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SESQUITERPENE LACTONES FROM BRAZILIAN *TITHONIA DIVERSIFOLIA*

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Key Word Index—*Tithonia diversifolia*; Asteraceae; Heliantheae; sesquiterpene lactones; heliangolides; guaianolide.

Abstract—Aerial parts of *Tithonia diversifolia* collected in São Paulo State afforded two new heliangolides in addition to the heliangolides tagitinin F and 1,2-epoxytagitinin C, one known guaianolide and the flavone hispidulin. Structures were established by spectroscopic studies. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

The genus *Tithonia* (Asteraceae, tribe Heliantheae), comprising 11 species and 13 taxa, ranges from the south-western United States to Panama, although one species, *T. diversifolia* (Hemsl.) A. Gray, has escaped to Africa, Australia, Asia and North America where it is locally abundant [1]. Flavonoid records are available for all taxa [2]. Some intensive chemical studies were carried out on seven species which furnished sesquiterpene lactones of various types [3–23] and in one instance diterpenes as well [21] (for correction of species misidentifications in early work, see [13]). We have now studied a collection of *T. diversifolia* from north-western São Paulo State, Brazil, where it has also become naturalized and is widespread. The aerial parts furnished hispidulin, tagitinin F (**1**) [7, 11], the guaianolide **2** and the epoxytifruticin epimer **3**, both previously found in *Greenmaniella resinosa* Sharp [24], and the new heliangolides **4a** and **5**.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum (Table 1) of **4a**, $C_{21}H_{30}O_7$, differed from those of **4b–d** [24] only in the presence of two methoxyl singlets at δ 3.34 and 3.12 which corresponded to ^{13}C NMR signals at δ 58.33 and 49.73, respectively, the former being allocated to the methoxyl at C-1 (δ 86.11), the latter to the methoxyl at C-3 (δ 106.4) by one bond and long range C-H correlation experiments. Values of $J_{1,2}$ (6.6, 10 Hz) indicated that the C-1 methoxyl was β-orientated while β attachment of the isobutyryl side chain to C-8 was clear from the values of $J_{7,8}$ and $J_{8,9}$ and $^3J_{CH}$ long range HMBC. Signals of the other carbon atoms were assigned by DEPT 90°, DEPT 135° and ^{1}H × ^{13}C (^1J_H) (Table 1).

The ^{1}H NMR spectrum (Table 2) of **5**, $C_{20}H_{28}H_7$, was similar to that of **4a** but exhibited only one methoxyl singlet at δ 3.45 corresponding to a carbon quartet at δ 57.90, and correlated with the broadened doublet of H-2 at δ 3.64 and the triplet of C-2 at δ 86.30. Further homo- and heteronuclear experiments established the relationship of the methoxyl, C-2 and H-2 to the triplet of C-1 at δ 41.20, the singlet of C-3 at δ 105.9 and the hemiacetal hydroxyl at δ 3.96, the latter being further related to C-4 at δ 139.87 and H-15 at δ 1.94. The sequence C-4 through C-9 and the attachment of the isobutyryl residue to C-8 were established in the usual way; compound **5** is therefore the 2-O-methyl derivative of tagitinin B [5, 11].

EXPERIMENTAL

General. NMR: 200 MHz (^{1}H) and 50 MHz (^{13}C), $CDCl_3$. Standard Varian software was used for 2D NMR COSY which aided structural assignments. IR: KBr; MS: Hewlett–Packard Model 5988-A, direct inlet at 70 eV; CC: silica gel D (Hiedel-de-Häen, 400 mesh), silica gel 60 (Merck, 70–130 mesh), florisil (Fisher, 60–100 mesh) and Sephadex LH 20 (Sigma); HPLC: Shim-pack PREP-ODS (20 × 250 mm, 5 μm) with UV detection.

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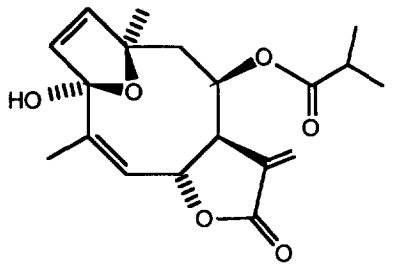
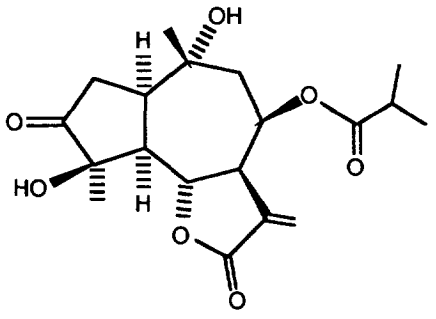


1



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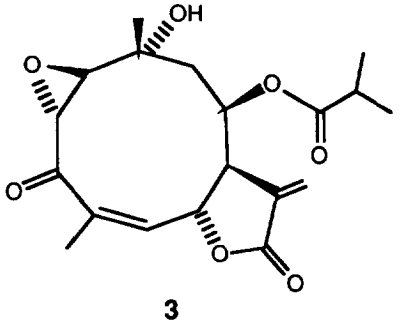

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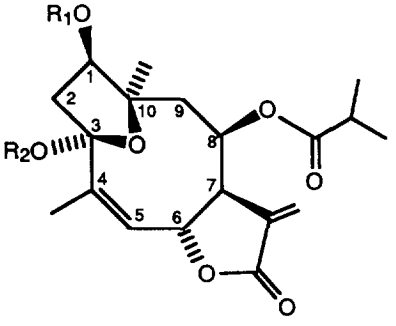
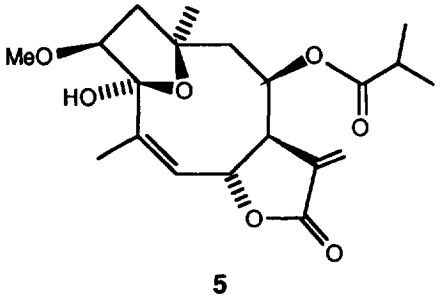


4 a R^1, R^2 = Me
b R^1, R^2 = H
c R^1 = H, R^2 = Me
d R^1 = Me, R^2 = H



5

Plant material. Aerial parts of *Tithonia diversifolia* (Hemsl.) A. Gray were collected near Ribeirão Preto, S.P., Brazil, in December 1990 and identified by the later Hermógenes de Freitas Leitão Filho, Departamento de Botânica, Universidade de Campinas (UNICAMP). A voucher specimen (Vichnewski # 311c) is on deposit in the herbarium of UNICAMP.

Extraction and isolation. Air dried and ground plant material (8.0 kg) was exhaustively extracted with hexane–EtOAc (4:1) at room temp. to give 750 g of crude extract which was suspended in MeOH–H_2O (19:1), H_2O being added to ppt fats and pigments. The sol was partitioned first with hexane and then with CH_2Cl_2. The CH_2Cl_2 extract on evapn at red. pres. furnished a residue (89.9 g) which was chromatographed over silica gel D (800 g) under vacuum, frs being eluted as follows, all frs being monitored by TLC: 1(*n*-hexane, 6 l), 2–33 (*n*-hexane–EtOAc, 19:1, 12 l), 33–44 (*n*-hexane–EtOAc, 93:7, 4 l), 45–90 (*n*-hexane–EtOAc, 9:1, 17 l), 91–108 (*n*-hexane–EtOAc, 17:3, 8 l), 109–162 (*n*-hexane–EtOAc, 4:1, 27.8 l), 163–215 (*n*-hexane–EtOAc, 3:1, 31 l), 216–291 (*n*-hexane–EtOAc, 7:3, 43 l), 292–316 (*n*-hexane–EtOAc, 3:2, 13 l), 317–345 (*n*-hexane–EtOAc, 1:1, 15.5 l), 346–381 (EtOAc, 20 l), 382–392 (EtOAc–MeOH, 9:1, 6 l) and 393–399 (MeOH, 4.2 l). Frs 48–100 (5.790 g) were recrystallized from hexane to give 180 mg of **4a**. Frs 121–138 (2.545 g) were placed on a florisil column (60 g) which was eluted with hexane containing increasing amounts of EtOAc and then with EtOAc–MeOH. Subfrs 33–72 (hexane–EtOAc, 19:1) furnished 0.52 g of solid whose recrystallization from EtOAc gave 200 mg of **5**. Frs 163–176 (800 mg) were subjected to RP-prep HPLC (ODS, 20 × 250 mm, flow rate 3.0 ml min^{-1}, UV detector operating at 210 nm) to give **1** (49.6 mg). Frs 191–215 (6.403 g) were chromatographed over Sephadex LH 20 (50 g), eluent MeOH, 10 ml frs. Subfrs 15–20 (5.525 g) were further purified by chromatography over silica gel 60 (200 g), eluent hexane–EtOAc. Frs eluted with hexane–EtOAc (4:1) were sepd by RP-prep HPLC (ODS 20 × 250 mm, flow rate 2.5 ml min^{-1}) using MeOH–H_2O (1:1) as eluent to provide **3** (27.8 mg, R_t 63–74 min) and **2** (34.6 mg, R_t 74–83 min). Subfrs 29–37 from frs 191–

Table 1. ^1H and ^{13}C NMR spectra of compound **4a** ($CDCl_3$)

Position	δ_H(*J* Hz)	$^1H \times {}^1H$	δ_C	$^1J_{CH}$	$^2J_{CH}$	$^3J_{CH}$
1	3.91 *dd*(6.6, 10)	2β, 2α	86.11 *d*	H-1		H-9α, H-14, OMe
2α	2.56 *dd*(6.6, 12.8)	1, 2β	41.94 *t*	H-2α, H-2β		
β	1.90 *dd*(10.0, 12.8)	1, 2α				
3			106.19 *s*			H-5, OMe′
4			139.15 *s*		H-15	H-2α
5	5.72 *m*	6, 15	130.42 *d*	H-5		H-15
6	5.37 *m*	7, 5, 15	75.90 *d*	H-6		
7	4.20 *m*	6, 8, 13a,b	49.19 *d*	H-7	H-8	H-13a
8	5.67 *m*	7, 9α	70.46 *d*	H-8	H-9α	
9α	2.00 *m*	8, 9β	35.11 *t*	H-9α, H-9β		
β	1.78 *m*	8, 9α				
10			81.79 *s*		H-14, H-9α	H-2β
11			136.39 *s*		H-13a	
12			169.38 *s*			
13a	6.24 *d*(2.6)	7	122.17 *t*	H-13a, H-13b		
13b	5.61 *d*(2.2)	7				
14	1.51 *s*		25.94 *q*	H-14		
15	1.74 *m*	5, 6	21.77 *q*	H-15		
1′			175.79 *s*		H-3′, H-4′	
2′	2.41 *m*	3′, 4′	33.84 *d*	H-2′		
3′	1.07 *d*(7.0)	2′	18.44 *q*	H-3′		
4′	1.02 *d*(7.0)	2′	19.93 *q*	H-4′		
OMe	3.36 *s*		58.33 *q*	OMe		
OMe′	3.12 *s*		49.73 *q*	OMe′		

Table 2. ^1H and ^{13}C NMR spectra of compound **5** ($CDCl_3$)

Position	δ_H(*J* Hz)	$^1H \times {}^1H$	δ_C	$^1J_{CH}$	$^2J_{CH}$	$^3J_{CH}$
1α	2.45 *m*	2, 1β	41.20 *t*	H-1		
β	2.34 *m*	2, 1α				
2	3.64 *d*(3.3)	1α, 1β, OMe	86.30 *d*	H-2		
3			105.90 *s*			H-15
4			139.87 *s*		H-15	OH
5	5.60 *m*	6, 15	128.30 *d*	H-5		
6	5.33 *m*	5, 7, 15	74.91 *d*	H-6		
7	4.20 *m*	6, 8, 13a, 13b	49.72 *d*	H-7		H-13a
8	5.60 *m*	6, 7, 9	71.02 *d*	H08		
9	1.84 *m*	8, 14	39.48 *t*	H-9		
10			85.09 *s*		H-14	
11			135.89 *s*		H-13b	
12			169.68 *s*			
13a	6.24 *d*(2.6)	7	122.76 *t*	H-13a,b		
13b	5.60 *d*(2.3)	7				
14	1.51 *s*	9	21.93 *q*	H-14		
15	1.84 *m*	5, 6	21.93 *q*	H-15		
1′			175.80 *s*			H-13′, H-4′
2′	2.40 *m*	3′, 4′	33.89 *d*	H-2′	H-3′, H-4′	
3′	1.06 *d*(7.0)	2′	18.50 *q*	H-3′		
4′	1.03 *d*(7.0)	2′	18.89 *q*	H-4′		
OH	3.96 *s*	OMe				
OMe′	3.45 *s*	OH	57.90 *q*	OMe		

215 furnished 51.6 mg of hispidulin. Compounds **2** and **3** were identified by ^1H and ^{13}C NMR spectroscopy using homonuclear correlation spectrometry. This showed, in the case of **2** that the literature assignments [24] for C-2 and C-9 must be inverted, i.e. δ C-2 = 39.32, δ C-9 = 46.97.

(R*, 3R*, 6S*, 7R*, 8R*, 10R*)-1,3-*Dimethoxy*-3,10-*epoxy*-8-(2-*methylpropanoyloxy*)-*germacra*-4,11

(13)-*dien*-6,12-*olide* (**4a**). White needles, mp 120–122°; IRν^{KBr} cm^{-1} 1760 (γ-lactone), 1725 (-CO_2R); MS PCI *m/z* (rel. int.) 395 ($[M+H]^+$ 0.6), 306 [M-$C_4H_8O_2$]6), 274 (5.7) 141 (93), 109 (64), 71 ($[C_3H_7CO]^+$); ^1H and ^{13}C NMR: Table 1.

(2S*, 3S*, 6S*, 7R*, 8R*, 10R*)-3,10-*Epoxy*-3,10-*hydroxy*-2-*methoxy*—8-(2-*methylpropanoyoxy*)-*germacra*-4,11(13)-*dien*-6,12-*olide* (**5**). White needles, mp 190–193°; IRν^{KBr} cm^{-1} 3500 (OH), 1750 (γ-lactone), 1720 (CO_2R); MS PCI *m/z* (rel. int.) 381 ($[M+H]^+$ 12), 292 (3.8), 71 ($[C_3H_7CO]^+$ 100); ^1H and ^{13}C NMR: Table 2.

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