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A New Steroid and α-glucosidase Inhibitors from *Anthocleista schweinfurthii*

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Abstract: The dichloomethane/methanol extract of the roots of *Anthocleista schweinfurthii* Gilg. has provided a new steroid, schweinfurthiin 1, two known compounds, bauerenone 2 and bauerenol 3 which were found to be highly promising α-glucosidase inhibitors. Along with these, two known xanthones, 1-hydroxy-3, 7, 8-trimethoxyxanthone 4 and 1, 8-dihydroxy-3, 7-dimethoxyxanthone 5 were also isolated.

Key words: *Anthocleista schweinfurthii*, schweinfurthiin, α-glucosidase inhibitors

INTRODUCTION

Anthocleista (Gentianaceae) is a genus of trees which grow in the tropical rain forest areas of Africa. These plants are used in traditional medicine for the treatment of various diseases. *A. djalonensis* is known for its antipyretic, analgesic and purgative actions (Keay *et al*., 1964). On the other hand *A. grandiflora* is known for its antimalarial activity (Watt and Breyer, 1962). Most importantly, the roots decoction of *A. djalonensis* and related species such as *A. vogelli* and *A. kerstingii*, have been used in the treatment of diabetes mellitus. Herbalists claim a high percentage of cures in their diabetic patients, treated with plants of genus *Anthocleista* (Amofo, 1977) *A. schweinfurthii* is used in Matachouom, Cameroon, to treat diabetes, malaria and stomach diseases. In order to establis the link between the traditional use and the chemical constituents of this species, we carried out the phytochemical and pharmacological investigation of *A. schweinfurthii* and we report here in the isolation and structure determination of a new steroid 1, four known compounds including two α-glucosidase inhibitors 2, 3 and two xanthones 4 and 5 from this natural source.

Glucosidases have drawn the attention of the scientific community for its wide role in the living biological systems. Glucosidases are involved in several biological processes, intestinal digestion, the biosynthesis of glycoproteins and the lyposomal catabolism of the glycoconjugates (Asano *et al*., 1997). Glucosidases inhibitors are of considerable current interest in view of potential aspects in the treatment of the AIDS (Acquired Immunodeficiency Virus), glucosidase inhibitors are of current considerable interest, because of its anti-HIV (Human Immunodeficiency Virus) activity shown by the natural competitive inhibitors nojirimycin (Josie *et al*., 1992). Intestinal α-glucosidases are involved in the final step of the carbohydrates digestion and convert them into monosaccharides, which are absorbed from the intestine thus its inhibitors could therefore suppress the postprandial hyperglycemia and can be used for the treatment of the type II diabetes (Sou *et al*., 2000). α-Glucosidases inhibitors have been also used as inhibitors of tumor metastasis, antiobesity drugs, fungistatic compounds, insects antifeedants, antiviral and immune modulators (El Ashry *et al*., 2000).

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We have focused to study on the discovery of effective inhibitors because of the multi dimensional scope of this enzyme. The acarbose is a very widely prescribed drug in the management of the type II diabetes.

Compounds 2 and 3 were found to be highly promising α-glucosidase inhibitors when compared with the standard inhibitors deoxynijorimicin and acarbose in the studies.

MATERIALS AND METHODS

General

This study was conducted in the HEJ Research Institute of Chemistry, University of Karachi, Pakistan from may to September 2005. IR Spectra were recorded on a Nicolet Magma 750 spectrophotometer as KBr disc. Optical rotation was determined on a JASCO DIP-181 polarimeter. ^{1}H-NMR spectra were recorded on Avance 400 MHZ Bruker NMR machine, while ^{13}C-NMR spectra were recorded at 100 MHZ. Chemical shifts are given in δ (ppm), taking TMS as reference and relative to the solvent used. NMR spectra were recorded in $CDCl_3$. EIMS and HREIMS were recorded on a JEOL HX110 MS. Silica gel (70-230 and 230-400 mesh) (Merck) was used for column chromatography. Melting points were recorded on BUCHI 535 melting points apparatus.

Plant Material

The stem bark of *Anthocleista schweinfurthii* Gilg. was collected from Dschang, Menoua Division, Western province of Cameroon, in February 2005. Plant material was identified by Dr. Onana, National Herbarium, Yaoundé, Cameroon and a voucher specimen No. WCS 2489b/26859/Ya. was deposited.

Extraction and Isolation

The air-dried and powdered plant material (800 g) was macerated in 4 L mixture of dichloromethane and methanol (1:1) for 3 days. Removal of the solvent in a rotary evaporator provided an organic extract (14 g). This extract was dissolved in methanol (500 mL) and re-extracted with petroleum ether (1 L) to obtain fraction A (700 mg). The resulting organic phase was then concentrated and dissolved in water (500 mL). This aqueous part was extracted with dichloromethane (1 L), ethyl acetate (1 L) and butanol (500 mL) to yield fractions B (6 g), C (2 g) and D (5 g). Fraction B was concentrated and subjected to column chromatography on silica gel (230-400 mesh), using a mixture of n-hexane-acetone, followed by dichloromethane-acetone as eluent. Fractions of 100 mL each were collected and combined on the basis of their TLC profiles. The fraction eluted with n-hexane-acetone, (3:1) (500 mg) was further purified by column chromatography on silica gel (70-230 mesh) to yield bauerenone 2 (32 mg) and bauerenol 3 (16 mg). The combined fractions (100 mg), eluted with n-hexane-acetone (95:5), was purified by column chromatography on silica gel (70-230) with n-hexane-chloroform as eluent to yield schweinfurthiin 1 (18 mg). Fraction eluted with dichloromethane-acetone (9:1) (300 mg) was further purified by column chromatography on silica gel (70-230 mesh) to yield 1-hydroxy -3,7,8- trimethoxyxanthone 4 (8 mg) and 1,8-dihydroxy-3,7-dimethoxyxanthone 5 (6 mg).

Enzyme Inhibition Assay

α-Glucosidase (E.C.3.2.1.20) enzyme inhibition assay has been performed according to the slightly modified method of Matsui *et al*. (1996). α-Glucosidase (E.C.3.2.1.20) from *Saccharomyces* species, purchased from Wako Pure Chemical Industries Ltd. (Wako 076-02841). The enzyme inhibition was measured spectrophotometrically through continuous monitoring of the nitropenyl produced by the hydrolysis of the substrate p-nitrophenyl α-D glucopyranoside (PNP-G) (0.7 mM) and 500 milli units mL^{-1} of the used enzyme. Whole enzymatic reaction was performed at 37°C for

30 min. The increment in absorption at 400 nm, due to the hydrolysis of PNP-G by α-glucosidase, was monitored continuously on microplate spectrophotometer (Spectra Max, Molecular Devices, USA). Phosphate saline buffer at pH 6.9 was used, which contains 50 mM sodium phosphate containing 100 mM NaCl. 1-Deoxynojirimycin (0.425 mM) and Acarbose (0.78 mM) were used as positive controls.

Schweinfurthiin 1, (campest-5-en-3β-ol tridecanoate)
White crystals from acetone mp: 76-77°C (uncorrected).

Rf: 0.4 (Hex-EtOAc, 92:8).
$(\alpha)_D^{27} = +2.4$, c: 50, $CHCl_3$
IR (KBr): 1720 cm^{-1}.
^1H and ^{13}C NMR (in $CDCl_3$ 400 and 100 MHZ, respectively, Table 1):
MS (EI, 70 eV): m/z (%): 393 (82), 241 (41) 229 (100), 129 (8), 71 (30);
HREIMS: (calculatded for $C_{41}H_{72}O_2$). 597.0186 found: 597.0154.

Bauerenone 2
White crystals from acetone mp: 230-231°C
IR (KBr): 1712, 1470, 1390, 1380 cm^{-1}; $C_{30}H_{48}O$,
MS (EI, 70 eV) m/z (%): 409 (11), 271 (13), 257 (17), 245 (100), 218 (12), 205 (22). Molecular formula $C_{30}H_{48}O$
^{13}C- and ^1H-NMR in agreement with literature.

Bauerenol 3
White crystals from acetone, mp: 192-193°C (uncorrected).
MS (EI, 70 eV) m/z (%): 411 (21), 393 (6), 273 (3), 259 (18), 247 (100), 229 (47), 207 (18). Molecular formula $C_{30}H_{50}O$.
^{13}C-and ^1H-NMR in agreement with literature.

Compound 1 was isolated as white crystals, mp: 76-77°C $[\alpha_D]^{27}$ 2.4, c: 50, $CHCL_3$ The molecular formula was determined to be $C_{41}H_{72}O_2$ from the High Resolution EI mass spectroscopy (HREIMS, m/z 597.0514). Mass fragments at m/z 393, 229, 241, 129, 73 and 71 were characteristics of steroids (Diekman and

Table 1: ^1H- and ^{13}C -NMR data of schweinfurthiin 1 recorded in $CDCl_3$

Position	δ ^1H (m, J (Hz))	δ ^{13}C	Position	δ^1H (m, J (Hz)	δ^{13}C
1		37.8	18	0.75 (s)	12.0
2	2.74 (t, 12.6)	31.9	19	0.97 (s)	23.6
3	4.50 (dd, 11.1, 4.3)	80.8	20	1. 64, m	35.4
4	2.37, m	38.0	21	1.02 (d, 6.5)	16.9
5	-	145.5	22	1.39, m	35.1
6	5.39 (d, 2.4)	116.3	23	1.23, m	32.1
7	1.88, m	34.8	24	1.12, m	35.4
8	1.22, m	29.7	25	1.49, m	31.9
9	1.42, m	48.2	26	0.93 (d, 6.8)	22.5
10	-	36.6	27	0.93 (d, 6.8)	22.7
11	1.12, m	22.7	28	0.82 (d, 6.4)	15.9
12	1.12, m	37.8	1'	-	173.6
13	-	41.3	2'	2.29 (t, 14.6)	34.8
14	1.22, m	50.6	3'	1.60 (br s)	25.2
15	1.12, m	24.3	4'-11'	1.23 (br s)	29.2-29.6
16	1.12, m	25.6	12'	1.23 (br s)	22.5
17	1.12, m	55.0	13'	0.87 (t, 7.1)	14.1

δ: Chemical shifft, m: Multiplet, d: Doublet, t: Triplet, s: Singlet, J: coupling constant, br: Broad, Hz: Hertz

Djerassi, 1967). Moreover several fragments were observed exhibiting a uniform difference of 14 mass units, revealing an aliphatic chain in the molecule (Misra *et al*., 1991; Akihisa *et al*., 1989). This suggested that compound 1 was a steroid with an aliphatic side chain. The IR spectrum of 1 showed carbonyl absorption at 1720 cm^{-1}. The ^{1}H- NMR spectrum of 1 (Table 1) displayed signal dues to four tertiary methyl groups at δ 0.82 (Me-28), 0.93 (Me -26/27) and 1.02 (Me-21). Two singlets appeared at δ 0.75 and 0.97, corresponding to Me-18 and Me-19 respectively. A triplet at δ 0.87 was due to Me-13' of the side chain while the other one at δ 2.29 was attributed to the methylene H-2' protons. A broad signal at δ 1.23 was attributed to many methylene protons. H-6 appeared as a broad doublet at δ 5.39 (J = 2.4 Hz). The doublet of doublets observed at δ 4.50 was attributed to C-3 proton characteristic of cholest-5-ene-3α H (Funel *et al*., 2004; Goad, 1991; Thompson, 1972). The ^{13}C- NMR spectrum (Table 1) showed the carbonyl carbon signal at δ 173.6, while C-3 resonated at δ 80.8, along with seven methyl carbons, 4 quaternary carbons and 29 methylene carbons. In the HMBC spectrum, correlations of H-3 with C-1, C-2, C-4 and C-1′; H-2′and H-3′ with C-1′ and H-12′ with C-13′ were observed (Fig. 1). Based on the data here described and on comparison with data of similar compounds (Rösecke and Konig, 2000), compound 1 was assigned structure 1 trivially named schweinfurthiin. Lubsy *et al*. (1984) has previously reported the same compound as a synthetic

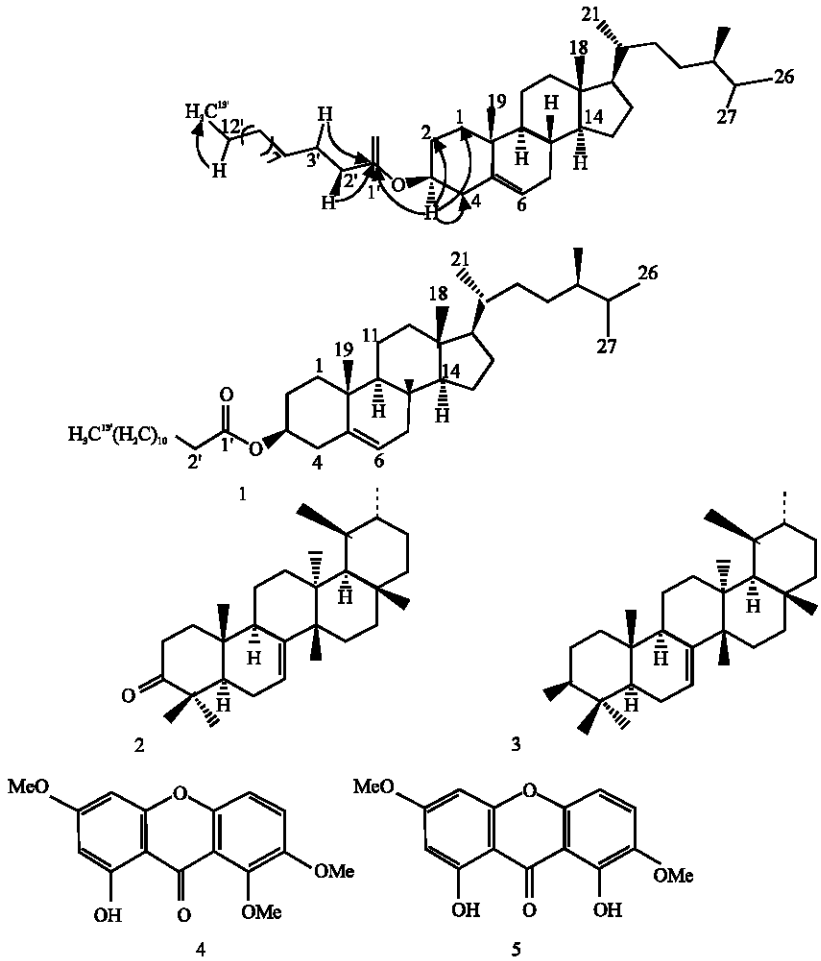

Fig. 1: Key HMBC correlations in compound 1

Table 2: The α-glucosidase inhibitory activity of compounds 1-4

No. of compound	Name	IC_{50}±SEM[μM]
1	Schweinfurthinn	NA
2	Bauerenone	9.8±0.04
3	Bauerenol	5.8±0.14
4	1-hydroxy-3, 7, 8-trimethoxyxanthone	75.0±1.04
Standard	Deoxynojirimycim*	425.0±8.14
	Acarbose*	780.0±028

*Standard drug; NA: Not Active

derivative from crystallization of soybean sterols. They gave neither physical nor spectral data of the compound. To best of our knowledge, its isolation and complete characterization (physical and spectral) from a natural source is here reported for the first time. Thus, it is a new compound.

Compound 2 was isolated as white crystals, mp: 230-231°C and gave a positive Liberman Buchar test. The IR spectrum in KBr revealed the presence of carbonyl group (1712 cm^{-1}); a double bond (1470 cm^{-1}) and germinal dimethyl group (1380-1390 cm^{-1}). The molecular formula of compound 2 was deduced to be $C_{30}H_{48}O$ on the basis of a molecular ion peak at m/z 424.3795 as shown by the HREIMS. Mass fragments at m/z 409, 271, 257, 245, 218 and 205 were observed. The ^{1}H-NMR spectrum in $CDCl_3$ suggested the presence of six tertiary methyl proton each singlets at δ 0.92, 0.98, 1.01, 1.02, 1.02 and 1.09. Two secondary methyl groups were observed at δ 0.89 and 1.04 as doublets. These data suggested the compound in hand to be a pentacyclic triterpenoid. The multiplet at δ 5.45 was attributed to H-7 and the doublet doublet of doublets at δ 2.73, was attributed to one of the C-2 methylene proton. In the ^{13}C-NMR spectrum of 2, 30 carbon signals were observed. The carbonyl carbon appeared at δ 216.9. The overall spectral behavior was identical to the reported compound, bauerenone 2 (Campello *et al*., 1975).

Compound 3 was obtained as white crystals, mp: 192-193°C. The EI mass spectrum showed the M^+ at m/z 426 for $C_{30}H_{50}O$. Fragment ions at m/z 411, 393, 273, 259 and 207. The ^{1}H-NMR spectrum of 3 exhibited eight methyl groups. In addition, the germinal proton to hydroxyl group was observed at δ 3.23. The hydroxyl bearing carbon appeared at δ 79.3. These spectroscopic data were identical to those reported by Khan *et al*. (1979) for bauerenol 3.

The compounds Bauerenone and Bauerenol showed approximately very similar IC_{50} values 9.8 and 5.8, respectively (Table 2). These results indicate that the basic skeleton of the compounds are responsible for the binding to the targeted enzymes, the action may be through some sorts of hydrophobic interaction with the binding sites of the enzyme.

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