

PUMILAIISOFLAVONES A AND B FROM THE SEED PODS OF *TEPHROSIA PUMILA*

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Abstract—Three flavonoids have been isolated from the pods of *Tephrosia pumila* collected in Ethiopia. The major compound was identified as the known β -oxygenated chalcone praecansone-A and attention is drawn to the need to revise the structure of this compound. The minor compounds have been characterized as novel isoflavones; pumilaisoflavone A (5-hydroxy-3',5'-dimethoxy-4'-(1'',1''-dimethylallyloxy)-2'',2''-dimethylpyrano[5'',6'':6,7]isoflavone) and pumilaisoflavone B (5-hydroxy-2''-isopropenyl-3',5'-dimethoxy-4'-(1'',1''-dimethylallyloxy)-2'',3''-dihydrofuro[4'',5'':6,7]isoflavone).

INTRODUCTION

Tephrosia pumila (Lam.) Pers. (Leguminosae, Papilionoideae) is a small annual or short-lived perennial herb of pan-tropical distribution [1, 2]. As far as we can ascertain this species has not previously been investigated. In this paper we report the results of an examination of the whole pods with the isolation of three flavonoids. One of these was identified as praecansone A, to which the structure 1 has been assigned [3]. On the basis of spectroscopic evidence the structure of this compound must be revised to 2. The other two compounds were characterized as novel isoflavones and have been assigned the trivial names of pumilaisoflavone A (3) and pumilaisoflavone B (4).

RESULTS AND DISCUSSION

Three flavonoids were isolated from a chloroform extract of the pods by a combination of column chromatographic techniques. The major component gave spectroscopic data identical to that reported for praecansone A [3]. A reinterpretation of ^1H NMR and the application of additional ^{13}C NMR data are in accord with structure 2 rather than 1*. The two major factors supporting this change are the ^{13}C NMR chemical shifts for C-1 (139.9 ppm) and C-4 (131.2 ppm) which indicate a benzoyl moiety and the occurrence of the base peak m/z 349 (5) in the EIMS, a rearrangement that can be envisaged from 2 but not 1. These findings support Pelter *et al.* [4] who suggested this revision but did not have access to authentic praecansone A. It is interesting to note that the related demethylpraecansone A isolated from *Lonchocarpus costaricensis* [5] showed ^{13}C NMR chemical shifts for C-1 and C-4 consistent with the 7-hydroxy tautomer (6) rather than the 9-hydroxy tautomer (7) (C-1 135.0; C-4

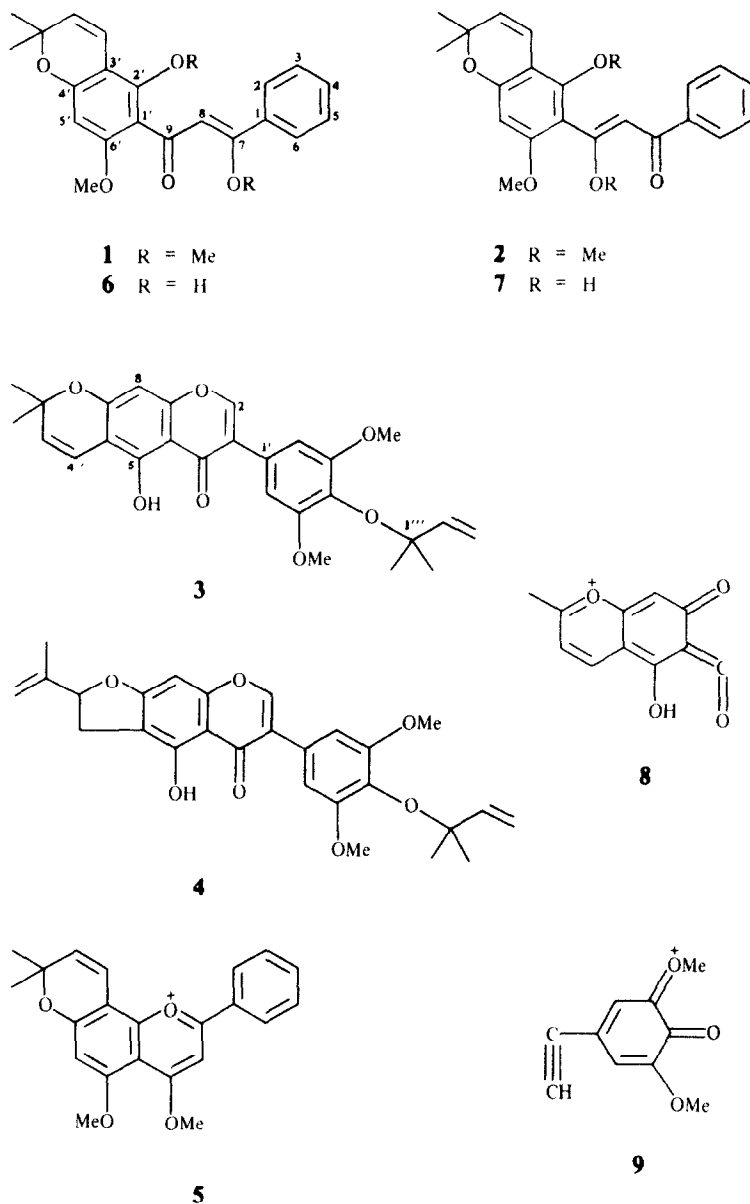
134.0 ppm; compare with values for structure 8 in ref. [4]).

Pumilaisoflavone A (3) analysed for $\text{C}_{27}\text{H}_{28}\text{O}_7$ and gave a UV spectrum typical of an isoflavone [4]. The ^1H NMR spectrum showed signals for 2,2-dimethylpyran, 1,1-dimethylallyloxy and two methoxyl substituents. The remaining signals were for aromatic protons at δ 7.86 (H-2 of isoflavone), δ 7.1 (2 equivalent H-ar), δ 6.33 (H-6 or H-8) and an H-bonded hydroxyl (δ 13.13 for 5-OH). The presence of the 2,2-dimethylpyran system was supported by a base peak at m/z 381 $[\text{M} - \text{Me}]^+$ and the dimethylallyloxy substituent by loss of $[\text{C}_5\text{H}_8]^+$ as a major fragment. The equivalent protons at δ 6.71 suggested a symmetrical B-ring, which is only possible if there is a 3',5'-dimethoxy-4'-(1',1'-dimethylallyloxy) or a 2',6'-dimethoxy-4'-(1',1'-dimethylallyloxy) system. This requires the 2,2-dimethylpyran system to be placed on ring-A between C-6 and C-7 (linear) or C-7 and C-8 (angular). Ions at m/z 203 (8) and 177 (9) support these assignments [6].

Of the two possible substitution patterns for ring B 3',4',5' is indicated by the relatively deshielded resonance for C-4' (134.7 ppm) which requires that the *ortho* positions also be oxygenated. The single A-ring aromatic proton at δ 6.33 is somewhat more deshielded than normal for H-6 [7] but not sufficiently deshielded to be unambiguously placed at H-8. By contrast the resonance for the tertiary A-ring carbon (94.7 ppm) is typical of C-8 [8] whereas a tertiary C-6 in 8-substituted 5-hydroxyflavones and isoflavones normally appears in the range of 97–98 ppm [9, 10]. An attempt of acetylate the 5-hydroxyl was not successful but further support for the proposed structure came from a positive Gibbs test [11] which requires that the 8-position (*para* to the 5-hydroxyl) be unsubstituted.

Pumilaisoflavone B (4), isolated in trace amounts only, gave the same empirical formula as 3. The ^1H NMR spectrum revealed an isoflavone with identical B ring substitution to 3 but lacking 2,2-dimethylpyran ring sig-

*The numbering system adopted for 1 has been retained for 2 to avoid confusion



nals. These were replaced by an ABX system for a $\text{CH}_2\text{CH}(\text{O})$ moiety together with signals at δ 1.87 (3H), 4.88, 4.99 (1H each) for an isopropenyl unit. Together these signals can be assigned to a prenyl-derived dihydrofuran. One noteworthy feature of **4** was the deshielding of H-8 to δ 6.48, typical of a linear 6-prenylated, 5,7-oxygenated isoflavone [7].

Pumilaiflavones A and B are unusual in two respects. In contrast to some allied genera of the Tephrosia isoflavones appear to be comparatively uncommon to *Tephrosia* [12]. Similarly A-ring prenylation of African *Tephrosia* flavonoids usually appears to involve C-8 rather than C-6 [13].

EXPERIMENTAL

Plant material. Pods of *Tephrosia pumila* were collected from Gibey Valley, Shoa Province, Ethiopia in August 1986. A

voucher specimen, MF-138, has been deposited at the Herbarium of Department of Botany, University of Addis Ababa.

Extraction and isolation of flavonoids. Powdered pods (300 g) were extracted using petrol (bp 60–80°) and then CHCl_3 . On concn the CHCl_3 extract gave 6 g of solid which was subjected to CC over silica gel. The fraction eluted with benzene–EtOAc (9:1) was then applied to a Sephadex LH-20 column. Elution of the Sephadex column with CHCl_3 –MeOH (9:1) gave a yellow oil which on addition of MeOH produced a ppt. Concn of the filtrate followed by prep TLC on silica gel (solvent, toluene–hexane–EtOAc 3:4:3) gave **2** (50 mg). Identical prep TLC treatment of the ppt gave **3** (11 mg). A second fraction from the original silica gel column, eluted with C_6H_6 –EtOAc (3:2), gave **4** (6 mg).

Praecansone-A (2) Yellow oil. UV, IR [3]. Found M^+ 380.1627, $\text{C}_{23}\text{H}_{24}\text{O}_5$ requires 380.1624. ^1H NMR (360 MHz, CDCl_3) δ 7.81 (2H, dd, $J=8, 2$ Hz, H-2, H-6), 7.42–7.30 (3H, m, H-3, H-4, and H-5), 6.45, 5.43 (2H, ABq, $J=9.6$ Hz, H-4'', H-3''),

6.40 (1H, s, H-8), 6.13 (1H, s, H-5'), 3.85, 3.69, 3.64 (3 × 3H, 3 × s, 2'-OMe, 6'-OMe and 9-OMe), 1.39 (6H, s, 2''-Me₂). ¹³C NMR (90.56 MHz, CDCl₃): *q* at 27.8 (2''-Me₂), 55.6, 55.8 (9-OMe, 6'-OMe), 61.9 (2'-OMe); *d* at 96.0 (C-5'), 101.4 (8-C), 116.7 (C-3''), 126.7 (C-4''), 127.6, 127.8, (C-2, C-3, C-5, C-6), 131.2 (C-4); *s* at 76.3 (C-2''), 107.5 (C-1'), 111.5 (C-3'), 139.9 (C-1), 154.6, 155.6 (C-2', C-6'), 157.7 (C-4'), 165.7 (9-C), 189.9 (7-C). EIMS *m/z* (rel. int.). 380 (9), 365 (25), 349 (100).

Pumilaiflavone A (3). Needles, mp 197–200°. Found: M⁺ 464.1823; C₂₇H₂₈O₇ requires 464.1835. UV λ_{max}nm: 280. IR ν_{max}cm⁻¹: 1630. ¹H NMR (360 MHz, CDCl₃) δ 13.13 (1H, s, 5-OH), 7.86 (1H, s, H-2), 6.72, 5.61 (2H, ABq, *J* = 10.1 Hz, H-4'', H-3''), 6.71 (2H, s, H-2', H-6'), 6.33 (1H, s, H-8), 6.22 (1H, *dd*, *J* = 17.5, 10.8 Hz, H-2''), 5.11 (1H, *dd*, *J* = 17.5, 1.2 Hz, H-3'''), 4.98 (1H, *dd*, *J* = 10.8, 1.2 Hz, H-3'''), 3.82 (6H, s, 3'-OMe, 5'-OMe), 1.47, 1.46 (2 × 6H, 2 × s, 2''-Me₂, 1'''-Me₂). ¹³C NMR (90.56 MHz, CDCl₃): *q* at 26.4 (1'''-Me₂), 28.2 (2''-Me₂), 55.9 (3'-OMe, 5'-OMe), *t* at 111.9 (C-3''); *d* at 94.7 (C-8), 106.3 (C-2', C-6'), 115.3 (C-3'), 128.1 (C-4''), 144.1 (C-2''), 152.9 (C-2), *s* at 78.0 (C-2''), 82.5 (C-1'''), 105.7, 106.0 (C-4a, C-6), 123.7 (C-3), 126.2 (C-1'), 134.7 (C-4'), 155.1 (C-3', C-5'), 156.9, 157.1 (C-5, C-8a), 159.7 (C-7), 180.6 (C-4). EIMS *m/z* (rel. int.): 464 [M]⁺ (9), 449 (5), 396 [M - C₅H₈]⁺ (86), 381 (100), 203 (4), 177 (2), 146 (2). Gibbs test. **3** (1 mg) was dissolved in borax buffer (pH 9.2, 10 ml) and an aq. soln of 2,6-dichloro-*p*-benzoquinone-4-chlorimine (10 drops) added. The development of a blue colour indicated a positive reaction.

Pumilaiflavone B (4). Cubes from CHCl₃, mp 126–129°. Found: M⁺ 464.1843; C₂₇H₂₈O₇ requires 464.1835. UV λ_{max}nm: 280. IR ν_{max}cm⁻¹: 1620. ¹H NMR (250 MHz, CDCl₃): δ 13.26 (1H, s, 5-OH), 7.89 (1H, s, H-2), 6.72 (2H, s, H-2', H-6'), 6.48 (1H, s, H-8), 6.24 (1H, *dd*, *J* = 17.6, 11.0 Hz, H-2''), 5.12 (1H, *dd*, *J* = 17.6, 1.0 Hz, H-3''' *trans*), 4.99 (1H, *dd*, *J* = 11.0, 1.0 Hz, H-3''' *cis*), 4.88, 4.99 (2 × 1H, 2 × *br s*, =CH₂), 4.44 (1H, *br d*, *J* = 7 Hz, H=2''), 3.83 (6H, s, 3'-OMe, 5'-OMe), 3.18 (1H, *dd*, *J* = 15.1, 2.2 Hz, H-3''), 2.94 (1H, *dd*, *J* = 15.1, 7.8 Hz, H-3''), 1.87 (3H, s, Me-C=), 1.47 (6H, s, 1'''-Me₂). EIMS

m/z (rel. int.). 464 [M]⁺ (4), 396 [M - C₅H₈]⁺ (13), 381 (19), 343 [C₁₈H₁₅O₇]⁺ (100).

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REFERENCES

- Gillet, J. (1971) *Flora of Tropical East Africa—Leguminosae, Papilionoideae*, p. 184, H.M.S.O., London.
- Thulin, M. (1983) *Opera Botanica* **68**, 78.
- Camele, G., Delle Monache, F., Delle Monache, G. and Marini-Bettolo, G. B. (1980) *Phytochemistry* **19**, 707.
- Pelter, A., Ward, R. S., Venkata Rao, E. and Ranga Raju, N. (1981) *J. Chem. Soc., Perkin Trans. I*, 2491.
- Waterman, P. G. and Mahmoud, E. N. (1985) *Phytochemistry* **24**, 571.
- Mabry, T. J. and Markham, K. R. (1975) *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds), p. 78. Chapman & Hall, London.
- Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Berlin.
- Markham, K. R. and Chari, V. M. (1982) *Recent Advances in Flavonoid Research 1975–1980* (Harborne, J. B. and Mabry, T. J. eds). Chapman & Hall, London.
- Oshima, Y., Konno, C., Hikino, H. and Matsushita, K. (1980) *Tetrahedron Letters* **21**, 3381.
- Osterdahl, B.-G. (1978) *Acta Chem. Scand.* **32B**, 93.
- Gibbs, H. D. (1927) *J. Biol. Chem.* **72**, 649.
- Gomes, C. M. R., Gottlieb, O. R., Marini-Bettolo, G. B., Delle Monache, F. and Polhill, R. M. (1981) *Biochem. Syst. Ecol.* **9**, 129.
- Waterman, P. G. and Khalid, S. A. (1980) *Phytochemistry* **19**, 909.