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FLAVONOIDS OF *TEPHROSIA POLYPHYLLA*

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Key Word Index—*Tephrosia polyphylla*; Papilionoideae; Leguminosae; 4'-demethyltoxicarol isoflavone; pyranoisoflavones; flavanone.

Abstract—The roots of *Tephrosia polyphylla* afforded a new isoflavonoid, 4'-demethyltoxicarol isoflavone, together with the known compounds toxicarol isoflavone and 7-methylglabranin.

INTRODUCTION

As part of our continued interest in the phytochemical studies of *Tephrosia* species from Ethiopia [1–4], we have investigated the constituents of *T. polyphylla*. Three compounds, namely 4'-demethyltoxicarol isoflavone (**1**), toxicarol isoflavone (**2**) and 7-methylglabranin (**5**), were isolated and characterized on the basis of their spectral data, and by chemical transformations in the case of **1**. Compound **1** is a new natural product although it has been synthesized recently by Tsukayama *et al.* [5].

RESULTS AND DISCUSSION

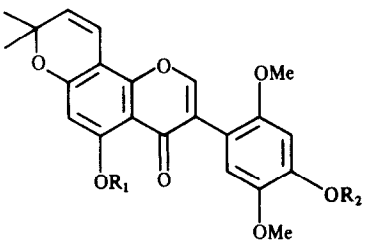
An ethanolic root extract of *T. polyphylla* afforded **1** and the known compounds toxicarol isoflavone (**2**) and 7-methylglabranin (**5**), after separation using silica gel 60 column chromatography.

Compound **1** analysed for $C_{22}H_{20}O_7$ (HRMS). The ^{1}H NMR spectrum confirmed the presence of an isoflavonoid nucleus with a 2',4',5'-substituted B ring, a dihydrodimethylpyran ring and a chelated hydroxyl group. The EIMS revealed a base peak for $[M-Me]^+$, typical of compounds with a 2,2-dimethylpyran system and a fragment ion at *m/z* 203 formed from an RDA fragmentation of **1** with the loss of ring B. Thus **1** must possess one hydroxyl and the 2,2-dimethylpyran substituent on ring A and one hydroxyl and two methoxyl substituents on ring B. That the pyran ring is angular was determined by converting **1** to the monomethyl ether **2** and the dimethyl ether **3**. Both **2** and **3** were identical to the previously reported [5] toxicarol isoflavone (**2**) and its methyl ether **3**, respectively. Moreover, a direct comparison of **1** with an authentic sample of the isomeric compound elongatin [6] proved the two to be different.

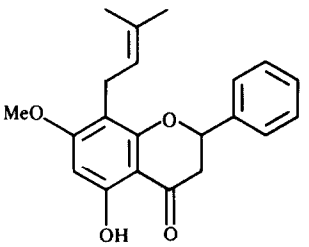
The substitution pattern on the B-ring was established by ^{1}H NMR experiments. Thus, comparison of the chemical shift of the 5-OH proton in **1** with that of the monomethyl derivative **2** showed no change. It has been demonstrated recently [7] that such a lack of difference in the chemical shift of the 5-OH resonance indicates the absence of a hydroxyl group at the 2'-position. Hence this allows the placement of one of the B-ring methoxyl groups at C-2'. Placement of the second methoxyl group at C-5' follows from an NOE experiment with **1**. Irradiation of the methoxyl signal at δ3.87 resulted in a 9% enhancement of the H-6' signal at δ6.88, thus establishing that the signal at δ3.87 was due to the 5'-OMe and consequently the signal at δ3.74 was attributed to the 2'-OMe. In agreement with this irradiation of the 2'-OMe signal (δ3.74) resulted in an 8% enhancement of the H-3' signal (δ6.66).

Consequently, **1** was characterized as 4',5-dihydroxy-2',5'-dimethoxy-2'',2''-dimethylpyrano-[6'',5''-h]isoflavone or 4'-demethyltoxicarol isoflavone and is reported here for the first time as a natural product.

Compound **2** had the composition $C_{23}H_{22}O_7$ as established by its HRMS. It was identified as toxicarol isoflav-



1 $R_1 = R_2 = H$
2 $R_1 = H$, $R_2 = Me$
3 $R_1 = R_2 = Me$
4 $R_1 = H$, $R_2 = Ac$



5

one on the basis of its spectral data and by comparison with literature values [5].

Compound **5** had a ^{1}H NMR spectrum characteristic of a flavanone. That the B-ring is unsubstituted and the A-ring contains C-prenyl, chelated hydroxyl and methoxyl groups was established from the ^{1}H and ^{13}C NMR spectra. Consequently, **5** was identified as 7-methylglabranin, a compound reported earlier from *Tephrosia villosa* [8].

EXPERIMENTAL

General. Mps were uncorr. ^{1}H NMR at 90 and 300 MHz, ^{13}C NMR at 22.5 MHz.

Plant material. Tephrosia polyphylla was collected 1 km from Melka Guba on the road to Negelle Borena at an altitude of 870 m and a voucher specimen (M. Gilbert 8686) was deposited in the National Herbarium of the Addis Ababa University.

Isolation of compounds from roots of T. polyphylla. Dried ground roots of *T. polyphylla* (500 g) were percolated with EtOH for 24 hr. Filtration and removal of the solvent yielded 26 g of crude extract. This was absorbed on silica gel, applied to a 300 g silica gel column and eluted with petrol with increasing amounts of EtOAc. A total of 33 frs were collected. Frs 5–7 were combined and rechromatographed on Sephadex LH-20 to afford 7-methylglabranin (**5**, 35 mg). Frs 10–13 afforded sitosterol. Frs 23–26 were combined and crystallization from MeOH provided 3 mg of toxicarol isoflavone (**2**). Frs 30–37 were pooled, solvent removed, the residue dissolved in MeOH giving a ppt of **1** (31 mg).

4′-Demethyltoxicarol isoflavone (**1**). Mp 178–180° (lit. [5] 173–175°); UV λ_{max}^{MeOH} nm: 247, 253, 260, 270, 300, 354; IR ν_{max}^{KBr} cm^{-1}: 3450, 1670, 1590, 1525, 1490, 1445, 1380, 1300, 1220, 1160, 1045; ^{1}H NMR (300 MHz, Me_2CO-d_6): δ1.47 (6H, *s*, Me), 3.72 (3H, *s*, 2′-OMe), 3.80 (3H, *s*, 5′-OMe), 5.75 (1H, *d*, J = 10 Hz, H-3″), 6.20 (1H, *s*, H-6), 6.64 (1H, *s*, H-3′), 6.72 (1H, *d*, J = 10 Hz, H-4″), 6.99 (1H, *s*, H-6′), 8.14 (1H, *s*, H-2), 13.15 (1H, *s*, 5-OH); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ154.8 (C-2), 120.6 (C-3), 189.8 (C-4), 105.8 (C-4a), 159.1 (C-5), 99.5 (C-6), 162.0 (C-7), 101.0 (C-8), 162.0 (C-8a), 110.1 (C-1′), 152.6 (C-2′), 101.0 (C-3′), 147.8 (C-4′), 141.3 (C-5′), 116.3 (C-6′), 77.9 (C-2″), 114.1 (C-3″), 127.3 (C-4″), 27.5 (C-5″, C-6″), 56.7 (OMe), 56.0 (OMe); HRMS *m/z* (rel. int.): 396.1211 [M]$^+$ (35) (calc. for $C_{22}H_{20}O_7$: 396.1203), 381 (100), 366 (5), 365 (2), 352 (5), 351 (31), 323 (11), 203 (3), 191 (4), 183 (3), 169 (5).

Methylation of **1**. Compound **1** was methylated using MeI–K_2CO_3 in dry Me_2CO. Leaving the reaction mixture at room temp. overnight resulted in toxicarol isoflavone (**2**) while refluxing for 3 hr yielded the dimethyl ether **3** as the major product.

Toxicarol isoflavone (**2**). Mp 213–214° (lit. [5] 219–220°); IR ν_{max}^{KBr} cm^{-1}: 1660, 1580, 1525, 1470, 1380, 1320, 1280, 1210, 1150, 1030; ^{1}H NMR (300 MHz, Me_2CO-d_6): δ1.48 (6H, *s*, Me), 3.77 (3H, *s*, OMe), 3.78 (3H, *s*, OMe), 3.88 (3H, *s*, OMe), 5.76 (1H, *d*, J = 9.9, H-3″), 6.21 (1H, *s*, H-6), 6.73 (1H, *d*, J = 9.9, H-4″), 6.81 (1H, *s*, H-3′), 6.98 (1H, *s*, H-6′), 8.16 (1H, *s*, H-2), 13.13 (1H, *s*, 5-OH); HRMS *m/z* (rel. int.): 410.1371 [M]$^+$ (63) (calc. for $C_{23}H_{22}O_7$: 410.1359), 395 (100), 379 (4), 381 (28), 365 (36), 351 (4), 337 (2), 326 (3), 253 (3), 203 (6), 190 (6), 176 (14).

2′,4′,5,5′-Tetramethoxy-2″,2″-dimethylpyrano-[6″,5″-h]isoflavone (**3**). Mp 179–180° (lit. [5] 179–180°); IR ν_{max}^{KBr} cm^{-1}: 1660, 1640, 1610, 1580, 1520, 1490, 1470, 1360, 1290, 1200, 1140, 1050, 1025; ^{1}H NMR (300 MHz, $CDCl_3$): δ1.49 (6H, *s*, Me), 3.74 (3H, *s*, OMe), 3.84 (3H, *s*, OMe), 3.92 (6H, *s*, 2 × OMe), 5.58 (1H, *d*, J = 10.2 Hz, H-3″), 6.32 (1H, *s*, H-6), 6.59 (1H, *s*, H-3′), 6.73 (1H, *d*, J = 10.2 Hz, H-4″) 6.95 (1H, *s*, H-6′), 7.80 (1H, *s*, H-2); MS *m/z* (rel. int.): 424 [M]$^+$ (100), 409 (99), 393 (70), 379 (31), 363 (5), 307 (3), 267 (3), 233 (4), 217 (12), 204 (26), 183 (18).

Acetylation of **1**. Compound **1** was acetylated using Ac_2O–pyridine following usual procedures to afford the monoacetate **4**. Mp 223–225°; IR ν_{max}^{KBr} cm^{-1}: 3500, 1780, 1670, 1590, 1520, 1500, 1460, 1440, 1380, 1320, 1300, 1220, 1190, 1140, 1025; ^{1}H NMR (90 MHz, $CDCl_3$): δ1.48 (6H, *s*, Me), 2.35 (3H, *s*, Ac), 3.75 (3H, *s*, OMe), 3.83 (3H, *s*, OMe), 5.60 (1H, *d*, J = 10 Hz, H-3″), 6.29 (1H, *s*, H-6), 6.67 (1H, *d*, J = 10 Hz, H-4″), 6.71 (1H, *s*, H-3′), 7.00 (1H, *s*, H-6′), 7.90 (1H, *s*, H-2), 12.83 (1H, *s*, 5-OH); HRMS *m/z* (rel. int.): 438.1319 [M]$^+$ (34) (calc. for $C_{24}H_{22}O_8$: 438.1308), 423 (33), 396 (33), 381 (100), 351 (18).

7-Methylglabranin (**5**). Mp 129–131° (lit. [8] 123–125°); IR ν_{max}^{KBr} cm^{-1}: 1635, 1600, 1505, 1470, 1460, 1380, 1310, 1285, 1220, 1180, 1100, 1080; ^{1}H NMR (300 MHz, $CDCl_3$): δ1.61 (3H, *s*, Me), 1.62 (3H, *s*, Me), 2.85 (1H, *dd*, J = 16.7, 3.6 Hz, H-3$_e$), 3.05 (1H, *dd*, J = 16.7, 12.5 Hz, H-3$_a$), 3.25 (2H, *d*, J = 7 Hz, H-1″), 3.85 (3H, *s*, OMe), 5.14 (1H, *t*, J = 7 Hz, H-2″), 5.42 (1H, *dd*, J = 12.5, 3.5 Hz, H-2), 6.10 (1H, *s*, H-6), 7.36–7.48 (5H, *m*, B-ring protons), 12.14 (1H, *s*, 5-OH); ^{13}C NMR (22.5 MHz, $CDCl_3$): δ78.8 (C-2), 21.7 (C-3), 196.0 (C-4), 103.2 (C-4a), 159.0 (C-5), 92.8 (C-6), 165.9 (C-7), 109.3 (C-8), 162.9 (C-8a), 131.0 (C-1′), 128.7 (C-2′), 126.0 (C-3′), 128.5 (C-4′), 126.0 (C-5′), 128.7 (C-6′), 43.4 (C-1″), 122.8 (C-2″), 139.2 (C-3″), 25.6 (C-4″), 17.6 (C-5″), 55.8 (OMe); MS *m/z* (rel. int.): 338 [M]$^+$ (100), 323 (63), 295 (26), 283 (15), 270 (23), 233 (12), 219 (76), 206 (25), 191 (49), 179 (34).

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