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Synthesis of 3′, 4′, 5, 6, 7-Pentamethoxy-8-C-Prenylflavone

M. Amzad Hossain[a]* and Zhari Ismail[b]

[a]Chemistry Division, Atomic Energy Centre, GPO Box No. 164, Ramna, Dhaka - 1000, Bangladesh
[b]School of Pharmaceutical Sciences, University Sains Malaysia, 11800 Pulau Pinang, Malaysia

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Abstract. 3′, 4′, 5, 6, 7-Pentamethoxy-8-C-prenylflavone, isolated from the leaves of Malaysian O*rthosiphon stamineus*, was synthesized starting with treating 2, 4, 5, 6-tetrahydroxyacetophenone with dimethyl sulphate which yielded several other minor compounds as well. The synthesized title compound is identical in all respects with the natural sample.

Keywords: flavones, medicinal plants, *Orthosiphon staminens*, methoxy-prenylflavone

Introduction

Orthosiphon stamineus Benth (Lamiaceae), is one of the popular traditional medicinal plants and is extensively used in southeast Asia for the treatment of a 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. (Hossain and Ismail, 2005a; Tezuka *et al*., 2000), in Vietnam, for urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis (Hossain and Ismail, 2005a; Tezuka *et al*., 2000) and in Malaysia, to alleviate diabetes and kidney stone diseaes. Owing to its pharmaceutical utility, it is under systematic cultivation in Malaysia and is locally known as 'Misai kucing' meaning 'Cat's whisker' and consumed as 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 its more than 60 components with different biological acitivies (Tezuka *et al*., 2000). Prenylated flavones have been found to display a variety of biological activities such as behavioural depression and muscle relaxation; they are also known to be antihypertensive and to have β_1-adrenergic inhibition and antimicrobial activities (Hossain *et al*., 2004). The title prenylated flavonoid, isolated from *O. stamineus*, was recently found to exhibit some important bioactivities including antioxidant and aldose reductase inhibitory effects (Tezuka *et al*., 2000). Such bioactivities, as well as the lack of synthesis routes, attracted our interest in this compound.

Materials and Methods

Melting points of the compounds were determined using an electrothermal melting point apparatus (Gallenkamp) and are uncorrected. IR spectra (ν_{max} per centimeter) were recorded (KBr discs) on FT-IR spectrophotometer, ^{1}HNMR and ^{13}CNMR spectra on Bruker R-32 (300 MHz) instrument in $CDCl_3$ with TMS as an internal standard (chemical shifts in δ, ppm) and UV spectra on a HATACHI, U-2000 spectrophotometer Ultrospeck in methanol (λ_{max} in nm). TLC was performed with silica gel GF_{254}. All solvents used were of analytical reagent grade.

2-Hydroxy-4, 5, 6-trimethoxyacetophenone (2). To 10 g a solution of 2, 4, 5, 6-tetrahydroxyacetophenone (**1**) in dry acetone (125 ml), dimethyl sulphate (9.03 g) and anhydrous K_2CO_3 (40 g) were added. The mixture was refluxed for 3 h. Acetone was removed by distillation and water was added to the residue. It was extracted with ether, dried over anhyd. Na_2SO_4 and evaporated to dryness. The ether extract, on column chromatography using petroleum spirit (40-60 °C), petroleum spirit-hexane (3:1, 3:2) and increasing quantities of hexane as eluent gave the major compound 2-hydroxy-4, 5, 6-trimethoxyacetophenon (**2**) and several other minor compounds. The compound **2** was obtained as white crystals (2.54 g), mp: 76 °C; (M^+, 226); R_f: 0.69 (choroform-methanol; 95:5); UV: 230, 245, 278 nm; IR: 3455, 2976, 2855, 1645, 1605, 1595, 1543, 1470, 1410, 1378, 1340, 1236, 1212, 1190, 1153, 1132, 1050, 1043, 1005, 945, 885, 765, 664 cm^{-1}; ^{1}HNMR (δ, DMSO-d_6): 2.48 (s, 3H, -$COCH_3$), 3.98, 3.99 and 4.00 (3s, 9H, -OCH_3x3), 6.41 (s, 1H, H-3), 12.71 (s, 1H, -OH); ^{13}CNMR (δ, DMSO-d_6): 158.06 (C-6), 89.06 (C-5), 156.43 (C-4), 102.62 (C-3), 131.47 (C-2), 98.04 (C-1), 182.07 (C=O), 60.76 (6- OCH_3), 55.69 (4- OCH_3), 54.40 (5- OCH_3), 19.43 (-CH_3). (Found: C, 58.4; H, 6.19. $C_{11}H_{14}O_5$ requires C, 58.4; H, 5.5%).

2-Hydroxy-4, 5, 6-trimethoxy-3-C-prenylacetophenone (3). Compound **2** (2.26 g) was added to cool solution of KOH (5 g) and absolute methanol (20 ml). The mixture was treated with prenyl bromide (0.98 g) slowly while shaking. After keeping

*Author for correspondence; E-mail: dramzadh@yahoo.com

the reaction mixture for 20 h at room temperature, it was diluted with ice-cold water, acidified and extracted with ether. The ethereal solution was successively extracted with 7% sodium carbonate and 1% KOH solution. The sodium carbonate fraction gave unreacted 2-hydroxy-4, 5, 6-trimethoxy-aceto-phenone (0.38 g), mp: 116 °C; (M^+, 294). The KOH fraction after acidification gave a solid product which after crystallization from petrol formed white shining plates (1.08 g), mp: 112 °C; (M^+, 294); UV: 234, 256, 348 nm; IR: 3510, 1640, 1605, 1593, 1376, 1363 cm^{-1}; ^{1}HNMR (δ, DMSO-d_6): 1.75 [s, 6H, >C(CH_3)$_2$], 2.43 (s, 3H, -$COCH_3$), 3.53 (d, 2H, *J*=7Hz, -CH_2-CH=), 3.95, 3.98 and 3.99 (3s, 9H, -OCH_3x3), 5.49 (t, 1H, *J*=7Hz, -CH_2-CH=), 12.78 (s, 1H, -OH); ^{13}CNMR (δ, DMSO-d_6): 158.26 (C-6), 88.36 (C-5), 156.49 (C-4), 101.68 (C-3), 131.56 (C-2), 94.44 (C-1), 181.17 (C=O), 60.26 (6-OCH_3), 55.12 (4-OCH_3), 54.49 (5-OCH_3), 19.43 (-CH_3), 76.10 (C-1′), 76.28 (C-2′), 76.68 (C-3′), 75.99 (C-4′), 77.88 (C-5′); (Found: C, 65.30; H, 7.48. $C_{16}H_{22}O_5$ requires C, 65.44; H, 7.5%).

2′-Hydroxy-3, 4, 4′, 5′, 6′-pentamethoxy-8-C-prenylchalcone (4). A mixture of **3** (1 g) and 3,4-dimethoxybenzaldehyde (0.43 g) in ethanolic solution of KOH (50%, 15 ml) was kept at room temperature for 3 days. The reaction mixture was diluted with ice-cold water, acidified with dil. HCl and extracted with ether. The ether layer was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was purified by preparative TLC over silica gel 60 using hexane-acetone (15:1) as developing solvent. The product was crystallized from dil. methanol to give yellow crystals (0.35 g), mp: 101 °C, (M^+, 442); R_f 0.76 (chloroform-methanol; 9.5:0.5); UV: 228, 254, 370 nm; IR: 3424, 2987, 2855, 2345, 1642, 1605, 1595, 1375, 1365 cm^{-1}; ^{1}HNMR (δ, DMSO-d_6): 1.73 [s, 6H, > C(CH_3)$_2$], 3.53 (d, 2H, *J*=7Hz, -CH_2-CH=), 3.94, 3.96, 3.97, 3.99 and 4.00 (5s, 15H, -OCH_3x5), 5.49 (t, 1H, *J*=7Hz, -CH_2-CH=), 6.94 (s, 1H, H-2), 7.32 (d, 2H, *J*=9Hz, H-5 and 6), 7.44 (d, 1H, *J*=9Hz, H-α), 8.01 (d, 1H, *J*=9Hz, H-β), 13.01 (s, 1H, -OH); ^{13}CNMR (δ, DMSO-d_6): 98.44 (C-1′), 131.86 (C-2′), 102.43 (C-3′), 156.88 (C-4′), 89.26 (C-5′), 158.66 (C-6′), 181.92 (C=O), 60.99(6′- OCH_3), 55.87 (4′ -OCH_3), 53.93 (5′- OCH_3), 55.64 (3-OCH_3), 47.22 (4-OCH_3), 151.41 (C-β), 125.79 (C-α), 118.51 (C-1), 124.78 (C-2), 111.23 (C-3), 144.43 (C-4), 111.77 (C-5), 118.55 (C-6), 77.45 (C-1″), 77.12 (C-2″), 77.62 (C-3″), 76.88 (C-4″), 77.95 (C-5″); (Found : C, 67.87; H, 6.78. $C_{25}H_{30}O_7$ requires C, 67.77; H, 6.63%).

5, 6, 7, 3′, 4′ -Pentamethoxy-8-C-prenylflavone (5). To a solution of compound **4** (1 g) in dry dioxan (25 ml), 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) (85 mg) was added. The mixture was refluxed for 3 h washed with water, dried over anhydrous sodium sulphate and evaporated to dryness. It was purified by preparative TLC over silica gel GF_{254} using hexane-acetone-ethyl acetate (7:5:1) as developing solvent. It was crystallized from methanol as orange needles (0.543 g); mp: 89 °C; (Hossain and Ismail, 2005b), mp: 134 °C); R_f 0.67 (hexane-methanol; 95:5), (M^+, 440); UV: 318, 288, 213 nm; IR: 3435, 2964, 2936, 2832, 2361, 1649, 1610, 1595, 1452, 1376, 1364, 1271, 1200, 1128, 1046, 1030, 879, 832, 800 cm^{-1}; ^{1}HNMR (δ, DMSO-d_6): 1.71 (s, 6H, - > C(CH_3)$_2$, 3.53 (d, 2H, *J*=7Hz, -CH_3-CH=), 3.93, 3.94, 3.97, 3.99 and 4.00 (5s, 15H, -OCH_3 x 5), 5.57 (t, 1H, *J*=7Hz, -CH_3-CH=), 6.99 (d, 1H, *J*=9Hz, H-2′), 7.35 (d, 2H, *J*=9Hz, H-5′ and H-6′); ^{13}CNMR (δ, DMSO-d_6): 153.47 (C-2), 126.27 (C-3), 183.07 (C-4), 159.16 (C-5), 90.97 (C-6), 159.16 (C-7), 104.95 (C-8), 153.47 (C-9), 111.10 (C-10), 119.50 (C-1′), 126.27 (C-2′), 112.75 (C-3′), 146.45 (C-4′), 112.75 (C-5′), 119.50 (C-6′), 77.80 (C-1″), 77.58 (C-2″), 77.38 (C-3″), 76.95 (C-4″), 77.38 (C-5″), 61.23 (5-OCH_3), 56.69 (7-OCH_3), 56.54 (6-OCH_3), 55.64 (3′-OCH_3), 47.38 (4′-OCH_3); (Found: C, 68.18; H, 6.36. $C_{25}H_{28}O_7$ requires C, 68.44; H, 6.30%).

Results and Discussion

In this paper, synthesis of 3′, 4′, 5, 6, 7-pentamethoxy-8-C-prenylflavone is described, starting with 2, 4, 5, 6-tetrahydroxy-acetophenone (**1**) which on treatment with dimethyl sulphate afforded 2-hydroxy-4, 5, 6-trimethoxyacetophenone (**2**) and several other minor compounds. Compound **2** on prenylation yielded 2-hydroxy-4, 5, 6-trimethoxy-3-C-prenylaceto-phenone (**3**) (Hossain, 1999). Alkaline condensation of **3** and 3, 4-dimethoxybenzaldehyde furnished 2′-hydroxy-3, 4, 4′, 5′, 6′-pentamethoxy-8-C-prenylchalcone (**4**) (Hossain *et al*., 1993). DDQ treatment of **4** gave the title compound 3′, 4′, 5, 6, 7-pentamethoxy-8-C-prenylflavone (**5**) (Fig. 1) (Hossain and Tarafdar, 1998). Its melting point and spectral characteristics agreed with those reported by Hossain and Ismail (2005b) for the natural sample.

The compound **1** was subjected to methylation to give compound **2** and several other minor compounds. The formation of **2** was ascertained by spectral studies and elemental analysis. Compound **2** had the molecular formula $C_{11}H_{14}O_5$ as evidenced by HR-EIMS. IR spectrum of compound **2** showed absorption frequencies at 3455 and 1645 cm^{-1} indicating the presence of hydroxyl and ketonic groups, respectively. In ^{1}HNMR spectrum, a singlet at δ 2.48 indicated the presence of one methyl group on the aromatic ring and a sharp singlet at δ 12.71 indicated the presence of one hydroxyl group on the aromatic ring. Other three singlets at δ 3.98, 3.99 and 4.00 indicated the presence of three -OCH_3 protons, which confirmed that the methylation has taken place. Compound **3** was obtained by the nuclear prenylation (methanolic KOH/

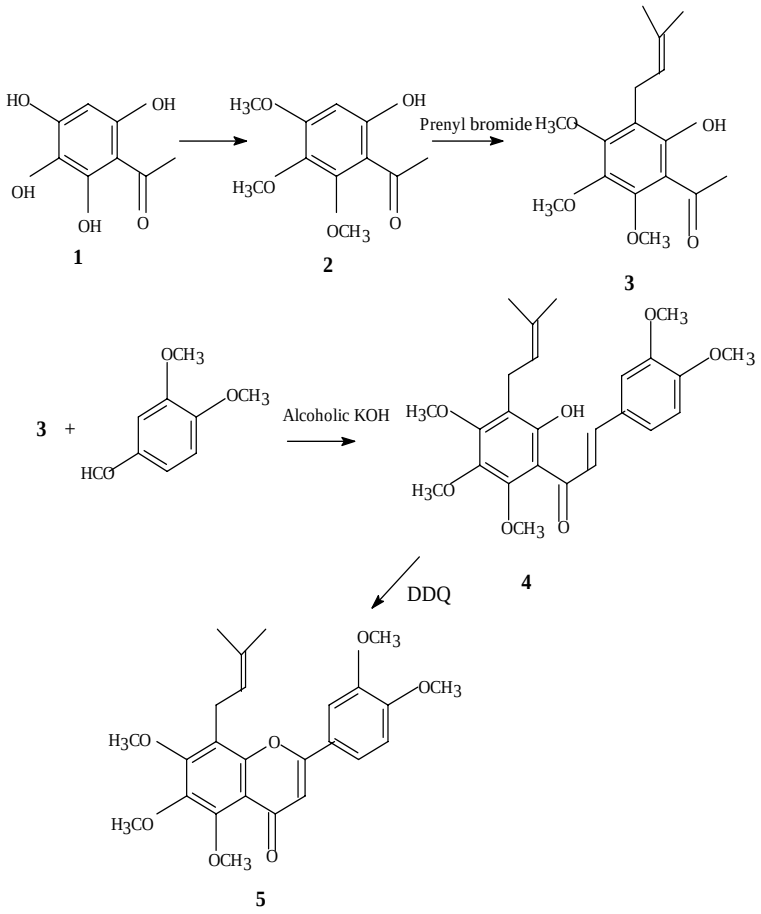

Fig. 1. Synthesis route of 3′, 4′, 5, 6, 7-pentamethoxy-8-C-prenylflavone

prenyl bromide) of **2**; its formation agrees with the data of spectral and elemental analysis. The ^{1}HNMR spectrum of the prenylated compound **3** indicated the presence of C-prenyl unit. A sharp singlet at δ 1.75 revealed the presence of gem-dimethyl group and the presence of -CH2- and -CH= protons attached to the aromatic ring was indicated by the doublet at δ 3.53 and a triplet at δ 5.49, respectively. Compound **3** on a cross-aldol condensation with 3, 4-dimethoxybenzal-dehyde in the presence of 50% ethanolic KOH afforded compound **4** after dehydration of the initial aldol product. The charcteristic IR absorption frequencies at 1642 cm^{-1} showed the presence of conjugated ketonic group and the absorption peaks at 1605 and 1595 cm^{-1} indicated the presence of unsymmetric ethylenic double bond and aromatic rings, respectively. The singlet for methyl protons of acetyl group disappeared while two new doublets at δ 7.44 and 8.01 appeared showing the presence of two vinylic protons (α and β protons). The elemental analysis for C and H showed satisfactory results (within ± 0.4%). DDQ treatment of **4** gave the corresponding title compound **5**. The formation of the title compound (**5)** was confirmed by comparing its spectral data and elemental analysis with that of the reported values of the natural sample.

In conclusion, a new and efficient synthesis of 3′, 4′, 5, 6, 7-pentamethoxy -8-C-prenylflavone has been described through the condensation of 2-hydroxy-4, 5, 6-trimethoxy-3-C-prenyl-acetophenone with 3, 4-dimethoxybenzaldehyde and finally cyclocondensation catalyzed by 2, 3-dichloro-5, 6-dicyano-1,4-benzoquinone (DDQ). The procedure is simple, rapid and high yielding, and will find many applications in organic synthesis. In addition, the CD data should usefully contribute towards the assessment of the absolute configuration of this class of flavonoids.

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