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Biflavonoid toxicarine, rotenoids and a flavanone from the roots of Tephrosia toxicaria

Biflavonóide toxicarina, rotenóides e uma flavanona das raízes de Tephrosia toxicaria

César Cornélio Andrei[1]; Terezinha de Jesus Faria[2]; Adriana Aparecida Bosso[3]; Pedro Renato Anizelli[4]; Raimundo Braz-Filho[5,6]

Abstract

For the first time the structural determination of the biflavonoid, 4-H-2,3-dihydro-1-benzopyran-4-one-6-[2-phenyl-8-(3-methyl-2-butenyl)-5, 7-dimethoxy-2-H-3,4-dihydro-1-benzopiran-4-yl)-5,7-dihydroxy-8-(3-methyl-2-butenyl)-2-phenyl, named toxicarine (**1**) is presented, joined the isolation of the flavanone 7-O-methylglabranine (**2**) and a mixture of the rotenoids tephrosin (**3**) and rotenolone (**4**) from the roots of *Tephrosia toxicaria* (Sw.) Pers. Their structures were established by spectroscopic methods, meanly spectral data obtained by analysis of ^{1}H and ^{13}C NMR (1D and 2D) spectra.

Keywords: *Tephrosia toxicaria*. Fabaceae. Flavonoids. Roots.

Resumo

O isolamento e a determinação estrutural do biflavonóide 4-H-2,3-diidro-1-benzopiran-4-ona-6-[2-fenil-8-(3-metil-2-butenil)-5,7-dimetoxi-2-H-3,4-diidro-1-benzopiran-4-il]-5,7-diidroxy-8-(3-metil-2-butenil)-2-fenil, denominada toxicarina (**1**) relatada pela primeira vez, a flavanona 7-O-metil glabranina (**2**) e a mistura de rotenóides tefrosina (**3**) e rotenolona (**4**) foram isolados e caracterizados, das raízes de *Tephrosia toxicaria* (Sw.) Pers. As estruturas foram determinadas por métodos espectroscópicos principalmente pelos espectros de ^{1}H e ^{13}C uni e bidimensionais.

Palavras-chave: *Tephrosia toxicaria*. Fabaceae. Flavonoides. Raízes.

[1] Prof. Dr., Depto. Química, UEL, Londrina, Pr, Brasil, E-mail: cesar2andrei@gmail.com
[2] Profa. Dra., Depto. Química, UEL, Londrina, Pr, Brasil, E-mail: tjfaria@uel.br
[3] Profa. Dra. Depto. de Química, UEMG, Divinópolis, MG, Brasil; E-mail: adriana.tomal@uemg.br
[4] Prof. Dr. IFPr, Campus Jacarezinho, Jacarezinho, PR, Brasil; E-mail: pedro.anizelli@ifpr.edu.br
[5] Prof. Dr. Depto. de Química, ICE, UFRRJ, Seropédica, RJ, Brasil; E-mail: braz@ufrrj.br
[6] Prof. Dr. Laboratório de Ciências Químicas, UENF, Campos dos Goytacazes, RJ, Brasil; E-mail: braz@uenf.br

Introduction

The genus *Tephrosia* of the family Fabaceae, is well-known to be a rich source of flavonoid compounds. This class of natural compounds presents a great variety of types and structures. Biological properties of flavonoids may be described by their capacity to act as antioxidants able to scavenge free radicals and reactive oxygen species (WILMSEN; DALLA SANTA; SALVADOR, 2005).

A review on the phytochemistry and pharmacology properties of this genus (TOUQEER; AJAIB, 2013) show flavonoids and rotenoids as the main classes of compounds. 47 of *Tephrosia* species presented around 219 flavonoids and 65 rotenoids cited in this paper.

Biflavonoids are dimmers which present several biological and pharmacological activities, in relation to different unit linkage moieties. A study of 28 biflavonoids distributed in four types of linkage constituted a semi-empirical method to calculate inhibitory activity against *Mycobacterium tuberculosis*. The results suggested that it is possible to select the most promising molecules from a series of untested biflavonoid molecules (DIAS; REBELO; ALVES, 2004).

Investigations were also conducted to develop more active anti-inflammatory biflavonoids having unique mechanisms of action. For this purpose, 5,7-dihydroxy[6,6"]biflavone was synthesized using C-C cross coupling reaction to link the different units (LIM *et al*., 2009).

Phytochemical study of *Rhus tripartitum* (Anacardiaceae) revealed the presence of myricetin and the new biflavonoid masazinoflavanone, **5**, which was active in anti-inflammatory assays (MAHJOUB *et al*., 2010).

Tepicanol A, **6**, was the first biflavanol with a 4,4"biflavanyl ether group to be isolated from *Tephrosia tepicana*, specially it presents the flavanol unit with the same pattern of the flavan part of toxicarine, described in this paper (GOMEZ-GARIBAY *et al*., 1997).

Previous phytochemical studies of *Tephrosia toxicaria* revealed the presence of flavanone, flavone, isoflavore, rotenoid and chalcone compounds. These compounds were isolated and tested for their potential chemopreventive properties (JANG *et al*., 2003), furthermore the structural and electronic characterization of the same isolated flavonoids showed that these effects are relevant to understand their biological activity (ARROIO *et al*., 2004).

Two other papers describe studies of the chemical composition of this plant. The first reports the essential oils composition of leaves, stems, and shoots using GC and GC/MS (RIBEIRO *et al*., 2006) and the second the chemical and biological study of the ethanol extracts from leaves, stems, pods and roots (VASCONCELOS *et al*., 2009).

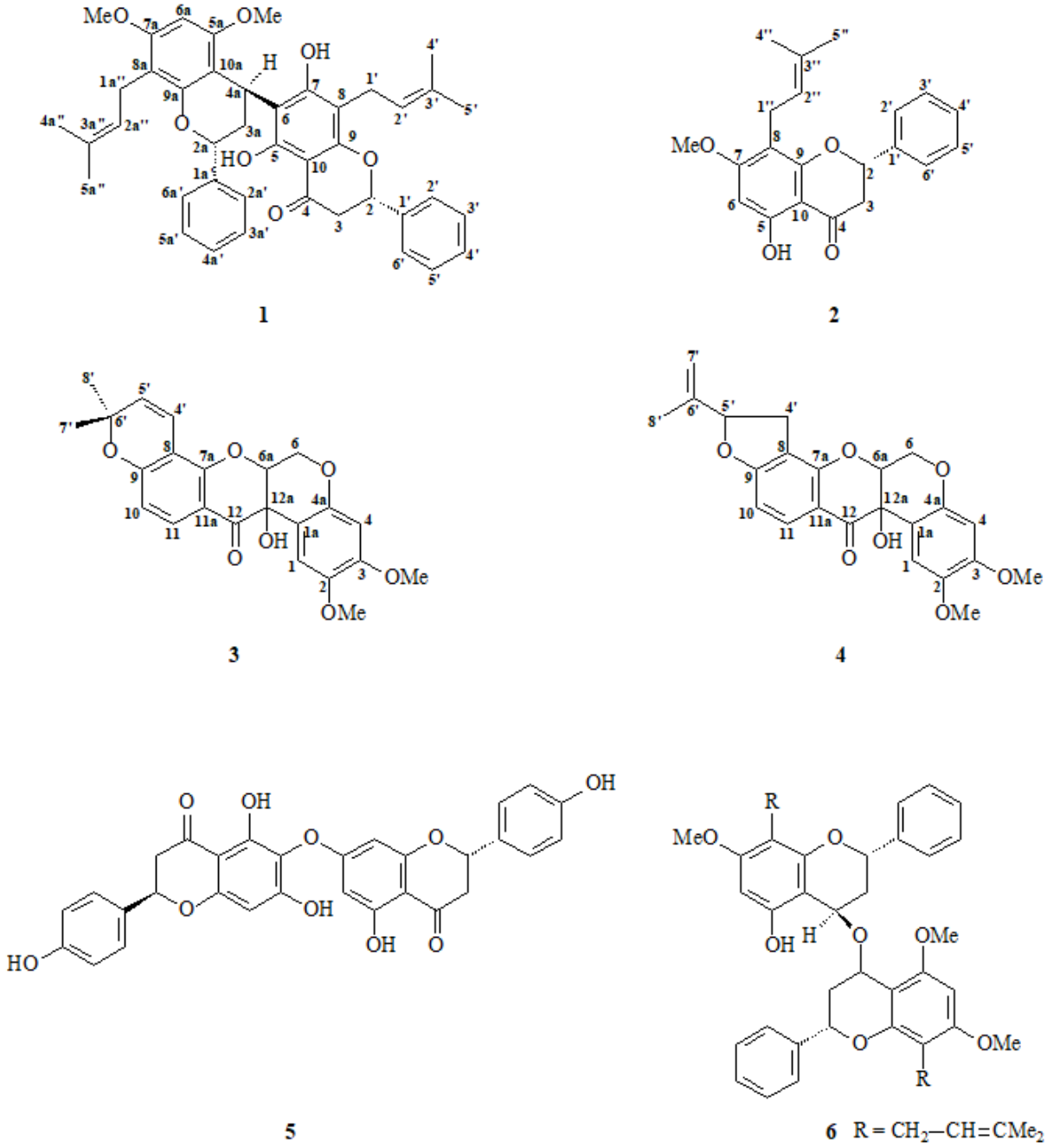
Tephrosia toxicaria cited as *Tephrosia sinapou*, in a synonyms case (DOMINGUES *et al*., 2019) has been used in South American countries in traditional medicine to alleviate pain and inflammation. Regarding to this studies have shown the analgesic effect of extracts of this plant in mice models, also in this paper it was presented for the first time the isolation of the biflavonoid toxicarine (MARTINEZ *et al*., 2013; MARTINEZ *et al*., 2016).

Results and Discussion

A new biflavonoid, named as toxicarine (**1**), constituted by the units of the flavanone glabranine and flavan 5-O-methylnitenin (respectively C-6:C-4a), the flavanone 7-O-methylglabranine (**2**) and a mixture of the rotenoids, tephrosin (**3**) and rotenolone (**4**), Figure 1, were isolated from the hexanic extract of the roots of *Tephrosia toxicaria*, through a combination of chromatographic techniques. The rotenoids were first identified with GC-MS analysis and and the structural confirmation was carried out by ^{1}H and ^{13}C NMR data and direct comparison with authentic samples isolated from *T. candida* (PEREIRA *et al*., 1998). The flavanone was identified by physical and spectral data comparison (mp, EIMS, IR, ^{1}H and ^{13}C NMR spectral values, (KISHORE *et al*., 2003). The position of the prenyl group was confirmed by HMBC correlations (Table 1), specially by heteronuclear interactions between C-5/HO-5 ($^{2}J_{CH}$) and C-6/C-10/HO-5, ($^{3}J_{CH}$). This NMR experiment is very useful to assure flavonoid A rings substitution pattern and applied to this compound, it's being reported for the first time. Compound **2** was first reported isolated from the roots of *Tephrosia* sp (CUCA-SUAREZ *et al*., 1980), and named as 5-hydroxyisoderricin. Subsequently the name 7-O-methylglabranine was used, for the first time, when this compound was synthesized (DAYAL, 1980).

The biflavonoid toxicarine (**1**), was characterized by ^{1}H, ^{13}C (^{1}H- and DEPT-), ^{1}H-^{1}H-COSY, ^{1}H-^{1}H-NOESY, ^{1}H-^{13}C-COSY–$^{n}J_{CH}$ (n = 1, direct correlation via one bond, HMQC or HETCOR, n = 2 and n = 3, long range, HMBC or COLOC) NMR spectra.

Figure 1 – Flavonoids and rotenoids isolated from *Tephrosia toxicaria* (Sw.) Pers: **1**-**4** and the biflavonoids **5**-**6** with biological activities.



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The flavanone unit was characterized by the chemical shifts of the carbonyl C-4 (δ_C 196.3), CH-2 [δ_C 78.6 and δ_H 5.42, dd, J =13.2 and 2.9 Hz (H-2_{ax})] and CH_2-3 [δ_C 43.7 and δ_H 2.83, dd, J =17.2 and 2.9 Hz, (H-3_{eq}) and 3.06, dd, J =17.2 and 13.2 Hz, (H-3_{ax})]; coherent with a ring C of a flavanone. COLOC spectra showed correlations among the carbons of the ring A at C-5 (^2J) and C-6 (^3J) with phenolic hydrogen HO-5 and C-7 (^2J) and C-8 (^3J) with HO-7, respectively. The correlations among C-7/C-9 (^3J) and C-8 (^2J) with H-1" indicated the position of the prenyl group at C-8. The B ring was characterized like as an unsubstituted ring, Table 1.

The flavan unit was characterized by the chemical shifts of the C ring. The saturated carbons CH-2a; CH_2-3a and CH-4a showed signals at δ_C 74.4; 37.0 and 26.9, respectively, which revealed correlation in HMQC spectrum (Table 1) with the correspondents hydrogen signals

Table 1 – NMR spectral data (^{1}H: 400 MHz; ^{13}C: 100 MHz; in $CDCl_3$), including chemical shifts, $^2J_{CH}$ and $^3J_{CH}$ of toxicarine **1**δ , $CDCl_3$ and TMS) and comparison with literature models. Chemical shifts in δ (ppm) and coupling constants (J in parentheses) in Hz.*

	Toxicarine				Models*	
C	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$	δ_C	δ_H
2	78.6	5.42 (dd, 13.2, 2.9)	H-3	H-2'-6'	79.0	5.58 (dd, 13.3)
3	43.7	2.83 (dd, 17.2, 2.9), 3.06 (dd, 17.2, 13.2)			43.0	2.80 (dd, 16.3), 3.13 (dd, 16.3, 13.9)
4	196.3	–		H-3	196.0	–
5	159.3	–	HO-5	H-4a	160.0	–
6	109.8**	–**	H-4a		97.0	6.04 (s)
7	162.8	–	HO-7	H-1"; H-4a	162.0	–
8	108.1	–	H-1"	HO-7	106.0	–
9	157.6	–		H-1"	164.0	–
10	102.8	–		HO-5; H-3	103.0	–
1'	139.1	–			135.0	–
2'/6'	125.9	7.28-7.48 (m)			128.0	7.59 (brd, 7.0)
3'/5'	128.3	7.28-7.48 (m)			128.0	–
4'	127.6	7.28-7.48 (m)			126.0	7.41-7.47 (m)
1"	21.8	3.19 (t, 5.5)			–	3.25 (d, 7.0)
2"	122.6	5.15 (t, 6.8)		3H-4"/3H-5"	–	5.21 (like m)
3"	130.9	–	3H-4"/3H-5"		–	–
4"	17.8	1.66 (s)			–	1.61 (brs)
5"	25.8	1.68 (s)			–	1.61 (brs)
HO-5	–	12.63 (s)			–	12.12 (s)
HO-7	–	6.80 (s)			–	9.57 (brs)
2a	74.4	5.06 (bd, 9.9)		H-4a	77.4	4.95 (d, 7.0)
3a	37.0	2.17 (ddd., 14.1, 11.5, 5.9) 2.32 (dbt, 13.9)			19.8	2.10 (m)
4a	26.9**	4.66 (dd, 4.0, 1.6)**			29.6	2.64 (m)
5a	157.5	–	H-6a	H-4a ; MeO-5a	156.1	–
6a	88.4	6.17 (s)			86.7	6.06 (s)
7a	158.7	–	H-6a	H-1"a ; MeO-5a	148.4	–
8a	110.9	–	H-1"a	H6a	112.7	–
9a	154.3	–		H-1"a; H-4a	154.7	–
10a	100.5	–	H-4a	H6a; H-3a	105.4	–
1'a	141.4	–			133.9	–
2'-6'a	126.0	7.28-7.48 (m)			125.6	7.30 (m)
3'a/5'a	128.7	7.28-7.48 (m)			113.9	7.30 (m)
4'a	128.5	7.28-7.48 (m)			159.3	7.30 (m)
1"a	22.0	3.35(d, 7.32)		–	3.25 (d)	
2"a	123.2	5.24 (t, 6.96)	3H-4"a/3H-5"a	–	5.2 (t)	
3"a	131.5	–	3H-4"a/3H5"a		–	–
4"a	17.8	1.57 (s)			–	1.6 (bs)
5"a	25.9	1.64 (s)			–	1.6 (bs)
MeO-5a	55.9	3.73 (s)			55.8	3.78 (s)
MeO-7a	55.8	3.87 (s)			–	3.80 (s)

* 5,7-dihydroxy-8-prenylflavan (glabranin): ^{1}H (400 MHz. Me_2CO-D_6) and ^{13}C ($CDCl_3$) values of C-2 to HO-7 (flavanone moiety) (TANAKA *et al*., 1997; AGRAWAL; THAKUR; BANSAL, 1989) and 5,7-dimetoxy-8-prenylflavan: ^{1}H values of C-2a to OMe-7a (flavan moiety) (GOMEZ-GARIBAY *et al*., 1997) and 2H-furo[2,3-h]-1-benzopyran. 3,4-dihydro-5-methoxy-2,8-bis (4-methoxyphenyl): ^{13}C (75 MHz $CDCl_3$) values of C-2a to OMe-7a values of C-2a to OMe-7a (flavan moiety) (BABA *et al*., 1986).

** Substituted position. Number of hydrogens bound to carbon atoms deduced by comparative analysis of ^{1}H- and DEPT-^{13}C NMR spectra. Chemical shifts and coupling constants (J. in parentheses) obtained from 1D-^{1}H NMR spectrum. 2D-^{1}H-^{1}H-COSY spectrum was also used in these assignments.

Source: The authors.

at δ_H 5.09 (brd, J =11.0 Hz, H-2a_{ax}), 2.35 (d, J =13.9 Hz, H-3a_{eq}), 2.22 (ddd, J =13.9, 11.0, 4.5, H-3a_{ax}) and 4.69 (d, J =4.5 Hz, H-4a_{ax}). The ring A of this unit showed two methoxyl groups at C-5a and C-7a. This pattern of substitution was confirmed by the HMBC (Table 1) and NOESY spectra, the interaction of H-6a (6.17, s) with both MeO-5a (δ_H 3.77, s) and MeO-7a (δ_H 3.90, s) indicating spatial proximity. Carbon atoms of the ring A of this unit showed similar type correlations (heteronuclear long range coupling, $^2J_{CH}$ and $^3J_{CH}$) between the C-5a/C-7a (^3J) with methoxy hydrogens (MeO-5a and MeO-7a, respectively) and also C-7a/C-9a (^3J) and C-8a (^2J) with H-1" suggesting the of the prenyl group at C-8a. As the same of the flavanone moiety, the flavan presents also an unsubstituted ring B.

The linkage of the two units was established by long range correlations observed in the HMBC spectrum, that revealed coupling of the C-5, C-6 and C-7 (δ_C 159.3, 109.8 and 162.8, respectively) with H-4a (δ_H 4.66, d, J =4.5 Hz), defining the linkage between C-6/C-4a.

The coupling constants of the signals of H-2 (dd, J =13.2 and 2.9 Hz) and H-2a (brd, J =9.9 Hz) were used to locate the phenyl groups at pseudo-equatorial position, since J =13.2 and 9,88 Hz indicated trans interactions between H-2/2a (αx) and H-3/3a (αx).

The relative configuration of the CH-4a (flavan moiety) was postulated by constants coupling (J =4.0 and 1.6 Hz) observed in the signal of the H-4a (δ_H 4.66, dd). These values of J allowed to eliminate the possibility of diaxial interaction involving this hydrogen H-4a. These arguments were used to locate the H-4a at pseudoequatorial position and, consequently, the flavanone moiety in pseudoaxial position, Figure 2.

Finaly, Table 1 shows the chemical shifts (^{1}H and ^{13}C), the correlations ($^2J_{CH}$ and $^3J_{CH}$) on COLOC spectra of toxicarine and comparison among chemical shifts (^{1}H and ^{13}C) with glabranin, (TANAKA *et al*., 1997; AGRAWAL; THAKUR; BANSAL, 1989), 5,7-dimetoxy-8-prenylflavan (GOMEZ *et al*., 1983) and 2H-furo[2,3-h]-1-benzopyran, 3,4-dihydro-5-methoxy-2,8-bis(4-methoxyphenyl) (BABA *et al*., 1986), that showed similar unit values in the comparison with the correspondent data of the flavonoid dimmer **1**.

Thus, structure of new flavonoid dimmer was established and the NMR spectra (1D and 2D) were also used to attribute the complete ^{1}H and ^{13}C chemical shift assignments, summarized at Table 1.

The mass spectra (GC-MS/EI) showed a fragmentation pattern of a very stable molecule. Toxicarine (**1**) presented the peak correspondent to molecular ion at *m/z* 660 ($M^{\cdot+}$, 100 %, base peak), and the other main fragments indicated loss of methoxy (*m/z* 629), isobutenyl (*m/z* 605) and isoprenyl (*m/z* 592) radicals. The peak at *m/z* 337 was attributed to the flavan moiety.

Experimental

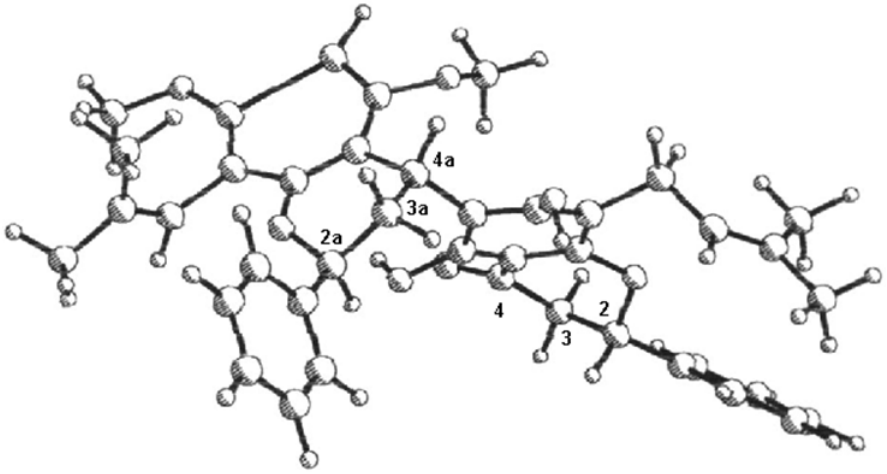
General experimental procedures

^{1}H (400 MHz), ^{13}C NMR (100 MHz) and HMBC spectra of **2** were recorded on a Bruker DRX-400 spectrometer (Chem. Dept. of University of Warwick, UK), ^{1}H (400 MHz), ^{13}C NMR (100 MHz), COSY, HMQC, HMBC and NOESY spectra of **1** were recorded on a JEOL Eclipse 400 spectrometer (LCQUI/ CCT-UENF) and ^{1}H (200 MHz), ^{13}C NMR (50 MHz), HETCOR and COLOC spectra of the mixture of **3** and **4** were recorded on a Bruker AC-200 spectrometer (Depto. de Química, ICE-UFRRJ). All of the RMN spectra used $CDCl_3$ as solvent and TMS as internal standard. EIMS were obtained on a Shimadzu QP 5000 (GC/MS), using direct inlet with 70 eV ionization to obtain spectrum of compound **1** (CCT- LCQUI/UENF) and EIMS on a Shimadzu QP 5000 (GC/MS), using DB-1 column, 70 eV ionization toobtain spectra of compounds **2**, **3** and **4** (LPMBA/DQ-CCE/UEL); CC Silica gel (Merck 0.05-0.70 mm); TLC: silica gel GF/PF_{254} (Merck); spots were visualised by UV (254 nm), by exposure to iodine vapour and after spraying with vaniline/MeOH/H_2SO_4. Uncorrected melting points were obtained on a Kofler apparatus type.

Plant material: Roots of *Tephrosia toxicaria* (Sw.) Pers. were collected and identified in the Instituto Agronômico do Paraná, PR, Brazil, in January 1988. Voucher specimen are deposited at the herbaruim of Instituto Agronômico, Londrina, PR, Brazil.

Isolation and purification of constituents: Exha-ustive extraction of the dry powdered roots (8.9 kg) with hexane at room temperature and the solvent removed under low pressure yielded 58.7 g of crude extract. 30g of hexane extract was applied to a silica gel (300 g) column, using hexane, hexane-CH_2Cl_2, CH_2Cl_2 and CH_2Cl_2-MeOH, as eluents, resulting in 20 groups, 8 oil and 12 solid, fractions. Column chromatography of the first solid fraction (1.86 g) using the same system of elution, re-

Figure 2 – Purpose of toxicarine relative configuration.



Source: The authors.

sulted in other 20 groups of refined fractions. Joined fractions 04-06 (1061.53 mg) with another CC, provided 825.3 mg of compound **2** and 35.4 mg of a mixture of **3** and **4**. Similar chromatographic methodology of joined fractions 08 and 09 (75.9 mg), followed by TLC as a final purification step afforded 23.67 mg of compound **1**.

Isolated compounds

Toxicarine (**1**): mp. 143 °C; ^{1}H, ^{13}C NMR and COLOC ($^{2}J_{CH}$ and $^{3}J_{CH}$) (Table 1); GC/EIMS *m/z* (rel. int. %): 660 ($M^{\cdot+}$, 100), 629 (5), 605 (5), 592 (10), 337 (48), 321 (32), 269 (30).

7-O-methylglabranine (**2**): mp. 123-125 °C; identified by ^{1}H (400 MHz) and ^{13}C (100 MHz) and HMBC NMR spectra and comparison with spectral data of literarure (KISHORE *et al*., 2003).

Tephrosin and rotenolone (mixture of **3** and **4**): identified by GC-MS analysis [tephrosin (**3**): 410 ($M^{\cdot+}$) 15 %; 392 ($M^{\cdot+}$-H2O) 20 %; 377 ($M^{\cdot+}$-H_2O–Me) 13 %; 208 (RDA) 100 %] and [rotenolone (**4**): 410 ($M^{\cdot+}$) 42 %; 392 ($M^{\cdot+}$-H_2O) 37 %; 208 (RDA) 100 %] and direct comparison with authentic samples isolated from *Tephrosia candida*. Structures confirmed by ^{1}H (200 MHz) and ^{13}C (50 MHz) NMR spectra from the mixture.

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