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A PHENOLIC GLYCOSIDE FROM *HYPOXIS OBTUSA*

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Key Word Index—*Hypoxis obtusa*; Hypoxidaceae; phenolic glycoside; obtusaside.

Abstract—A new phenolic glycoside was isolated from *Hypoxis obtusa*, together with the known compounds acuminoside, hypoxoside and nyasoside. In the new glycoside, 2,5-dihydroxybenzyl alcohol and 3-hydroxy-2,6-dimethoxybenzoic acid moieties are linked to glucose at the C-1 and C-6 positions respectively.

INTRODUCTION

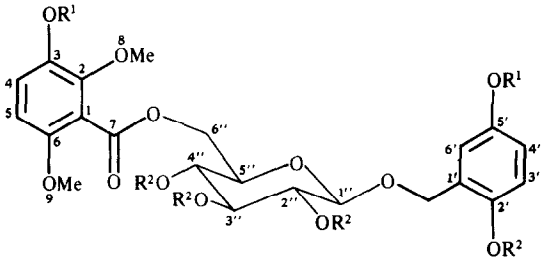
Pharmacological interest in the genus *Hypoxis* arises from its use in traditional medicine by people in eastern and southern Africa. Infusions of the tuber are employed as a remedy for prostate hypertrophy and uterine cancer [1]. The compounds so far isolated from various *Hypoxis* species are zeatin and zeatin glycoside [2], hypoxoside from *H. obtusa* [1], acuminoside from *H. acuminata* [3], nyasoside, mononyasine A, mononyasine B and nyasicoside from *H. nyasica* [4–6] and 1-(3″,4″-dihydroxyphenyl)-5-(4′-hydroxyphenyl)pent-1-en-4-yne from *H. rooperi* [7]. Some of these compounds show strong anticancer activity [7].

Phytochemical investigation of *Hypoxis obtusa* has led to the isolation of a new phenolic glycoside named obtusaside (**1**), together with three known compounds from the methanol extract of the whole plant. The structure of **1** was established by spectroscopic methods and chemical transformations.

RESULTS AND DISCUSSION

The whole fresh plant was cut into small pieces and extracted with methanol. Following evaporation, the methanol extract was washed with dichloromethane and *n*-butanol. The *n*-butanol residue was fractionated by column chromatography (silica gel, $CHCl_3$–MeOH–H_2O, 13:7:1), Sephadex LH-20 gel filtration (MeOH) and medium-pressure liquid chromatography (MPLC) (RP-8, MeOH–H_2O step gradient), yielding obtusaside (**1**), acuminoside, hypoxoside and nyasoside.

Compound **1**, an off-white amorphous powder, produced a dark blue coloration with $FeCl_3$ on TLC plates. The D/CI mass spectrum of **1** gave quasimolecular ion peaks at *m/z* 483 $[M+H]^+$ and *m/z* 500 $[M+NH_4]^+$ (positive ion mode, reagent gas NH_3) and the IR spectrum showed the presence of a hydroxyl group (3400 cm^{-1}), a conjugated carbonyl group (1700 cm^{-1}) and an aromatic moiety (1610 and 1500 cm^{-1}). The ^1H NMR (see Table 1) showed signals for two methoxy groups, five oxygenated methine protons, two methylene groups bearing an oxygen and five aromatic protons. The spin–spin correlations of the aromatic protons were confirmed as AB and ABX systems by a NMDR experiment. A NOE effect between a methoxy group and one aromatic proton (δ 6.60) of the AB system was observed in a difference NOE experiment. The ^{13}C NMR spectrum (see Experimental) not only contained 22 carbons, but also showed the presence of two aromatic rings and a glucose moiety.



1 $R^1 = R^2 = H$
1a $R^1 = R^2 = Ac$
1b $R^1 = OMe$, $R^2 = H$

The above spectral evidence suggested that **1** might be a phenolic glucoside. This conclusion was confirmed by the following chemical transformations: enzymatic hydrolysis of **1** with β-D-glucosidase afforded 2,5-dihydroxybenzyl alcohol; acidic hydrolysis (5% H_2SO_4–EtOH, 1:1) gave 3-hydroxy-2,6-dimethoxyethylbenzoate and glucose as the sole monosaccharide in the aqueous solution (TLC). The presence of glucose was confirmed by the formation of pentaacetyl glucitol and comparison with an authentic sample (GC).

Acetylation of **1** afforded the corresponding hexaacetate (**1a**). The ^1H NMR data are given in Table 1. Of interest are the signals for C-7′ and C-6″ methylene protons of the aromatic ring and glucose respectively. There was no observable downfield shift of the two signals on acetylation. This confirmed that there is no free $-CH_2OH$ group present and that the two aglycones, 2,5-dihydroxybenzyl alcohol and 3-hydroxy-2,6-dimethoxybenzoic acid, are connected to the glucose unit at the C-1″ and C-6″ carbon atoms respectively. Finally, the substitu-

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Table 1. ^{1}H NMR of phenolic glycoside **1** and its derivatives (pyridine-d_5)

H	1	1a	1b
Ester	—		
1	—	—	—
2	—	—	—
3	—	—	—
4	7.11 *d* (8.8)	7.07 *d* (9)	6.98 *d* (9)
5	6.6 *d* (8.8)	6.65 *d* (9)	6.69 *d* (9)
6	—	—	—
7	—	—	—
8	4.04 *s*	3.80 *s*	3.96 *s*
9	3.65 *s*	3.75 *s*	3.68 *s*
Alcohol			
1′	—	—	—
2′	—	—	—
3′	7.59 *d* (8.7)	7.10 *d* (8.8)	7.57 *d* (9)
4′	7.06 *dd* (8.7, 2.9)	6.99 *dd* (8.8, 2.7)	6.85 *dd* (9, 2.9)
5′	—	—	—
6′	7.67 *d* (2.9)	7.25 *d* (2.9)	7.44 *d* (2.9)
7′	6.10, 6.01, each *d* (13.6)	5.39, 5.30, each *d* (13.6)	6.03, 5.94, each *d* (13.6)
Glucose			
1″	5.48 *d* (6.7)	5.03 *d* (7.6)	5.47 *d* (7)
2″			
3″	4.29–4.33 *m*	5.12–5.31 *m*	4.28–4.36 *m*
4″			
5″	4.06 *m*	3.84 *m*	4.08 *m*
6″	4.56 *dd* (12.2, 2.2)	4.29 *dd* (12.4, 5.4)	4.37 *dd* (12.4, 5)
	4.40 *dd* (12.2, 5.3)	4.17 *dd* (12.4, 2.6)	4.57 *dd* (12.4, 2.2)
OMe (C-3)	—	—	3.69 *s*
OMe (C-5′)	—	—	3.65 *s*
OAc	—	2.04, 2.05, 2.07, 2.09, 2.28, 2.31	—

J (Hz) in parentheses.

tion pattern of obtusaside was confirmed by difference NOE experiments on the tetramethyl ether (**1b**). Effects were observed between the C-3 methoxy group and H-4; the C-6 methoxy group and H-5; the C-5′ methoxy group, H-4′ and H-6′; and between the C-7′ methylene group and H-1″ and H-6′.

Nyasoside, previously isolated from *H. nyasica*, has *cis* geometry at the internal double bond, as deduced from the coupling constants ($J = 11.5$ Hz) in its ^{1}H NMR spectrum. Obtusaside (**1**) and hypoxoside are the major constituents, whereas acuminoside and nyasoside are present in *H. obtusa* in minor amounts. Compound **1** represents a new type of molecule isolated from the genus *Hypoxis*. The substitution pattern of the methoxy groups in the ester moiety is also unique.

EXPERIMENTAL

General. ^{1}H and ^{13}C NMR: 200 and 50.1 MHz respectively. Chemical shifts are given in δ values (ppm) with TMS as int. standard; UV: MeOH; IR: KBr; MS: DC/I MS (NH_3); silica gel: Merck Art. 9385: All solvents were spectral grade or distilled prior to use.

Plant material. Hypoxis obtusa Burch was collected in Mzimba District, Northern Region of Malawi, in November 1988 and was identified by Mr E. A. K. Banda. A voucher specimen is deposited in the National Herbarium and Botanic Gardens of Malawi, Zomba.

Extraction and purification. Whole plant material (1.5 kg) was cut into small pieces and extracted with MeOH. The MeOH extract (141 g) was successively washed (× 3) with CH_2Cl_2 (yield, 4.3 g) and then (× 3) with *n*-BuOH (yield, 33.6 g). The *n*-BuOH extract (20 g) was fractionated on a silica gel column ($CHCl_3$–MeOH–H_2O, 13:7:1; TLC, same solvent system, Godin spray reagent) to give 5 fractions (1–5). Hypoxoside (4.97 g), identified by comparison of spectral data and physical constants with literature values [1], was obtained from fr. 5. A portion (6 g) of fr. 2 (9.32 g) was further purified by CC (silica gel, $CHCl_3$–MeOH–H_2O, 13:7:1), yielding seven fractions (I–VII). The known compounds acuminoside (19 mg) and nyasoside (100 mg), identified by comparison with literature data [3, 4], were obtained from frs I and II, respectively. Fr. IV was further purified on RP-8 MPLC (step gradient solvent system; MeOH–H_2O 50→60→70% MeOH) to give a pure compound, named obtusaside (**1**) (760 mg) as an off-white amorphous powder. IR ν^{KBr}; 3400 (OH), 1710 (C=O), 1610, 1500 (aromatic ring), 1290, 1260, 1215, 1090 cm^{-1} (C=O); UV λ^{MeOH} nm (log ε); 205 (5.33), 230 (5.05), 292 (4.64). ^{13}C NMR (in pyridine-d_5): Me: 56.47 and 61.30; CH_2: 62.49, 62.76*; CH: 71.31*, 74.99*, 78.59*, 78.78*, 104.45*, 108.07, 116.14, 116.36, 118.42, 118.74; C: 120.23, 128.12, 145.64, 146.44, 149.17, 149.92, 154.26, 166.67 ppm (C=O) (*Glc). For ^{1}H NMR (in pyridine-d_5) see Table 1.

Acid hydrolysis of obtusaside (**1**). Obtusaside (**1**) (39 mg) in 5% H_2SO_4–EtOH (1:1) (15 ml) was refluxed for 2 hr, poured into cold H_2O, neutralised with $BaCO_3$ and filtered. After extraction (×3) with Et_2O and (×3) with EtOAc, the combined extracts were dried over Na_2SO_4 and evapd to dryness. The product mixture was purified by CC (silica gel $CHCl_3$–MeOH 4:1) to give 3-hydroxy-2,6-dimethoxyethylbenzoate (12 mg); R_f 0.8 ($CHCl_3$–MeOH, 4:1, Godin). The aq. layer was filtered and lyophilised to give glucose (8 mg), co-TLC with authentic sample (EtOAc–HOAc–MeOH–H_2O, 13:4:3:3; anisidine phthalate). The glucose was converted to its pentaacetyl glucitol and co-injected with authentic sample (GC). The same retention times were shown.

Enzymatic hydrolysis of **1**. Obtusaside (55 mg) was treated with β-D-glucosidase (110 mg) in NaOAc–HOAc buffer solution (10 ml) (pH 5.5) at 35° with stirring over a weekend, extracted (×3) with *n*-BuOH and evapd. The *n*-BuOH extract was purified by CC (silica gel $CHCl_3$–MeOH 4:1) to give three fractions. Fraction 2 yielded 2,5-dihydroxybenzyl alcohol (22 mg) [R_f 0.5 ($CHCl_3$–MeOH–H_2O, 80:20:3); positive $FeCl_3$ test], directly converted to its triacetate using Ac_2O in the presence of pyridine.

Peracetylation of **1**. Obtusaside (120 mg) in dry pyridine (3 ml) was treated with Ac_2O (10 ml) and stirred at room temp. overnight, poured into cold H_2O, extracted (×3) with Et_2O, dried over Na_2SO_4 and evapd to give the hexaacetate (**1a**) (125 mg). For ^{1}H NMR (in pyridine-d_5) see Table 1.

Permethylation of **1**. Obtusaside (84 mg) was treated with MeI (3 ml) and K_2CO_3 (1.25 g). The mixture was dissolved in Me_2CO (1.5 ml) and refluxed. Usual work-up afforded a product mixture (90 mg) which was purified by CC (silica gel, C_6H_6–EtOAc, 4:1) to give the tetramethyl ether (**1b**) (40 mg) [R_f 0.3 (C_6H_6–EtOAc, 4:1) Godin, $FeCl_3$]; IR (neat): 3400 cm^{-1} (OH). For ^{1}H NMR (in pyridine-d_5) see Table 1.

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PLICATIN A AND B, TWO PHENOLIC CINNAMATES FROM *PSORALEA PLICATA*

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Key Word Index—*Psoralea plicata*; Leguminosae; phenolic cinnamates; 4-hydroxy-3-(2′-hydroxy-3′-methyl-3′-butenyl)-*trans*-methyl cinnamate and 4-hydroxy-3-(3′-methyl-2′-butenyl)-*trans*-methyl cinnamate.

Abstract—Two new phenolic cinnamates, plicatin A and plicatin B, have been isolated from *Psoralea plicata*. Structures have been assigned to these through chemical and spectroscopic studies.

INTRODUCTION

Psoralea plicata Del is a small shrub commonly found in Sindh, Pakistan. It is highly regarded for its medicinal properties in the indigenous system of medicine [1]. Previously we have reported a new triterpenoid from this plant [2]. Following further studies on the fresh and undried plant material, two new phenolic cinnamates have been isolated and named plicatin A and B.

RESULTS AND DISCUSSION

Plicatin A (**1**) crystallized from acetone, mp 182°, $[\alpha]_D$ −59.7°. Its HR mass spectrum gave a molecular ion peak at *m/z* 262.2016 corresponding to the molecular formula $C_{15}H_{18}O_4$ (calcd 262.3050) indicating seven double bond equivalents. Other fragments were observed at *m/z* 247 $[M-CH_3]^+$, 244 $[M-H_2O]^+$, 229 $[M-Me-H_2O]^+$, 192 $[M-C_4H_6O]^+$, 191 $[M-C_4H_7O]^+$ and 160