VIMINALOL FROM *ORTHOSIPHON STAMINEUS*

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ABSTRACT

Viminalol and some known compound were isolated have been isolated from the leaves of Malaysian tropical medicinal plant of O*rthosiphon stamineus.* Viminalol was obtained for the first time from this plant. All the isolated compounds were identified on the basis of UV, IR, ^{1}H-NMR, ^{13}C-NMR, Dept 90 and 135^{o}, cosy and mass spectral data.

INTRODUCTION

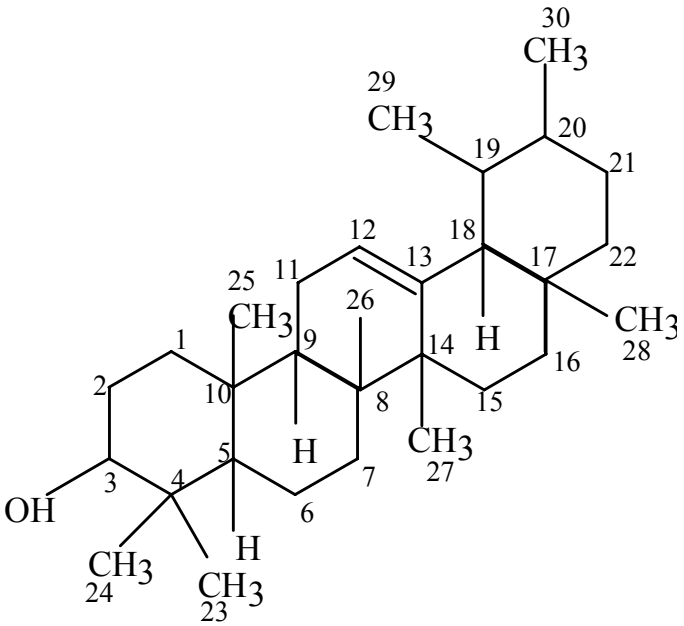
Orthosiphon stamineus, Benth belong to the Lamiaceae family and are common in the rain forests of several tropical countries. The leaves of this plant are used as a diuretic and to treat rheumatism, diabetes, urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis, and hypertension[1-3]. 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 *Orthosiphon stamineus* are now widely used in Malaysia as drugs for the treatment of diabetes and kidney stone diseaes[4-5]. The recent surge of interest in chemistry of this plant has led to the isolation of more than 50 components including flavonoids[6], terpenoids[7] and caffeic acid derivatives[8] with different biological acitivies.They are also known to be antihypertensive and to have b_1-adrenergic inhibition and antimicrobial activities[9]

The present work deals with the isolation, structure elucidation and identification of the new pentacyclic terpenoid viminalol (**1**) from the chloroform fraction of the leaves of local *Orthosiphon stamineus.*

RESULTS AND DISCUSSION

The leaves of the Malaysian tropical medicinal plant *Orthosiphon stamineus* was extracted with 50% methanol. The crude extract was dissolved in water and further extracted successively with hexane, chloroform, ethyl acetate, and butanol. The chloroform extract was subjected to column chromatography on silica gel eluted with a chloroform-methanol mixture. By repeated chromatography and preparative TLC, two compounds were isolated from one fraction of the chloroform extract.

Viminalol (**1**) was obtained as a orange amorphous solid. High resolution mass spectrum exhibited molecular ion at m/z 424, which is consistent with the molecular formula $C_{30}H_{48}O$. **UV** spectra displayed characteristic absorption bands for double bond at 249 nm. IR spectra of compound (**1**) showed frequencies at **ν**3435 cm^{-1} and **ν** 2964-2734 cm^{-1} indicating the presence of hydroxyl group and C-H in conjugation and the absorption peaks at **ν**1665, **ν** 1452 and 1369 cm^{-1} indicated the presence of unsymmetric ethylenic double bond and –CH_3 group, respectively. The **^{1}H-NMR** spectrum showed five tertiary methyl singlets at δ 0.82 , 0.84, 0.86, 0.88 and 1.09 indicating the triternoidal nature of the compound. It also exhibited one double bond proton multiplet at δ 5.13 along with two secondary methyl signal at δ 1.26 and 1.30 indicating the isopropenyl residue of the skeleton.



Viminalol (1)

The secondary hydroxyl group was assigned to C-3 on biogenetic grounds[10] in b- and equatorial configuration based on larger coupling constants at δ 2.75. The downfield shift of one of the methyl groups at δ 1.30, compared with 3-hydroxyurs-12-ene[4], led to assignment of one methyl group to C-19. Further evidence for the structure was obtained from **^{13}C-NMR** analysis in which the **DEPT** experiment showed that the isolated compounds had eight CH_3 groups, ten CH_2 groups, four –CH groups and one –CH-C group. Viminalol (**1**) was also obtained as on orange amorphous solid showing a dark pink colour on TLC silica gel

plate when heated with concentrated sulphuric acid at R_f (0.66) in chloroform–benzene (1:4), suggests a triterpene group. It is known that to viminalol (**1**) is widely distributed in the plant kingdom [7-9], however, to the best of our knowledge, the isolation of viminalol from *Orthosiphon stamineus* has not been reported elsewhere.

MATERIALS AND METHODS

General

IR spectra were recorded (KBr discs) on a FT-IR spectrophotometer, validation (ν_{max} in cm^{-1}). **^{1}H-NMR** spectra were recorded on a Bruker R-32 (300 MHz) instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts in δ, ppm). **UV** spectra were recorded on HATACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). Mass spectra were recorded on Varian GC-MS/MS 3800. **TLC** was performed with silica gel GF_{254}. All solvents were analytical reagent grade.

Plant Material

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

Extraction

Dried leaves of the plant (1.5Kg) were milled into powder and then extracted with direct methanol (10L) in a Soxhlet extractor for 36 hours. 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. Chloroform soluble fraction (8 g) was subjected to chromatography on silica gel (60-120 mesh, Merck) eluted with ethyl acetate-hexane (7:3) solvent system. By repeated chromatography to give 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-3

Fraction-3 was obtained from column chromatography was further purified by preparative TLC over silica gel GF_{254} using chloroform-ethyl acetate-hexane (3:2:1) as developing solvent to give unknown compound **1** (4 mg) and some other minor known compounds.

Compound 1: Viminalol (1)

It could not be crystallized from any solvent. It was an orange amorphous solid. R_f 0.66 (chloroform-benzene; 1:4); **MS**: (M^+, 424; base peak 218). **UV** : 249 nm. **IR** (KBr) : 3435, 2964, 2920, 2854, 2734, 1665, 1452, 1369, 1315, 1260, 1178, 1090, 1019, 964., 887, 832, 794, 728, 580 cm^{-1}. **^{1}H-NMR** (d values, $CDCl_3$) **:** 0.86 (s, 3H, -CH_3), 0.88 (d, 3H, J=7Hz, -CH_3), 1.08 (d, 3H, J=7.0 Hz, -CH_3), 1.26 –1.29 (d, 6H, J=7 Hz, two secondary -CH_3), 1.33 (s, 3H, -CH_3 at H-29) 1.47 (m, 2H, H-16), 1.51 (m, 2H, H-21), 1.60 (d, 1H, J=11.5 Hz, H-18), 1.68 (s, 7H, -CH_3 x 2 and H-5), 1.80 (m, 1H, H-19), 1.83 (m, 2H, H-22), 1.85 (m, 2H, H-15), 2.01 (d, 2H, J= 11.5 Hz, H-1), 2.04 (s, 1H, H-9), 2.17-2.24 (d, 2H, J=11.5 Hz, H-1 and H-16), 3.53 (s, 2H, H-7), 3.67 (m, 2H, H-2), 5.11 (s, 2H, H-11), 5.13 (s, 1H, H-12); **^{13}C-NMR** (d values, $CDCl_3$) **:** 14.47 (C-24), 15.84 (C-25), 20.05 (C-5) 20.12 (C-26), 22.68 (C-11), 23.00 (C-30), 23.06 (C- 27), 23.08 (C-15), 23.80 (C-16), 24.26 (C-2), 26.78 (C-23), 26.99 (C-28), 28.35 (C-20), 28.48 (C-17), 29.10 (C-7), 29.56 (C-29), 29.63 (C-10), 32.30(C-1), 32.59 (C-8), 33.18 (C-14), 37.68 (C-19), 37.83 (C-22), 39.76 (C-18), 40.09 (C-9), 76.97 (C-5), 77.39 (C-3), 125.28 (C-12), 135.58 (C-13).

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