FLAVONOIDS FROM THE SEED PODS OF TEPHROSIA PUMILA

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Abstract—In addition to the known pumilaisoflavones A and B two further isoflavonoids have been isolated from the seed pods of Tephrosia pumila collected in Ethiopia. These have been characterized, on the basis of spectral analysis, as pumilaisoflavone C (5,7,4'-trihydroxy-3',5'-dimethoxy-6,2'-d(3,3-dimethylallyl)isoflavone) and pumilaisoflavone D (5,4'-dihydroxy-3',5'-dimethoxy-2',2'-dimethylpyrano[5',6'; 6,7]isoflavone)

INTRODUCTION

Tephrosia pumila (Lam.) Pers. (Leguminosae, Papilionoideae) is a small annual or short-lived perennial herb of pan-tropical distribution [1]. In a previous study [2] we reported the presence in the seed pods of two new isoflavones, pumilaisoflavone A (1) and pumilaisoflavone B (2) together with the known β-oxygenated chalcone praecansone A. We have now had the opportunity to examine the seed pods of a further collection of T. pumila and in addition to 1, 2, praecansone A and an isomer of praecansone A, which is still under investigation, we obtained two further isoflavones of the pumilaisoflavone series. The identification of these new isoflavones, named pumilaisoflavone C and pumilaisoflavone D, is reported here.

RESULTS AND DISCUSSION

Extraction of the seed pods of T. pumila with petrol followed by chromatographic separation yielded 1, 2, praecansone A and an isomer of praecansone A. Similar treatment of the chloroform extract of the defatted seed pods gave more praecansone A, 2 and two further isoflavones.

The less polar of the new compounds analysed for C27H30O7 and gave the typical UV spectrum of an isoflavone. The 1H NMR spectrum showed the presence of a chelated 5-OH, two further hydroxyl, two methoxyl and two 3,3-dimethylallyl substituents. Of the three unsubstituted positions on the isoflavone nucleus H-2 (δ 7.94) and H-8 (δ 6.36) were indicated by analogy with 1 [2]. The 13C NMR spectrum revealed a pattern of oxygenation identical to 1 [2]. Thus, on comparison with 1, the presence of an additional 3,3-dimethylallyl unit, the absence of the 1,1-dimethylallyloxy substituent and the loss of one unsubstituted position together with the introduction of asymmetry into the resonances for ring B, suggested structure 3. This was substantiated by the EIMS which revealed several ions for the fragmentation of the 3,3-dimethylallyl side-chains and significant fragments at m/z 191 (3a) and m/z 165 (3b) which could be assigned to rings B and A, respectively. The positions of the methoxyl substituents was resolved by means of an NOE study in which irradiation of the δ 3.86 methoxyl signal led to an enhancement of 16% in H-6. By contrast irradiation of the δ 3.51 methoxyl gave only small enhancement for the methylene and methine resonances of one of the 3,3-dimethylallyl units. Compound 3 has been assigned the trivial name pumilaisoflavone C.

The second new compound analysed for C22H26O7. The 1H NMR spectrum revealed the presence of a 2,2-dimethylpyrano ring system, a single A-ring proton (δ 6.34, H-8), a chelated 5-hydroxy substituent and a symmetrical 4'-hydroxy-3',5'-dimethoxy substituted B-ring. These data indicated that this compound was directly comparable to 1 except for the absence of the 1,1-dimethylallyl substituent on C-4'. This conclusion was supported by the 13C NMR spectrum which showed close correspondence to 1 except for C-1', C-3' and C-5' and the EIMS which revealed the anticipated ions at m/z 203 (4a) and m/z 178 (4b). Thus, this compound can be assigned structure 4 and the trivial name of pumilaisoflavone D.

The isolation of pumilaisoflavone C (3) sheds some light on the likely biosynthetic route to pumilaisoflavones A (1) and B (2). In both 1 and 2 the presence of the 4' 1,1-dimethylallyloxy substituent can be attributed to a Claisen rearrangement of the 2'-3,3-dimethylallyl moiety of 3 while the C-6 prenyl substituent of 3 has undergone different cyclisation reactions to give the 2,2-dimethylpyrano system in 1 and the 2-isopropenylidihydrofuran system in 2, respectively. Pumilaisoflavone D (4) can be generated by hydrolysis of 1.
then with CHCl₃ for 5 days. Evapn of the petrol yielded an oily
residue which was taken up in Me₂CO and filtered. The Me₂CO
soluble fraction was chromatographed on silica gel (100 g)
eluting with petrol-EtOAc mixtures of increasing polarity. Forty
100 ml fractions were collected. Fractions 10/20 (5% EtOAc)
gave 1 (7 mg), fractions 21/30 (10% EtOAc) contained a mixture
which was separated by circular PTLC on silica gel (solvent,
petrol-C₆H₆-EtOAc 3:2:1) to give praeunone A (300 mg) and a
second β-oxygenated chalcone (24 mg). Fractions 31/40 (15% 
EtOAc) gave 2 (16 mg).

The CHCl₃ extract was first applied to a Sephadex LH-20
column and eluted with CHCl₃-MeOH (1:1) to remove chloro-
phyll and fatty materials. The residue was treated by column
chromatography in an identical manner to the petrol extract
Fractions 13/14 (10% EtOAc) showed one major spot purified
by PTLC (system as above) to give 3 (5 mg). Fractions 15/19
(10% EtOAc) gave further praeunone A (150 mg), fractions
20/25 (15% EtOAc) yielded 2 (6 mg) and fractions 26/31 (15%
EtOAc) afforded 4 (20 mg).

Pumlasioflavone C (3). Needles from MeOH, mp 180-182°
Found [M]+ 466 1930; C₁₇H₁₃O₆ requires 466 1991 UV λmax (nm)
263 (4.47), 290 (4.15), 340 sh (3.38) IR νmax cm⁻¹
3600-3200 (OH), 1660 (CO). 1H NMR (360 MHz, CDCl₃)
δ 1.20 (3H, s, 5-OH), 7.93 (1H, s, H-2), 6.74 (1H, s, H-6'), 6.56 (1H,
s, OH), 6.36 (1H, s, H-8), 5.86 (1H, s, OH), 5.26 (2H, 2 x d, J
= 7 Hz, H-1', H-1''), 3.86 (3H, s, 5'-OMe), 3.51 (3H, s, 3'-OMe),
3.47 (4H, 2 x d, J = 7 Hz, CH₂-2', CH₂-2''), 1.84, 1.78, 1.71, 1.69
(4 x 3H, 4 x s, 2''-Me₂, 2'''-Me₂) 13C NMR (22.5 MHz, CDCl₃)
ppm δ at 17.7 (C-2), 23.5, 25.6 (3''-Me₂, 3'''-Me₂), 56.1 (C-3''-Me),
61.2 (3''-OMe), δ at 21.4, 23.4 (C-1'', C-1'''), δ at 93.4 (C-8), 113.3
(C-6'), 121.7, 122.5 (C-2'', C-2'''), 154.7 (C-2), δ at 105.5 (C-4a),
111.6 (C-6, C-2), 114.3 (C-1'), 120.0 (C-3), 131.6, 132.7 (C-3'',
C-3''), 142.9 (C-4), 144.6 (C-5), 150.9 (C-3'), 151.5, 159.1, 161.6 (C-5,
C-7, C-8), 181.0 (C-4) EIMS m/z (rel int) 466 [M]+ (100), 435
[M-OMe]+ (19), 424 [M-C₆H₆]⁺ (4), 411 [M-C₆H₅]+ (34),
395 [M-C₆H₅-Me-Me]⁺ (17), 379 [M-C₆H₅-OMe]⁺ (14),
245 (4), 191 (13), 165 (14).

Pumlasioflavone D (4). Needles from MeOH, mp 174-176°
Found [M]+ 396 1209; C₁₉H₁₆O₅ requires 396 1209 UV λmax (nm)
280 (4.47) IR νmax cm⁻¹
3600-3300 (OH), 1660 (CO). 1H NMR (360 MHz, CDCl₃)
δ 13.15 (1H, s, 5-OH), 7.84 (1H, s, H-2), 6.74, 5.63 (2H, ABq, J
= 10 Hz, H-4'', H-3''), 6.74 (2H, s, H-2', H-6'), 6.34 (1H, s, H-8),
3.94 (6H, s, 3'-OMe, 5'-OMe), 1.48 (6H, s, 2''-Me₂) 13C NMR (22.5 MHz, CDCl₃)
ppm δ at 28.4 (2''-Me₂), 56.5 (3''-OMe, 5'-OMe), δ at 94.8 (C-8), 106.2 (C-2', C-6'),
115.5 (C-3''), 128.1 (C-4'), 152.6 (C-2), δ at 78.0 (C-2''), 105.6 (C-
4a, C-6), 128.8 (C-3'), 133.9 (C-1'), 135.4 (C-4'), 147.1 (C-5'),
157.1, 157.2, 157.5 (C-5, C-7, C-8), 180.8 (C-4) EIMS m/z (rel
int) 396 [M]+ (45), 381 [M-Me]+ (100), 203 [C₁₁H₈O₆]⁺
(11), 178 [C₁₁H₆O₄]⁺ (12).

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EXPERIMENTAL.

Plant material. Pods of Tephrosia punica were collected from
Gibe Valley, Ethiopia, in September 1987. For authentication
of material see ref. [2].

Extraction and isolation. Ground pods of T. punica (1 kg) were
extracted by percolation with petrol (bp 60-80°C) for 3 days and
then with CHCl₃ for 5 days. Evapn of the petrol yielded an oily
residue which was taken up in Me₂CO and filtered. The Me₂CO
soluble fraction was chromatographed on silica gel (100 g)
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100 ml fractions were collected. Fractions 10/20 (5% EtOAc)
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