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VIRIDIFLORIN, AN ISOFLAVONE FROM *TEPHROSIA VIRIDIFLORA**

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Key Word Index—*Tephrosia viridiflora*, Leguminosae, prenyl isoflavone, 4',5,7-trihydroxy-2',5'-dimethoxy-6-prenylisoflavone, viridiflorin

Abstract—A new isoflavone, viridiflorin, has been isolated from *Tephrosia viridiflora* Its structure was established as 4',5,7-trihydroxy-2',5'-dimethoxy-6-prenylisoflavone based on spectral evidence and chemical transformation

INTRODUCTION

Several reports have indicated that the extract of some species of the genus *Tephrosia* have piscicidal, insecticidal, repellent [1] and anti-cancer properties [2] As part of our chemical systematic study of the genus *Tephrosia* we have previously investigated *T madrensis*, *T watsoniana* and *T nitens* and isolated a number of novel flavonoids [3, 4] In cognizance of these results we have undertaken the study of a new species of this genus, *T viridiflora* a herbaceous plant endemic to western Mexico

From the roots and aerial parts of *Tephrosia viridiflora* we have isolated a new isoflavone, viridiflorin (**2a**), in addition to the known elongatin (**1**) [5], the rotenoids villosinol [6] and 11-hydroxytephrosin [7], and the sterols sitosterol and stigmasterol

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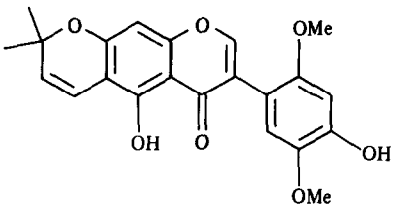
RESULTS AND DISCUSSION

Extraction of the roots and aerial parts of the plant with petrol, ethyl acetate and methanol, followed in each case by CC and prep TLC over silica gel (see Experimental), gave two isoflavonoids One of them was identified as elongatin (**1**), an isoflavone previously isolated from *T elongata* E Mey [5] The second flavonoid was a new compound which we named viridiflorin

Viridiflorin (**2a**), $C_{22}H_{22}O_7$ ([M]$^+$ *m/z* 398) was an isoflavone which showed a strong hydroxyl absorption at 3420 cm^{-1} in the IR spectrum (positive ferric chloride)



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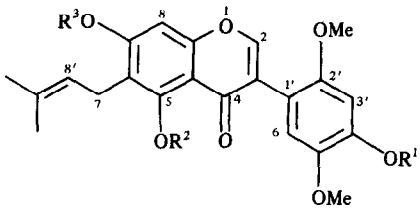
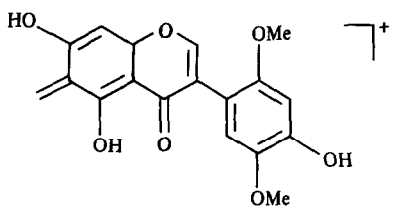


2a $R^1 = R^2 = R^3 = H$
2b $R^1 = R^2 = R^3 = Ac$
2c $R^1 = R^3 = Ac$, $R^2 = H$
2d $R^1 = R^3 = Me$, $R^2 = H$



3

The presence of a hydrogen bonded hydroxyl group at C-5 was indicated by the carbonyl absorption at 1650 cm^{-1} [8] and the typical hydroxyl proton signal at δ13 00 in the ^1H NMR spectrum (Table 1) The compound also showed a downfield singlet at δ7 8 characteristic of the C-2 proton of the isoflavone nucleus [9] Since the ^1H NMR spectrum of viridiflorin (**2a**) exhibited three further singlets at δ6 30, 6 42 and 6 80, accounting for three isolated skeletal protons, viridiflorin must be a hexasubstituted isoflavone The nature of the substituents was evident from the ^1H NMR spectrum, which showed two methoxyl singlets at δ3 70 and 3 82 and the typical resonances of a prenyl group, observed as two broad singlets at δ1 66 (3H) and 1 78 (3H), a broad doublet at δ3 30 (2H) and a broad triplet at δ5 20 (1H) Two broad singlets interchangeable with deuterated water at δ8 35 and 10 06 indicated the presence of two extra phenolic hydroxyl groups As in elongatin (**1**), three substituents, one hydroxyl and two methoxyl groups, can be placed in the B-ring at C-4′, C-2′ and C-5′, respectively, and the remaining prenyl and hydroxyl groups at C-6 and C-7 in the A-ring Based on the above data, viridiflorin can be represented by structure **2a**

As expected, the mass spectrum of viridiflorin (**2a**) showed a weak peak at *m/z* 177 which can be assigned to the fragments $[B_1-H]^+$ and for $[A_1-43]^+$ derived from the RDA process [10] Besides the $[M]^+$ at *m/z* 398 (66%), other intense peaks were observed at *m/z* 355 $[M-43]^+$ (100%) and 343 $[M-55]^+$ (88%) due to the fragmentation of the prenyl group The lost of 55 mass units can give rise to the ion, **3**, which would be in accord with the prenyl group being attached at C-6 The presence of three phenolic groups was confirmed by acetylation of **2a** which provided the corresponding triacetate, **2b**, and the diacetate derivative, **2c** The chelated hydroxyl group at C-5 was also evident when **2a** was treated with dimethyl sulphate giving the derivative, **2d** This result is in accordance with the presence of the bulky prenyl group at C-6 rather than at C-8

Table 1 ^1H NMR data of viridiflorin (**2a**) and its derivatives (80 MHz, deuterochloroform, TMS as int standard)

	2a*	**2b**	**2c**	**2d**
H-2	7 8 *s*	7 8 *s*	7 9 *s*	7 83 *s*
H-8	6 42 *s*	6 7 *s*	6 72 *s*	6 38 *s*
H-3′	6 6 *s*	6 93 *s*	6 72 *s*	6 6 *s*
H-6′	6 8 *s*	7 18 *s*	6 97 *s*	6 87 *s*
H-7″†	3 3 *d*	3 28 *d*	3 31 *d*	3 35 *d*
H-8″‡	5 2 *t*	5 03 *t*	5 13 *t*	5 09 *t*
gem-Me_2	1 65 *s*	1 69 *s*	1 69 *s*	1 67 *s*
	1 77 *s*	1 79 *s*	1 77 *s*	1 78 *s*
OH	13 0 *s*	—	13 02 *s*	12 9 *s*
	10 07 *s*			
	8 35 *s*			
OMe	3 7 *s*	3 7 *s*	3 73 *s*	3 76 *s*
	3 81 *s*	3 8 *s*	3 8 *s*	3 84 *s*
				3 89 *s*
				3 92 *s*
OAc	—	2 33 *s*	2 35 *s*	—
		2 35 *s*		
		2 42 *s*		

*Run in deuterochloroform–DMSO
†*d*, $J_{7',8''}$ = 7 Hz
‡*t*, $J_{8'',7'}$ = 7 Hz

Confirmation of structure **2a** was achieved by cyclodehydrogenation with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) All the spectral data (IR, UV, NMR and mass spectra) of the obtained product were identical to those of elongatin (**1**)

The isolation of viridiflorin (**2a**) is of particular interest since it is probably the biogenetic precursor of elongatin (**1a**)

EXPERIMENTAL

Tephrosia viridiflora Tellez, was collected in Jalisco, Mexico, *ca* 60 km, north of Melaque in 1983 A voucher is deposited at the Herbarium of Instituto de Biología (UNAM), Mexico

Extraction Air-dried leaves and flowers (270 g) were extracted successively with heptane, EtOAc and MeOH After evaporation of solvents the green syrups A (17 3 g), B (15 1 g) and C (27 1 g), respectively, were obtained In the same way, from the air-dried roots (1 320 kg), yellow pastes D (18 8 g), E (12 9 g) and F (41 8 g) were obtained

The heptane extract A (17 3 g) was percolated on a column packed with 170 g Tonsil and eluted with heptane and mixtures of heptane–EtOAc From the fractions eluted with heptane, a mixture of sitosterol and stigmasterol was obtained

The EtOAc extract B (15 1 g) was fractionated on Tonsil (150 g) using heptane and EtOAc The heptane fraction (7 5 g) was chromatographed on silica gel (80 g) using heptane and heptane–Me_2CO mixtures Fractions eluted with heptane–Me_2CO (19 1) were combined and crystallized from CH_2Cl_2–MeOH giving **1** (198 mg) mp 180–182° (lit [5] 181–182°), identified by IR, ^1H NMR, MS and comparison with an authentic sample TLC of fractions eluted with heptane–Me_2CO (8 2) afforded 25 mg **2a**

In the same way, extract D (18 8 g) afforded 1 36 g of **1** Extract E (12 9 g) afforded 27 mg **2a** and 36 mg **1** Finally, from extract F (41 8 g), 10 g **1**, 58 3 mg **2a** and 22 mg of a mixture of villosinol [6] and 11-hydroxytephrosin [7] were obtained

Viridiflorin (**2a**) $C_{22}H_{22}O_7$, colourless needles, mp 220–222° (CH_2Cl_2–hexane) UV λ_{max}^{MeOH} nm (ε) 204 (12 595), 263 (7414), 295 (4815) IR ν_{max}^{KBr} cm^{-1} 3420, 1650, 1515, 1300 EIMS (probe) 70 eV, *m/z* (rel int) 398 $[M]^+$ (62), 355 $[M-43]^+$ (100), 343 $[M-55]^+$ (82), 177 $[B_1-H]^+$ and/or $[A_1-43]^+$ (11)

Acetylation of viridiflorin (**2a**) To a soln of **2a** (16 mg) in pyridine (1 ml) was added Ac_2O (0 5 ml) and the soln heated at 80° for 1 5 hr The reaction mixture was poured over crushed ice and extracted with CH_2Cl_2, washed with H_2O, dried and evaporated to afford after prep TLC [hexane–Me_2CO (4 1), × 6] the triacetate, **2b** (10 mg), and the diacetate, **2c** (5 mg)

Viridiflorin triacetate (**2b**) $C_{28}H_{28}O_{10}$, colourless needles, mp 200–202° UV λ_{max}^{MeOH} nm (ε) 215 (8559), 250 (5362), 296 (3825) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1770, 1650 EIMS (probe) 70 eV, *m/z* (rel int) 524 $[M]^+$ (17), 482 $[M-C_2H_2O]^+$ (18), 440 $[M-C_4H_4O_2]^+$ (25), 397 $[M-C_4H_4O_2-C_2H_3O]^+$ (43)

Viridiflorin diacetate (**2c**) $C_{26}H_{26}O_9$, colourless needles, mp 140–144° UV λ_{max}^{MeOH} nm (ε) 210 (31 206), 262 (18 420), 297 (12 111) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1770, 1765, 1645, 1600, 1510 EIMS (probe) 70 eV, *m/z* (rel int) 482 $[M]^+$ (28), 440 $[M-C_2H_2O]^+$ (34), 398 $[M-C_4H_4O_2]^+$ (12), 397 $[M-C_4H_5O_2]^+$ (52), 385 $[M-C_6H_9O]^+$ (20), 343 $[M-C_8H_{11}O_2]^+$ (41), 43 (100)

Methylation of viridiflorin (**2a**) A mixture of **2a** (24 mg) dry K_2CO_3 (30 mg) and Me_2SO_4 (1 ml) in dry Me_2CO (20 ml) was refluxed for 3 hr and worked-up as usual Purification by TLC [hexane–Me_2CO (4 1) × 6] of the reaction residue afforded **2d** (9 mg), mp 193–195° UV λ_{max}^{EtOH} nm (ε) 213 (16 111), 263 (11 389), 290 (7543) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1650, 1590, 1510, 1300 EIMS

(probe) 70 eV, *m/z* (rel int) 426 $[M]^+$ (51), 395 $[M-31]^+$ (22), 383 $[M-43]^+$ (98), 371 $[M-55]^+$ (100), 191 $[C_{10}H_7O_4]^+$ (18)

Conversion of viridiflorin (**2a**) *to elongatin* (**1**) A soln of 34 mg **2a** and 30 mg DDQ in 40 ml dry C_6H_6 was refluxed for 2 hr After usual work-up followed by prep TLC [hexane–Me_2CO (3 2) × 3] **1** was obtained identical in all respects (TLC, IR, ^1H NMR, mp) with authentic **1**

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