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NATURAL OCCURRENCE OF *TEPHROSIA* FLAVONES

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Key Word Index—*Tephrosia apollinea*; *T. semiglabra*; *T. purpurea*; *T. multijuga*; Leguminosae; semiglabrin; pseudosemiglabrin; lanceolatin A; semiglabrinol; pseudosemiglabrinol; multijugin; multijuginol.

Abstract—A new flavone, pseudosemiglabrinol has been isolated from *T. apollinea* along with semiglabrinol isolated for the first time from the same plant. ^{1}H NMR of some of the above mentioned flavones isolated from *Tephrosia semiglabra* and *T. multijuga* casts some doubt on their authenticity.

INTRODUCTION

During work on poisonous plants of Saudi Arabia, [1], we isolated the three known flavonoids semiglabrin (**1**), pseudosemiglabrin (**5**) and lanceolatin A [2] from the whole plant of *Tephrosia apollinea*. Also semiglabrinol (**2**), earlier reported from *T. semiglabra* [3], has been isolated from *T. apollinea* along with a new natural product to which structure **6** has been assigned on the basis of spectral evidence and named pseudosemiglabrinol. However, the ^{1}H NMR spectra of these flavones reported by some workers are not consistent with the proposed structures. This casts some doubt on the authenticity of these natural products [3]. Therefore, by direct comparison of ^{1}H NMR data of these flavones, we have also shown the true identity of these natural products.

RESULTS AND DISCUSSION

The plant material after defatting with petrol was extracted with chloroform. The chloroform extract yielded colourless needles, mp 244–248°. Spectral data of the natural product was in close agreement with the data reported for semiglabrin (**1**) by Waterman and Khalid [2]. The residue from the chloroform extract (after removal of

Table 1. ^{13}C NMR spectra semiglabrinol (**2**) and pseudosemiglabrinol (**6**) recorded in DMSO-d_6 and pseudosemiglabrin (**5**) recorded in $CDCl_3$

Carbon No.	Ahmad **6**	Ahmad **2**	Pelter **2**	Ahmad **5**	Rao and Raju **5**
2	162.12	161.6	162.1	162.6	162.86
3	106.39	106.58	106.9	107.66	107.59
4	176.60	176.30	177.1	177.4	177.54
5	126.36	127.06	127.4	129.14	128.70
6	111.38	112.69	112.8	111.82	111.80
7	164.18	163.02	163.5	164.80	164.60
8	113.68	114.82	114.5	111.53	111.51
9	153.71	152.56	153.0	153.9	153.85
10	117.74	117.72	118.2	118.46	118.71
1′	131.51	131.62	131.4	131.73	131.75
2′,6′	126.54	126.22	126.2	126.2	126.21
3′,5′	129.03	129.05	129.0	128.72	129.04
4′	131.51	131.10	131.4	131.46	131.39
2″	108.15	108.42	108.6	108.92	108.94
2‴	85.01	88.16	88.4	84.62	84.65
3‴	77.10	79.30	80.0	76.90	76.86
3″	48.79	54.37	54.8	48.01	47.97
Gem-Me2	22.92	23.09	23.2	23.2	23.15
	27.38	27.14	27.4	27.7	27.61
Ac				20.30	20.02
				169.76	169.81

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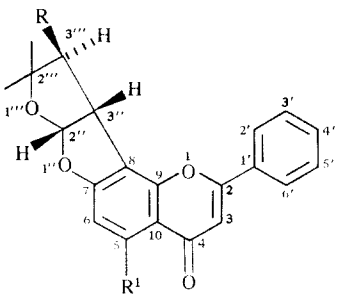
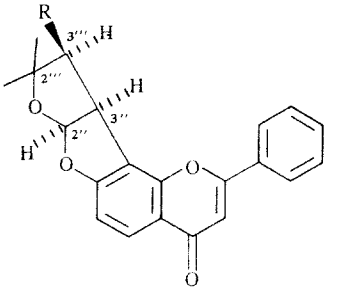
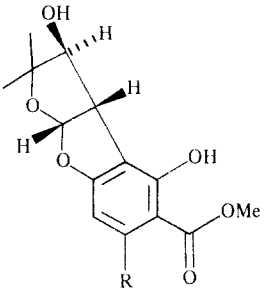


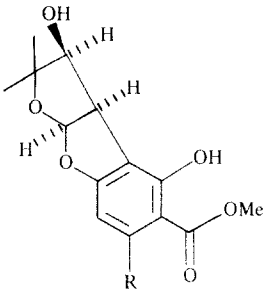
1 R = OAc, R^1 = H Semiglabrin
2 R = OH, R^1 = H Semiglabrinol
3 R = OAc, R^1 = OMe Multijugin
4 R = OH, R^1 = OMe Multijuginol



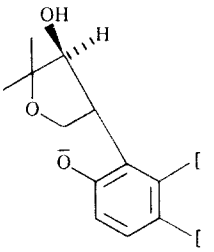
5 R = OAc Pseudosemiglabrin
6 R = OH Pseudosemiglabrinol



7 R = H Methylsemiglabrinate
8 R = OMe Methylmultijuginate



9 R = H Methyl pseudosemiglabrinate
10 R = OMe Methyl pseudomultijuginate



11 Intermediate ion

semiglabrin) on chromatography gave colourless plates of a compound mp 171–174°. Spectral data and optical rotation $[\alpha]_{25}^{D} - 328°$ confirmed the identity of this compound as pseudosemiglabrin (**5**). The ^{13}C NMR of pure **5** which we recorded for the first time was in close agreement with the spectra reported recently on the basis of substraction analysis of mixtures of isomers (Table 1) [4].

I would like to point out that Waterman and Khalid [2] and later Pelter *et al.* [5] made no comment on the considerable difference of ^1H NMR and the mp of semiglabrin (**1**) isolated by them and Smalberger *et al.* [3] (253–256° and 176–178°, respectively), particularly when the latter's mp is so close to pseudosemiglabrin (181–183°) but Smalberger reports a ^1H NMR spectrum identical to semiglabrin (Table 2). Also the alkaline hydrolysis of semiglabrin performed by Smalberger *et al.* led them to the isolation of a mixture (after methylation) of methyl semiglabrinate (**7**) and methylpseudosemiglabrinate (**9**). They attribute this to the isomerization of the inter-

Table 2. Direct comparison of melting points and acetyl protons in the ^{1}H NMR spectra of semiglabrin (**1**) and pseudosemiglabrin (**5**)

	Smalberger *et al.*	Waterman and Khalid		Pelter *et al.*	Ahmad	
	1	**1**	**5**	**1**	**1**	**5**
Mp	176–178°	253–256°	181–183°	254–255°	244–248°	171–174°
^{1}H NMR, Ac	2.22	2.22	1.51	2.18	2.20	1.47

mediate ion **11** during alkaline hydrolysis of semiglabrin. We believe that the main contributing factor in the isolation of two isomers was the mixture of starting material of **1** and **5**. Further support for this view is obtained by the isolation of only (after methylation) one isomer, methyl multijuginate (**8**) from alkaline hydrolysis of multijugin (**3**) by Vleggar *et al.* [6]. If the postulated isomerization of the intermediate ion were correct, one would have expected also the formation of methyl pseudomultijuginate (**10**) and its subsequent isolation. However, no methylpseudomultijuginate was reported by Vleggar *et al.* [6]. Obviously, in this case, their starting material was a pure single isomer multijugin (**3**). I, therefore, believe that semiglabrin isolated by Smalberger *et al.* was a mixture of both the isomers with a predominance of **1**.

Elution of the column with chloroform–benzene (4:1) gave colourless needles of a compound, mp 270–272°. It analysed for $C_{21}H_{18}O_5$. The IR spectrum of this compound showed hydroxyl absorption at 3362 cm^{-1} and its ^{1}H NMR spectrum showed a typical 3-H signal of flavones at δ6.70. The compound was readily acetylated which showed the CO absorption at 1733 cm^{-1}, and ^{1}H NMR gave a signal at δ1.47 which was reminiscent of the acetate signal in **5**. The ^{13}C NMR spectrum of the alcohol showed typical signals of carbon atoms at 2‴, 3‴, 3″ which were 3–6 ppm upfield in comparison with **2** and as expected showed little change when the spectra is compared with the same carbon atoms of **5** (Table 1). However, the ^{1}H NMR (Table 3) of the natural alcohol was quite complex at the asymmetric centres. The proton at 2″ appeared as a doublet at δ6.42 (*d*, *J* = 7 Hz), while the protons at 3″ and 3‴ in addition to coupling with each other (as in **5**) are split respectively with the protons at 2″ and the hydroxyl proton, hence an unresolved multiplet at δ4.44. However, on addition of D_2O the doublet of

Table 3. ^{1}H NMR spectra of semiglabrinol (**2**) and pseudosemiglabrinol (**6**)

	$CDCl_3$–DMSO-d_6 (3:1)			Pyridine-d_5		
	Petler *et al.* **2**	Ahmad **2**	Ahmad **6**	Smalberger *et al.* **2**	Ahmad **2**	Ahmad **6**
3	6.75	6.77	6.85	6.90	7.13	7.02
5	8.20	8.18	8.17	8.32	8.37	8.39
				d, *J* = 8.5	*d*, *J* = 8.5	*d*, *J* = 8.5
6	6.82	6.84	6.88	6.97	7.05	7.00
	J = 9	*J* = 9	*J* = 9	*J* = 8.5	*J* = 8.5	*J* = 8.5
B ring protons	7.8–8.0	7.98	7.98	8.13	8.19	8.27
	m, 2H	*m*, 2H	*m*, 2H	*m*, 2H	*m*, 2H	*m*, 2H
	7.4–7.6	7.56	7.53	7.43	7.48	7.57
	m, 3H	*m*, 3H	*m*, 3H	*m*, 3H	*m*, 3H	*m*, 3H
2″	6.53	6.56	6.42	6.67	6.91	6.78
	d, *J* = 7	*d*, *J* = 7	*d*, *J* = 7	*d*, *J* = 6.5	*d*, *J* = 6.5	*d*, *J* = 6.5
3″	4.22	4.24	4.44 *m*	4.63	4.60	4.75 *m*
	4.22	4.24	2H	*d*, *J* = 6.5	*d*, *J* = 6.5	2H
3‴	(*d*, *J* = 6)*	(*d*, *J* = 6)*	4.44 *	4.68	4.71	4.75 *
Me_2	0.92, 1.42	0.93, 1.31	1.02, 1.30	1.38, 1.44	1.15, 1.65	1.35, 1.50
OH	5.62	5.72	5.43	not reported	5.05	4.94
	d, *J* = 6	*d*, *J* = 6	*d*, *J* = 6			
		Disappears on addition of D_2O	Disappears on addition of D_2O		Disappears on addition of D_2O	Disappears on addition of D_2O
Mp	245–247°	245–246°	270–272°	273–275°		
Optical rotation	$[\alpha]_{29}^{D}$ −270	$[\alpha]_{28}^{D}$ −267	$[\alpha]_{28}^{D}$ −293	$[\alpha]_{24}^{D}$ −289.7		

*Broad signal after D_2O exchange.

hydroxyl proton disappears and the multiplet is reduced to a broad signal (Table 3). Finally the acetate of the natural alcohol was found to be identical with **5** in all respects (mp, UV, IR, NMR). The isolated compound must therefore be the corresponding stereoisomer of **2** and by analogy with **5** is named pseudosemiglabrinol and assigned structure **6**. Further elution of the column with chloroform gave a colourless compound mp 245–246°. It was identified as semiglabrinol (**2**) on the basis of comparison of ^{1}H and ^{13}C NMR spectra of the natural alcohol and its acetate which was found to be in good agreement with the known spectra of **2** and **1**, respectively (Tables 1 and 3).

Semiglabrinol (**2**) (mp 273–275°) was first isolated from *T. semiglabra* [3] and later from *T. purpurea* [5] (mp 245–247°). The notable difference reported for the melting points of semiglabrinol and their closeness to ours for pseudosemiglabrinol (270–272°) and semiglabrinol (245–246°) prompted us to measure the ^{1}H NMR spectra of both compounds in $CDCl_3$–DMSO (3:1) [5] and in deuterated pyridine [3] because different solvents were used by two workers. The ^{1}H NMR spectrum of semiglabrinol isolated by us was in close agreement with Pelter's data [5] (Table 3). However, the ^{1}H NMR spectra of **2** and **6** in deuterated pyridine, on comparison with Smalberger's semiglabrinol showed significant differences for the 2″-H signal and the signals for the *gem* dimethyl protons in **2** and considerable difference in the chemical shifts of protons at 2″, 3″ and 3‴ in **6** (Table 3). Therefore, we believe that **2** isolated by Pelter *et al.* is authentic but Smalberger's 'semiglabrinol' is in fact a mixture of isomers **2** and **6**. Also Waterman and Khalid's semiglabrinol (**2**), a product of hydrolysis of **1**, is also authentic as it is derived from authentic semiglabrin. Further elution of this column gave small amounts of a compound, mp 184°, which was identified as lanceolation A [2].

Another compound multijuginol has been isolated from *T. multijunga* [6] to which structure **4** has been assigned. The ^{1}H NMR spectrum reported for this compound and its comparison with **2** and **6** shows a distinct difference in the chemical shift of the alcoholic hydroxyl. The signal of the hydroxyl group in **2** and **6** occurs in the range of δ4.94–5.05 in pyridine, and 5.43–5.72 in $CDCl_3$–DMSO (3:1). Introduction of the 5-methoxyl in the molecule of **2** is unlikely to shift the hydroxyl signal from 5.62 in **2** or to 1.74 in **4** as reported by Vleggar *et al.* [6]. The structure of multijuginol (**4**), therefore, warrants further investigation.

To our knowledge this is the first report of the natural occurrence of two parirs of stereoisomers in *Tephrosia* species. As discussed earlier my findings strongly suggest the presence of both pairs of isomers also in *T. semiglabra*.

EXPERIMENTAL

UV and IR spectra were recorded in EtOH and Nujol, unless otherwise stated. ^{1}H NMR spectra were run at 100 MHz using TMS as int. standard. ^{13}C NMR spectra were recorded at 50.3 MHz using the same standard. MS were obtained at 70 eV.

Plant material. Whole plants of *T. apollinea* (Del). Link were collected from Jizan (south-western Saudi Arabia) in September, 1982. A voucher specimen has been deposited at the National Herbarium for Saudi Arabia (RIY) at the Regional Agriculture and Water Research Center, P.O. Box 17285, Riyadh, Saudi Arabia 11484.

Isolation of flavonoids. Powdered whole plant material (575 g) was successively extracted with petrol (60–80°) and $CHCl_3$ in a Soxhlet apparatus. The $CHCl_3$ extract on concn. gave 100 g of a dark green extract. Twenty grams of the crude extract was chromatographed on silica gel (200 g).

Semiglabrin (**1**). Elution with $CHCl_3$–C_6H_6 (4:1) gave semiglabrin, mp 244–248°. (lit. 253–256°, 254–255°, 176–178°); $[\alpha]_{25}^{D}$ −265° (*c* 0.08; $CHCl_3$) (lit. −293°, −273°, −369° [2, 5, 3]). IR, UV, NMR and MS as in ref. [2].

Pseudosemiglabrin (**5**). Elution with $CHCl_3$–C_6H_6 (1:1) gave colourless plates from MeOH of pseudosemiglabrin, mp 171–174° (lit. 181–183°); $[\alpha]_{25}^{D}$-328° (*c* 0.23; $CHCl_3$) (lit −384° [2]). IR, UV, ^{1}H NMR and MS as in ref. [2]. For ^{13}C and ^{1}H NMR see Tables 1 and 2.

Lanceolation A. Elution with $CHCl_3$ gave colourless needles from MeOH of lanceolation A, mp 184–185° (lit. 187–189°). UV, IR and NMR as in ref. [2].

Pseudosemiglabrinol (**6**). Elution of the column with $CHCl_3$–C_6H_6 (4:1) gave colourless needles from MeOH–$CHCl_3$ of pseudosemiglabrinol, mp 270–272°. $[\alpha]_{28}^{D}$ −293°. IR ν_{max}^{Nujol} cm^{-1}: 3362, 1638. UV $\lambda_{max}^{CHCl_3}$ nm: 210, 245, 255 and 307. (Found: C, 72.00; H, 5.09, $C_{21}H_{18}O_5$ requires C, 71.99; H, 5.16%.) Acetylation of 50 mg of **6** with C_5H_5N–Ac_2O gave the acetate which was identical in all respects (mp, UV, IR, NMR) to natural pseudosemiglabrin (**5**).

Semiglabrinol (**2**). Elution of the column with $CHCl_3$ gave colourless needles from MeOH–$CHCl_3$ of semiglabrinol, mp 245–246°. $[\alpha]_{28}^{D}$ −267°. IR ν_{max}^{Nujol} cm^{-1}: 3360, 1641. UV $\lambda_{max}^{CHCl_3}$ nm: 210, 243, 255, 308. (Found: C, 72.05; H, 5.05, $C_{21}H_{18}O_5$ requires C, 71.99; H, 5.16%.) The ^{1}H and ^{13}C NMR spectra of this compound was in close agreement with the data reported for semiglabrinol [5] (Tables 1 and 3). Acetylation in the usual manner gave the acetate which was identical to semiglabrin in all respects (mp, UV, IR, NMR).

Mild alkaline hydrolysis. Compound **5** (50 mg) was dissolved in 25 ml of 0.1 N KOH–MeOH soln which was stirred overnight at room temp. Excess H_2O was added and the mixture extracted with $CHCl_3$ to give colourless needles of **6** from MeOH–$CHCl_3$, mp 270–272°. Compound **6** was identical in all respects to natural pseudosemiglabrinol (mp, UV, IR, NMR). Similar treatment of 50 mg of **1** gave **2**, mp 245–246° identical in all respects to natural semiglabrinol (mp, UV, IR, NMR).

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