

### Three New Heliangolides from *Tithonia rotundifolia*

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Further studies on metabolites of *Tithonia rotundifolia* (Mill.) Blake have furnished three new germacranolides, tithonin (**1a**), *O*-methyltithonin (**1b**), and 14-deacetyl-17,18-dihydrorotundin (**2**). Tithonin is a novel hemiacetal heliangolide oxygenated at C-14.

As part of our search for secondary metabolites of Salvadorian Compositae we studied *Tithonia rotundifolia* (Mill.) Blake (Heliantheae, subtribe Helianthinae), a native plant of Central America. Collections of the same species from Panama,<sup>1</sup> Brazil,<sup>2</sup> and different regions of Mexico<sup>3,4</sup> have been studied by several authors. The wide diversity of products obtained and their variation according to the place of collection led us to analyse a sample of *T. rotundifolia* collected in El Salvador.

The sesquiterpene lactones reported earlier were eudesmanolides of type (3) and heliangolides with an oxirane group at the 1,10-position, such as leptocarpin.<sup>5</sup>

Tirotundin (4) which bears an hemiacetal group not present in the former lactones, has been isolated from samples of *T. rotundifolia* collected in Panama.

*Tithonia rotundifolia* from El Salvador afforded three new heliangolides, tithonin (**1a**), its methyl ether (**1b**), and a rotundin derivative<sup>3</sup> (**2**).

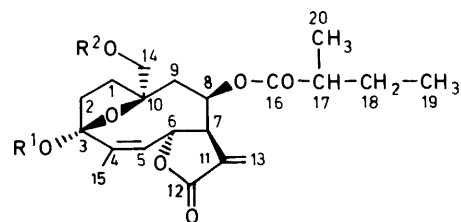
Tithonin (**1a**), m.p. 134–135 °C; C<sub>20</sub>H<sub>28</sub>O<sub>7</sub> (mass spectrum and elemental analysis);  $[\alpha]_D^{20} -121.3^\circ$  (MeOH), showed i.r. bands at 1760 and 1665 cm<sup>-1</sup> attributed to a  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone. The <sup>1</sup>H n.m.r. spectrum (Table 1) exhibits structural features typical for a heliangolide framework. The occurrence of a multiplet at low frequency ( $\delta_H$  3.87) suggested that tithonin might be a saturated hemiacetal of the woodhousin type (5).<sup>6</sup> The spectrum differs in that the methyl group attached to C-10 of woodhousin is oxidized to a hydroxymethyl group. This is shown by the AB part of an ABX system which collapses to two doublets by D<sub>2</sub>O exchange. This deduction was supported by the <sup>13</sup>C n.m.r. spectrum (Table 2) which showed a signal at  $\delta_C$  106.9, typical of a carbon carrying two oxygens, and another at  $\delta_C$  85.8 for the carbon atom attached to oxygen of the furan ring.

The signal at  $\delta_C$  68.1 corresponds to methylene C-14, and the signals at  $\delta_C$  37.5 and 39.1 correspond to C-1 and C-2; these signals are not present in woodhousin which has an acetate group on C-2.<sup>7</sup> The nature of the ester group was revealed by the peaks at  $\delta_H$  0.84 (methyl triplet), 1.04 (methyl doublet), and 1.38, 1.56 (two multiplets) and by the fragmentation under electron impact (diagnostically important peaks at *m/z* 261, 85, and 57, the first being base peak) showed that the ester chain was 2-methylbutyrate.

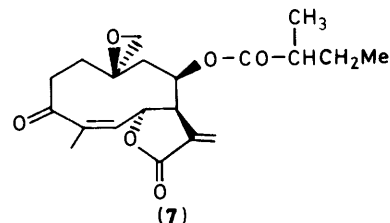
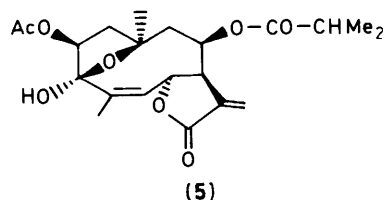
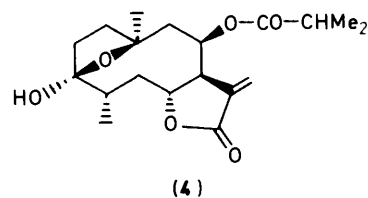
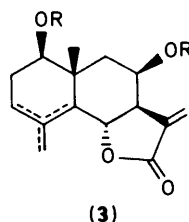
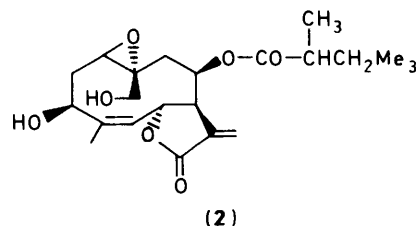
We have tried opening the hemiacetal group in tithonin (**1a**) by different chemical methods. However, tithonin has great stability towards boron hydrides<sup>8</sup> or oxalic acid-dioxane,<sup>9</sup> typical reagents used in the cleavage of hemiacetals.

Another approach was the tosylation of tithonin (**1a**) to afford the C-14 tosylate (**6**) in low yield (28%). The tosyl derivative was treated with NaH in tetrahydrofuran (THF) to give the product (**7**) (Table 1). The behaviour of tithonin (**1a**) towards these reagents can be rationalized by assuming the existence of hydrogen bonding between the hydroxy groups on C-3 and C-14.

*O*-Methyltithonin (**1b**), a gum, is the corresponding methyl ether at C-3 of tithonin. Its m.s. gave the parent peak at *m/z* 364, which differed from the parent of tithonin by fourteen mass



(1a) R<sup>1</sup> = R<sup>2</sup> = H  
(1b) R<sup>1</sup> = Me, R<sup>2</sup> = H  
(6) R<sup>1</sup> = H, R<sup>2</sup> = Ts



units, and an *M*<sup>+</sup> - OCH<sub>3</sub> peak, thus strongly suggesting that compound (**1b**) represented a methyl ether derivative of tithonin. Further evidence supporting the structure (**1b**) was provided by the <sup>1</sup>H and <sup>13</sup>C n.m.r. spectra. The first showed a singlet at  $\delta_H$  3.23, characteristic of a methoxy group ( $\delta_C$  50.4),

**Table 1.**  $^1\text{H}$  N.m.r. spectral data of compounds (1a), (1b), (2), and (7) (200 MHz;  $\text{SiMe}_4$  as internal standard;  $\text{CDCl}_3$ )  $J$  values in parentheses

	(1a)	(1b)	(2)	(7)
1-H			2.92 dd (5.1, 10.3)	
2-H			2.50 dt (15, 5), 1.53 m	
3-H			4.45 dd (3.5, 5)	
5-H	5.57 dq (4.9, 1.5)	5.71 dq (3.4, 1.6)	5.26 br d (1.5, 10.9)	5.59 dq (7.1, 1.6)
6-H	5.46 ddq (4.9, 1.5, 3.3)	5.40 ddq (3.6, 2.0, 3.9)	6.52 dd (2.5, 10.9)	5.42 br d (7.1)
7-H	3.87 dddd (3.4, 2.2, 2.4, 3.3)	4.12 dddd (2.5, 2.6, 3.6, 3.6)	2.87 m	3.26 m
8-H	5.44 ddd (3.4, 10.9, 6.1)	5.65 ddd (3.6, 10.5, 5.2)	5.14 m	4.74 ddd (12.2, 11.0, 4.3)
9-H	2.01 dd (10.9, 15), 2.04 dd (6.1, 15)	1.97 dd (10, 15), 2.15 dd (5.5, 15)	1.18 br d (15.6), 3.01 dd (4.7, 15.6)	2.06 dd (11.0, 15.6), 2.16 dd (4.3, 15.6)
13-H	5.64 d (2.2), 6.28 d (2.4)	5.63 d (2.3), 6.30 d (2.3)	5.71 d (2.0), 6.31 d (2.2)	5.77 (d (2.0), 6.34 d (2.5)
14-H	3.51 d (11.2) (exchanged with $\text{D}_2\text{O}$ ), 3.72 d (11.2)	3.65 d (11.3), 3.79 d (11.3)	3.90 d (14.5), 3.96 d (14.5)	2.77 (d (7.1), 2.88 d (7.1)
15-H	1.87 dd (1.5, 1.5)	1.79 dd (1.6, 2.0)	1.81 (d (1.5)	1.96 br s
17-H	2.36 m	2.28 m	2.39 m	2.45 m
18-H	1.38 m, 1.56 m	1.40 m, 1.58 m	1.37 m, 1.55 m	1.37 m, 1.54 m
19- $\text{H}_3$	0.84 t (7.2)	0.87 t (7.2)	0.81 t (7.5)	0.83 d (7.4)
20- $\text{H}_3$	1.04 d (7)	1.07 d (7)	1.05 d (7.2)	1.02 d (7.2)
MeO		3.23 s		

**Table 2.**  $^{13}\text{C}$  N.m.r. spectral data of compounds (1a), (1b), and (2) (50.32 MHz;  $\text{SiMe}_4$  as internal standard;  $\text{CDCl}_3$ )

Carbon	(1a)	(1b)	(2)
1	37.5* $\text{CH}_2$	37.7*	63.8 $\text{CH-O}$
2	39.1* $\text{CH}_2$	37.9*	32.2 $\text{CH}_2$
3	106.9 $\text{C-O}$	110.4	75.0* $\text{CH-O}$
4	141.6 $\text{C=}$	139.9	141.0 $\text{C=}$
5	128.6 $\text{HC=}$	130.9	126.8 $\text{HC=}$
6	73.2 $\text{CH-O}$	72.2	75.4* $\text{CH-O}$
7	49.7 $\text{CH}$	49.7	48.4 $\text{CH}$
8	75.5 $\text{CH-O}$	76.1	73.7 $\text{CH-O}$
9	31.5 $\text{CH}_2$	29.7	39.1 $\text{CH}_2$
10	85.8 $\text{C-O}$	86.5	60.5 $\text{C-O}$
11	137.2 $\text{C=}$	136.9	137.2 $\text{C=}$
12	169.8 $\text{C=O}$	169.6	169.5 $\text{C=O}$
13	123.2 $\text{C=}$	122.6	124.8 $\text{C=}$
14	68.1 $\text{CH}_2\text{-O}$	68.7	72.4 $\text{CH}_2\text{-O}$
15	21.1 $\text{CH}_3$	32.2	23.0 $\text{CH}_3$
16	175.6 $\text{C=O}$	175.7	175.5 $\text{C=O}$
17	41.3 $\text{CH}$	41.2	41.5 $\text{CH}$
18	26.5 $\text{CH}_2$	26.5	26.3 $\text{CH}_2$
19	11.5 $\text{CH}_3$	11.6	11.6 $\text{CH}_3$
20	16.6 $\text{CH}_3$	16.7	16.6 $\text{CH}_3$
OMe		50.4	

\* Assignments may be interchanged.

and the signals for 7-H and 8-H are shifted +0.25 and +0.21 p.p.m., respectively.

The stereochemistry depicted in formulae (1a) and (1b) for carbons C-3 and C-10 (3*S*,10*S*) has been tentatively assigned in relation to that of the structurally related compound woodhousin.<sup>10</sup> In order to ascertain this point, we have carried out an n.o.e. difference experiment on compound (1b) between 3-MeO and 4-Me. Unfortunately we observed only an 8% enhancement in the integrated intensity of the 4-Me signal when the methoxy group was irradiated and this result does not provide any conclusive stereochemical information as to the absolute configuration at C-3.

Compound (1b) was correlated with tithonin by reaction of the latter with MeOH in acid medium.

14-Deacetyl-17,18-dihydrorotundin (2) is a minor constituent of this species, and has the formula  $\text{C}_{20}\text{H}_{28}\text{O}_7$ . Its  $^1\text{H}$  n.m.r. spectrum shows again the hydroxymethyl group as an AB system at  $\delta_{\text{H}}$  3.90 and 3.96. The major differences seen in the spectrum are the presence of a proton at  $\delta_{\text{H}}$  4.45 and the shift of

the 7-H to higher field.\* This suggests the presence of a hydroxy group at C-3. Both the  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. spectra (see Tables 1 and 2) (as compared with those of rotundin<sup>3</sup>) were fully consistent with the assigned structure (2).

### Experimental

M.p. were determined with a Büchi apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 141 polarimeter at 20–25 °C and i.r. spectra were recorded on a Pye Unicam SP 1100 spectrophotometer. Mass spectra were obtained with an Hewlett Packard GC-MS 5985B instrument, with high-resolution spectra being obtained on a Kratos MS9 instrument, c.i. spectra were run with methane as the carrier gas; only relevant ions in the mass spectra are listed and their relative intensities are indicated in parentheses as a percentage of the base peak.  $^1\text{H}$  and  $^{13}\text{C}$  n.m.r. spectra were recorded on a Bruker SY-200 spectrometer at 200 MHz and 50.3 MHz, respectively, the chemical shifts being given in p.p.m. from  $\text{SiMe}_4$  ( $\delta$  0) as internal standard. Microanalyses were performed at the Servicio de Microanálisis del Centro Nacional de Química Orgánica de Madrid.

*Isolation of T. rotundifolia constituents.*—Above-ground parts of dried (1.4 kg) *Tithonia rotundifolia* (Mill.) Blake (aerial part) collected in Nov. 1979 near volcán de Izalco, El Salvador, were extracted with hexane. The defatted plant was then extracted with chloroform. The crude gum (38 g) obtained from the second extract was chromatographed over silica gel (800 g) eluted with toluene–EtOAc mixtures, then with  $\text{Et}_2\text{O}$  and  $\text{Et}_2\text{O}$ –MeOH mixtures. The fractions eluted with  $\text{Et}_2\text{O}$  gave a mixture of three components; the mixture was rechromatographed over silica gel ( $\text{Et}_2\text{O}$ ) and afforded compounds (1b) (0.1 g) and (1a) (1.2 g), while the intermediate fractions were separated by preparative t.l.c. ( $\text{Et}_2\text{O}$ –MeOH; 95:5) to yield compounds (2) (6 mg) and (1a) (0.1 g).

*Tithonin (1a).*—(1.3 g, 3.5%), m.p. 134–135 °C (from diethyl ether–acetone);  $[\alpha]_{\text{D}}^{20}$  –121.3° ( $c$  0.45 in MeOH);  $R_{\text{F}}$  0.28 (diethyl ether), 0.59 (diethyl ether–acetone; 10:1), and 0.16 (toluene–acetone; 10:1);  $\nu_{\text{max}}$  (KBr) 3 600, 1 760, 1 735, 1 665,

\* The deshielding effect of 7-H is due to the oxygen atom attached to C-10 in hemiacetals with configuration *R* at C-10.<sup>1</sup>

1 290, 1 150, 1 020, and 980  $\text{cm}^{-1}$ ; the positive c.i. m.s. exhibited ions at  $m/z$  (%) 381 ( $M + H^+$ , 19.9), 363 (35.3), 345 (1.3), 261 (100), 85 (35.1), and 57 (80.0) (Found: C, 63.2; H, 7.4.  $\text{C}_{20}\text{H}_{28}\text{O}_7$  requires C, 62.95; H, 7.65%).

3-O-Methyltithonin (**1b**).—(0.1 g, 0.26%), an oil;  $R_F$  0.48 (diethyl ether), 0.79 (diethyl ether–acetone; 10:1), and 0.34 (toluene–acetone; 10:1);  $\nu_{\text{max.}}$ ( $\text{CHCl}_3$ ) 3 650, 1 770, 1 740, 1 610, 1 110, and 1 020  $\text{cm}^{-1}$ ;  $m/z$  (%) 394 ( $M^+$ , 1.9), 363 (1.8), 309 (1.4), 293 (3.5), 261 (21.9), 85 (20.4), and 57 (100) (Found:  $M^+$ , 294.1974.  $\text{C}_{21}\text{H}_{30}\text{O}_7$  requires  $M$ , 394.1991).

14-Deacetyl-17,18-dihydrotundin (**2**).—(6 mg, 0.016%), an oil;  $R_F$  0.66 (diethyl ether), 0.84 (diethyl ether–acetone; 10:1), and 0.48 (toluene–acetone; 10:1);  $\nu_{\text{max.}}$ ( $\text{CHCl}_3$ ) 3 510, 1 760, 1 735, and 1 650  $\text{cm}^{-1}$ ;  $m/z$  (%) 380 ( $M^+$ , 2.1) 363 (1.9), 259 (18.3), 85 (25.1), and 57 (100) (Found: C, 62.9; H, 7.55.  $\text{C}_{20}\text{H}_{28}\text{O}_7$  requires C, 62.95; H, 7.65%).

Tosylation of Tithonin (**1a**).—A solution of the lactone (**1a**) (400 mg, 1.05 mmol) and toluene-*p*-sulphonyl chloride (175 mg, 0.92 mmol) in dry pyridine (7.2 ml) was stirred for 72 h at 0 °C. The solution was poured into ice-water, and extracted with diethyl ether. The combined extracts were washed successively with 2% sulphuric acid (3  $\times$ ), water (1  $\times$ ), and brine (1  $\times$ ). The ether layer was dried over magnesium sulphate, and the solvent was removed. Chromatography on silica gel with  $\text{Et}_2\text{O}$  as eluant yielded the tosyl derivative (**6**) (0.160 g, 28.5%) as a slightly yellow viscous oil,  $\nu_{\text{max.}}$ (KBr) 3 450, 1 770, 1 735, 1 601, 1 305, 1 285, 1 195, 1 180, 1 150, 985, and 815  $\text{cm}^{-1}$ ;  $\delta_{\text{H}}$  (100 MHz;  $\text{CDCl}_3$ ) 7.45 (dd,  $J$  9 Hz, ArH), 6.30 (1 H, d,  $J$  2.3 Hz, 13-H), 5.64 (1 H, d,  $J$  2.3 Hz, 13-H'), 5.60 (1 H, dq,  $J$  5 and 1.5 Hz, 5-H), 5.45 (1 H, ddq,  $J$  5, 1.5, and 3.3 Hz, 6-H), 5.43 (1 H, ddd,  $J$  3.35, 10, and 6 Hz, 8-H), 4.20 (2 H, s, 14-H<sub>2</sub>), 3.85 (1 H, m, 7-H), 2.40 (3 H, s, ArMe), and 1.85 (3 H, t,  $J$  1.5 Hz, 15-H<sub>3</sub>);  $m/z$  (%) 517 ( $M^+ - 17$ , 0.6), 432 (1.8), 165 (6.7), 85 (39.1), and 57

(100) (Found: C, 60.5; H, 6.3.  $\text{C}_{27}\text{H}_{34}\text{O}_9\text{S}$  requires C, 60.70; H, 6.36%).

Reaction of Tosyl Compound (**6**) with NaH.—A solution of compound (**6**) (0.1 g, 0.19 mmol) in dry THF (1 ml) was added during 5 min to a stirred suspension of sodium hydride (4.5 mg, 0.19 mmol) (mineral oil free) under  $\text{N}_2$ . The reaction mixture was stirred for 30 min more and was then treated with water (20 ml) and extracted with diethyl ether. The combined extract was dried over magnesium sulphate, and the solvent was removed. Chromatography on silica gel with  $\text{Et}_2\text{O}$  as eluant yielded the epoxide (**7**) (0.026 g, 38.5%) as an oil,  $\nu_{\text{max.}}$ (KBr) 3 500, 1 770, 1 750, 1 700, 1 665, and 1 275  $\text{cm}^{-1}$ ;  $m/z$  (%) 362 ( $M^+$ , 0.3), 277 (1.9), 85 (21.3), and 57 (100) (Found: C, 66.1; H, 7.1.  $\text{C}_{20}\text{H}_{26}\text{O}_6$  requires C, 66.32; H, 7.18%).

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