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DIMETHYLCHROMENE ROTENOIDS FROM *TEPHROSIA CANDIDA*

CESAR C. ANDREI,* PAULO C. VIEIRA,† JOÃO B. FERNANDES, M. FÁTIMA DAS G. F. DA SILVA and EDSON RODRIGUES FO.

Departamento de Química, Universidade Estadual de Londrina, CP 6001, CEP 86051-970, Londrina, PR, Brazil; * Departamento de Química, Universidade Federal de São Carlos, CP 676, CEP 13565-905, São Carlos, SP, Brazil

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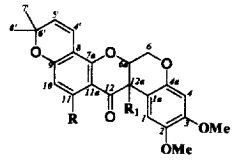
Key Word Index—*Tephrosia candida*; Fabaceae; roots; rotenoids; dimethylchromene rotenoids.

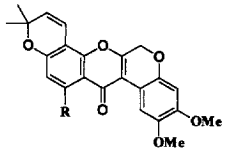
Abstract—The hexane and ethyl acetate extracts of roots of *Tephrosia candida* afforded a new rotenoid, 12a-hydroxy-β-toxicarol, together with a series of known ones, identified as: deguelin, α-toxicarol, tephrosin, 6a,12a-dehydrodeguelin, 12a-hydroxy-α-toxicarol, 6a,12a-dehydro-α-toxicarol and 6a,12a-dehydro-β-toxicarol. The possibility of four of these rotenoids to be linear or angular is discussed on the basis of HMBC experiments. Angular rotenoids are very common, however the linear are restricted, and to the best of our knowledge only two linear dimethylchromene rotenoids are described in the literature. © 1997 Elsevier Science Ltd

INTRODUCTION

The genus *Tephrosia* is rich in flavonoids including the isoflavonoid rotenoids. *T. candida* has been investigated extensively [4–14], however the main parts of the plant to be studied have been the seeds and aerial parts. Rotenoids show activity against insects, and exhibit strong ictiotoxic activity. Rotenone, the first rotenoid to be identified, was used as an insecticide before the advent of the organosynthetic insecticides [1]. Recently, rotenoids have been found to have anti-carcinogenic properties and tephrosin and amorphospirone isolated from *Amorpha* species have been shown to be active against tumours including skin cancer [2, 3]. In this paper, we report on the isolation and identification of some rotenoids in the roots of this plant.



1 R = R1 = H
2 R = OH; R1 = H
3 R = H; R1 = OH
5 R = R1 = OH



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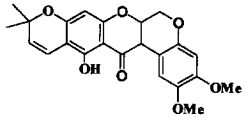
7 R = OH



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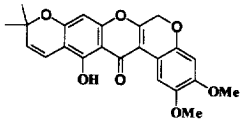

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RESULTS AND DISCUSSION

The hexane extract and ethyl acetate extract of the dried roots afforded the dimethylchromene rotenoids deguelin (**1**) [15], α-toxicarol (**2**) [15], tephrosin (**3**) [12], 6a,12a-dehydrodeguelin (**4**) [12], 12a-hydroxy-α-toxicarol (**5**) [16], 12a-hydroxy-β-toxicarol (**6**), 6a,12a-dehydro-α-toxicarol (**7**) [17] and 6a,12a-dehydro-β-toxicarol (**8**) [17]. Compounds **3** and **5** were obtained as mixtures with other isopropenyl-dehydrofuranerotenoids. They were purified by mean of recycling preparative HPLC [18].

Analysis of the spectral data for the above compounds allowed us to assign structures **1**–**8**. Tables 1–3 show the NMR (^{1}H and ^{13}C) data for these compounds.

All of them, except **6** have already been reported in the literature. However, the possibilities of geometric dimethylchromene isomerism, when C-11 is substituted by a hydroxyl group, led us to examine the use of HMBC for determining this isomerism.

Compounds **5**–**8** have very similar ^{1}H NMR spectra (Table 2). The dimethylchromene system is well characterized by two doublets (δ 6.29 ± 0.13; 5.54 ± 0.06) for the vinyl protons and a singlet integrating for 6 protons (σ 1.44 and 1.47) for compounds **7** and **8**, and two singlets (δ 1.37 ± 0.01; 1.43 ± 0.01) for compounds

* Author to whom correspondence should be addressed.

Table 1. ^{1}H NMR data for rotenoids ($CDCl_3$, 400 MHz)*

H	1	2	3	4
1	6.72(*s*)	6.86(*s*)	6.49(*s*)	8.45(*s*)
4	6.38(*s*)	6.46(*s*)	6.41(*s*)	6.56(*s*)
6ax	4.56(*dd* 12.4 3.2 Hz)	4.60(*dd* 12.03/3.2 Hz)	4.55(*dd* 12.0 2.4 Hz)	—
6eq	4.11(*d* 12.4 Hz)	4.16(*d* 12.0 Hz)	4.42(*dd* 12.0 1.2 Hz)	—
6a	4.84(*m*)	4.87(*t* 3.2 Hz)	4.49(2dd 2.4 1.2 Hz)	5.02(*s*)
10 (8-β)	6.38(*d* 8.8 Hz)	5.95(*s*)	6.39(*d* 8.8 Hz)	6.87(*d* 8.8 Hz)
11	7.67(*d* 8.8 Hz)	—	7.65(*d* 8.8 Hz)	8.04(*d* 8.8 Hz)
12a	3.77(*d* 4.0 Hz)	3.84(*d* 4.0 Hz)	—	—
4′	6.57(*d* 10.0 Hz)	6.55(*d* 10.0 Hz)	6.52(*d* 10.0 Hz)	6.77(*d* 10.0 Hz)
5′	5.48(*d* 10.0 Hz)	5.47(*d* 10.0 Hz)	5.48(*d* 10.0 Hz)	5.76(*d* 10.0 Hz)
7′/8′	1.32/1.38(*s*)	1.37/1.43(*s*)	1.31/1.37(*s*)	1.50(*s*)
OMe(2/3)	3.70/3.73(*s*)	3.79/3.81(*s*)	3.65/3.74(*s*)	3.87/3.96(*s*)
OH(†11 and ‡12a)	—	12.19(*s*)†	4.32(*s*)‡	—

* δ in ppm.
† Value for OH (11).
‡ Value for OH (12).

5 and **6**. All of the compounds contained a chelated hydroxyl (δ 12.40±0.78). Compounds **7** and **8** displayed three singlet signals for aromatic protons at δ 8.25±0.01, 6.45 and 6.27±0.01, while compounds **5** and **7** showed signals for these at δ 6.71±0.01, 6.49 and 5.93±0.06. The remaining signals typically represent the protons for either a dehydrorotenoid compound having a 6a-12a double bond (compounds **7** and **8**), or a 12a-hydroxy compound e.g. **5** and **6**. Compounds **7** and **8** were acetylated yielding the corresponding acetates **7a** and **8a**. The ^{1}H NMR spectra (Table 2) for the acetates showed more accentuated differences for H-4′ (δ 6.72 and 6.49) for the angular and linear forms, respectively. Also one aromatic proton H-8/H-10 displayed a chemical shift (δ 6.40 and 6.68) for the angular and linear forms, respectively. A complete assignment of all chemical shifts and the corresponding $\Delta\delta$ for each proton in compounds **5**–**8** are shown in Table 2.

The ^{13}C NMR data for compounds **5**–**8** (Table 3) is also inconclusive as regards to the possible isomeric forms of these compounds. The only carbons showing differences and easily assigned were C-7a, C-8, C-10, C-11 and C-11a. However, a complete distinction between the four compounds was obtained from NMR one bond and long range heteronuclear correlation C-H experiments. C-10 which is a C-H carbon showed a correlation with the phenolic proton in the case of angular chromenes. Linear chromenes showed a correlation between the phenolic proton and two non-hydrogenated aromatic carbons (C-10 and C-11a). Correlations involving phenolic hydrogens chelated to carbonyl have already been described before [19]. The HMBC experiments were carried out with compounds **2**, **7** and **8** (Table 4), while for compounds **5** and **6** the isomerism was only observed by comparison of the differences in the ^{1}H and ^{13}C NMR spectra of the isolated compounds and also in mixture.

The occurrence of linear rotenoids containing a dimethylchromene raises an interesting question about the 11-hydroxychromene or furanerotenoids. Most of known rotenoids have been described an angular, however from our NMR results it is clear that it is very difficult to assign each isomer based only on data ^{1}H and ^{13}C NMR chemical shift data.

Tentative use of ^{1}H NMR to address this problem has been made in the past. However, in the case of 11-OH this technique was not considered the best choice [20]. Therefore, the differentiation between linear or angular rotenoids is best made by using the photometric Gibbs reaction [21]. ^{13}C NMR has also been used to decide the geometry of 11-OH dimethylchromene rotenoids [22].

The relative positioning of the chromene ring [17], was deduced from a NOE (3.1%) observed between an acetyl group and a chromene proton (H-4′) in the case of 6a,12a-dehydro-β-toxicarol.

Our conclusion is that the ^{1}H NMR and ^{13}C NMR data of these compounds show only slight differences between possible isomers, and that HMBC experiments appear to be a secure way to distinguish between the isomeric rotenoids.

EXPERIMENTAL

T. candida (Roxb.) DC was identified and collected in the Instituto Agronômico do Paraná, January, 1988. The roots were dried in an open stove at 60° and powdered. Exhaustive extraction of the powdered extract (2.8 kg) with hexane and EtOAc gave respectively, 22.82 g and 36.52 g of crude extracts. The hexane extract was chromatographed on a silica gel column (70–230 mesh) with pure hexane and then with increasing amounts (4, 7, 20 and 50%) of EtOAc. The fr. eluted with hexane–EtOAc (93:7) (8.26 g) was submitted to silica gel (70–230 mesh) CC and eluted with hexane–EtOAc and MeOH in increasing polarity. Further purification of the resultant frs by

Table 2. ^{1}H NMR data for rotenoids ($CDCl_3$, 400 MHz)*

H	**5**	**6**	$\Delta\delta$	**7**	**8**	$\Delta\delta$	**7ac**	**8ac**	Δ
1	6.72(*s*	6.70(*s*)	0.02	8.26(*s*)	8.23(*s*)	0.03	8.35	8.34	0.01
4	6.49(*s*)	6.49(*s*)	0.00	6.54(*s*)	6.51(*s*)	0.03	6.54	6.53	0.01
6ax	4.61(*dd* 12.0 2.4 Hz)	4.57(*dd* 12.8 2.4 Hz)	0.04	—	—		—	—	—
6eq	4.54(*dd* 12.0 0.8 Hz)	4.44(*d* 12.8 Hz)	0.10	—	—	—	—	—	—
6a	4.48(*dd* 2.4 0.8 Hz)	4.52(*d* 2.4 Hz)	0.04	4.98(*s*)	4.92(*s*)	0.06	4.97	4.93	0.02
10(α)/8(*β*)	5.98(*s*)	5.87(*s*)	1.10	6.28(*s*) –	6.25(*s*)	0.03	6.50**	6.68*	0.18
11	—	—	—	—		—	—	—	—
12a	—	—	—	—	—		—	—	—
4′	6.52(*d* 10.0 Hz)	6.57(*d* 10.0 Hz)	0.05	6.62(*d* 10.0 Hz)	6.69(*d* 10.0 Hz)	0.07	6.72 (*d* 10.0 Hz)	6.49(*d* 10.0 Hz)	0.23
5′	5.47(*d* 10.0 Hz)	5.49(*d* 10.0 Hz)	0.02	5.59(*d* 10.0 Hz)	5.59(*d* 10.0 Hz)	0.00	5.70(*d* 10.0 Hz)	5.77(*d* 10.0 Hz)	0.07
7′/8′	1.37/1.44(*s*)	1.38/1.42(*s*)	0.01/0.02	1.47(*s*)	1.44(*s*)	0.03	1.57	1.58	0.01
OMe(2/3)	3.77/3.83(*s*)	3.75/3.82(*s*)	0.02/0.01	3.87/3.94(*s*)	3.84/3.91(*s*)	0.03	3.87/3.94	3.87/3.94	0.00
OH(11)	11.61(*s*)	11.78(*s*)	0.17	12.99(*s*)	13.18(*s*)	0.19	—	—	—
OAc	—	—	—	—	—	—	2.47	2.52	0.05

* δ in ppm.

Table 3. ^{13}C NMR data for rotenoids ($CDCl_3$, 100.2 MHz)

C	1	2	3	4	5	6	7	8
1a	105.0	108.5*	104.4	109.1*	108.4	108.4	109.9	109.8
1	110.7*	109.3†	110.3	110.0	109.4	109.4	109.7	109.9
2	144.1	143.8	143.9	144.1	144.1	144.1	144.2	144.1
3	149.8	148.3‡	149.6	149.0	151.4	151.3	149.2	149.1
4	101.2	100.9	101.0	100.4	101.2	101.2	100.5*	100.5
4a	147.7	150.9‡	147.3	146.3	148.4	148.4	146.3	146.3
6	66.5	66.7	66.0	64.8	63.6	63.6	64.7	64.8
6a	72.7	75.9	71.9	156.2†	75.7	75.6	156.8	157.0*
7a	158.0	156.5	155.9	151.1	155.5	158.5	150.9	155.9
8	109.4	109.0*	101.8	110.5*	102.0	96.6	101.1	94.8
9	160.3	160.6	162.8	157.2†	163.6	161.1*	159.3	159.2
10	111.7*	111.7†	97.8	114.7‡	98.0	99.8	100.6*	105.8†
11	128.8†	128.4§	164.5	130.6	164.0	163.4*	162.3	157.1
11a	113.0	111.0	101.2	118.5	99.9	103.3	106.0	106.0†
12	189.4	191.3	194.3	174.4	194.8	195.0	179.3	179.2
12a	44.65	67.36	43.51	111.77	66.77	66.94	110.83	110.7
4′	116.0	115.3	115.4	115.4‡	115.1	114.9	114.4	115.4
5′	128.9†	128.7	126.4	126.5	126.6	127.6	127.7	128.2
6′	77.9	77.9	78.3	77.8	78.6	78.8	78.1	78.1

* † ‡ §-δ interchangeable values in each column

Table 4. HMBC (^2J and ^3J) data correlations of α-toxicarol (**2**), 6a,12a-dehydro-α-toxicarol (**7**) and 6a,12a-dehydro-β-toxicarol (**8**) ($CDCl_3$, 400 and 100.2 MHz)

	C					
	2		**7**		**8**	
H	^2J	^3J	^2J	^3J	^2J	^3J
1	1a, 2	12a	1a	3, 4a		3, 4a, 12a
4	3, 4a	1a, 2	4a	1a, 2		1a, 2
6ax or 6*	6a	4a, 12a	*6a	*4a, 12a	*6a	*4a, 12a
6a		1a				
10 or 8†	9	11a			†7a, 9	†10 and/or 11a
12a						
4′	8	7a, 9, 6′		9, 6′		9, 6′
5′	6′	8, 7′/8′	6′		6′	10
7′/8′	6′	5′	6′	5′	6′	5′
OCH_3(2)		2		2		2
OCH_3(3)		3		3		3
OH(11)	11	10, 11a	11	10, 11a	11	10 and/or 11a

CC, prep. TLC, and recrystallization from MeOH gave: deguelin (**1**) (28 mg), α-toxicarol (**2**) (27.8 mg), tephrosin (**3**) (200 mg), 6a,12a-dehydrodeguelin (**4**) (13.2 mg), 12a-hydroxy-α-toxicarol (**5**) (400 mg), 6a,12a-dehydro-α-toxicarol (**7**) (47 mg) and 6a,12a-dehydro-β-toxicarol (**8**) (14 mg). Compounds **3** and **5** were purified by prep. recycling HPLC on a silica (5 μm) column with CH_2Cl_2–hexane–MeOH (70:28.5:1.5) as the mobile phase.

The EtOAc extract (8.0 g) was submitted to DCCC using a mobile phase system of hexane–$CHCl_3$–MeCN (10:3:7). Purification of the frs by silica gel (230–400 mesh) CC and prep. HPLC with recycling in the same mobile phase as described above afforded 36 mg of 12a-hydroxy-β-toxicarol (**6**). The acetates of compounds **7** and **8** were obtained by reaction of the natural products with Ac_2O/Py followed by silica gel CC.

12a-Hydroxy-β-toxicarol (**6**). Mp (C_6H_6): 157–160°, $[\alpha]_D$ −24.3° (C_6H_6, *c* 0.0015). EIMS *m/z* (rel. int.): 426 $[M]^+$ (7), 411 (2), 219 (13), 208 (100), 207 (65), 193 (7), 191 (5), 179 (4), 177 (3), 165 (16), 147 (3); ^1H NMR: Table 2; ^{13}C NMR: Table 3.

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