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A CHALCONE AND AN ISOFLAVONE FROM *MILLETTIA PACHYCARPA* SEEDS

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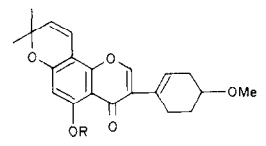
Key Word Index—*Millettia pachycarpa*; Leguminosae; Lotodoideae; seeds; prenylated isoflavones; prenylated chalcone.

Abstract—Chemical examination of the seeds of *Millettia pachycarpa* has yielded a new prenylated isoflavone and a new prenylated chalcone in addition to the previously reported isoflavones 5-hydroxy-4′-methoxy-6″,6″-dimethylpyrano(2″,3″:7,8)isoflavone, 5,7,4′-trihydroxy-6,8-diprenylisoflavone, 5,7,3′,4′-tetrahydroxy-6,8-diprenylisoflavone and pomiferin.

INTRODUCTION

We have earlier reported the chemical examination of the leaves and roots of *Millettia pachycarpa* collected from two different localities [1–3]. Extracts and aqueous suspensions of finely ground seeds of *M. pachycarpa* are reported to possess considerable insecticidal activity when used in sprays against a variety of insects, e.g. houseflies, bean aphids, pentatomids, leaf beetles and cabbage worms. They act both as stomach and contact poisons and are also ovicidal [4, 5]. Investigation of the seeds of *M. pachycarpa* furnished the already known **1a**, 5,7,4′-trihydroxy-6,8-diprenylisoflavone (**2d**), 5,7,3′,4′-tetrahydroxy-6,8-diprenylisoflavone (**2e**) and pomiferin (**5**). In addition, a new prenylated isoflavone, (**2a**) and a new chalcone (**3a**) have been isolated whose structure determination form the subject of this paper.



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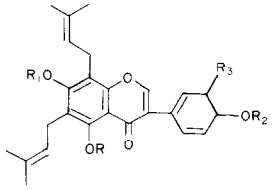
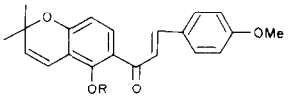
1b R=Ac



2a R_1=H, R_2=Me, R_3=OH
2b R_1=Ac, R_2=Me, R_3=OAc
2c R_1=R_2=Me, R_3=OMe
2d R_1=R_2=R_3=H
2e R_1=R_2=H, R_3=OH



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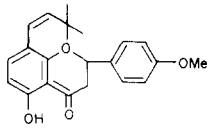
3b R=Ac



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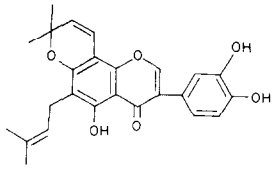

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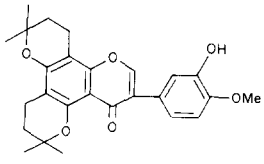

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RESULTS AND DISCUSSION

Compound **2a** was a gum which could be purified through its acetate (**2b**) [6]. The NMR spectrum of **2a** exhibited three singlets at δ 7.8 (1H), 3.45 (3H) and 12.11 (1H, disappearing on D_2O exchange), suggesting that it was a 5-hydroxymonomethoxyisoflavone. Methylation of **2a** with diazomethane gave a compound which was identical with **2c** [1] proving that **2a** was a monomethoxy-derivative of **2e**. Heating **2a** with formic acid resulted in cyclization of both the prenyl units as indicated by the NMR spectrum (see Experimental) which led us to conclude that the methoxy group was present in ring B. This was further supported by the low resolution mass spectrum which gave a M^+ at *m/z* 436 and other significant peaks at *m/z* 288 and 148 formed as a result of retro-Diels–Alder cleavage [2]. Alkaline hydrogen peroxide degradation of the formic acid reaction product of **2a** gave 3-hydroxy-4-methoxybenzoic acid. Therefore, structure **2a** was assigned to isoflavone and **6** to its formic acid reaction product.

Compound **3a**, mp 130° was a monomethoxychalcone (UV λ_{max}^{MeOH} nm: 231, 280 and 360; NMR α- and β-protons at δ 7.76 and 7.40, each as a doublet with J = 16 Hz and a methoxyl at δ 3.70). The NMR spectrum of **3a** showed an AB system at δ 5.47 and 6.79 with J = 10 Hz which, together with the presence of a singlet at δ 1.30 integrating for six protons, suggested the presence of a cyclized dehydrogenated prenyl side chain. That ring B was monosubstituted at C-4 was indicated by the presence of an AB system at δ 7.80 (2H, *d*) and 6.85 (2H, *d*) with J = 9 Hz in the NMR spectrum. A singlet at δ 12.53 which disappeared on D_2O exchange confirmed the presence of a chelated hydroxyl in **3a**. The low resolution mass spectrum exhibited a M^+ at *m/z* 336 and other significant peaks were at *m/z* 321, 202, 187, 149 and 134. The peaks at *m/z* 202 and 134 suggested the presence of a prenyl side chain in ring A and a methoxyl group in ring B. Because of

the presence of an AB system at δ 7.64(*d*) and 6.28(*d*), with $J = 9$ Hz in the NMR spectrum, structures **3a** and **4** were considered possible for the chalcone. Proof in favour of structure **3a** was forthcoming from a comparison of the NMR spectrum of **3a** with that of its acetate (**3b**). One of the olefinic protons of the chromene ring had undergone a diamagnetic shift from δ 6.79 in **3a** to 6.30 in **3b** and the other had undergone a paramagnetic shift from δ 5.47 in **3a** to 5.70 in **3b** [1]. Therefore, structure **3a** was assigned to the chalcone.

EXPERIMENTAL

Extraction. M. pachycarpa Benth. seeds (500 g) collected from the Lumding area of District Nowgaon, Assam, India, were cold extracted with $CHCl_3$ for 72 hr. Evaporation of the solvent at red. pres. gave 5 g of a residue which was chromatographed over 250 g Si gel. Fractions (200 ml) were collected in the following order: 1–10 (C_6H_6); 11–15 (C_6H_6–EtOAc, 9:1); 16–20 (C_6H_6–EtOAc, 7:1); 21–25 (C_6H_6–EtOAc, 4:1); 26–30 (C_6H_6–EtOAc, 2:1); 31–35 (C_6H_6–EtOAc, 1:1); 36–40 (C_6H_6–EtOAc, 1:2); 41–45 (C_6H_6–EtOAc, 1:4); 46–50 (EtOAc); 51–55 (EtOAc–MeOH, 19:1).

Fractions 2–5, which showed a single spot by TLC (C_6H_6) were combined to give 50 mg **1a** as a gum [7], ^{1}H NMR signals at δ 12.25 (OH-5), 7.62 (H-2), 7.30 (*d*, $J = 8$ Hz, H-2′ and H-6′), 6.78 (*d*, $J = 8$ Hz, H-3′ and H-5′), 6.6 (*d*, $J = 10$ Hz, H-4″), 5.4 (*d*, $J = 10$ Hz, H-3″), 6.15 (H-6), 3.65 (OMe), 1.35 (2 Me of pyran). Angular cyclization in **1a** was confirmed by making its acetate (**1b**) whose NMR spectrum did not show any change in the chemical shift of H-3″ and H-4″ when compared with **1a** [1].

Fractions 7–15 (200 mg) showed one major spot on TLC. However, all attempts to obtain it in a pure state by prep. TLC failed. Therefore, it was purified by making its acetate [6]. A soln of 150 mg of combined fractions 7–15 in 0.5 ml pyridine and 1 ml Ac_2O was left at room temp. for 48 hr. After usual work-up the major product was purified by prep. TLC (EtOAc–petrol, 1:9) to give 100 mg of a crystalline compound (**2b**), mp 72° (EtOAc). IR bands at 3525, 1755 and 1640 cm^{-1}; ^{1}H NMR spectrum: δ 12.03 (OH-5), 7.70 (H-2), 7.12–7.0 (H-2′ and H-6′), 6.78 (H-5′), 5.0 (*t*, $J = 7$ Hz, vinyl protons), 1.98 (2 Ac), 1.45 (2 Me), 1.35 (2 Me); MS *m/z* 520 $[M]^+$, 478, 460, 435, 421, 404, 379, 367, 351, 337, 310, 252, 250, 235 and 187. Hydrolysis of **2b**. A soln of 50 mg **2b** in 5 ml 60% aq. MeOH was stirred with 20 mg imidazole. After 20 hr TLC indicated that no starting material was left. Dilution with H_2O followed by extraction with $CHCl_3$ and evaporation of the washed and dried extract at red. pres. gave 35 mg **2a** as a gum, IR bands at 3500, 1640 and 1580 cm^{-1}. ^{1}H NMR signals at δ 12.11 (OH-5), 7.60 (H-2), 6.80 6.45 (3H, H-2′, H-5′ and H-6′), 5.0 (vinyl protons), 3.45 (OMe), 3.10 (*m*, methylenes), 1.40 (four methyls); MS *m/z* 436 $[M]^+$, 404, 393, 391, 381, 379, 365 and 337. (Calc. for $C_{26}H_{28}O_6$: MW, 436.1884. Found: MW(MS) 436.1896.) Cyclization of **2a**: A soln of 40 mg **2a** in 3 ml 90% HCO_2H was heated at 100° for 3 hr. Dilution with H_2O, extraction with $CHCl_3$, evaporation of the washed and dried extract and purification of the residue by prep. TLC (EtOAc–C_6H_6, 1:1) gave 30 mg **6** as a gum. ^{1}H NMR signals at δ 7.50 (H-2), 6.8–6.6 (H-2′, H-5′ and H-6′), 3.58 (OMe), 2.35(*m*) and 1.40(*m*) (methylenes), 1.0 (flow methyls). Alkaline hydrogen peroxide oxidation of **6**: To a soln of 30 mg **6** in 7 ml EtOAc and 5 ml 12% aq. NaOH was added 1 ml 30% H_2O_2 with stirring. Stirring was continued while three portions of 1.5 ml each of H_2O_2 were added over 4.5 hr. The mixture was allowed to stand at room temp. for 60 hr, diluted with H_2O and extracted with Et_2O. The aq. layer was acidified with dilute H_2SO_4 and extracted with Et_2O. The Et_2O layer was extracted with 3 × 50 ml of 10% $NaHCO_3$ and the latter again extracted with Et_2O. Acidification of the basic layer followed by extraction with Et_2O gave, after evaporation of the washed and dried Et_2O layer, 8 mg 3-hydroxy-4-methoxybenzoic acid, mp 251°, reported mp 255° [8].

Fractions 17–20, showing a single spot on TLC, were combined to give 100 mg **3a** as a crystalline solid (petrol) mp 130°. UV λ_{max}^{MeOH} nm: at 231, 280 and 360; IR ν_{max}^{KBr} cm^{-1}: bands at 1630, 1600, 970 and 790; ^{1}H NMR spectrum: δ 12.53 (OH-6′), 7.76 (*d*, J = 16 Hz, H-α), 7.64 (*J* = 9 Hz, H-2′), 7.50 (*d*, $J = 9$ Hz, H-2 and H-6), 7.40 (*d*, $J = 16$ Hz, H-β), 6.85 (*d*, $J = 9$ Hz, H-3 and H-5), 6.79 (*d*, $J = 10$ Hz, H-4″), 6.28 (*d*, $J = 9$ Hz, H-3′), 5.47 (*d*, J = 10 Hz, H-3″), 3.70 (OMe), 1.30 (2 Me of pyran); MS peaks at *m/z* 336 $[M]^+$, 321, 319, 202, 187 (base peak), 149 and 134. (Calc. for $C_{21}H_{20}O_4$: MW, 336.1360. Found: MW(MS), 336.1378.) Acetylation of **3a**: To a soln of 50 mg **3a** in 0.5 ml pyridine was added 1 ml Ac_2O. After keeping the reaction mixture overnight, usual work-up provided 48 mg **3b** as a gum, ^{1}H NMR signals at δ 7.6–7.0 (overlapping signals integrating for five protons, H-α, H-β, H-2, H-6 and H-2′), 6.70 (*d*, $J = 9$ Hz, H-3′), 6.90 (*d*, J = 9 Hz, H-3 and H-5), 6.30 (*d*, $J = 10.5$ Hz, H-4″), 5.70 (*d*, J = 10.5 Hz, H-3″), 3.80 (OMe), 2.20 (Ac), 1.40 (2 methyls of pyran); MS peaks at *m/z* 378 $[M]^+$, 363, 336, 321 (base peak), 319, 202, 187, 160, 149 and 121.

Fractions 22–24 which showed a single spot on TLC were combined to give 150 mg 5,7,4′-trihydroxy-6,8-diprenylisoflavone (**2d**), mp 142° reported [1] mp 142°, identical in all respects (mmp, TLC, IR, NMR and MS) with an authentic sample.

Fractions 25–28 which showed a single spot on TLC were combined to give 400 mg 5,7,3′,4′-tetrahydroxy-6,8-diprenylisoflavone (**2e**), mp 155°, reported [1] mp 155°, identical in all respects (mmp, TLC, IR, NMR and MS) with an authentic sample.

Fractions 30–33 were combined to give 100 mg **5**, mp 200.5°, reported [9] mp 200.5°, identical spectral data (IR, NMR and MS) with that reported for pomiferin (**5**).

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