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Chemical constituents of *Drypetes gossweileri* and their enzyme inhibitory and anti-fungal activities

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ABSTRACT

Phytochemical studies on the methanolic extract of *Drypetes gossweileri* afforded *N*-β-D-glucopyranosyl-*p*-hydroxyphenylacetamide (**1**), *p*-hydroxyphenylacetic acid (**2**), *p*-hydroxyphenyl-acetonitrile (**3**), *p*-hydroxyacetophenone (**4**), 3,4,5-trimethoxyphenol (**5**), dolichandroside A (**6**), and β-amyrone (**7**). Compounds **1**–**7** were identified with the aid of extensive NMR and MS spectroscopic studies. Compound **1** was a new natural product and was isolated for the first time from plant containing *N*-glucose moiety incorporated in its structure. Compounds **1**–**7** exhibited moderate to the weak source anti-α-glucosidase inhibitory activity. Compound **7** exhibited moderate anti-acetylcholinesterase (AChE) activity while the rest of the isolates were weakly active in this bioassay. Compounds **1**–**7** also showed moderate anti-fungal activity.

1. Introduction

The genus *Drypetes* (Euphorbiaceae) is composed of over 200 species worldwide; many of which are found in Africa, as well as other tropical and subtropical regions of the world. These plants are extensively used in African folk medicine to treat various diseases such as bronchitis, ailments of the digestive tract, fever, rheumatism and kidney pain (Bouquet and Debray, 1974; Schmelzer and Gurib-Fakim, 2008). *Drypetes gossweileri* is a large tree found in the dense humid forests of Africa and is known for its smell of horseradish. The crude extract of the stem bark has shown anti-microbial and phytotoxic properties against *Lemna minor* L. (Schmelzer and Gurib-Fakim, 2008). Our recent phytochemical studies on the methanolic extract of *D. gossweileri* of Nigerian origin afforded *N*-β-D-glucopyranosyl-*p*-hydroxyphenylacetamide (**1**), *p*-hydroxyphenylacetic acid (**2**), *p*-hydroxyphenylacetonitrile (**3**), *p*-hydroxyacetophenone (**4**), 3,4,5-trimethoxyphenol (**5**), dolichandroside A (**6**), and β-amyrone (**7**). Spectroscopic methods were used to identify compounds **1**–**7**. As part of our ongoing efforts in discovering new natural products exhibiting bioactivities including anti-GST (Ata and Udenigwe, 2008; Ata et al., 2009a,b), anti-AChE (Ata et al., 2007; Ata and Andersh, 2008; Ata, 2009), anti-oxidants (Ata et al., 2009b), anti-α-glucosidase, anti-microbial and anti-leishmanial activities, we evaluated isolated compounds for anti-α-glucosidase, anti-AChE and anti-microbial activities using hit and miss approach. Compounds **1**–**7** exhibited a wide range of α-glucosidease inhibitory, and anti-microbial activities. Compounds **1**–**7** were moderately to weakly active in AChE inhibition assay. In this manuscript, we describe the isolation and structure elucidation of compounds **1**–**7** as well as their bioactivity data.

2. Results and discussion

Compound **1** was isolated as a brown amorphous solid. Its UV spectrum showed absorption maxima at 226, 279, and 383 nm indicating the presence of a phenolic chromophore (Pavia et al., 2009). The IR spectrum showed intense absorption bands for hydroxyl (3423 cm^{-1}), amide carbonyl (1653 cm^{-1}) and benzene (1557 and 1416 cm^{-1}) functionalities. A combination of HR-FAB-MS and ^{13}C NMR spectral data of **1** provided its molecular formula as $C_{14}H_{19}NO_7$ (calcd. 313.1162).

The ^1H NMR spectrum (CD_3OD, 400 MHz) of **1** showed a set of AB doublets in the aromatic region, at δ 7.22 (J = 8.3 Hz) and 6.76 (J = 8.3 Hz) due to H-2/H-6 and H-3/H-5, respectively. Another set of AB doublets at δ 4.15 (J = 15.7 Hz) and 3.95 (J = 15.7 Hz) was ascribed to the C-7 methylene protons flanked by a benzene ring and an amide group. A one-proton doublet at δ 4.55 (J = 9.3 Hz) was due to the anomeric proton (H-1′) of the glucose moiety. Other signals resonating between δ 3.10 and 4.56 were ascribed to the

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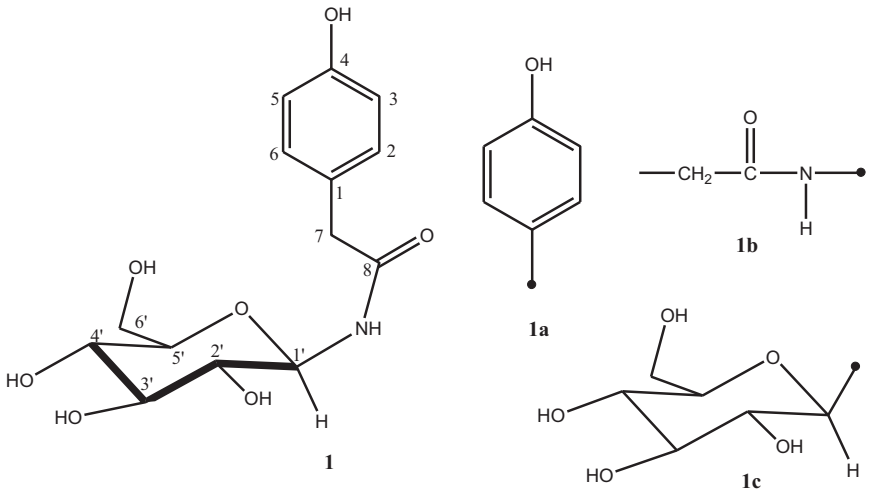

Fig. 1. Structure of compound (**1**) and partial structures (**1a**–**1c**) of **1**.

sugar moiety. The COSY 45° spectrum displayed the presence of three isolated spin systems (**1a**–**1c**, Fig. 1): one being the benzene ring, the second due to the C-7 methylene protons, and the third spin system comprised of a glucose moiety. The benzene spin system (**1a**) showed vicinal couplings between H-2/H-6 (δ 7.22) and H-3/5 (δ 6.76), indicating the presence of *para* substituted benzene ring in **1**. The H_2-7 (δ 4.15 and 3.95) a exhibited geminal couplings in the COSY spectrum and represented the second spin system (**1b**). The third spin system (**1c**) was due to the glucose moiety that showed ^1H–^1H spin-spin correlations between H-1′ (δ 4.55) and H-2′ (δ 3.15). The latter exhibited cross-peaks with H-3′ (δ 3.13) that in turn showed vicinal coupling with H-4′ (δ 3.24). The COSY 45° interaction of H-4′ with H-5′ (δ 3.23) was also observed in the spectrum. The H-5′ displayed cross-peaks with H_2-6′ (δ 3.88 and 3.64). The broad-band ^{13}C NMR spectrum (CD_3OD, 100 MHz) of **1** showed the resonance of all 14 carbons (C-2/C-6 and C-3/C-5 resonated as two signals at δ 130.5 and 116.8, respectively). A combination of ^{13}C NMR and DEPT spectra indicated the presence of two methylene, nine methine (C-2/C-6 and C-3/C-5 resonated as two signals) and three quaternary carbons in **1**. The ^{13}C NMR spectrum showed the resonance of three quaternary carbons, C-1 (δ 128.0), C-4 (δ 157.9), and C-8 (δ 161.5), respectively. The down-field chemical shift value of C-4 was indicative of the presence of a geminal hydroxyl group and C-8 was an amide carbonyl carbon. The anomeric carbon resonated at δ 82.9. The sugar moiety in compound **1** was determined to be glucose based on the comparison of the ^1H and ^{13}C NMR data with those of glucose reported in the literature (Nenkeo et al., 2008). Complete ^1H and ^{13}C NMR chemical shift assignments of **1** and its HSQC spectral data are shown in Table 1. The HMBC spectrum of **1** showed $^1H/^{13}C$ long-range couplings of H–2/H-6 (δ 7.22) with C-7 (δ 39.1) and C-4 (δ 157.9). The H-3/H-5 (δ 6.76) showed HMBC couplings with C-1 (δ 128.0) and C-4 (δ 157.9). The H_2-7 (δ 4.15 and 3.95) showed couplings with C-1 (δ 128.0), C-2/C-6 (δ 130.5), and C–8 (δ 161.5). These HMBC spectral data led us to substitute C-7 between benzene and amide moieties. The H-1′ anomeric proton (δ 4.55) showed HMBC cross-peaks with C-2′ (δ 79.5), C-3′ (δ 74.4) and C-8 (δ 161.5). These HMBC observations suggested a bond between C-1′ and C-8 *via* an amide linkage. Important HMBC interactions are shown in Fig. 2. The anomeric proton of glucose moiety exhibited large coupling constants (J = 9.3 Hz) indicating the β-linkage for glucose moiety in **1**. The sugar moiety was also identified by the acid hydrolysis of compound **1** and this yielded aglycone and glucose. The ^1H NMR data of purified aglycone was in agreement with its structure. Analytical TLC of purified glucose displayed the same R_f value as that of glucose purchased from the Sigma–Aldrich.

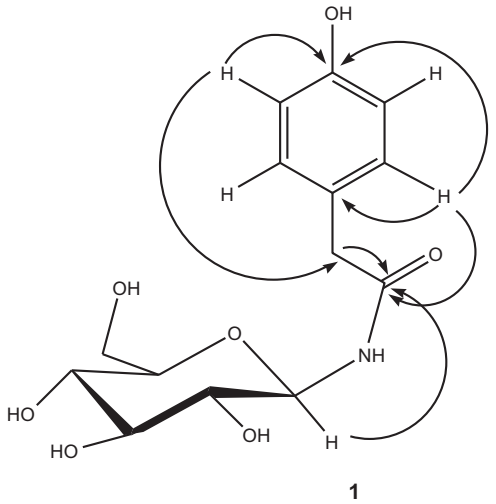

Fig. 2. Important HMBC interactions of compound **1**.

Table 1
^1H and ^{13}C NMR chemical shift assignments of **1**, and $^1H/^{13}C$ one-bond-shift correlations of **1**, as determined from HSQC spectrum.

Carbon number	**1**	
	^1H	^{13}C[a]
	δ	δ
1	–	128.0 (–C–)
2	7.22 (d, 8.3)	130.5 (CH)
3	6.76 (d, 8.3)	116.8 (CH)
4	–	157.9 (–C–)
5	6.76 (d, 8.3)	116.8 (CH)
6	7.22 (d, 8.3)	130.5 (CH)
7	3.95 (d, 15.7)	39.1 (CH_2)
	4.15 (d, 15.7)	–
8	–	161.5 (–C–)
1′	4.55 (d, 9.3)	82.9 (CH)
2′	3.15 (m)	79.5 (CH)
3′	3.13 (m)	74.4 (CH)
4′	3.24 (m)	71.4 (CH)
5′	3.23 (m)	82.3 (CH)
6′	3.64 (m)	62.9 (CH_2)
	3.88 (m)	–

[a] Multiplicity was determined by DEPT spectra.

The absolute configuration of isolated glucose were determined to be D by comparing the optical rotation value with that of commercially purchased sugar. Optical rotation of glucose $[\alpha]_D^{20} = 48^\circ$ (mutarotation equilibrium value) obtained from the acid hydrolysis of **1** was found to be nearly identical as that of commercially available D-glucose $[\alpha]_D^{20} = 49^\circ$, treated with the acid under identical conditions.

Additionally these studies afforded six known natural products, *p*-hydroxyphenylacetic acid (**2**) (Ding et al., 2008), *p*-hydroxyphenylacetonitrile (**3**) (Nagatsu et al., 2004), *p*-hydroxyacetophenone (**4**) (Dhami and Stothers, 1965), 3,4,5-trimethoxyphenol (**5**) (Xu et al., 2008), dolichandroside A (**6**) (Aparna et al., 2009) and β-amyrone (**7**) (Ramadan et al., 2009). Compounds **2**–**7** were isolated for the first time from genus *Drypetes*. Structures of these known compounds were established by comparing their ^{1}H, ^{13}C NMR, and mass spectral data with those of previously reported compounds in the literature.

2.1. Bioactivities of compounds 1–7

Compounds **1**–**7** were evaluated for anti-α-glucosidase, anti-AChE and anti-microbial activities. In α-glucosidase inhibition assay, compound **1** was found to be significantly active with an IC_{50} value of 12 μM compared to the rest of the isolates. The IC_{50} values for α-glucosidase inhibitory activity of compounds **1**–**7** are shown in Table 2. Compounds **1**–**7** also exhibited anti-fungal activity against *Candida albicans* with minimum inhibitory concentrations (MIC) values in the range of 8–16 μg/ml (Table 2). All of the isolates were weakly active in AChE inhibition assays with an exception of β-amyrone (**7**) that was moderately active in this bioassay with an IC_{50} value of 23.0 μM. Anti-AChE inhibitory activity data of compounds **1**–**7** are presented in Table 2. Among all of the isolates, compound **1** displayed significant α-glucosidase inhibitory and antifungal activities than other isolated natural products where as β-amyrone (**7**) exhibited potent anti-AChE activity compared to the rest of the isolates but this activity was moderate when compared with galanthamine, a positive control. Further studies on the structure-activity relationships on compounds **1** and **7** are warranted.

3. Experimental

3.1. Spectral analysis

The UV spectra were recorded in methanol on Shimadzu UV-250 PC spectrophotometer. Michelson–Bomen Hartmann and Braun (MB-series) spectrometer was used to acquire IR spectra. ^{1}H NMR experiments including 1D and 2D were performed on a Bruker Avance 400 MHz spectrometer. The ^{13}C NMR spectra were also recorded on the same instrument at 100 MHz. The mass spectral studies were carried out on Micro-TOF II and Hewlett–Packard 5989B MS. A Haensch–Schmidt polarimeter was used to measure the optical rotations (Na lamp, 589 nm). Anti-glucosidase and anti-AChE inhibitory activities were measured α spectrophotometrically using a Synergu HT multidetection BioTek microplate reader. Column chromatography was carried out on silica gel (200–400 mesh).

Table 2
Anti-α-glucosidase, anti-AChE and anti-fungal activities data of **1–7**.

Compounds	α-Glucosidase	AChE	Anti-fungal
	IC_{50} (μM)[a]	IC_{50} (μg/ml)	MIC (μg/ml)
1	12.0 ± 2.0	78.1 ± 1.3	8.0
2	50.0 ± 6.0	127.2 ± 7.5	16
3	48.0 ± 4.0	207.9 ± 10	16
4	50.0 ± 1.0	210.0 ± 3.0	16
5	56.2 ± 3.0	220.0 ± 5.0	16
6	20.0 ± 2.0	189.2 ± 2.5	16
7	25.0 ± 1.0	23.0 ± 1.0	8.0
Acarbose	0.6 ± 0.2	–	–
Galanthamine	–	0.53±0.5	–
Flucanazol	–	–	<8.0

±Represents Standard error of mean (each bioassay was performed in triplicate). Acarbose, galanthamine and flucanazol were used as positive controls.

[a] IC_{50} value represents the concentration of compounds required to inhibit the 50% activity of enzyme.

3.2. Drypetes gossweileri

The bark of *D. gossweileri* was collected from Nigeria in 2008. The plant was identified by one of the authors (AA) and a voucher specimen (NPL 4) was deposited in the Natural Product Chemistry Lab, The University of Winnipeg.

3.3. Isolation of compounds 1–7

The bark (2 kg) of *D. gossweileri* was collected and dried. The dried bark (1.5 kg) was extracted three times with methanol (3 L) at room temperature. This extract was filtered and solvent was removed *in vacuo* to afford a gum (100 g). This methanolic extract (100 g) was loaded onto a silica gel column for further fractionations. This column was eluted with chloroform–methanol (0–100%, v:v) to afford several fractions. These fractions were pooled on the basis of analytical TLC results. Four fractions, F_1–F_4 showed the presence of natural products on analytical TLC. The fraction F_1 was obtained on elution of the silica gel column with chloroform–methanol (96:4, v:v) and its preparative TLC (PTLC) using chloroform–methanol–acetic acid (50:50:5, v:v:v) as a mobile phase was carried out to purify compound **1** (12.0 mg). Compound **2** (81.4 mg) was isolated from the fraction F_2, obtained on elution of the silica gel column with chloroform-methanol (95:5, v:v), after performing its PTLC using chloroform–methanol (94:6, v:v) as developing solvent. The PTLC of fraction F_3, obtained on elution of the silica gel column with chloroform–methanol (93:5, v:v), using chloroform–methanol (98:2, v:v) as a mobile phase afforded compound **3** (14.9). The fraction F_4 was obtained on elution of the silica gel column with chloroform-methanol (90:10, v:v) and showed the presence of four compounds on analytical TLC using ethyl acetate–methanol (99:1, v:v) as a developing solvent. The PTLC of this fraction using same mobile phase as that of analytical TLC afforded compounds **4** (5.8 mg), **5** (8.0 mg), **6** (10.0 mg) and **7** (5.0 mg).

3.3.1. N-β-D-glucopyranosyl-p-hydroxyphenylacetamide (1)

Brown amorphous solid; IR (KBr): ν_{max} 3423, 1653, 1557, 1416 cm^{-1}: UV (MeOH): λ_{max} 383, 279 and 226 nm; HR-FAB-MS *m/z* 313.1166 (calcd. for $C_{14}H_{19}NO_7$, 313.1162); ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13} C NMR ($CDCl_3$, 100 MHz) see Table 1.

3.3.2. Acid hydrolysis of 1

Compound **1** (8 mg) was refluxed with 10% HCl (15 ml) at 90 °C for 7 h. TLC was used to monitor the reaction progress. After 7 h, TLC showed the two distinct spots indicating the completion of this reaction. Based on these TLC results, the reaction was stopped by the addition of cold water (20 ml). This reaction mixture was extracted with ethyl acetate in order to isolate aglycone. The aqueous phase was concentrated under reduced pressure to afford sugar moiety.

3.3.3. Biological assays

α-Glucosidase inhibitory activity of **1**–**7** was determined by method described by Hou et al. (2009). Anti-AChE activity of all isolated compounds was measured using modified Ellman's assay (Ellman et al., 1961; Babar et al., 2006; Ata et al., 2006). Anti-fungal

assays on compounds **1**–**7** were performed by using serial dilution method (Ata et al., 2006, 2009a,c).

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