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PONGAGLABOL, A NEW HYDROXYFURANOFLAVONE, AND AURANTIAMIDE ACETATE, A DIPEPTIDE FROM THE FLOWERS OF *PONGAMIA GLABRA*

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Key Word Index—*Pongamia glabra*; Leguminosae; furanoflavone; pongaglabol; karanjin; lancheolatin B; kanjone; pinnatin; kanugin; (−)-isolonchocarpin; pongamol; ovalitenone; aurantiamide acetate.

Abstract—Pongaglabol, a new hydroxyfuranoflavone, and aurantiamide acetate, a rarely occurring modified phenylalanine dipeptide, have been isolated together with 4 furanoflavones, karanjin, lancheolatin B, kanjone and pinnatin, a simple flavone, kanugin, a chromenoflavanone (−)-isolonchocarpin, two furanodiketones pongamol and ovalitenone, and β-sitosterol from the petrol and chloroform extracts of the flowers of *Pongamia glabra*. The structure of pongaglabol has been established as 5-hydroxyfurano(8,7-4″,5″)flavone on the basis of spectral and chemical evidence.

INTRODUCTION

Pongamia glabra Vent (Leguminosae) is one of the commonest and most useful [1] trees of India. All its parts have been extensively studied and are known to yield furanoflavones and chromenoflavones [2–6], simple flavones [2], chromenochalcones [2, 7], a chromenoflavanone [8], furanodiketones [2, 9] and amino acids [10, 11]. Kaempferol and β-sitosterol are reported [12] from the flowers of this plant. Our reinvestigation of the flowers resulted in the isolation of one new hydroxyfuranoflavone, designated pongaglabol, a recently reported modified phenylalanine dipeptide aurantiamide acetate and a number of known polyphenolics. The present paper gives an account of the isolation of all the constituents and the establishment of the structure of pongaglabol as 5-hydroxyfurano(8,7-4″,5″)flavone.

RESULTS AND DISCUSSION

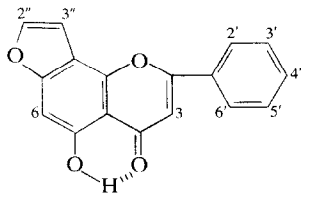
Extensive chromatography over Si gel of the petrol and $CHCl_3$ extracts of the dried flowers of *P. glabra* afforded a new furanoflavone pongaglabol (**1**) in addition to 4 known ones, viz. karanjin (**3**), lancheolatin B (**4**), kanjone (**5**) and pinnatin (**7**), one simple flavone kanugin (**8**), one chromenoflavanone (−)-isolonchocarpin (**9**), two furanodiketones pongamol (**10**) and ovalitenone (**11**), β-sitosterol and one dipeptide aurantiamide acetate (**12**).

Pongaglabol, mp 198°, $C_{17}H_{10}O_4$ (M^+ 278), $[\alpha]_D \pm 0°$, has been assigned structure **1** from the following spectral and chemical evidence. A yellow colouration of **1** in the Shinoda test and its UV spectrum [λ_{max}^{EtOH} nm (log ε): 221 (4.48), 257 (4.29) and 281 (4.47)] are characteristic of a furanoflavonoid chromophore. It gave a green colouration with $FeCl_3$ but no IR band for OH, indicating the presence of a chelated phenolic OH at C-5. This was supported by (i) a bathochromic shift of its UV absorption maxima with dry $AlCl_3$ [λ_{max}^{EtOH} nm (log ε): 223.5 (4.43), 265 (4.29) and 306 (4.48)] but no shift with dilute alkali, (ii) a relatively weaker IR band at 1650 cm^{-1} (chelated CO), and (iii) an appropriately deshielded phenolic proton signal at δ12.73 (1H, *s*), exchangeable with D_2O. The ^1H NMR spectrum displayed the following other signals: δ 6.8 (1H, *s*, H-3); 6.95 [1H, *d*, $J = 0.9$ Hz, H-6 for an angular furanoflavone (**1**) or H-8 for a linear furanoflavone (**2**)]; 7.04 (1H, *dd*, $J_{3'',2''} = 2.1$ and $J_{3'',6(or\ 8)} = 0.9$ Hz, H-3″); 7.53–7.61 (4H, *m*, H-2″, H-3′, H-4′ and H-5′), 7.90–8.01 (2H, *m*, H-2′ and H-6′). The MS showed the stable aromatic M^+ peak at *m/e* 278 as the most abundant peak and only one other intense peak at 176 ($M^+ - PhC{\equiv}CH$, 48%).

The alternative structure **2** for pongaglabol was eliminated in the following way. Pinnatin (**7**), mp 176° was isolated from the marc and characterized by its spectral properties [3]. Demethylation of **7** (dry $AlCl_3/Et_2O$) gave the flavonoid **2**, mp 200–201°, which is different from pongaglabol as evident from the depressed mmp, co-TLC* and non-superimposable IR. Furthermore, methylation (Me_2SO_4–K_2CO_3) of pongaglabol gave the Me ether **6**, mp 181–182°, which was different from pinnatin (mmp, co-TLC*, IR); the UV spectrum of **6** closely resembled that of 5-methoxyfurano(8, 7−4″, 5″)-flavone reported [13] earlier as a synthetic compound.

A comparison of the ^1H NMR signals of **1** and **2** and the corresponding Me ethers **6** and **7** shows that H-6

* R_f values were the same but the colour of the spots were different on iodine absorption.



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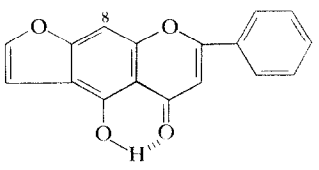

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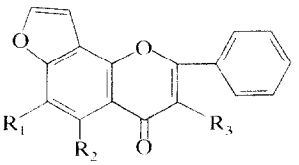
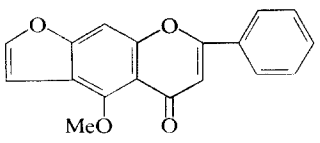


3 $R_1 = R_2 = H$, $R_3 = OMe$
4 $R_1 = R_2 = R_3 = H$
5 $R_1 = OMe$, $R_2 = R_3 = H$
6 $R_1 = R_3 = H$, $R_2 = OMe$



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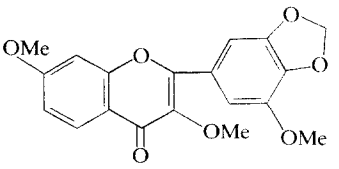

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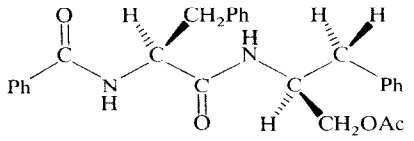

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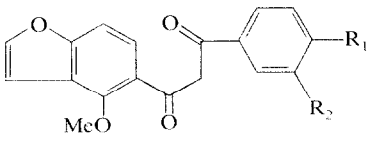
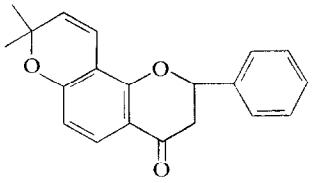


10 $R_1 = R_2 = H$
11 $R_1, R_2 = -O-CH_2-O-$



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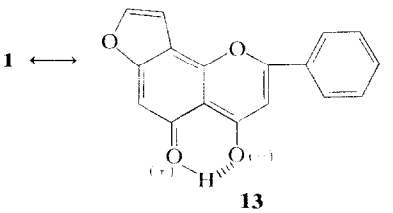

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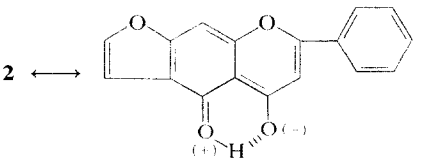

14

of **1** and **6** absorbs at a higher field [δ 6.95 in **1** and 7.01 in **6**] than H-8 of the corresponding linear isomers [δ 7.17 in **2** and 7.39 in **7**]. This may be due to the fact that in **2** and **7** both the oxygen atoms flanking H-8 are in aromatic rings, and are thus deactivated; hence their shielding effect on H-8 is decreased. On the other hand, of the two oxygen atoms flanking H-6, only the furan oxygen is deactivated in **1** and **6**. Again, the proton of the chelated phenolic OH of **1** absorbs at δ 12.73, whereas that of **2** absorbs at 13.61; these chemical shifts were found to be independent of concentration. The greater deshielding of the chelated OH in **2**, may be due to the greater participation of its canonical form **14** than the canonical form **13** of **1**. This may be attributed to the fact that the aromaticity of both benzene and furan rings are lost in **13** whereas the aromaticity of the furan ring is retained in **14**.

Other flavonoids **3–5** and **7–9** and the furanodiketones **10** and **11** were identified by UV, IR, NMR and MS. The spectral data of the dipeptide, mp 185–186°, $[\alpha]_D - 63.1°$ are similar to those of aurantiamide acetate [14], $[\alpha]_D - 23.6°$ isolated from *Piper auranticum* Wall, asperglaucide, $[\alpha]_D - 39.9°$ a metabolite of *Aspergillus glaucus* [15] and a dipeptide of the same structure, $[\alpha]_D - 74°$ isolated from an alga *Cystoseira corniculata* [16]. The difference in the specific rotation values of this dipeptide obtained from different sources is noteworthy. The decreased rotation values may be due to the presence of the racemic

variety to different extents along with the (−)-variety of the dipeptide.

EXPERIMENTAL

Mps are uncorr.; IR were recorded in KBr, ^{1}H NMR at 80 or 90 MHz using TMS int. standard. MS at 70 eV, and optical rotations in $CHCl_3$. Si gel (100–200 mesh) was used for chromatography, unless otherwise stated; spots were visualized in UV light and with I_2 vapour.

Extraction. Dried and powdered flowers (2 kg) of *P. glabra* collected from Midnapur, West Bengal during 1977, were extracted exhaustively in a Soxhlet apparatus with petrol (60–80°) and then $CHCl_3$. The petrol extract on concn deposited a solid which was filtered. The filtrate (fraction **A**) the residue (fraction **B**) and the $CHCl_3$ extract (fraction **C**) were separately chromatographed over Si gel (60–120 mesh) using solvents and solvent mixtures of increasing polarity. Similar fractions as indicated by TLC (Si gel G) were combined. The identity of the previously reported compounds were established by IR, UV, ^{1}H NMR and MS.

Chromatography of fraction A. Isolation of pongamol (**10**) *and* (−)-*isolonchocarpin* (**9**). The later petrol–C_6H_6 (1:1) eluates showing two spots (one major) in TLC were subjected to repeated chromatography over Si gel to afford pure pongamol (**10**), crystallizing from $CHCl_3$–petrol in light brown plates, mp 127–128° (Cu-chelate mp 225°), and (−)-isolonchocarpin (**9**), crystallizing from $CHCl_3$–petrol in colourless needles, mp 117–118°, $[\alpha]_D^{28}$ −120.2° ($CHCl_3$, *c* 0.065).

Isolation of pongaglabol (**1**) *and ovalitenone* (**11**). The yellow residue obtained from the first C_6H_6 eluate fraction (1 l.), showing two major yellow spots on TLC, was rechromatographed over Si gel. The early petrol–C_6H_6 (1:2) eluates afforded pongaglabol (**1**), crystallizing from $CHCl_3$–petrol as fine yellow needles, mp 198°, yellow colouration with Mg/HCl, green colouration with $FeCl_3$; λ_{max}^{EtOH} nm (log ε): 221 (4.48), 257 (4.29) and 281 (4.47), $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ε): 223.5 (4.43), 265 (4.29) and 306 (4.48); ν_{max}^{KBr} cm^{-1}: 1650 (conjugated C=O), 1605, 1442, 1418, 1347, 1280, 1128, 1055 (benzofuran), 800 and 760; ^{1}H NMR (80 MHz, $CDCl_3$): δ 6.8 (1H, *s*, H-3), 6.95 (1H, *d*, $J_{3'',6}$ = 0.9 Hz, H-6), 7.04 (1H, *dd*, $J_{3'',2''}$ = 2.1 Hz, $J_{6,3''}$ = 0.9 Hz, H-3''), 7.53–7.61 (4H, *m*, H-2'', H-3' H-4' and H-5'), 7.90–8.01 (2H, *m*, H-2' and H-6'), 12.73 (1H, *s*, exchangeable with D_2O, chelated OH on C-5); MS: *m/e* (rel. intensity): 278 (100, M^+), 176 (48, M^+ − PhC≡CH), 148 (3.1, 176 − CO), 139 (4.4), 125 (3.8), 120 (4.4), 102 (2.6), 92 (5.5) and 77 (3.0). The later fractions of the petrol–C_6H_6 (1:2) eluate afforded ovalitenone (**11**), crystallized from $CHCl_3$–petrol as yellow needles, mp 118–119°; orange-red colouration with Mg/HCl, greenish violet colouration with $FeCl_3$; λ_{max}^{EtOH} nm (log ε): 239.5 (4.21), 288 (4.25) and 363 (4.36); ν_{max}^{KBr} cm^{-1}: 1585 (C=O of 1,3-diketone), 1060 (benzofuran), 1027 and 920; ^{1}H NMR (90 MHz, $CDCl_3$) and MS data are similar to reported data [9, 17].

Isolation of β-sitosterol, karanjin (**3**) *and lancheolatin B* (**4**). The light brown residues obtained from the 2nd to 5th C_6H_6 eluates (1 l. each), showing two major spots in TLC, were combined and subjected to rechromatography over Si gel. β-Sitosterol, obtained from C_6H_6 eluates, crystallized from $CHCl_3$–MeOH in flakes, mp 139°, $[\alpha]_D^{30}$ −37° ($CHCl_3$, *c* 0.120); acetate, mp 134°, $[\alpha]_D^{30}$ −40° ($CHCl_3$, *c* 0.084); identical with an authentic sample. The C_6H_6-$CHCl_3$ (1:1) eluates afforded karanjin (**3**), crystallized from $CHCl_3$–petrol in colourless plates, mp 161°; yellow colouration with Mg/HCl. The light brown solid obtained from the later C_6H_6 eluate fractions on careful rechromatography over Si gel followed by crystallization from EtOAc–petrol furnished lancheolatin B (**4**) as colourless needles, mp 137°, yellow colouration with Mg/HCl.

Isolation of kanjone (**5**) *and kanugin* (**8**). The residue from the first $CHCl_3$ eluate (1 l.) on rechromatography over Si gel followed by crystallization from $CHCl_3$–EtOH furnished kanjone (**5**) as colourless needles, mp 187–188°; orange colouration with Mg/HCl. The residue obtained from the later $CHCl_3$ eluates on repeated chromatography over Si gel followed by crystallization from $CHCl_3$–petrol afforded kanugin (**8**) as colourless needles, mp 204°; pink colouration with Mg/HCl.

Isolation of pinnatin (**7**). The residue obtained from the early $CHCl_3$–MeOH (97.5:2.5) eluates on rechromatography over Si gel followed by crystallization from $CHCl_3$–petrol furnished pinnatin (**7**) as light yellow needles, mp 176°; yellow colouration with Mg/HCl; λ_{max}^{EtOH} nm (log ε): 221 (4.57), 270 (4.49) and 305 (4.16) [lit [3] 269 (4.52) and 305 (4.19)]; ν_{max}^{KBr} cm^{-1}: 3100 (w), 1632 (conjugated C=O), 1468, 1368, 1337, 1147, 1070 (benzofuran), 840 and 763; ^{1}H NMR (80 MHz, $CDCl_3$): δ 4.18 (3H, *s*, $-OCH_3$), 6.7 (1H, *s*, H-3), 7.05 (1H, *dd*, $J_{3'',2''}$ = 2.2 Hz and $J_{8,3''}$ = 0.9 Hz, H-3''), 7.39 (1H, *br s*, H-8), 7.48–7.56 (3H, *m*, H-3', H-4' and H-5'), 7.63 (1H, *d*, $J_{2'',3''}$ = 2.2 Hz, H-2''), 7.88–7.98 (2H, *m*, H-2' and H-6'); M^+ 292.

Isolation of aurantiamide acetate (**12**). The residue obtained from the later $CHCl_3$–MeOH (97.5:2.5) eluates on rechromatography over Si gel followed by crystallization from $CHCl_3$-petrol furnished aurantiamide acetate (**12**) as fine colourless needles, mp 185–186°, $[\alpha]_D^{30}$ −63.1° ($CHCl_3$, *c* 0.062); λ_{max}^{EtOH} nm (log ε): 206 (4.4) and 227 (*sh.* 4.09); ν_{max}^{KBr} cm^{-1}: 3292 (N—H), 1727, 1660, 1632, 1540 (br), 1220, 1050 and 700; ^{1}H NMR (80 MHz, $CDCl_3$) and MS are similar to those reported in refs [14–16].

Chromatography of fraction B and fraction C. Fraction B yielded aurantiamide acetate (**12**) and pinnatin (**7**) and fraction C yielded all the constituents of fraction A except aurantiamide acetate. Yields of the constituents of the three fractions are presented in Table 1.

5-Hydroxyfurano (6, 7-4'', 5'') *flavone* (**2**). A soln of pinnatin (**7**) (20 mg) in Et_2O (30 ml) was treated at 0° with a soln of dry $AlCl_3$ (150 mg) in Et_2O (5 ml) and the mixture kept at room temp. for 48 hr. The complex was decomposed

Table 1. Yields of constituents from flowers of *Pongamia glabra* (mg/100 g dry wt)

Fraction	**1**	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**11**	**12**	β-Sitosterol
A	0.53	41.4	13.3	6.6	0.93	1.2	0.53	30.0	3.7	3.7	6.8
B	—	—	—	—	3.7	—	—	—	—	14.7	—
C	0.4	26.6	14.7	5.3	4.6	1.8	1.6	13.3	4.14	—	6.4

with ice and dil HCl, warmed at 100° for 30 min and extracted with $CHCl_3$. The yellow solid obtained after removal of the solvent was chromatographed over Si gel. Petrol–C_6H_6 (1:2) elution afforded 5-hydroxyfurano (6, 7–4″,5″) flavone (**2**), crystallizing from $CHCl_3$–petrol as yellow needles (10 mg), mp 200–201°, mmp with pongaglabol 165–170°; λ_{max}^{EtOH} nm (log ϵ): 214 (4.48), 260 (4.39) and 278 (4.56); $\lambda_{max}^{EtOH+AlCl_3}$ nm (log ϵ): 213 (4.44), 268 (4.33) and 295 (4.52); ν_{max}^{KBr} cm^{-1}: 1630, 1470, 1348, 1148, 1063, 800, 758 and 713, non-superimposable with that of pongaglabol (**1**): ^{1}H NMR (80 MHz, $CDCl_3$): δ 6.72 (1H, *s*, H-3), 7.02 (1H, *dd*, $J_{3'',2''} = 2.2$ and $J_{8,3''} = 0.9$ Hz, H-3″), 7.17 (1H, *br s*, H-8), 7.53–7.59 (4H, *m*, H-2″, H-3′, H-4′ and H-5′), 7.89–7.98 (2H, *m*, H-2′ and H-6′), 13.61 (1H, *s*, exchangeable with D_2O, chelated 5-OH).

Pongaglabol Me ether (**6**). Pongaglabol (**1**) (5 mg) was refluxed in Me_2CO with Me_2SO_4 (0.1 ml) in the presence of dry K_2CO_3 (0.2 g) for 10 hr. The light yellow solid obtained on working up the product in the usual way was chromatographed over Si gel. $CHCl_3$–MeOH (49:1) elution afforded pongaglabol Me ether (**6**) crystallizing from $CHCl_3$–petrol in light yellow needles (3 mg), mp 181–182° (lit. [13] 182–183°), mmp with pinnatin 140–150°; λ_{max}^{EtOH} nm (log ϵ): 221.5 (4.51), 228 (4.50), 256 (4.37), 274 (4.49) and 321 (sh, 3.76) (lit. [13] 271 and 320 nm); ν_{max}^{KBr} cm^{-1}: 1640, 1400, 1303, 1140, 1108, 1065 and 765; ^{1}H NMR (80 MHz, $CDCl_3$) δ 4.0 (3H, *s*, $-OCH_3$), 6.80 (1H, *s*, H-3), 7.01 (1H, *d*, $J_{3'',6} = 0.9$ Hz, H-6), 7.09 (1H, *dd*, $J_{3'',2''} = 2.3$ Hz and $J_{6,3''} = 0.9$ Hz, H-3″), 7.49–7.61 (3H, *m*, H-3′, H-4′ and H-5′), 7.64 (1H, *d*, $J_{2'',3''} = 2.3$ Hz, H-2″), 7.92–8.0 (2H, *m*, H-2′ and H-6′).

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