New Spirorotenoids from *Tephrosia candida*

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The ethyl acetate extract of roots of *Tephrosia candida* afforded three new spirorotenoids belonging to a new class of spirocompounds, named tephrospirolactone, tephrospiroketone I, and tephrospiroketone II. The structures of these compounds were determined mainly based on spectral analysis. The only known spirorotenoid described in the literature is amorphispironone, isolated from *Amorpha fruticosa*.

Introduction

The genus *Tephrosia* belongs to the family Fabaceae, which is well-known to be a rich source of flavonoids, besides the rotenoids among their secondary metabolites. Rotenoids, an interesting class of compounds showing mainly activity against insects, have also shown powerful ictiotoxic activity, fish poison. Rotenone was the first rotenoid identified and had been used as insecticide before the advent of the organosynthetic insecticides (Jacobson, 1971). Recently, properties as anticarcinogenic activities have also been attributed to rotenoids. Tephrosin and amorphispironone (**1**) isolated from *Amorpha* species have shown a potential use against tumours including skin cancer (Konoshima *et al*., 1993 and Li *et al*., 1993).

We have been interested in the chemistry of *T. candida* and have described the isolation of rotenoids from this plant (Andrei *et al*., 1997 and Pereira *et al*.,1998). In continuation of the study of the roots of *T. candida* we now describe the isolation and identification of three spirorotenoids, which represent a new spiroclass system formed by the rings B and C, a different spiro system type of amorphispironone, the only known spirorotenoid (Li *et al*.,1991).

Materials and Methods

General experimental procedures

A Bruker DRX-400 spectrometer, operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C NMR was used. All spectra were run in $CDCl_3$ and with TMS as internal standard. Optical rotations were measured with a Perkin-Elmer polarimeter. Mass spectra were obtained with a HP 5987A spectrometer.

Plant material

Roots of *T. candida* (Roxb.) DC were collected and identified in the Instituto Agronômico do Paraná, PR, Brazil, January 1988. Voucher specimens are deposited at the Herbário of Instituto Agronômico, Londrina, PR, Brazil.

Extraction and isolation of compounds

The roots were dried in an open stove at 60 °C and powdered affording 2.7 kg. Exhaustive extraction with ethyl acetate after hexane extraction, gave 36.5 g of crude extract (dry weight). A part of the ethyl acetate extract (8.0 g) was submitted to droplet countercurrent chromatography (DCCC) using an ascending system obtained from the mixture of the following solvents hexane:$CHCl_3$:CH_3CN (10:3:7 v/v/v). A fraction (2275 mg) was submitted to column chromatography (70–230 mesh) eluted with hexane and ethyl acetate with increasing polarity. Purification of the fractions with silica gel columns (230–400 mesh), preparative *tlc* on silica eluted with hexane:CH_2Cl_2:MeOH (50:49:1 v/v/v) and recycling preparative HPLC on silica gel using as eluent a mixture hexane:CH_2Cl_2:*i*-PrOH (80:10:1 v/v/v) afforded tephrospirolactone (**2**)

(7.3 mg), tephrospiroketone *I* (**3**) (5.2 mg) and tephrospiroketone *II* (**4**) (1.2 mg).

Tephrospirolactone (**2**): $[\alpha]_D^{25}$ +9.5° (4.5 mg/ml – CH_2Cl_2). IR ν_{max} KBr cm^{-1}: 1748, 1681, 1611, and 1583. EIMS *m/z* (rel. int.): 424(4), 222(3), 203(30), 202(27), 187(100) and 180(14). ^{1}HNMR ($CDCl_3$, 400 MHz): Table I. ^{13}CNMR ($CDCl_3$, 100 MHz): Table II. HMBC ($CDCl_3$, 100/400 MHz): Table III.

Thephrospiroketone *I* (**3**): $[\alpha]_D^{25}$ +30.9° (4.2 mg/ml – CH_2Cl_2). IR ν_{max} KBr, cm^{-1}: 1716, 1675, 1607, and 1507. EIMS: *m/z* (rel. int.): 408(32), 393(17), 213(26) and 180(100). ^{1}H NMR ($CDCl_3$, 400 MHz): Table I. ^{13}C NMR ($CDCl_3$, 100 MHz): Table II.

Tephrospiroketone *II* (**4**): ^{1}H NMR ($CDCl_3$, 400 MHz): Table I. ^{13}C NMR ($CDCl_3$, 100 MHz): Table II.

Results and Discussion

The infrared spectra showed absorption of carbonyls at 1681, 1748 and 1675 cm^{-1}, and 1716 cm^{-1} for **2** and **3** respectively. The absorption 1748 cm^{-1} was attributed to a carbonyl of an α,β-dihydro-δ-lactone ring and the one 1716 cm^{-1} to the α,β-dehydro-cyclopentanone, while absorptions close to 1680 cm^{-1}, are usually observed for carbonyls of 6a, 12a-dihydrorotenoids

The ^{1}H NMR of the three compounds displayed an AB coupling system for two geminal hydrogens, neighbouring the quaternary carbon of the spirorings (Table I). This system has never been observed before for any rotenoids. Other signals showed to be characteristic of hydrogens in the rings A, D and E, exhibiting differences of the chemical shifts, in comparison with deguelin (**5**) (Andrei *et al.* 1997) and rotenone (**6**) (Birch *et al.* 1985) as models. The chemical shifts observed for H-1 and H-4 in the ring A (Table I) exhibited accentuated differences indicating the presence of an electron withdrawing substituent at the aromatic system. The hydrogens in the D and E rings showed closer chemical shifts when compared with those of the above deguelin and rotenone. The pair of doublets attributed to H-6 in the spirocompounds showed similar chemical shifts with those of the homoisoflavanone scillascillin (**7**). The diastereotopic protons of this compound (H-2) appear as an AB system, displayed at δ 4.52 and 4.62 (J = 11.0 Hz) (Heller and Tamm, 1981).

The ^{13}C NMR chemical shifts were attributed to the carbons of compounds **1**, **2** and **3**, having rotenone (**6**) (Crombie *et al.*, 1975) and deguelin (**5**) (Andrei *et al.*,1997) as models (Table II). The chemical shifts of C-6a and the additional carbonyl in C-12a of the new compounds have never been described for common rotenoid skeleta before. The data of Table II are in agreement with the proposed structures.

When compared with the model compounds the main differences observed were: deshielding of C-3 and C-4a ($\Delta\delta$ ~ 8 ppm), suggesting the addi-

Table I. ^{1}H NMR data for spirocompounds ($CDCl_3$, 400 MHz). Rotenone (Birch *et al.*, 1985) and deguelin (Andrei *et al.*,1997) were used as models.

H	Tephrospirolactone (**2**)	Deguelin (**5**)	Tephrospiroketone I (**3**)	Tephrospiroketone II (**4**)	Rotenone (**5**)
1	7.18 *s*	6.72 *s*	7.27 *s*	7.27 *s*	6.77 *s*
4	6.51 *s*	6.38 *s*	6.53 *s*	6.53 *s*	6.46 *s*
6α	4.54 *d* (12)	4.11 *d* (12.4)	4.63 *d* (12.4)	4.63 *d* (12.4)	4.19 *d* (12)
6ß	4.78 *d* (12)	4.56 *dd* (12.4, 3.2)	4.69 *d* (12.4)	4.70 *d* (12.4)	4.62 *dd* (12, 3)
10	6.53 *d* (8.8)	6.38 *d* (8.8)	6.57 *d* (8.8)	6.65 *d* (8.8)	6.52 *d* (8.6)
11	7.74 *d* (8.8)	7.67 *d* (8.8)	7.44 *d* (8.8)	7.56 *d* (8.8)	7.84 *d* (8.6)
4′	6.36 *d* (10)	6.57 *d* (10)	6.55 *d* (10)	3.03/3.43 *dd* (15.6, 8.0/12.8, 9.6)	2.95/3.33 *dd* (15.8, 8.0/15.8, 9.8)
5′	5.55 *d* (10)	5.48 *d* (10)	5.62 *d* (10)	5.38 *t* (8.0)	5.25 *t* (8.8)
7′	1.41 *s*	1.32 *s*	1.49 *s*	4.98/5.11 *bs*	4.94/5.08 *s*
8′	1.43 *s*	1.38 *s*	1.52 *s*	1.79 *s*	1.77 *s*
OMe (2)	3.85 *s*	3.70 *s*	3.88 *s*	3.88 *s*	3.77 *s*
OMe (3)	3.96 *s*	3.73 *s*	3.96 *s*	3.96 *s*	3.81 *s*

Coupling constants (Hz) in parentheses.

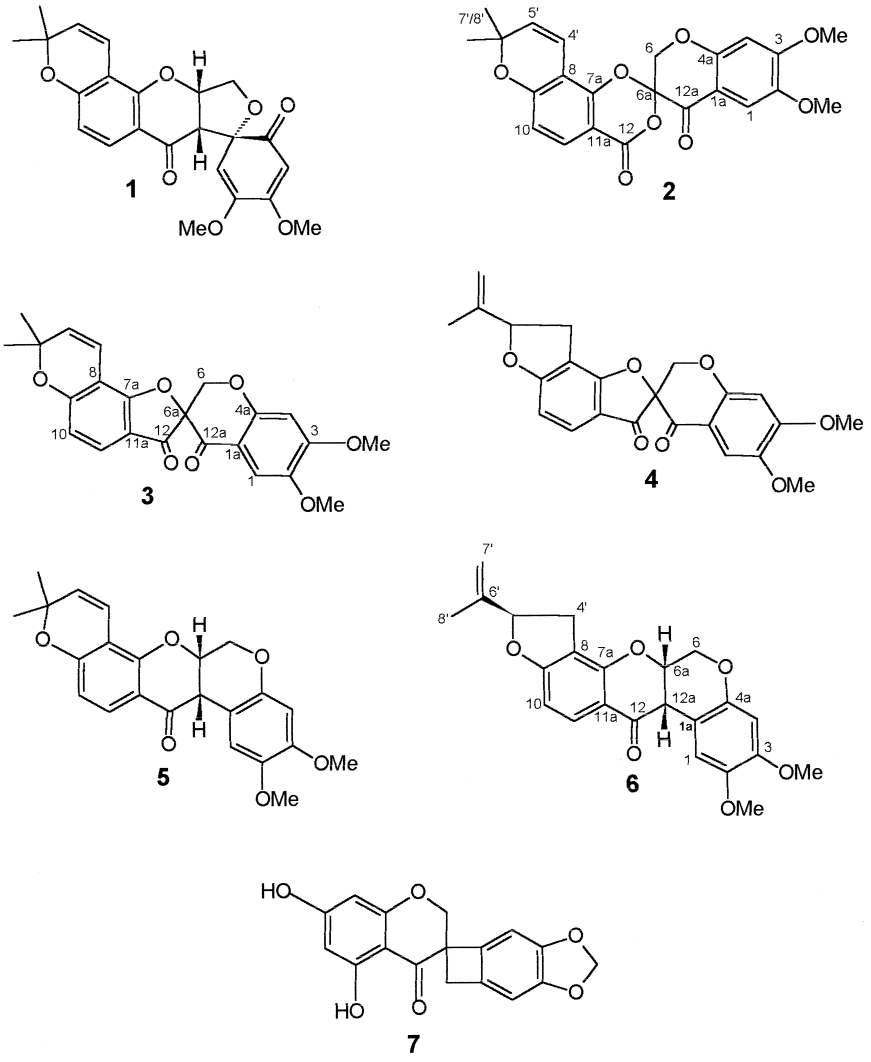

Fig. 1. Structures of rotenoids amorphispironone (**1**), tephrospirolactone (**2**), tephrospiroketone *I* (**3**), tephrospiroketone *II* (**4**), deguelin (**5**), rotenone (**6**) and scillascillin (**7**).

tional withdrawing effect of the conjugated carbonyl at C-12a; C-6 is deshielded ($\Delta\delta \sim 5$ ppm) due to β-effect of the quaternary carbon (6a); C-7a of the spiroketones showed deshielding of 9 ppm due to their presence in a five membered ring; the spirolactone with a six member ring showed a shielding for C-11a ($\Delta\delta = 6.1$ ppm) that can be explained by the electron donor effect of the lactone oxygen to C-12 and the chemical shifts for C-7a , C-8, C-9 and C-10 of spiroketones are deshielded in comparison with the models, while C-11 are shielded of 4 ppm in both cases.

The HMBC spectrum of tephrospirolactone (**2**)(Table III) showed cross peaks between hydrogens and carbons (^2J and ^3J) that confirmed the proposed structure. The main correlation was observed between C-6a and the diasterotopic hydrogens at C-6.

EI/MS also corroborated the structures proposed for the spirocompounds. The spectrum showed two possibilities of a retro Diels-Alder rearrangement. The base peak for **2**, could be explained by a retro Diels-Alder between C and D rings, followed by a common loss of a methyl

Table II. ^{13}C NMR data* for spirorotenoids ($CDCl_3$, 100 MHz). Rotenone (Crombie *et al.*, 1975) and deguelin (Andrei *et al.*, 1997) were used as models.

C	Tephrospiro-lactone (**2**)	Deguelin (**5**)	Tephrospiro-ketone I (**3**)	Tephrospiro-ketone II (**4**)	Rotenone (**6**)
1[a]	109.8	105.3	106.4	107.0	104.7
1	107.1	110.7	106.9	106.6	110.4
2	145.5	144.1	145.2	145.3	143.9
3	157.4[†]	149.8	157.2	157.3	149.5
4	100,0	101.2	100.1	100.2	100.9
4[a]	157.7[†]	147.7	158.2	158.3	147.4
6	71.7	66.5	71.0	71.0	66.3
6[a]	95.6	72.7	86.7	86.7	72.2
7[a]	150.5	150.0	161.8	168.8[†]	156.1
8	108.8	109.4	112.2[‡]	113.1[‡]	113.0
9	159.3[‡]	160.3	169.0	169.7[†]	167.4
10	112.3	111.7	113.3	109.5	104,7
11	130.0	128.9	125.1	126.5	130.0
11[a]	106.9	113.0	113.2[‡]	114.2[‡]	114.7
12	159.6[‡]	189.4	182.9	183.0	188.9
12[a]	181.3	44.6	193.0	192.7	44,6
4′	114.5	116.0	114.4	31.5	31.3
5′	130.0	128.9	129.2	88.3	87.8
6′	77.9	77.9	78.3	142.6	143.0
7′/8′	28.2/28.5	28.4/28.7	28.4/28.5	112.2/17.1	112.6/17.2
OMe(2)	56.2	56.1	56.2	56.3	55.8
OMe(3)	56.5	56.7	56.4	56.5	56.3

* Multiplicity obtained by PENDANT.
† and ‡ – δ values interchangeable in each column.

Table III. Correlation observed in the HMBC spectrum for tephrospirolactone (**2**) ($CDCl_3$, 100/400 MHz).

H/C	^2J	^3J
6α	6a	4[a]
6ß	6a	4a and 12a
11	—	7a, 9 and 12
4	3 and 4a	1a and 2
1	—	3 and 4a
4′	—	9 and 6′
5′	6′	8
Me (7′/8′)	6′	5′
OMe (2)	—	2
OMe (3)	—	3

group of the chromene ring. Mass spectrum of **3**, also presented the base peak due to the only one retro Diels-Alder type fragmentation.

Rotenoid biosynthesis is already well-known (Crombie, 1984 and Crombie and Whitting, 1998). These two last reviews show the main biosynthetic pathways. The biosynthetic pathway for amorphispironone **1**, isolated from *Amorpha fruticosa* (Li *et al.*, 1991; Terada *et al.*,1993) was discussed by Li (1991), and also mentioned by Crombie (1998). For the spiroketones **3** and **4**, a similar first step mechanism with an epoxidation, like in amorphispironone, can be suggested. Therefore, the starting compound should be the 6a,12a-dehydrodeguelin. The epoxidation should occur between C-6a and C-12a. In a hydrate form at C-12 carbonyl, a pinacol-pinacolone type rearrangement could lead to the spiro compounds, after interconversion to the carbonylated form and oxidation of C-12a into a keto group. Subsequent oxidation by a Baeyer-Villiger reaction type at C-12 and expansion to a six membered lactone ring could explain the formation of the spirolactone **2**.

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