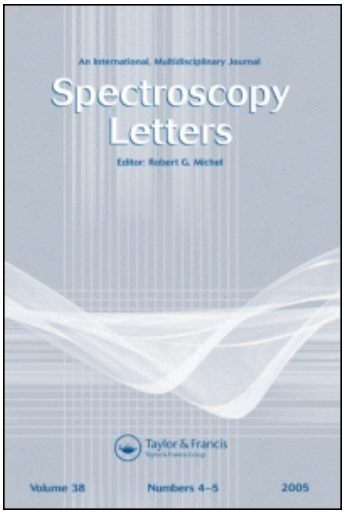

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A NEW ANTIBACTERIAL SESQUITERPENE GLYCOSIDE AND OTHER BIOACTIVE COMPOUNDS FROM *BIEBERSTEINIA HETEROSTEMON*

Key Words: *Biebersteinia heterostemon*, Geraniaceae, monoterpenes, iridoids, (-)-anymol glycoside, antibacterial activity

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ABSTRACT

In addition to the plant sterols β-sitosterol and daucosterol, a new bisabolane-typed sesquiterpene glycoside and three bioactive compounds (artemetin, geniposide and 6β-hydroxygeniposide) were characterized from the whole plant of *Biebersteinia heterostemon* endemic to the Tibetan area. The structure determination of the novel glycoside and identification of the known phytochemicals were accomplished by a combination of modern spectroscopic

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methods. Tests of all isolates for the antimicrobial activity indicated that the new sesquiterpene glycoside exhibited pronounced antibacterial activities against *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas sp.* with MICs at 50, 50 and 70 μg/ml, respectively.

INTRODUCTION

Biebersteinia heterostemon Maxim. is being used in traditional Chinese medical practice to treat a wide range of diseases such as fever, convulsions, encephalitis and dysentery. Furthermore, the ethanol extract of the plant was shown to be hypotensive, analgesic and immunity-regulatory [1,2]. However, very little is known concerning the antimicrobial principle of this species. This gave us impetus to reinvestigate the species hopefully to characterize structurally novel and/or antimicrobial phytochemicals. The results are presented in this paper.

EXPERIMENTAL

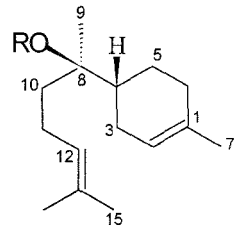
All NMR experiments were performed on a Bruker AM 400 FT-IR spectrometer. Mass spectra were run on a VG-ZAB-HS mass spectrometer. Optical rotations were measured on a DXP-118 instrument. All chemicals used in this study were of analytical grade.

The whole herb of *B. heterostemon* were collected in July 18, 1995, in the suburb of Tianshui City, Gansu Province, China. A voucher specimen (GC-82E), identified by Prof. G. L. Zhang, was deposited in the Herbarium of Lanzhou University, Lanzhou 730000, China. The air-dried and roughly pulverized plant material (1.1 kg) was extracted thrice at room temperature with petroleum/Et_2O/MeOH (1:1:2). Evaporation of the solvent from extract under reduced pressure gave a syrup (28.3 g) which was chromatographed over silica gel column with petroleum-acetone mixtures of growing polarity. Five fractions (F-

1~F-5) were combined according to the TLC fractions. F-1 and F-5 contained nothing of interest, and repetitious chromatography of F-2~F-4 over silica gel and filtration over Sephadex LH-20 gave eventually **1** (19 mg), β-sitosterol (59 mg), artemetin (23 mg), daucosterol (55 mg), geniposide (54 mg) and 6β-hydroxygeniposide (33 mg).

The antimicrobial activity of all isolates was assayed by the method described previously [3] using as the tested microorganisms *Candida albicans*, *Aspergillus niger*, *Epidermophyton floccosum* and *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas sp.* Compound **1** was found to be inhibitory to the growth of *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas sp.* with the MIC values being 50, 50 and 70 μg/ml, respectively.

Compound **1**: a colorless gum; $[\alpha]_D^{20}$ = -10.3° (*c* 0.001, EtOH); FAB-MS: *m/z* 355 ($M+H^+$), 223 ($M+H^+$-lyx), 132; ^1H and ^{13}C NMR data: Table 1.



1 R = β-D-lyxopyranosyl
1a R = H

RESULTS AND DISCUSSION

Compound **1** was assigned as a sesquiterpene glycoside by spectroscopic methods. The molecular formula $C_{20}H_{34}O_5$ was inferred from the quasimolecular ion peak at *m/z* 355 ($M+H^+$) in conjunction with the ^1H and ^{13}C NMR data and DEPT experiments. In the ^1H NMR spectrum of **1**, the bisabola-1,12-diene moiety was demonstrated by a set of methyl singlets at δ 1.19, 1.65, 1.60 and 1.68 (the

Table 1. ^{1}H and ^{13}C NMR data of Compound **1** in $CDCl_3$.

C	^{1}H (J in Hz)	^{13}C (DEPT)
1		133.7 (C)
2	5.37 br d (2.7)	120.9 (CH)
3α	1.76 m	31.1 (CH_2)
3β	2.03 m	
4	1.89 m	41.1 (CH)
5α	1.44 m	24.0 (CH_2)
5β	1.83 m	
6α	1.73 m	26.4 (CH_2)
6β	1.50 m	
7	1.65 s	23.3 (CH_3)
8		81.1 (C)
9	1.19 s	21.0 (CH_3)
10	1.50 , 1.55 m	36.0 (CH_2)
11	1.91 m	22.1 (CH_2)
12	5.08 br t (6.0)	124.2 (CH)
13		131.6 (C)
14	1.60 s	17.6 (CH_3)
15	1.68 s	25.6 (CH_3)
1'	5.10 d (2.3)	94.4 (CH)
2'	3.64 br s	72.6 (CH)
3'	3.92 m	70.0 (CH)
4'	3.86 m	66.0 (CH)
5'α	3.73 dd (12, 1.5)	63.3 (CH_2)
5'β	4.01 d (12)	

The assignment was accomplished by a combination of COSY, HMQC, HMBC and NOESY experiments.

latter three signals were broadened discernibly owing to the allylic and homoallylic couplings) as well as the olefinic proton resonances at δ 5.08 (br t, J=6.0 Hz), 5.37 (br d, J=2.7 Hz) [4]. This hypothesis was further confirmed by its ^{13}C NMR data edited by the DEPT pulse sequences (Table 1). As tabulated, all proton and carbon NMR signals were assigned unequivocally by the 2D NMR techniques (COSY, HMQC, HMBC and NOESY). The presence of an oxygen atom on C-8 was revealed by the 9-methyl singlet at δ 1.19 and the quaternary

carbon signal of C-8 at δ 81.1. Moreover, an anomeric doublet (d, J=2.3 Hz) at δ 5.10 in the ^{1}H NMR spectrum and a set of oxygenated carbon resonance lines at δ 94.4 (CH), 72.6 (CH), 70.0 (CH), 66.0 (CH) and 63.3 (CH_2) suggested the β-D-lyxopyranosyloxy residue [5] which could only be assumed to be on C-8. This assumption was further confirmed by the FAB mass spectrum of **1** which gave an intense peak at *m/z* 223 produced through elimination of the lyxose moiety from the protonated molecular ion at *m/z* 355. The proposed attachment of the sugar moiety was also reinforced by the observed long range correlation of C-8 with the anomeric proton (H-1') in its HMBC spectrum. In order to establish the stereochemistry at the chiral centers, compound **1** was subjected to acid hydrolysis affording the aglycone **1a** and D-lyxose which was identified by co-PC (paper chromatography) with the authentic sample. The aglycone **1a** was identical to (-)-anymol in the optical rotation, ^{1}H and ^{13}C NMR spectral data [4,6]. Accordingly, compound **1** also possessed 4S,8R-configurations. In conclusion, glycoside **1** was (-)-anymol-8-O-β-D-lyxopyranoside which, to our knowledge, is the first glycoside of the bisabolane-typed sesquiterpene.

The plant sterols were identified as β-sitosterol and daucosterol by direct comparisons with authentic materials (IR, MS and TLC). The flavone was shown to be artemetin (5-hydroxy-3,3',4',6,7-pentamethoxyflavone) by comparing its ^{13}C NMR data with those in the literature [7]. This compound was reported to be antimicrobial [8], antitumor [9], antimalarial [10], and potentiating the antimalarial activity of artemisinin [11]. The iridoid glycosides were disclosed to be geniposide and 6β-hydroxygeniposide by comparing their spectral data (IR, MS, ^{1}H and ^{13}C NMR) with those reported previously [12]. Geniposide was found to be protective against a decrease in libido, memory loss [13], and hepatotoxicity [14]. Moreover, both iridoid glycosides were inhibitory to the growth of rice and lettuce seedlings [15].

The *in vitro* antimicrobial assay of the isolates mentioned above revealed that the new glycoside **1** was active against *Bacillus subtilis, Staphylococcus*

aureus, and *Pseudomonas sp.* with MICs (Minimum Inhibitory Concentration) being 50, 50 and 70 μg/ml, respectively.

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REFERENCES

1. Jiangsu New Medical College *Dictionary of Traditional Chinese Medicines*, Shanghai: Shanghai People's Press 1977; 1902-1903.

2. Zhang XF., Hu BL., Zhou BN. Studies on the Active Constituents of Tibetan Herb *Biebersteinia heterostemon* Maxim. Yaoxue Xuebao 1995; **30**: 211-214.

3. Zheng WF., Tan RX., Yang L., Liu ZL. A New Antimicrobial Sesquiterpene Lactone from *Artemisia giraldii*. Spectroscopy Letters 1996; **29**: 1589-1597.

4. Georgo WO., Maurice DC. Terpenoid Chemistry. XXIX. (-)-Anymol from *Myoporum crassifolium* Forst., the C7 Epimer of (-)-α-Bisabolol from Camomile. Aust. J. Chem. 1989; **42**: 2021-2034, and related references cited therein.

5. Olah AV., Harangi J., Liptak A. Synthesis of Everninose, A Non-reducing Disaccharide Component of the Orthosomycin-type Oligosaccharide Antibiotics. Carbohydr. Res. 1988; **174**: 113-120.

6. Chen XJ., Archelas A., Furstoss R. Microbiology Transformations, 27. The First Examples for Preparative-Scale Epoxide Hydrolysis Using

Microorganisms. An Unequivocal Access to All Four Bisabolol Stereoisomers. J. Org. Chem. 1993; **58**: 5528-5532.

7. Iinuma M., Matsuura S., Kusuda K. Carbon-13 Nuclear Magnetic Resonance(NMR) Spectral Studies on Polysubstituted Flavonoids. I. Carbon-13-NMR Spectra of Flavones. Chem. Pharm. Bull. 1980; **28**: 708-716.

8. Nadir MT., Hatam NAR., Abdul-Khaliq N., Yousif NT. The Constituents of *Achillea Conferta*: Phytochemical and Antimicrobial Studies. Int. J. Pharmacogn. 1991; **29**: 89-93.

9. Tan RX., Zheng WF., Tang HQ. Biologically Active Substances from the Genus *Artemisia*. Planta Med. 1998; **64**: 295-302.

10. Liu KCSC., Yang SL., Roberts MF., Elford BC., Phillipson JD. Antimalarial Activity of *Artemisia annua* Flavonoids from Whole Plant and Cell Culture. Plant. Cell. Rep. 1992; 11: 637-640.

11. Elford BC., Roberts MF., Phillipson JD., Wilson PJM. Potentiation of the Antimalarial Activity of Qinghaosu by Methoxylated Flavone. Trans. R. Soc. Trop. Med. Hyg. 1987; **81:** 434-436.

12. Christie AB., Frank RS. Iridoids, An Updated Review. J. Nat. Prod. 1990; **53**: 1055-1147.

13. Imai T., Kishi T., Inoue H., Nishiyama N., Saito H. Effects of Iridoids on Sex and Learning-behaviors in Chronic Stressed Mice. Yakugaku Zasshi 1988; **108:** 572-585.

14. Wang JC., Wang SW., Lin JK. Suppressive Effect of Geniposide on the Hepatotoxicity and Hepatic DNA Binding of Aflatoxin B_1 in Rats. Cancer Lett. (Shannon, Irel) 1991; **60**: 95-102.

15. Komai K., Nakasugi T., Tujii I., Miura M., Hamada M. Plant Growth Inhibitory Activity of Iridoid Glucosides . Zasso Kenkyu 1990; **35**: 44-52 , and related references cited therein.

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