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FLAVONOIDS FROM THE SEED PODS OF *TEPHROSIA PUMILA*

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Key Word Index—*Tephrosia pumila*, Leguminosae, Papilionoideae, isoflavones, pumilaisoflavone-C, pumilaisoflavone-D

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′-di(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 $C_{27}H_{30}O_7$ and gave the typical UV spectrum of an isoflavone. The ^{1}H 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 (δ7.94) and H-8 (δ6.36) were indicated by analogy with **1** [2]. The ^{13}C 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 δ3.86 methoxyl signal led to an enhancement of 16% in H-6′ at δ6 74. By contrast irradiation of the δ3 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 $C_{22}H_{20}O_7$. The ^{1}H NMR spectrum revealed the presence of a 2,2-dimethylpyrano ring system, a single A-ring proton (δ6 34, H-8), a chelated 5-hydroxyl 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 ^{13}C 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-isopropenyldihydrofuran system in **2**, respectively. Pumilaisoflavone D (**4**) can be generated by hydrolysis of **1**.

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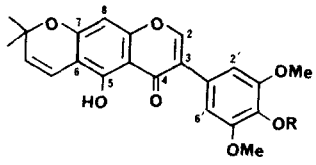
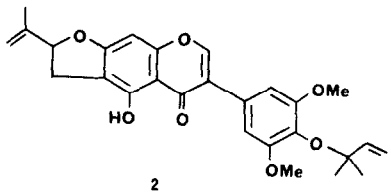


1 R = $C(Me)_2CH=CH_2$
4 R = H



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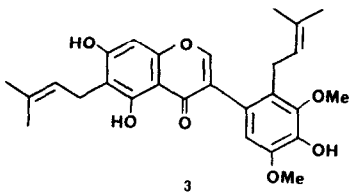

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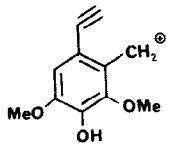

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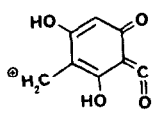

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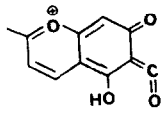

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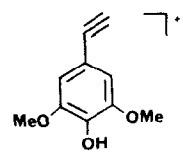

4b

EXPERIMENTAL

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

Extraction and isolation Ground pods of *T pumila* (1 kg) were extracted by percolation with petrol (bp 60–80°) for 3 days and then with $CHCl_3$ for 5 days Evapn of the petrol yielded an oily residue which was taken up in Me_2CO and filtered The Me_2CO-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–C_6H_6–EtOAc 3 2 1) to give praecansone A (300 mg) and a second β-oxygenated chalcone (24 mg) Fractions 31/40 (15% EtOAc) gave **2** (16 mg)

The $CHCl_3$ extract was first applied to a Sephadex LH-20 column and eluted with $CHCl_3$–MeOH (1 1) to remove chlorophyll 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** (25 mg). Fractions 15/19 (10% EtOAc) gave further praecansone A (150 mg), fractions 20/25 (15% EtOAc) yielded **2** (6 mg) and fractions 26/31 (15% EtOAc) afforded **4** (20 mg)

Pumilaisoflavone C (**3**). Needles from MeOH, mp 180–182° Found $[M]^+$ 466 1930; $C_{27}H_{30}O_7$ requires 466 1991 UV λ_{max} nm (log ε) 263 (4 47), 290 (4 15), 340 sh (3 38) IR ν_{max} cm^{-1} 3600–3200 (OH), 1660 (CO) ^1H NMR (360 MHz, $CDCl_3$) δ 13 20 (1H, *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 × *t*, *J* = 7 Hz, H-1'', H-1'''), 3 86 (3H, *s*, 5'-OMe), 3.51 (3H, *s*, 3'-OMe), 3 47 (4H, 2 × *d*, *J* = 7 Hz, CH_2-2'', CH_2-2'''), 1 84, 1 78, 1 77, 1 69 (4 × 3H, 4 × *s*, 2''-Me_2, 2'''-Me_2 ^{13}C NMR (22 5 MHz, $CDCl_3$) ppm *q* at 17 9 (2C), 25 5, 25 6 (3''-Me_2, 3'''-Me_2), 56 1 (5'-OMe), 61 2 (3'-OMe), *t* at 21 4, 23 4 (C-1'', C-1'''), *d* at 93 4 (C-8), 111 3 (C-6'), 121 7, 122 5 (C-2'', C-2'''), 154 7 (C-2), *s* 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'), 155 5, 159 1, 161 6 (C-5, C-7, C-8a), 181 0 (C-4) EIMS *m/z* (rel int) 466 $[M]^+$ (100), 435 $[M-OMe]^+$ (19), 424 $[M-C_3H_7]^+$ (4), 411 $[M-C_4H_7]^+$ (34), 395 $[M-C_4H_7-Me-H]^+$ (17), 379 $[M-C_4H_7-OMe]^+$ (14), 245 (4), 191 (13), 165 (14)

Pumilaisoflavone D (**4**) Needles from MeOH, mp 174–176 Found $[M]^+$ 396 1189, $C_{22}H_{20}O_7$ requires 396 1209 UV λ_{max} nm (log ε) 280 (4 47) IR ν_{max} cm^{-1} 3600–3300 (OH), 1660 (CO) ^1H NMR (360 MHz, $CDCl_3$) 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''-Me_2) ^{13}C NMR (22 5 MHz, $CDCl_3$) ppm *q* at 28 4 (2''-Me_2), 56 5 (3'-OMe, 5'-OMe), *d*, at 94 8 (C-8), 106 2 (C-2', C-6'), 115 5 (C-3''), 128 1 (C-4''), 152 6 (C-2), *s*, 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-3', C-5'), 157.1, 157 2, 159 5 (C-5, C-7, C-8a), 180 8 (C-4) EIMS *m/z* (rel int) 396 $[M]^+$ (45), 381 $[M-Me]^+$ (100), 203 $[C_{11}H_7O_4]^+$ (11), 178 $[C_{10}H_{10}O_3]^+$ (2)

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2 Dagne, E, Dinku, B, Gray, A I and Waterman, P G (1988) *Phytochemistry*, **27**, 1503