4'-Methoxy-2-Phenyl-4-Chromone-3-O-D-Glucoside. A Novel Compound from *Millettia zechaina* (Papilionaceae)

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Summary: A new flavonol glucoside, 4'-methoxy-2-phenyl-4- chromone-3-O-D-glucoside was isolated and identified from the flowers of Millettia zechiana. The aglycone, 4'-methoxy-3-hydroxyl-2-phenyl-4-chromone was synthesized by a novel method and its structure established on the basis of physical and chemical characteristics. In addition some known glycosides of kaempferol, quercetin, malvidin, cyanidin and pelargonidin were also found, which were identified by standard procedures.

Introduction

Mellettia zechiana Harmas. (Ir. 1957), genus *Millettia* of family Papilionaceae, is found in coastal grassy planes from Guinea to Cameroons, bears reddish purple, silky golden brown outside, flowers (October - December) [1]. The bark pulp, with sea-salt water and Guinea grains diluted with warm water is used as a gargle for rhino-pharyngal and bronchial troubles and purple leaves are rubbed on painful parts [2]. In the present work, we report the isolation and identification of a novel flavonol glycoside and the synthesis of its aglycone by a new method.

The known glycoside, 3-rhamnoside of kaempferol; 3-glucoside of quercetin; 7-glucoside of 8-OH-quercetin and three anthocyanins viz. 3,5-diglucoside of malvidin and cyanidin and 3-rhamnoside of pelargonidin, isolated and purified by standard procedures were characterized on the basis of colour reactions, hRf values, UV spectral analysis, acid hydrolysis to a glycone and sugar and by direct comparison with authentic markers. In all cases structural assignments were consistant with appropriate model compound described by Mabry *et al* [3].

Experimental

Plant Material

The fresh flowers of *M. zechiana* were collected in December 1987 from the rain forest around Ekpoma, Headquaters of Okpebho Local Government Area of Bendel State University.

Extraction, Isolation and Identification

Fresh petals (200 g) of *M. zechiana* were extracted with MeOH for 72 hrs. (3x150cm^3). The combined dilute extract filtered, conc. in vacuo at 30°C and conc. extract was re-extracted successively with EtOAc and n-BuOH. The n-BuOH extract was chromatographed IDPC on Whatman No.3 paper in BAW, n-BuOH- HOAc-H_2O (4:1:5, top layer) and 15% HOAc for the fraction of anthocyanins and afforded three known anthocyanins which were identified by standard procedures as 3-*O*, 5-*O*-diglucosides of cyanidin and malvidin and 3-*O*-rhamnoside of pelargonidin. The EtOAc extract was chromatographed over Sephadex LH-20 in EtOAc and afford three flavonoids, two of which were known and identified on the basis of colour reactions, hRf values, UV spectral analysis and acid hydrolysis to aglycone and sugar to be 3-*O*-rhamnoside and 3-glucoside of kaempferol and quercetin respectively. The third compound was afforded as a light yellow crystalline solid purified by preparative TLC (20x20cm, glass plates coated with 0.5 mm layer of silica gel 60 F_{250}) and identified to be 4'-OCH_3-2-phenyl-4-chromone-3-*O*-D-glucoside as under; appeared purple on IDPC with and without UV + NH_3 indicating 4'-OCH_3 group [4,5], hRf values: BAW, n-BuOH-HOAc-H_2O (4: 1: 5, top layer) 65; HOAc-conc-HCl-H_2O(3:30:10)75; PhOH, H_2O-PhOH (3:1) 72; BAFW, n-BuOH-HOAc-HCO_2H-H_2O (5:1:1:3)68; BFTW, n-BuOH-HCO_2H- $C_6H_5CH_3$-H_2O (3:1: 1:5)64. Fluorescent yellow with UV + 5% $AlCl_3$; purple with Na_2CO_3 and 30% aq. NaOH showing the presence of a flavonol skeleton; no orange colour with conc. soln. of 2-amino ethyldiphenyl borate in MeOH and no colour with conc.solution of 2-aminoethyldiphenyl borate in MeOH and no colour with MgOAc solution indicating the absence of 5,4' -5'-OH and any other aromatic-OH group [4,6]; red colour with Mg-HCl and Zn-HCl indicated a flavonol with 3-*O*-glycosylation [3,7], m.p. 182- 184°C, mol.wt. 430. Found: C, 61.39; H, 5.11; O, 33.48%, calc. for $C_{22}H_{22}O_9$; C, 61.4;H, 5.1; 0.33.5% UV $\lambda_{max.}$ nm (MeOH) 256, 380; -ve bathochromic shift with NaOAc and (NaOEt) 258, 413. After acid hydrolysis (2M HCl, 100°C and 30 min.) the aglycone in the acid hydrolysate was identified to be 4'-OCH_3-3-OH-2-phenyl-3-chromone and sugar as D-glucose as under: aglycone, purple on IDPC with and without UV + NH_3 indicating 4'-OCH_3 group; gave the colour reactions of fvlanonols and negative reaction with conc. solution of 2-aminoethyldiphenyl borate and MgOAc indicating the absence of any nuclear-OH group. M.p. 260-262°C, mol. wt. 268. Found C, 71.64; H, 4.47; O, 23.94%, calc. for $C_{16}H_{12}O_4$: C, 72; H, 4.0; O.24.0% UV $\lambda_{max.}$ nm (MeOH) 253, 370; - ve bathochromic shift with NaOAc, (NaOEt) 256, 409. IR $\nu_{max.}$ cm^{-1} 3400 (OH); 2916 (C-H); 1656 (C=O); 1590 (C=C); 1064 (C=O); 800-700 (O-O-p, bending for substituted benzene ring), hRf values: BAW, 92; HOAc, 92; HOAc-conc. HCl-H_2O, 85; PhOH, 90; BAFW; 90; BFTW; 92. These data show that the compound is 4'-OCH_3-3-OH-2-phenyl-4-chromone. This aglycone was synthesized by dissolving 3.9g (0.025 mol) of o,w-di-OH-acetophenone in 25 cm^3 of dry, redistilled pyridine in a 100 cm^3 round bottom flask. The contents were stirred vigorously and heated to 50°C. 7.2 g (0.025 mol) p-OCH_3-benzoic acid anhydride and 120 cm^3. 2M HCl were added. Stirring continued for 15 min. system was heated under reflux on oil bath, temperature (150-160°C) for 50 min. The reaction mixture was poured with stirring on to 1kg of crushed ice and allowed the ice to melt.

Crystals of the product which separated as light yellow solid, were filtered off. The yield was 3.6 g (92%).

Product had; m.p. 260-261°C, UV $\lambda_{max.}$ nm. (95% EtOH) 253, 370; - ve bathochromic shift with NaOAc and ($NaOCH_3$) 256, 409. IR $\nu_{max.}$ cm^{-1} 1064 (C-O); 1590 (C=O); 1656 (C=O); 2916 (C-H); 3400 (OH), hRf values: BAW, 92; Forestal, 85; PhOH, 90; BAFW, 90; BFTW, 92. These data are identical with those reported for the natural products. The synthetic pathway and the structure of 4'-OCH_3-3-OH-2-phenyl-4- chromone is given in Fig. 1.

The sugar of the glycoside was identified in acid hydrolysate by chromatography and co-chromatography in t-BuOH-HOAc-H_2O (3:1:1);

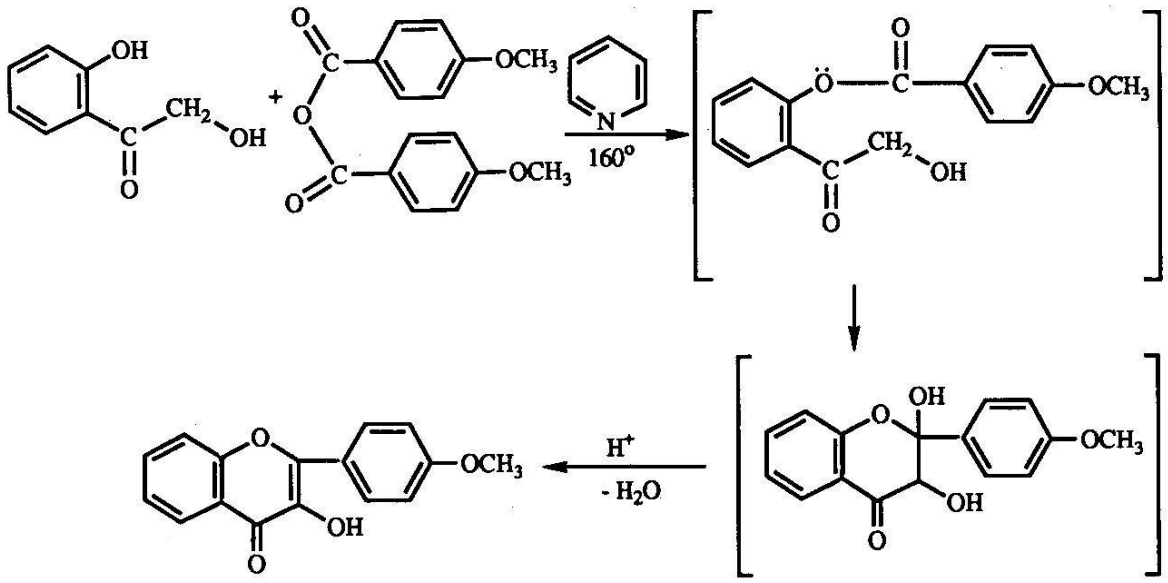

Fig. 1

EtOAc-Py-H_2O (10:4:3) and n-BuOH-EtOH-H_2O (4:1:1) with authentic marker to be D-glucose. The colour reactions, measurement of chromatographic and spectral chracteristics and elemental analysis etc. confirmed that the compound is 4'-OCH_3-2-phenyl-4-chromone-3-*O*-D-glucoside (Fig. 2).

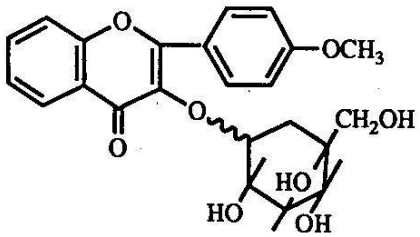

Fig. 2

Results and Discussion

The conc. methanolic extract of the fresh flowers of *M. zechiana* was extracted successively with EtOAc and n-BuOH The n-BuOH extract was paper chromatographed on Whatman No. 3 paper and yielded 3,5-diglucoside of malvidin; 3,5-diglucoside of cyanidin and 3-rhamnoside of pelargonidin identified on the basis of colour reactions, chromatographic and spectral properties, m.p. and comparison with authentic samples. The EtOAc extract was column chromatographed over Sephadex LH-20 gave 3-rhamnoside of kaempferol, 3-glucoside of quercetin, these two known glycosides were identified according to standard procedures and a third compound, m.p. 182-184°C, analysed for $C_{22}H_{22}O_9$ and appeared from colour reactions to be a flavonol glycoside with 4'- OCH_3 group and no free aromatic-OH group. The UV spectrum had a strong absorption at 256 nm. It did not show any shift with NaOAc but with NaOEt 258, 413 nm. These observations indicated a flavonol skeleton with C-3 glycosylation without any free-OH group.

Acid hydrolysis yielded 4'-OCH_3-3-OH-2-phenyl 4-chromone and D-glucose which were identified as explained in experimental section. Hence the glycoside obtained from the fresh flower of *M. zechiana* is 4'-methoxy-2-phenyl-4-chromone-3-*O*-D-glucoside.

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