1**).

Table 1
^{1}H and ^{13}C NMR data (measured in DMSO-d6) of 6-hydroxy-galanthindole (**1**) and 6-hydroxy-galanthindolyloside A (**2**).

No.	^{1}H NMR of **1**	^{13}C NMR of **1**	^{1}H NMR of **2**	^{13}C NMR of **2**
2	6.95 (1H, *d*, *J* = 3.1)	130.9	6.95 (1H, *d*, *J* = 3.1)	130.9
3	6.52 (1H, *d*, *J* = 3.1)	101.1	6.52 (1H, *d*, *J* = 3.1)	101.1
3a	–	127.8	–	129.3
4	7.83 (1H, *d*, *J* = 8.3)	120.6	7.83 (1H, *d*, *J* = 8.3)	120.6
5	6.94 (1H, *d*, *J* = 8.3)	108.6	6.57 (1H, *d*, *J* = 8.3)	109.5
6	–	151.4	–	149.4
7	–	104.8	–	106.1
7a	–	131.0	–	131.0
2′	6.03 (1H, *s*); 6.04 (1H, *s*)	101.2	6.03 (1H, *s*); 6.04 (1H, *s*)	101.2
3′a	–	148.5	–	148.5
4′	6.82 (1H, *s*)	110.7	6.82 (1H, *s*)	110.7
5′	–	131.8	–	131.8
6′	–	132.0	–	132.0
7′	7.04 (1H, *s*)	107.9	7.04 (1H, *s*)	107.9
7′a	–	148.6	–	148.6
8′	4.20 (2H, *s*)	64.5	4.20 (2H, *s*)	64.5
N-Me	3.29 (3H, *s*)	35.8	3.29 (3H, *s*)	35.8
1″	–	–	4.89 (1H, *d*, *J* = 7.1)	105.1
2″	–	–	3.54 (1H, *dd*, *J* = 7.1, 8.9)	74.7
3″	–	–	3.62 (1H, *t*, *J* = 8.9)	77.4
4″	–	–	3 43 (1H, *t*, *J* = 8.9)	71.5
5″	–	–	3.49 (1H, *m*)	78.6
6″	–	–	3.97 (1H, *dd*, *J* = 12.1, 2.3), 3.71 (1H, *dd*, *J* = 12.1, 5.3)	64.7

Compound **1** showed an optical activity ($[\alpha]_D^{23}$ +174). This is due to the rotational barrier of biaryl derivatives, which depends on the nature, position, and number of the substituents. The unsubstituted biphenyl has a rotational barrier of approximately 8.4 kJ/mol (2 kcal/mol). Increasing steric bulk of substituents in the *o*-positions causes increasing conformational stability as a result of steric interactions in the coplanar transition state. The majority of tetra-*o*-substituted biaryls have a rotational barrier sufficiently high to prevent racemization of the atropisomers at room temperature. The separation of stereoisomers at room temperature requires energy barriers of at least 92.1 kJ/mol (22 kcal/mol) (Leroux, 2004). For this reason we computed (B3LYP functional and 6-31G* basis set, see below) the rotational barrier of our biaryl derivative (**1**) by computing the energy of conformers whose dihedral angle of aryls differed by 10°. The rotational barrier was then calculated as the difference between the maximum and minimum of conformer energy and the result for (**1**) was calculated to be 260 kJ/mol. This clearly shows that at room temperature compound **1** cannot isomerize from *M* to *P* atropisomer and vice versa. This is in conformity with previously published results (Arbain et al., 1998; Pezzella et al., 2003) for similar compounds.

Therefore, to elucidate the absolute configuration of **1** a quantum chemical calculation (Diedrich and Grimme, 2003) of CD was carried out with Gaussian 03 software (Frisch et al., 1998) using time-dependent density functional theory with B3LYP functional and 6-31G* basis set with 40 electronic transitions. Unfortunately, no quantum chemical calculation has as yet been used for this type of compounds. The conformer used for CD calculation was the minimum-energy conformer. A comparison of theoretically predicted data with the corresponding experimental data has allowed us to elucidate the absolute configuration of **1**. Experimental and calculated spectra (Fig. 2) are in good agreement. Hence the absolute configuration of **1** is *M* (i.e. *R*), see Fig. 3.

The three isolated more polar components (**2**, **3** and **4**) (retention times = 16.37, 9.56 and 8.11 min, respectively) were tentatively identified as glycosides of **1**.

Compound **2** was obtained as a colorless, semicrystalline mass, with the molecular formula $C_{23}H_{25}NO_9$, based on the $[M+H]^+$ peak at *m/z* 460.1610 in the HR-FAB-MS, and confirmed by ^1H and ^{13}C NMR (DEPT) data (Table 1). Acid hydrolysis of **2** afforded D-Glc and aglycone (**1**). The ^1H NMR spectrum of **2** showed the presence of all H-atoms observed in the aglycone and Glc units, with its anomeric H- and C-atoms resonating at δ 4.89 (*d*, *J* = 7.1 Hz, 1H) and at δ 105.1, respectively. The corresponding coupling constants

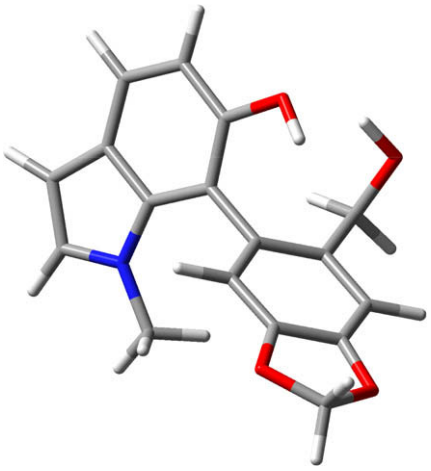

Fig. 3. Model of (*R*)-6-hydroxy-galanthindole (**1**) shows that rotation about the 7,5′ bond is hindered.

(*J* = 7.1 Hz) suggested that Glc unit has β-configuration. NOEs were observed between H-1″ (δ 4.89, *d*, *J* = 7.1 Hz) and H-5 (δ 6.57, *d*, *J* = 8.3 Hz), which suggested that the Glc unit was attached to the hydroxyl at C-6. Also the HMBC spectrum of **2** showed an important correlation between H-1″ (δ 4.89) and C-6 (δ 149.4) of aglycone, which confirmed the bond of Glc to aglycone. From the above data, the structure of compound **2** was elucidated as 6-hydroxy-galanthindolyl 6-β-D-glucopyranoside, and this new constituent was named 6-hydroxy-galanthindolyloside A.

Compound **3** was obtained as a pale-yellow, amorphous powder. HR-FAB-MS showed the pseudo-molecular-ion peak $[M+H]^+$ peak at *m/z* 606.2186, in accord with the molecular formula $C_{29}H_{35}NO_{13}$, as supported by the ^1H and ^{13}C NMR data (Table 2). The negative ESIMS of **3** showed the quasimolecular ion peak $[M-H]^-$ at *m/z* 604 with prominent fragments at *m/z* 458 $[M-H-146]^-$ and *m/z* 296 $[M-H-146-162]^-$, due to the sequential loss of one deoxyhexose unit and one hexose unit, respectively. The identification of the sugar units as D-glucose and L-rhamnose was confirmed by GLC analyses and optical rotation after acid hydrolysis.

The structure of the aglycone of **3** was established on the basis of its HMBC spectrum, in which ^{13}C–^1H long-range correlation signals were observed at C-6/H-1″; C-1″/H-3″ and H-5″; C-6″/H-1‴;

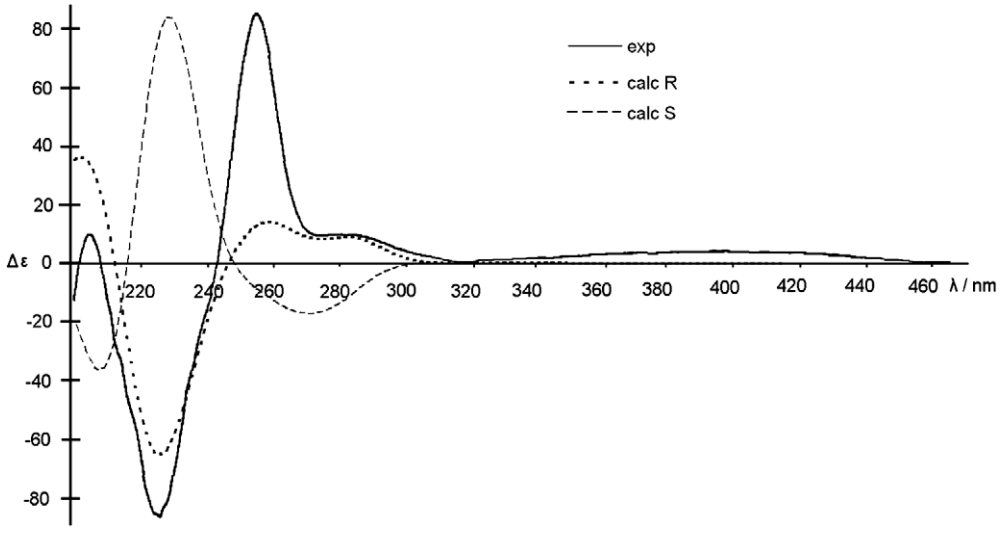

Fig. 2. B3LYP/6-31G*-calculated CD spectrum for (*R*)-**1**, (*S*)-**1**, and experimental CD spectrum of **1**.

Table 2
^{1}H and ^{13}C NMR data (measured in perdeuterated DMSO) of saccharide moieties of 6-hydroxy-galanthindolylosides B (**3**) and C (**4**).

No.	^{1}H NMR of **3**	^{13}C NMR of **3**	^{1}H NMR of **4**	^{13}C NMR of **4**
Glc			Glc	
1″	4.69 (1H, *d*, *J* = 7.8)	104.6	5.19 (1H, *d*, *J* = 7.5)	100.2
2″	3.48 (1H, *dd*, *J* = 7.8, 9.3)	74.9	3.86 (1H, *dd*, *J* = 7.5, 9.0)	82.4
3″	3.44 (1H, *t*, *J* = 9.3)	78.0	3.64 (1H, *t*, *J* = 9.0)	76.0
4″	3 33 (1H, *t*, *J* = 9.3)	72.6	3.49 (1H, *dd*, *J* = 9.0, 10.0)	71.1
5″	3.52 (1H, *m*)	77.4	3.42 (1H, *m*)	77.0
6″	4.02 (1H, *dd*, *J* = 11.5, 2.0),	68.4		
	3.61 (1H, *dd*, *J* = 11.5, 6.3)		3.73 (1H, *dd*, *J* = 12.0, 5.0)	68.6
			3.92 (1H, *dd*, *J* = 12.0, 3.0)	
Rha			Xyl	
1‴	4.72 (1H, *d*, *J* = 1.6)	102.3	4.88 (1H, *d*, *J* = 7.6)	105.2
2‴	3.86 (1H, *dd*, *J* = 1.6, 3.5)	72.5	3.42 (1H, *dd*, *J* = 7.6, 9.0)	74.8
3‴	3.70 (1H, *dd*, *J* = 9.2, 3.5)	72.6	3.36 (1H, *t*, *J* = 9.0)	77.7
4‴	3.38 (1H, *t*, *J* = 9.2)	74.1	3.58 (1H, *ddd*, *J* = 10.0, 9.0, 5.0)	70.5
5‴	3.64 (1H, *m*)	69.9	3.89 (1H, *dd*, *J* = 10.0, 12.0)	66.5
			3.24 (1H, *dd*, *J* = 5.0, 12.0)	
6‴	1.23 (1H, *d*, *J* = 6.9)	18.3	–	–
			Rha	
1⁗	–	–	5.03 (1H, *d*, *J* = 1.5)	104.6
2⁗	–	–	3.87 (1H, *dd*, *J* = 1.5, 3.0)	73.8
3⁗	–	–	3.75 (1H, *dd*, *J* = 3.0, 9.0)	72.4
4⁗	–	–	3.49 (1H, *t*, *J* = 9.0)	73.7
5⁗	–	–	3.87 (1H, *m*)	70.5
6⁗	–	–	1.28 (3H, *d*, *J* = 6.5)	17.8

and C-1‴/H-6″. The structure of **3** was further confirmed by the NOE correlation signals between H-5 and H-1″ and H-6″ and H-1‴ in its spectrum. The anomeric proton signal at δ 4.69 (*d*, *J* = 7.8 Hz, H-1″) revealed that the glucose was present in a β glycosidic linkage. The α configuration of Rha was deduced from the NOE between H-1″ and H-4″ and from small $J_{1'''2'''}$ = 1.6 Hz. In the ^{13}C NMR spectrum, the anomeric C-atoms appeared at δ 104.6 and 102.3, respectively. Analysis of the HMBC spectrum of **3** showed the key correlations between H-1″ (δ 4.69, *d*, *J* = 7.8 Hz) and (C-6, δ 149.4), and between H-1‴ (δ 4.71, *d*, *J* = 1.6 Hz) and C-6″ (δ 68.4), which confirmed the glycosidic linkages. From the above data, the structure of compound **3** was determined as 6-hydroxy-galanthindolyl-6-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside, and this new compound was named 6-hydroxy-galanthindolyloside B.

Compound **4** was obtained as colorless syrup. Its molecular formula was established as $C_{34}H_{43}NO_{17}$ by the pseudo-molecular ion peak at *m*/*z* 738.2609 $[M+H]^+$ in the HR-FAB-MS. The negative ESIMS of **4** showed the quasimolecular ion peak $[M-H]^-$ at *m*/*z* 736 with prominent fragments at *m*/*z* 604 $[M-H-132]^-$ and *m*/*z* 590 $[M-H-146]^-$, due to the sequential loss of one pentose unit and/or one deoxyhexose unit, respectively. Further ions were observed at *m*/*z* 458 $[M-H-146-132]^-$, due to the sequential losses of one pentose and one deoxyhexose unit, and ion at *m*/*z* 296 $[M-H-132-146-162]^-$, i.e. aglycone. The sequential losses in the fragmentation pattern suggested both the pentose and deoxyhexose to be terminal sugars to the hexose. Complete acid hydrolysis of **4** afforded aglycone, which was identified as described above by TLC and comparison of its NMR and MS data; D-glucose, D-xylose, and L-rhamnose were identified by co-GLC comparison with authentic samples and their optical rotations.

Three sugar residues were clearly indicated by signals at δ 100.2, 105.2, and 104.6 in the ^{13}C NMR spectrum and signals at δ 5.19 (1H, *d*, *J* = 7.5 Hz), 4.88 (1H, *d*, *J* = 7.6 Hz), and 5.03 (1H, *d*, *J* = 1.5 Hz) in the ^{1}H NMR spectrum. The above data, together with the results of the 2D NMR, indicated that the saccharide moiety was composed of β-D-glucose, β-D-xylose, and α-L-rhamnose residues.

As observed in the HMBC spectrum (Fig. 4), the long-range correlations between H-1″ (δ 5.19) of the glucose and C-6 (δ 149.4) of the aglycone, H-1″ (δ 4.88) of the xylose and C-2″ (δ 82.4) of the glucose, and H-1‴ (δ 5.03) of the rhamnose and C-6″ (δ 68.6) of glucose established the sequences and linkage sites of a trisaccharide chain at C-6 as [β-D-xylopyranosyl(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranose. In its ^{1}H NMR spectrum, a usual pattern of glycosylation was indicated by downfield shifts of H-5 (ca. +0.37 ppm) with respect to the aglycone. Similarly, in the ^{13}C NMR spectra of **2–4** (see also the Section 3), 6-*O*-glycosylation was confirmed by the diagnostic upfield shift of C-6 (−2.0 ppm) and by downfield shifts of the *ortho*-related C-5 (+0.9 ppm) and C-7 (+1.3 ppm) and the *para*-related C-3a (+1.5 ppm) carbons with respect to aglycone.

Thus, the structure of **4** was established as 6-hydroxy-galanthindolyl-6-*O*-[β-D-xylopyranosyl(1 → 2)]-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-glucopyranoside, and this new compound was named 6-hydroxy-galanthindolyloside C.

As expected, the steric factors caused by the 7,5′-disubstitution force the rings out of coplanarity, resulting in a drastically diminished resonance interaction between the two aryl moieties. This is clearly reflected in the absence of the K-band in the UV spectrum of **1**.

Compound **1** and its glycosides **2–4** are the first benzodioxoleindol alkaloids having biaryl chirality that were isolated from nat-

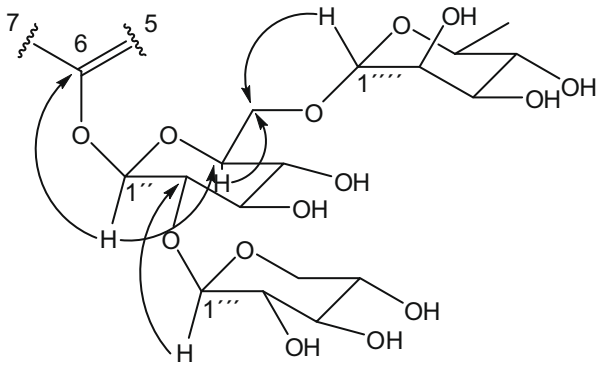

Fig. 4. The key HMBC correlations of saccharide moiety of glycoside **4**.

ure, since chiral benzodioxole-indol alkaloids, whether as aglykones or as glycosides, have not yet been described.

3. Experimental

UV–vis spectra were measured in MeOH within the range of 210–500 nm in a Cary 118 (Varian) apparatus. A Perkin–Elmer (Perkin–Elmer, Norwalk, CT, USA) model 1310 IR spectrophotometer was used for scanning IR spectroscopy as neat films. Circular dichroism measurement was carried out under dry N_2 on a Jasco-500A spectropolarimeter at 24 °C. High resolution MS were recorded using a VG 7070E-HF spectrometer (70 eV). HR-FAB-MS (positive ion mode) were obtained with a PEG-400 matrix. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C). Optical rotations were measured with a Perkin–Elmer 243 B polarimeter.

HPLC equipment consisted of a 1090 Win system, PV5 ternary pump and automatic injector (HP 1090 series, Hewlett Packard, USA), and Ascentis® Express HILIC HPLC column 2.7 μm particle size, L × I.D. 15 cm × 2.1 mm (Supelco, Prague) was used. LC was performed at a flow rate of 300 μl/min with a linear gradient from mobile phase containing methanol/acetonitrile/aqueous 1 mM ammonium acetate (60:20:20, v/v/v) to methanol/acetonitrile/aqueous 1 mM ammonium acetate (20:60:20, v/v/v) for 40 min. The whole HPLC flow (0.3 ml/min) was introduced into the ESI source without any splitting.

The detector was an Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) using electrospray mass spectra. The ionization mode was negative, the nebulizing gas (N_2) pressure was 345 kPa and the drying gas (N_2) flow and temperature were 9 l/min and 300 °C, respectively. The electrospray needle was at ground potential, whereas the capillary tension was held at 4000 V. The cone voltage was kept at 250 V. The mass resolution was 0.1 Da and the peak width was set to 6 s. For an analysis, total ion currents (full scan) were acquired from 100 to 1000 Da. The instrument was interfaced to a computer running Applied Biosystems Analyst version 1.4.1 software.

Gas chromatography–mass spectrometry of sugar derivatives was done on a GC–MS system consisting of Varian 450-GC, Varian 240-MS ion trap detector with external ionization (EI), and CombiPal autosampler (CTC, USA). The sample was injected onto a 25 m × 0.25 mm × 0.1 μm Ultra-1 capillary column (Supelco, Czech Republic) under a temperature program: 5 min at 50 °C, increasing at 10 °C min^{-1} to 320 °C and 15 min at 320 °C. Helium was the carrier gas at a flow of 0.52 ml min^{-1}. All spectra were scanned within the range *m*/*z* 50–600.

Saccharides were converted to *O*-trimethylsilyl ethers. Trimethysilylation was achieved by treating the dry samples with 100 μl of *bis*(trimethylsilyl) trifluoroacetamide for 30 min at 60 °C. TMS-ethers were analyzed by GC–MS (Rezanka and Mares, 1987).

The bulbs of *Narcissus* cultivar 'Dutch Master' were obtained from local gardening (http://www.bakker-cz.com). A voucher specimen is kept in our institute (voucher No. TR-150609b). Minced bulbs (11.2 kg) were exhaustively extracted with 95% EtOH by cold percolation. The combined extracts were concentrated and then defatted with hexane, acidified with tartaric acid to ca. pH 2, filtered and alkalinized with NH_4OH to ca. pH 10 and extracted successively with *n*-BuOH. The extract was washed with water, dried and concentrated. A total *n*-BuOH extract was separated on Sephadex LH-20 by elution with the mixture $CHCl_3$–MeOH and then separated by RP-HPLC on a Discovery C18 column (Supelco) particle size 5 mm, L × I.D. (250 mm × 21.2 mm) using a linear gradient from 90% H_2O and 10% acetonitrile to 10% water and 90% acetonitrile over 50 min, with a flow rate of 9.0 ml/min and monitored by a variable wavelength detector at 290 nm, to give compound **1** 13.2 mg (1.18×10^{-4}%), **2** 6.5 mg (5.8×10^{-5}%), **3** 14.2 mg (1.27×10^{-4}%) and **4** 7.2 mg (6.42×10^{-5}%).

Each compound **2**, **3**, or **4** (~2.5 mg) was heated in 10% HCl/dioxane 1:1 (5 ml) at 80 °C for 4 h. After evaporation of the dioxane, the solution was extracted with AcOEt (3 × 3 ml). The aqueous layer was neutralized with $NaHCO_3$ and concentrated. The solid residue was extracted by a mixture of CH_2Cl_2–MeOH (90:10) and then chromatographed on a column of silica gel (10 g), using CH_2Cl_2–MeOH–H_2O (90:10:1) to provide the aglycone **1** (~1 mg) for further analysis. The aqueous phase was lyophilized and the residue obtained after lyophilization was purified on a Sepharon SGX NH_2 column (7 μm, 3 × 150 mm) eluted with 90% MeCN (flow rate 0.7 ml/min) to yield: from **2** D-Glc ($[\alpha]_D^{25}$ +50°, equilib.), from **3** D-Glc and L-Rha $[\alpha]_D^{25}$ +8° (equilib.), and from **4** D-Glc, L-Rha, and D-Xyl ($[\alpha]_D^{25}$ +19°, equilib.).

Compound (**1**): colorless amorphous solid; $[\alpha]_D^{23}$ +174 (*c* 0.02, MeOH); UV λ_{max} (MeOH, nm) (log ε): 222 (4.11), 288 (3.64) and 296 (3.19) nm; IR (KBr, cm^{-1}): ν_{max} 3380, 2920, 2850, 1670, 1615, 1600, 1575, and 940 cm^{-1}; CD (*c*, 0.9 dioxan): λ ($\Delta\varepsilon$) 205 (11.5), 227 (−2.34), 251 (−87.6), 254 (83.2), 283 (13.2), 388 (0), 394 (3.4) nm, see also Figs. 1 and 3; HR-FAB-MS (*m*/*z*): 298.1082 $[M+H]^+$, calc. for $[C_{17}H_{15}NO_4 + H]^+$ 298.1079; NMR data see Table 1.

Glycoside (**2**): colorless semicrystalline mass, $[\alpha]_D^{20}$ +178 (*c* 0.04, MeOH). HR-FAB-MS (*m*/*z*): 460.1610 $[M+H]^+$, calc. for $[C_{23}H_{66}NO_9 + H]^+$ 460.1607; negative FABMS *m*/*z* 458 $[M-H]^-$, *m*/*z* 296 $[M-H-162]^-$; NMR data see Table 1.

Glycoside (**3**): amorphous pale-yellow powder, $[\alpha]_D^{20}$ +171 (*c* 0.05, MeOH). HR-FAB-MS (*m*/*z*): 606.2191 $[M+H]^+$, calc. for $[C_{29}H_{35}NO_{13} + H]^+$ 606.2186; negative ESIMS $[M-H]^-$ at *m*/*z* 604, *m*/*z* 458 $[M-H-146]^-$ and *m*/*z* 296 $[M-H-146-162]^-$; NMR data see Table 2.

Glycoside (**4**): colorless syrup, $[\alpha]_D^{20}$ +179 (*c* 0.03, MeOH); HR-FAB-MS (*m*/*z*): 738.2611 $[M+H]^+$, calc. for $[C_{34}H_{43}NO_{17} + H]^+$ 738.2609; negative ESIMS $[M-H]^-$ at *m*/*z* 736, *m*/*z* 604 $[M-H-132]^-$, *m*/*z* 590 $[M-H-146]^-$, *m*/*z* 458 $[M-H-146-132]^-$, and *m*/*z* 296 $[M-H-132-146-162]^-$, i.e. aglycone; NMR data see Table 2. The signals in the ^{1}H and ^{13}C spectra of aglycone were identical with values measured for glycosides **2** and/or **3**, see Table 1.

3.1. Computations

The calculated CD spectrum presented in Fig. 2 was scaled down by a factor of 0.95 to the wavelength scale. The spectral intensity was scaled up by a factor of 2. Gaussian band shapes and 20 nm half-width (at 1/*e* of peak height) were used to simulate the predicted spectrum.

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