

NOTE

A NEW PRENYLATED FLAVANONE FROM THE ARIAL PART OF *Orthosiphon stamineus*M. Amzad Hossain^{1*}, Atiqur Rahman^{2,3}, and Sun Chul Kang²¹ Chemistry Division, Atomic Energy Centre, GPO Box 164, Ramna, Dhaka-1000, Bangladesh² Department of Biotechnology, College of Engineering, Daegu University, Kyungsan 712-714, Korea³ Department of Applied Chemistry & Chemical Technology, Islamic University, Kushtia-7003, Bangladesh

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ABSTRACT

Phytochemical investigations on the chloroform extract of the arial part of *Orthosiphon stamineus* resulted in isolation of one new prenylated compound 5,7-dimethoxy-3,4'-dihydroxy-3',8-di-C-prenylflavanone together with four known flavonoids compounds, sinensetin, eupatorin, 5,6,7,4'-tetramethoxyflavone and 3-hydroxy-5,6,7,4'-tetramethoxyflavone. The structures were deduced on basis of different analytical methods such as UV, IR, ¹H-NMR, ¹³C-NMR, DEPT, COSY, HMBQ and GC-MS. The prenylated compound is reported for the first time from this plant.

Keywords: *Orthosiphon stamineus*, prenylated flavanone, flavonoids

INTRODUCTION

Traditional medicines are widely used alongside with modern medicine in many countries of Southeast Asia and play an important role in promoting a health care system [1].

Orthosiphon stamineus, Benth (Lamiaceae) is one of the popular traditional folk medicine extensively used in Southeast Asia for the treatment of wide range of diseases: in Indonesia it is used for rheumatism, diabetes, urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis, and hypertension etc [2], in Vietnam for urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis [3], and in Malaysia to alleviate diabetes and kidney stone disease. Owing to its beneficial pharmaceutical utility, it is under systematic cultivation in Malaysia and is locally known as Misai kucing meaning 'Cats whisker' and consumed as a healthy Java tea to facilitate body detoxification.

In particular, extracts of *O. stamineus* are now widely used in Malaysia as drugs for the treatment of diabetes and kidney stone diseases. The recent surge of interest in chemistry of this plant has led to the isolation of more than 60 components with different biological activities [4-8]. Prenylated flavanone has been found to display a variety of biological activities such as behavioural depression and muscle relaxation; this is also known to be anti hypertensive and to have β_1 -adrenergic inhibition and antimicrobial activities [4,9]. In this paper, we describe the isolation and structural elucidation of the isolated compounds from the chloroform extract of the arial part of *O. stamineus*. All the isolated compounds were identified by their spectral data.

EXPERIMENTAL SECTION

General

Melting points were determined using an electro-thermal melting point apparatus (Gallenkamp) and are uncorrected. IR spectra (ν in cm^{-1}) were recorded (KBr discs) on a FT-IR spectrophotometer, ¹H-NMR and ¹³C-NMR spectra on Bruker R-32 (300 MHz) instrument in CDCl₃ with TMS as an internal standard (chemical shifts in δ , ppm); and UV spectra on a HITACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). TLC was performed with silica gel GF₂₅₄. All solvents used were of analytical reagent grade.

Plant material

Orthosiphon stamineus Benth (Lamiaceae) leaves were collected from Penang, Malaysia. The plant was identified and voucher specimen has been deposited in the herbarium of the School of Biology, University Sains Malaysia.

Extraction and Isolation

Dried leaves of the plant (1 kg) were milled into powder and then extracted with methanol (8 L) in a Soxhlet extractor for 36 hr. The extract was evaporated in a rotatory evaporator and dried by vacuum pump. The methanolic extract (50 g) was suspended on water and extracted successively with hexane, chloroform, ethyl acetate, and butanol to yield hexane (4 g), chloroform (10.5 g), ethyl acetate (7.4 g) and BuOH-soluble (4.23 g) fractions, respectively. The chloroform soluble fraction (8 g) was subjected to chromatography on silica gel (60-120

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mesh, Merck) and was eluted with ethyl acetate-hexane (7:3) solvent system. Repeated chromatography gave five major fractions (Fraction-1, 0.110 g; Fraction-2, 0.143g; Fraction-3, 1.229 g; Fraction-4, 0.059 g; Fraction-5, 0.125 g).

Fraction-2: Obtained from column chromatography was further purified by preparative TLC over silica gel GF₂₅₄ using benzene-acetone (85:15) as the developing solvent to give one new prenylated compound **1** (2.2 mg) and other four known compounds [6-9], sinensetin (**2**, 2 mg), eupatorin (**3**, 2.6 mg), 5,6,7,4'-tetramethoxyflavone (**4**, 1.8 mg) and 3-hydroxy-5,6,7,4'-tetramethoxyflavone (**5**, 1.5 mg).

Compound 1: 5,7-Dimethoxy-3,4'-dihydroxy-3',8-di-C-prenylflavanone (1)

The purified compound was crystallized from *n*-hexane-methanol to give yellowish powder (1.9 mg), mp 49°C; *R_f* 0.59 (*n*-hexane-chloroform-acetone; 85:12:3); (*M⁺*, 452), UV: 232, 340, 357 nm; IR (KBr): 3445, 3250, 2910, 2840, 2343, 2131, 1645, 1600, 1599, 1478, 1365, 1050 cm⁻¹; ¹H-NMR (CDCl₃): 1.69 [s, 12H, -C(CH₃)₂×2], 2.94 (d, 1H, J=9Hz, H-3), 3.43 (d, 4H, J=7Hz, -CH₂-CH= x2), 3.94 (s, 3H, -OCH₃), 3.98 (s, 3H, -OCH₃), 5.41 (t, 2H, J=7Hz, -CH₂-CH= x2), 5.55 (t, 1H, H-2), 6.96 (s, 1H, H-5), 7.10 (d, 1H, J=9Hz, H-5'), 7.45 (s, 1H, H-2'), 7.68 (d, 1H, J=9Hz, H-6'), 12.55 (s, 2H, -OH×2). [Found: C, 71.7; H, 7.1. C₂₇H₃₂O₆ requires. C, 71.6; H, 7.0]. It was identified as 5,7-dimethoxy-3,4'-dihydroxy-3',8-di-C-prenylflavanone (**1**)

Sinensetin (2)

The isolated purified compound was further purified by preparative TLC over silica gel GF₂₅₄ using chloroform-methanol (95:5) as developing solvent. It was crystallized from petroleum ether as white needles (**2**, 1.8 mg). It was crystallized from ethyl acetate as yellow needles. mp 175-176 °C (lit.[6] mp 177°C). (*M⁺*, 373). UV: 327, 282, 213 nm. IR (KBr) : 2850, 1645, 1600, 1590, 1476, 1378, 1215, 1189, 870 cm⁻¹. ¹H-NMR (δ, DMSO-d₆): 3.76 (s, 3H, 5-OCH₃), 3.78 (s, 3H, 6-OCH₃), 3.84 (s, 3H, 4'-OCH₃), 3.88 (s, 3H, 3'-OCH₃), 3.96 (s, 3H, 7-OCH₃), 6.48 (s, 1H, H-8), 6.78 (s, 1H, H-3), 7.12 (d, 1H, J= 8.5 Hz, H-5'), 7.52 (s, 1H, H-2'), 7.68 (d, 1H, J= 8.5 Hz, H-6').

Eupatorin (3)

It was crystallized from methanol as yellow crystals. mp 196-7°C (lit.[6] mp 198°C); (*M⁺*, 344). UV: 340, 275, 210 nm. IR (KBr): 3470, 3250, 2840, 1650, 1605, 1599, 1478, 1365, 1050 cm⁻¹. ¹H-NMR (δ, DMSO-d₆): 3.72 (s, 3H, 6-OCH₃), 3.88 (s, 3H, 4'-OCH₃), 3.96 (s, 3H, 7-OCH₃), 6.47 (s, 1H, H-8), 6.76 (s, 1H, H-3), 7.10 (d, 1H, J=8.5Hz, H-5'), 7.45 (s, 1H, H-2'), 7.68 (d, 1H, J=8.5Hz, H-6'), 9.48 (s, 1H, 3'-OH), 12.55 (s, 1H, -OH).

3.6. 5,6,7,4'-Tetramethoxyflavone (4)

It was crystallized from benzene-petroleum ether as yellow needles (**4**, 8 mg); mp: 142°C (lit.^[8] mp 142°C) (*M⁺*, 360). UV: 345, 285, 229. IR : 3421, 2985, 2937, 2908, 1658, 1600, 1571, 1506, 1456, 1361, 1295, 1234, 1132, 1091, 1062, 1043, 1012, 943, 908, 879, 805. ¹H-NMR (δ, DMSO-d₆): 3.93, 3.97, 3.99., 4.00 (4s, 12H, -OCH₃×4), 6.48 (s, 1H, H-8), 6.81 (s, 1H, H-3), 6.96 (d, 1H, J=9Hz, H-6'), 7.34 (d, 1H, J=9Hz, H-5'), 7.85 (d, 2H, J=8.5Hz, H-2' and H-3').

3.7. 3'-Hydroxy-4',5,6,7-tetramethoxyflavone (5)

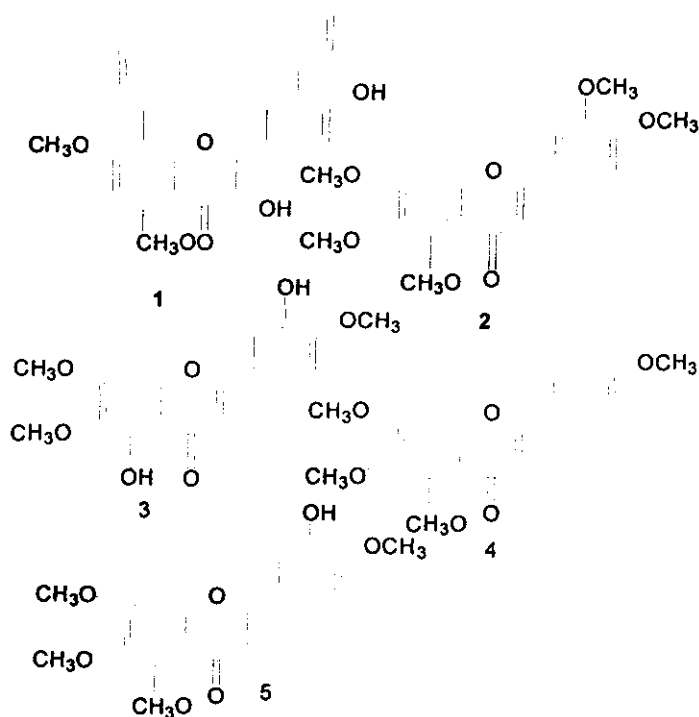
It was crystallized from methanol as yellow tiny needles (**5**, 1.6 mg). mp 189°C. (lit.[6-9] mp 188-9°C) (*M⁺*, 458). UV: 335, 308, 217 nm. IR (KBr): 3512, 3065, 2942, 2832, 1627, 1600, 1572, 1468, 1424, 1358, 1304, 1265, 1227, 1200, 1172, 1138, 1112, 1013, 986, 876, 833, 695 cm⁻¹. ¹H-NMR (δ, DMSO-d₆): 3.76, 3.80, 3.90., 3.98 (4s, 12H, -OCH₃×4), 6.51 (s, 1H, H-8), 6.78 (s, 1H, H-3), 7.09 (d, 1H, J= 8.5 Hz, H-5'), 7.45 (s, 1H, H-2'), 7.72 (d, 1H, J= 8.5 Hz, H-6'), 9.37 (s, 1H, -OH).

RESULT AND DISCUSSION

The dried leaves of local *O. stamineus* were extracted with methanol in a Soxhlet extractor. The crude extract was suspended in water and extracted successively with hexane, chloroform, ethyl acetate, and butanol. The chloroform extract was subjected to column chromatography on silica gel and eluted with a chloroform-methanol mixture. By repeated chromatography and preparative TLC, one new prenylated compound together with four known compounds were isolated from one fraction of the chloroform extract. 5,7-Dimethoxy-3,4'-dihydroxy-3',8-di-C-prenylflavanone (**1**) was isolated from this plant for the first time (Scheme 1).

The compound (**1**) was obtained as a yellowish powder. It had the molecular formula C₂₇H₃₂O₆ as determined by HREIMS and confirmed by ¹³C-NMR (see experimental) and DEPT analysis.

The UV spectra displayed characteristic absorption bands for a conjugated double bond at 232 nm. IR spectra of compound (**1**) showed frequencies at ν 3445 cm⁻¹ and ν 2910-2840 cm⁻¹ indicating the presence of hydroxyl group and C-H in conjugation and the absorption peaks at ν 1645, ν 1599, 1463 and 1380 cm⁻¹ indicated the presence of unsymmetric ethylenic double bond, and aromatic -CH₃ group, respectively. The ¹H-NMR spectrum of the compound (**1**) indicated the presence of C-prenyl unit. A sharp singlet at δ 1.69 (12H, 4 x CH₃) revealed the presence of gem-dimethyl group whereas the presence of -CH₂- and -CH= protons attached to the



Scheme-1

aromatic ring was indicated by a doublet at δ 3.43 (4H, $J=7\text{Hz}$) and a triplet at δ 5.41 (2H, $J=7\text{Hz}$) respectively. A doublet at δ 2.94 indicated the presence of H-3 and a triplet at 5.55 indicating the presence of H-2 for the flavanone nucleus. Two sharp singlets of three protons each at δ 3.94 and 3.98 indicated the presence of two methoxy group on the aromatic rings. Another two singlets at δ 6.96 and δ 7.45 indicated the presence of H-5 and H-2' protons. A doublets at δ 7.10 ($J=9\text{Hz}$) and a doublet of doublet at δ 7.68 indicated the presence of H-5' and H-6' protons. The ^{13}C -NMR and DEPT spectra showed 27 carbon atoms for the molecules consisting of

four $-\text{CH}_3$, two $-\text{OCH}_3$, two $-\text{CH}_2-$, eight CH and eleven fully substituted carbons. Thus on the basis of above studies the structure of compound (1) was established as 5,7-dimethoxy-3,4'-dihydroxy-3',8-di-C-prenylflavone (1). All the known compounds spectral data were identical with the natural samples of sinensetin, eupatorin, 5,6,7,4'-tetramethoxyflavone and 3-hydroxy-5,6,7,4'-tetramethoxyflavone [6-9] (super-imposable TLC, mixed melting point, super imposable UV, IR and ^1H -NMR).

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