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SYNTHESIS OF SINENSETIN, A NATURALLY OCCURRING POLYMETHOXYFLAVONE

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5, 6, 7, 3′, 4′-Pentamethoxyflavone **(8)** isolated from the leaves of *Orthosiphon stamineus* has been synthesized by following an unambiguous route. All the new products have been characterised on the basis of spectral data and microanalysis.

Key words: Synthesis, Characterisation, Chalcone, Flavone.

Introduction

Flavonoids constitute an important group of natural products and some of them possess a wide range of biological activities such as antibacterial (Conn 1981), antifungal (Ghosal and Chaudhuri 1975), anti-inflammatory, antimicrobial, anti-tumour anti-cancer, prostaglandin binding (Conn 1981) and insect antifeedant (Ghosal and Chaudhuri 1975). Kielland *et al* reported the isolation of 5,6,7, 3′,4′-pentamethoxyflavone **(8)**, from the leaves of *Orthosiphon stamineus.* The structure for sinensetin was assigned on the basis of spectral data and synthesis (Matsuura *et al* 1973). This paper describe a new synthesis of sinensetin **(8)** (Scheme I) in better yield (59%). Methoxymethylation (Hossain *et al* 2001) of 2,4,5,6-tetrahydroxyacetophenone **(1)** using methoxymethyl chloride and K_2CO_3 afforded 2, 4, 5, 6-tetra (methoxymethoxy) acetophenone **(2)**. Similarly methyoxymethylation of 3, 4-dihydroxybenzaldehyde **(3)** yielded 3, 4-di (methoxymethoxy) benzaldehyde (Hossain and Islam 1993) **(4)**. Alkaline codensation of **(2)** and **(4)** gave 2′, 4′, 5′, 6′, 3, 4-hexa (methoxymethoxy) chalcone **(5)**. Dimethoxymethylation (Hossain *et al* 2001) of **(5)** using methalonic 3NHCl and boiled afforded 2′, 4′, 5′, 6′, 3, 4-hexahydroxychalcone **(6)**. DDQ treatment (Hossain 1997) of **(6)** yielded 5,6,7,3′,4′-pentahydroxyflavone **(7)**. Finally, methylation of **(7)** using dimethyl sulphate and potassium carbonate furnish the title compound **(8)**, which was identical with the natural sample of sinensetin (co-TLC, co-IR, co-^{1}H-NMR and m.p).

Experimental

Melting points were determined using an electrothermal melting point apparatus (Gallenkamp). 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 (400 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). TLC was performed using silica gel GF_{254}.

*2,4,5,6-Tetra (methoxymethoxy) acetophenone **(2)***. To a solution of 2, 4, 5, 6-tetrahydroxyacetophenone (**1**, 5g) in dry acetone (75 ml) were added methoxy methyl chloride (2 g) and anhyd. K_2CO_3 (40 g). 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 petrol (40-60°), petrol-benzene (4:1), petrol-benzene (4:3) and increasing quantities of benzene as eluents gave the major compounds **(2)** and several other minor compounds. It was oily liquid (3.99 g), (M^+, 360); R_f 0.69 (benzene-acetone; 25:1); UV: 230, 245, 278 nm; IR: 2855, 2479, 1645, 1605, 1599, 1543, 1469, 1410, 1375, 1345, 1234, 1212, 1190, 1156, 1132, 1050, 1043, 1005, 945, 885, 765, 664 cm^{-1}; ^{1}H-NMR: 2.48 (s, 3H, -COC$\underline{H}_3$), 3.42 (s, 12H, -$CH_2OC\underline{H}_3$x4), 5.53 (s, 8H, -$C\underline{H}_2OCH_3$x4), 6.41 (s, 1H, H-3).

*3,4-Di(methoxymethoxy)benzaldehyde **(4)***. A mixture of 3, 4-dihydroxybenzaldehyde (**3**, 2.8 g) in dry acetone (40 ml), methoxymethyl chloride (3.20 g) and anhydrous potassium carbonate (10 g) was refluxed for 15 min. The reaction mixture was worked-up as above and the solid obtained was crystallized from petroleum ether was white crystals (1.98 g), m.p. 54°C (Hossain and Islam 1993, m.p. 54°C).

*2′,4′,5′,6′,3,4-Hexa (methoxymethoxy) chalcone **(5)***. A mixture of 2, 4, 5, 6-tetra methoxymethoxy acetophenone (**2**, 1.76 g) and 3, 4-di (methoxymethoxy) benzaldehyde (0.58 g) in ethanolic solution of KOH (50%, 20 ml)

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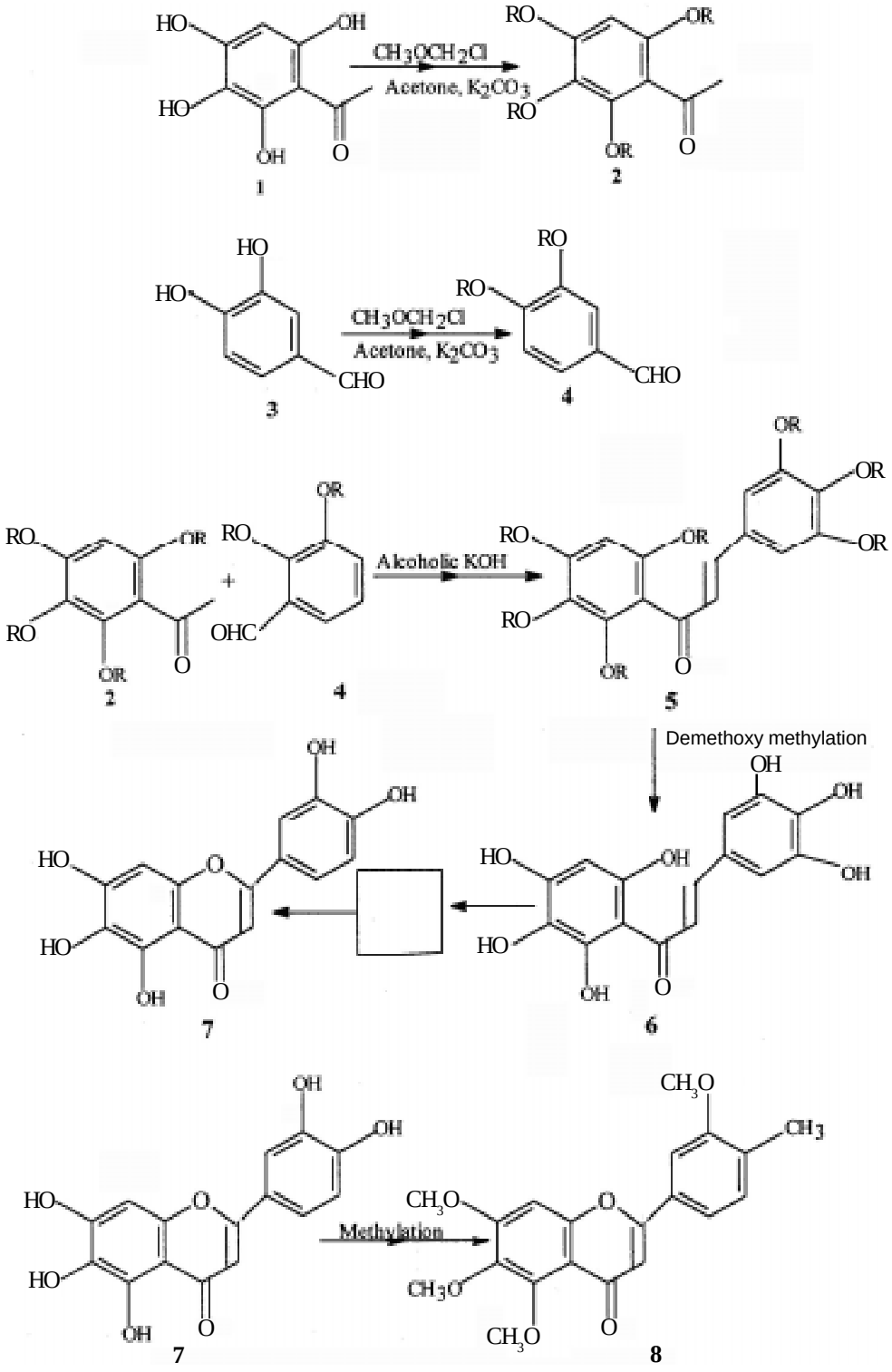

Scheme 1

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 60G using benzene-acetone (15:1) as developing solvent. The product was crystallized from petroleum spirit (40-60°C) to give yellow crystals (1.55 g),

m.p. 85°C, (M^+, 600); R_f 0.76 (benzene-acetone; 10:1); UV: 228, 254, 370; IR : 2855, 2345, 1642, 1605, 1595, 1375, 1365; ^{1}H-NMR: 3.48 (s, 18H, -$CH_2OC\underline{H}_3$x6), 5.48 (s, 12H, -$C\underline{H}_2OCH_3$x6), 6.45 (s, 1H, H-3′), 6.99 (m, 3H, H-2, H-5 and H-6), 7.45 (d, 1H, J = 9Hz, H-α), 8.03 (d, 1H, J = 9Hz, H-β). [Found : C, 54.00.; H, 6.00. $C_{27}H_{36}O_{15}$ requires: C, 54.31, H, 6.1.3%].

*2′,4′,5′,6′,3,4-Hexahydroxychalcone **(6)***. To a solution of the above methoxymethylated chalcone (**5**, 1 g) in methanol (25 ml), HCl (3N, 75 ml) was added and boiled in water bath for 15 min. It was diluted with water (100 ml) and extracted with ethyl acetate. The ethylacetate extract was washed with water, dried over anhydrous Na_2SO_4 and concentrated. TLC examination of the residue showed several spots and the major product was purified by preparative TLC using ethyl acetate-benzene (1:1) as developing solvent. It was crystallized from methanol as yellow needles (0.345 g) m.p.124°C, (M^+, 304); R_f 0.66 (acetone-benzene; 5:1). It gave positive ferric chloride test. UV: 232, 254, 365; IR:3540, 1645, 1605, 1595, 1385, 1355; ^{1}H-NMR : 6.48 (s, 1H, H-3"), 7.45 (d, 1H, J = 9Hz, H-α), 7.01 (m, 3H, H-2, H-5) and H-6), 8.01 (d, 1H, J = 9Hz, H-β), 8.88 (s, 3H, -OHx3), 12.71 (s, 3H, -OHx3); [Found : C, 59.21; H, 3.95. $C_{15}H_{12}O_7$ requires : C, 59.11; H, 3.91%].

*5,6,7,3′,4′-Pentahydroxyflavone **(7)***. To a solution of 2′, 4′, 5′, 6′, 3, 4-hexahydroxychalcone (**6**, 1g) in dry dioxan (50 ml) was added DDQ. The mixture was refluxed for 3 h. Dioxan was removed by distillation, water was added to the residue. It was extracted with ether, dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude mass on fractionation over a silica gel 60-120 mesh column with petroleum spirit, petroleum spirit-benzene (4:3) and benzene-petroleum spirit (9:4) gave two fractions A and B. Crystallization of fraction A from dil. alcohol gave 5, 6, 7, 3′, 4′-pentahydroxyflavone **(7)**, m.p. 153°C, (M^+, 302); UV: 234, 264, 378 nm; IR: 3450, 2984, 2432, 1643, 1604, 1600, 1475, 1365 cm^{-1}; ^{1}H-NMR : 6.48 (s, 1H, H-3″), 6.71 (s, 1H, H-3), 7.01 (m, 3H, H-2, H-5 and H-6), 8.88 (s, 2H, -OHx2), 12.71 (s, 3H, -OHx3); [Found : C, 59.60; H, 3.31. $C_{15}H_{10}O_7$ requires : C, 59.81; H, 3.98%]. On the other hand crystallization of fraction B from methanol yielded 2′,4′,5′,6′,3,4-hexahydroxychalcone **(6)**.

*5,6,7,3′,4′-Pentamethoxyflavone **(7)***. A mixture of 5,6, 7,3′, 4′-pentahydroxyflavone (**7**, 0.85 g), dimethyl sulphate (0.308 g) and anhydrous K_2CO_3 (5 g) in acetone (20 ml) was refluxed for 2 h. Acetone was removed by distillation, water was added to the residue and extracted with ether. The ether layer was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dryness. The product purified by preparative TLC over silica gel GF_{254} using methanol-chloroform (10:1) as developing solvent. It was crystallized from petrol to give yellow crystals (0.200 g); m.p. 178°C (Matsuura *et al* 1973, m.p. 177-179°C); R_f 0.61 (methanol-chloroform; 10:1); (M^+, 373); UV:327, 282, 213; IR: 2850, 1645, 1600, 1590, 1476, 1378, 1215, 1189, 870; ^{1}H-NMR (DMSO-d_6): 3.76 (s, 3H, 5-OCH_3), 3.78 (s, 3H, 6-OCH_3), 3.84 (s, 3H, 4′-OCH_3), 3.88 (s, 3H, 3′-OCH_3); 3.96 (s, 3H, 7-OCH_3), 6.78 (s, 1H, H-3), 7.12 (d, 1H, H-5′) , 7.18 (s, 1H, H-8), 7.52 (s, 1H, H-2'), 7.68 (d, 1H, H-6′).; [Found : C, 64.51; H, 5.5.7 $C_{20}H_{20}O_7$ requires: C, 64.66; H, 5.81%].

Results and Discussion

The compounds **(2)** and **(3)** have been prepared by following the literature procedure (Hossain and Islam 1993; Hossain *et al* 2001).The formation of product **(4)** has been confirmed by comparing the melting point with the reported values (Hossain and Islam 1993). The compounds **(1)** and **(3)** were subjected to methoxymethylation (methoxymethyl chloride/ K_2CO_3/acetone) to give compounds **(2)** and **(4)** the formation of which were ascertained by spectral studies and elemental analysis. Infrared spectrum of **(2)** showed the absorption frequency at 1645 cm^{-1} indicating the presence of ketonic group and the absorption peaks at 1605 and 1595 cm^{-1} indicated the presence of conjugated double bond and aromatic rings, respectively. In ^{1}H-NMR spectrum a singlet at δ 2.48 indicated the presence of methyl protons of acetyl group. Two singlets at δ 3.42 and δ 5.53 indicated the presence of 3 protons of -OCH_3 group and 2 protons of -CH_2 group, respectively which confirmed that the methoxymethylation has taken place. In addition, no peak for hydroxyl group was obtained which also confirms same. The compound **(2)** on a cross-aldol condensation with **(4)** in the presence of 50% ethanolic KOH afforded the compound **(5)** after dehydration of the initial aldol product. The characteristic IR absorption frequencies at 1642 cm^{-1} showed the presence of conjugated ketonic group and the absorption peaks at 1600 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 7.45 and δ 8.03 appeared showing the presence of two vinylic protons (α and β protons; i.e cis isomer). The elemental analysis for C and H showed satisfactory results (within ± 0.4%). The compound **(6)** was obtained from **(5)** by dimethoxymethylation (MeOH/3N HCl). IR absorption frequencies at 3540 and 1645 cm^{-1} showed the presence of -OH (phenolic) group and ketonic group and the absorption peaks at 1605 and 1590 cm^{-1} indicated the presence of unsymmetric ethylenic double bond

and the aromatic rings, respectively. Two singlets at δ 8.88 and δ 12.71 indicated the presence of -OH protons which confirming the completion demethoxymethylation. The B-ring protons have their usual chemical shift value. DDQ treatment of **(6)** gave the corresponding flavone **(7)**. Two doublets at δ 7.45 and δ 8.01 for vinylic protons disappeared. The title compound **(8)** was finally obtained by methylation of its precursor. The formation **(8)** was ascertained by spectral studies and elemental analysis.

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