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SESQUITERPENE LACTONES FROM *TITHONIA ROTUNDIFOLIA**

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Key Word Index—*Tithonia rotundifolia*; Compositae; Heliantheae; sesquiterpene lactones; heliangolides; eudesmanolides; 15-hydroxyleptocarpins; tirotundifolins.

Abstract—A chemical investigation of *Tithonia rotundifolia* afforded seven new sesquiterpene lactones in addition to three known ones. The structures of the new compounds 3-acetyl-15-hydroxyleptocarpin, 15-hydroxyleptocarpin and tirotundifolins A, B, C, D and E were established by spectroscopic and chemical means.

INTRODUCTION

Tithonia rotundifolia (Miller) S. F. Blake, a plant native of Mexico and Central America has been introduced to other parts of the world [1]. Previous studies analysed the sesquiterpene lactones of *T. rotundifolia* from north-eastern Brazil [2] and the Mexican high plateau [3]. Now we report our results on the study of *T. rotundifolia* from four different regions of México.

T. rotundifolia from Tepic, Nayarit, furnished leptocarpin (**1a**) [4]. The plant from Iguala, Guerrero, yielded two new heliangolides, 3-acetyl-15-hydroxyleptocarpin (**1b**) and 15-hydroxyleptocarpin (**1c**). Samples of *T. rotundifolia* collected in Coyuca de Benitez and Agua de Obispo, Guerrero, contained in addition to the previously reported **3a** and **4a** [2], five new eudesmanolides: tirotundifolin A, B, C, D and E (**3b**, **4b**, **4c**, **5a** and **5c**).

RESULTS AND DISCUSSION

T. rotundifolia from Tepic, Nayarit, afforded leptocarpin (**1a**), mp 202–204°, identified by its typical physical constants [4] and confirmed by direct comparison of one of its hydrogenation products. Leptocarpin afforded on hydrogenation dihydroheliangin (**2a**) and dihydroleptocarpin (**2b**). The latter compound was identical with an authentic sample of dihydroleptocarpin.

The two new heliangolides **1b** and **1c** were constituents of the plant from Iguala, Guerrero. 3-acetyl-15-hydroxyleptocarpin (**1b**), $C_{22}H_{28}O_8$, mp 213–214°, showed IR bands at 1765, 1745 and 1725 cm^{-1} attributed to a γ-lactone, an acetate and another ester group. The ^1H NMR spectrum (Table 1) is typical for a heliangolide esterified at C-3, resembling that of acetyl leptocarpin, except for the absence of the C-15 methyl which in **1b** is substituted for a hydroxymethylene group. This is shown by the signal at δ4.18 (AB system) which moves downfield on acetylation (δ4.62). The C-15 position for the free hydroxyl was confirmed by a mild oxidation (MnO_2) to an aldehyde conjugated with a *cis*-double bond as shown by the signal of the aldehyde proton which resonates at δ9.45 [5].

The second compound 15-hydroxyleptocarpin (**1c**), is the corresponding desacetyl derivative of **1b**, since it afforded **1d** on acetylation. Catalytic hydrogenation transformed **1c** into dihydroheliangin **2a**. This reaction implies the saturation of the exocyclic methylene group, isomerization of the angelate and hydrogenolization of the C-15 hydroxyl group. The same compound (**2a**) was obtained by catalytic hydrogenation of leptocarpin (**1a**), thus confirming structures **1b** and **1c**.

A collection of *T. rotundifolia* from Coyuca de Benitez, was extracted with chloroform and the extract percolated through tonsyl (see Methods). Two main fractions were separated: (*a*) the chloroform fraction and (*b*) the ethyl acetate fraction. Chromatography of fraction (*a*) gave three crystalline eudesmanolides (**3a**, **4a** and **5a**). The lactones **3a** and **4a** were previously found in a Brazilian collection of *T. rotundifolia* [2]. The third constituent was the new eudesmanolide **5a** which we have called tirotundifolin A.

Tirotundifolin A (**5a**) $C_{20}H_{26}O_6$, mp 90–92°, contains a γ-lactone (IR bands at 1765 and 1675 cm^{-1}). The position and stereochemistry of the lactone ring was inferred from its ^1H NMR spectrum (Table 2), which was almost superimposable upon that of reynosin (**6**) [6, 7], except for an epoxyangelate group attached at C-8 on the β-face, since the H-8 signal appeared as a broadened doublet of doublets at δ5.88 (J = 3, 6 Hz). The signals corresponding to the epoxyangelate were identical to those for **3a** and **4a**.

Tirotundifolin A (**5a**) contains a β-hydroxyl group at C-1 which was shown by an IR band at 3500 cm^{-1} and confirmed by formation of the acetate **5b**. In both **5a** and **5b** the β-orientation of the sustituent at C-1 was deduced from their coupling constants (Table 2).

A catalytic hydrogenation of **4a** provided dihydroisotirotundifolin A (**7**) and tetrahydrotirotundifolin A (**8**). The latter was identical with the hydrogenation product of **5a** thus indicating that **4a** and **5a** have the same stereochemistry at C-1, C-5, C-6, C-7, C-8 and C-10.

The fraction (*b*) provided four new eudesmanolides (**3b**, **4b**, **4c** and **5c**). Tirotundifolin B (**3b**, $C_{20}H_{26}O_6$, mp 163–165°) had a γ-lactone conjugated with an exocyclic methylene group as indicated by its IR bands (1770 and

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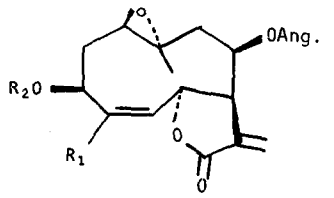
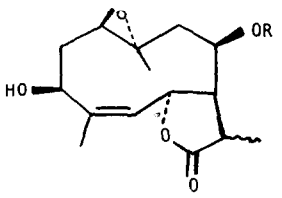
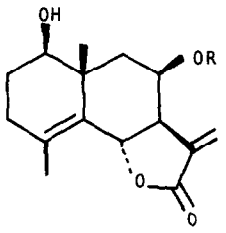


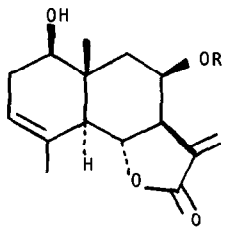
1a R_1=Me, R_2=H
1b R_1=CH_2OH, R_2=Ac
1c R_1=CH_2OH, R_2=H
1d R_1=CH_2OAc, R_2=Ac
1e R_1=CHO, R_2=Ac



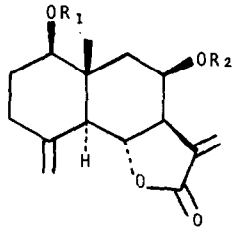
2a R=Tig.
2b R=Ang.



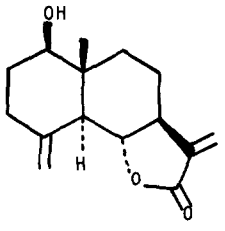
3a R=Epang.
3b R= A



4a R=Epang.
4b R= A
4c R=Sarrac.



5a R_1=H, R_2=Epang.
5b R_1=Ac, R_2=Epang.
5c R_1=H, R_2= B



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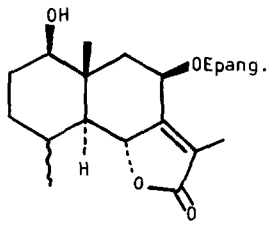

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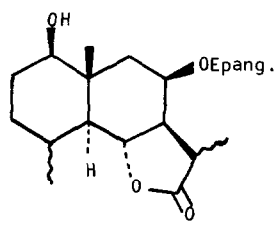

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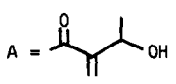

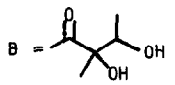

1670 cm^{-1}), and by characteristic low field doublets in its ^1H NMR spectrum (Table 2). A broadened doublet signal at δ5.07 (J = 12 Hz) is attributed to the allylic proton of the lactone closure at C-6. The hydroxyl group at C-1 was established by the doublet of doublets at δ3.55 (J = 7, 8 Hz) which was identical to the corresponding signal in santamarine [8].

The ester group was shown by the IR band at 1715 cm^{-1} and by the ^1H NMR signals of two broadened singlets at δ6.1 and 5.95 for the hydrogens of the conjugated methylene in the side chain. Furthermore, a doublet at δ 1.36 (3H, J = 7 Hz) and a quartet at 4.57 (1H, J = 7 Hz) corresponded to the methyl and hydrogen attached to C-3′ in the 2-methylidene-3-hydroxybutyrate ester. This was in accord with the mass spectrum, which in addition to the molecular peak at m/z 362 and the base peak at m/z 246 $[M-C_5H_8O_3]^+$, displayed diagnostically important peaks at m/z 99 $[C_5H_7O_2]^+$ and 81 $[C_5H_5O]^+$ which are compatible with a hydroxyl-containing $C_5H_7O_2$ acyl side chain in **3b**.

Tirotundifolin C and D (**4b** and **4c**) showed the same gross structure as **4a**. The ^1H NMR signals of **4b** at δ6.1 (1H, *br s*), 5.9 (1H, *br s*), 4.57 (1H, *br q*, J = 7 Hz) and 1.36 (3H, *d*, J = 7 Hz) corresponded to the two vinylic hydrogens and the methyl of the ester at C-8. The sarracinic ester of **4c** was identified by the typical ^1H NMR signals at δ6.36 (*br q*), 4.2 (*br s*) and 2.03 (*br d*).

Tirotundifolin E (**5c**) is a eudesmanolide with the same gross structure as **5a**. The ^1H NMR spectra of both compounds showed a great similarity except for the presence of three signals interchangeable with D_2O in **5c** instead of only one in **5a**. The presence of two methyl

Table 1. ^{1}H NMR spectral data for sesquiterpene lactones obtained from *Tithonia rotundifolia*

	1b	1c	1d	1e	2a
H-1	2.77 *dd* (5,6)	2.8 *m*	2.85 *m*	2.64 *d br* (5)	2.75 *m*
H-2$_a$	2.6 *dt* (5,17)	2.38 *dt* (3,14)	2.55 *dt* (5,16)	2.50 *dt* (5.16)	
H-2$_b$	1.77 *m*	2.0 *m*			
H-3	5.35 *dd* (2,5)	4.52 *br*	5.32 *dd* (2,5)	5.95 *dd* (3,5)	4.45 *dd* (3,4)
H-5	5.52 *d br* (11)	5.47 *d br* (11)	5.52 *d br* (11)	6.3	5.47 *d br* (11)
H-6	6.09 *dd* (2,11)	6.62 *d br* (11)	6.1 *dd* (2,11)	6.28 *br*	6.55 *d br* (11)
H-7	2.95 *m*	2.9 *m*	2.93 *m*	3.0 *m*	2.4 *m*
H-8	5.22 *m*	5.15 *m*	5.2 *m*	5.26 *m*	5.1 *m*
H-9$_a$	2.75 *m*	2.75 *m*	2.85 *m*	2.84 *dd* (5,16)	
H-9$_b$	1.36 *dd* (2,15)	1.0 *m*	1.35 *dd* (2,16)	1.35 *dd* (2,16)	
H-13$_a$	6.37 *d* (2)	6.32 *br*	6.37 *d* (2)	6.45 *d* (2)	1.07* *d* (7)
H-13$_b$	5.76 *d* (2)	5.8 *br*	5.78 *d* (2)	5.89 *d* (2)	
H-14$_a$	1.52	1.45	1.49	1.5	1.43
H-15	4.18† *br*	4.0† *br*	4.62† *br*	9.45	1.82* *br*
OAc	2.1*		2.1‡	2.1*	
OCOR	6.15 *q br* (7)	6.08 *q br* (7.5)	6.15 *m*	6.16 *d br* (7.5)	6.85 *q br* (7.5)
	2.0* *d br* (7)	1.93* *d br* (7.5)	2.0* *d br* (7.5)	2.0* *d br* (7.5)	1.82* *br*
	1.86* *m*	1.81* *br*	1.86* *m*	1.87* *m*	1.8* *d br* (7.5)

Run at 80 MHz in $CDCl_3$ with TMS as an internal standard. Values are in ppm. Unmarked signals are singlets. Values in parentheses are coupling constants in Hz.
*Intensity of three protons.
†Intensity of two protons.
‡Intensity of six protons.

signals (δ1.17, *s* and 1.26, *d*) and a quartet (δ3.77, 1H) suggested the occurrence of a 1,2-dihydroxy-1-methyl-butanoate in tirotundifolin E. This was confirmed by the mass spectrum, since it exhibited peaks at *m/z* 381 $[(M+1)-C_5H_{10}O_4]^+$, 117 $[C_5H_9O_3]^+$, 89 $[C_4H_9O_2]^+$ and 45 $[C_2H_5O]^+$.

Esterification *in situ* (TAI) of tirotundifolin E (**5c**) afforded a tricarbamate, evidenced by three N–H signals in its ^{1}H NMR spectrum. This spectrum also showed paramagnetic shifts of the signals due to methyl groups (δ1.72, *s* and 1.48, *d*) and H-3′ (δ5.45, *q*), thus confirming the positions of two hydroxyl groups at C-2′ and C-3′ as depicted in **5c**.

A collection of *T. rotundifolia* from Agua de Obispo, Guerrero, was shown to contain the same constituents (in smaller yield) as those found in the plant from Coyuca de Benítez.

EXPERIMENTAL

Mps. are uncorr. Elementary analysis were performed by Mr. Egmont Pascher, Bonn, Germany. The tonsyl employed consisted of SiO_2 (72.5%), Al_2O_3(13.0%), Fe_2O_3(5.0%), MgO (1.5%), CaO (0.8%) humidity (8.5%) with pH 3. Vouchers of the plants were deposited in the herbarium of the Biology Institute, U.N.A.M.

Extraction of Tithonia rotundifolia *collected in Tepic, Nayarit.* The air-dried plant material (1.54 kg) collected in July, 1980 (voucher: ARV 0026 MEXU 282540) was extracted with $CHCl_3$ (3 × 2 l.) and the resulting residue (60 g) was percolated on tonsyl using solvents of increasing polarity (hexane, $CHCl_3$). The $CHCl_3$ fraction (20 g) was chromatographed through silica gel eluting with $CHCl_3$ and increasing proportions of Me_2CO. Leptocarpin (**1a**) crystallized in the fraction eluted with $CHCl_3$–Me_2CO (9:1). Recrystallization from $CHCl_3$–hexane (1.3574 g), mp 202–204°, $[\alpha]_D = -108.92°$ ($CHCl_3$, *c* 0.213). The ^{1}H NMR and IR data were identical with those published for leptocarpin [4].

Hydrogenation of **1a**. A soln of 100.7 mg compound **1a** in 10 ml EtOAc was hydrogenated for 6 hr over 10 mg 10% Pd–C at atm. pres. The usual work up furnished 66.9 mg of **2a** as white crystals from $CHCl_3$–hexane, mp 209–211°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1760, 1710; EIMS 70 eV, *m/z* (rel. int.): 364 $[M]^+$ (1.3), 265 $[M-AngOH]^+$ (3.0), 247 $[M-AngOH-H_2O]^+$ (4.0), 83

Table 2. ^{1}H NMR spectral data for sesquiterpene lactones obtained from *Tithonia rotundifolia*

	3b	4b	4c	5a	5b	5c	8
H-1	3.55 *dd* (7,8)	3.62 *dd* (7,10)	3.65 *dd* (7,8)	3.52 *dd* (5,11)	4.76 *dd* (5,11)	3.47 *dd* (5,11)	3.25 *m*
H-2	1.7 *m*	2.4 *m*	2.45 *m*	1.7 *m*	2.25 *m*	1.6 *m*	2.25 *m*
H-3	2.2 *m*	5.35 *m*	5.35 *m*	2.25 *m*	1.8 *m*	2.25 *m*	1.6 *m*
H-5	2.2 *m*	2.45 *m*	2.45 *m*	2.25 *m*	2.25 *m*	2.25 *m*	1.6 *m*
H-6	5.07 *d br* (12)	4.42 *t* (11)	4.4 *t* (11)	4.52 *t* (11)	4.47 *t* (11.5)	4.5 *t* (11)	3.72 *t* (11)
H-7	2.93 *dq br* (3.5,12)	2.82 *dq br* (3,12)	2.82 *dq br* (3,11)	2.85 *dq br* (3,11)	2.83 *dq br* (3,11.5)	2.83 *dq br* (3,11)	2.5 *m*
H-8	5.82 *dd br* (3.5,5)	5.87 *dd br* (3,5.5)	5.82 *dd br* (3,6)	5.88 *dd br* (3,6)	5.76 *dd br* (3,6)	5.67 *dd br* (3,5)	5.6 *dd br* (2,6)
H-9	2.42 *dd* (3.5,15)	2.4 *m*	2.38 *dd* (2,16)	2.25 *m*	2.35 *m*	2.4 *m* 1.7 *m*	2.5 *m* 1.6 *m*
H-13$_a$	6.2 *d* (3.5)	6.2 *d* (3.5)	6.17 *d* (3.5)	6.13 *d* (3.5)	6.13 *d* (3.5)	6.13 *d* (3.5)	1.25* *d* (7)
H-13$_b$	5.5 *d* (3.5)	5.5 *d* (3.5)	5.46 *d* (3.5)	5.42 *d* (3.5)	5.38 *d* (3.5)	5.56 *d* (3)	
H-14$_a$	1.23	1.04	1.05	1.0	1.1	0.95	1.17
H-15	1.85* *br*	1.87* *br*	1.89* *br*	5.02 *br* 4.94 *br*	5.03 *br* 4.95 *br*	4.97 *br* 4.89 *br*	1.07* *d* (7)
OCOR	4.57 *q br* (7) 1.36 *d* (7) 6.1 *br* 5.85 *br*	4.57 *q br* (7) 1.32 *d* (7) 6.1 *br* 5.9 *br*	6.36 *q br* (7.5) 2.02 *d br* (7.5) 4.2† *br*	3.03 *q* (5.5) 1.27 *d* (5.5) 1.55*	3.01 *q* (5) 1.26 *d* (5) 1.53*	3.77 *q* (7) 1.17 *d* (7) 1.27*	3.04 *q* (5.5) 1.33 *d* (5.5) 1.55*
OAc					2.05*		

*Intensity of three protons.
†Intensity of two protons.

$[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (43.4).

A soln of 156.5 mg compound **1a** in 15 ml EtOAc was hydrogenated for 30 min over 16.9 mg 5% Pd–C under atm. pres. The usual work up gave 103.9 mg of white crystals from $CHCl_3$–hexane, mp 144–147°. Direct comparison with an authentic sample of dihydroleptocarpin established the identity.

Extraction of T. rotundifolia *from Iguala, Guerrero.* The air-dried plant material (2.370 kg) collected in October, 1981, (voucher AR V 0052 ME XU 34 0 474) was extracted with $CHCl_3$ (3 × 3 l.) and the resulting extract (125.5 g) was percolated on tonsyl with solvents of increasing polarity: hexane, $CHCl_3$, EtOAc, Me_2CO. In the EtOAc fractions yielded crystallized **1b**. The remaining uncrystalline materials from the EtOAc fractions were combined and chromatographed over silica gel. Elution with $CHCl_3$–Me_2CO (7:3) gave **1b** and **1c**. 3-Acetyl-15-hydroxyleptocarpin (4.750 g) (**1b**) was obtained as white crystals from $CHCl_3$–*i*-propyl ether, mp 213–214°; $[\alpha]_D = -118.25°$ ($CHCl_3$, *c* 0.16); UV λ_{max}^{EtOH} nm: 221 (ε19950); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3615, 3510, 1765, 1745, 1725, 1665. EIMS 70 eV, *m/z* (rel. int.): 420 $[M]^+$ (0.1), 402 $[M-H_2O]^+$ (35.5), 360 $[M-HOAc]^+$ (5.5), 337 $[M-C_5H_7O]^+$ (51.1), 321 $[M-AngO]^+$ (12.2), 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (55.2), 43 $[C_2H_3O]^+$ (43.1). (Found: C, 62.44; H, 6.69. $C_{22}H_{28}O_8$ requires: C, 62.84; H, 6.71%.)

The later fractions yielded 5.3 g of 15-hydroxyleptocarpin (**1c**) as colourless gum. $[\alpha]_D = -89.49°$ (MeOH, *c* 0.238); UV λ_{max}^{EtOH} nm: 212 (ε18430); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410, 1750, 1710, 1650, 1635. CIMS (CH_4) 200 eV, *m/z* (rel. int): 379 $[M+1]^+$ (60.7), 279 $[M+1-AngOH]^+$ (33.9), 261 $[M+1-AngOH-H_2O]^+$ (78.6), 243 $[M+1-AngOH-2H_2O]^+$ (35.7).

Acetylation of **1b**. Compound **1b** (100 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) for 1 hr on the steam bath. Work-up in the usual manner afforded **1d** (78 mg) as white crystals from hexane–*i*-propyl ether, mp 149–150°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1740, 1715, 1650. EIMS 70 eV, *m/z* (rel. int.): 462 $[M]^+$ (0.1), 402 $[M-HOAc]^+$ (1), 302 $[M-HOAc-AngOH]^+$ (1), 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (31.8), 43 $[C_2H_3O]^+$ (37.6). (Found: C, 62.8; H, 6.54. $C_{24}H_{30}O_9$ requires: C, 62.32; H, 6.34%.)

Oxidation of **1b**. A soln of 100 mg **1b** in 10 ml $CHCl_3$ was stirred at room temp. with 1 g of activated MnO_2. The reaction was being followed by TLC. After 2 hr the mixture was filtered and the solvent evaporated. Addition of *i*-propyl ether gave **1e** (40 mg), mp 149–161°. UV λ_{max}^{EtOH} nm: 217 (ε12 300); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1750, 1725, 1700, 1660, 1650; EIMS 70 eV, *m/z* (rel. int.): 400 $[M-H_2O]^+$ (2.19), 259 $[M-HOAc-AngO]^+$ (7.7), 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (51.7), 43 $[C_2H_3O]^+$ (32.8). (Found: C, 62.40; H, 6.25. $C_{22}H_{26}O_8$ requires: C, 63.15; H, 6.26%.)

Acetylation of **1c**. Compound **1c** (100 mg) was acetylated with Ac_2O (1 ml) and pyridine (1 ml) for 5 hr on the steam bath. Work-up in the usual manner afforded 48 mg of **1d**.

Hydrogenation of **1c**. A soln of 1 g of **1c** in 15 ml EtOAc was hydrogenated for 3 days over 100 mg 10% Pd–C at atm. pres. The usual work-up furnished a mixture which was purified by prep. TLC ($CHCl_3$–Me_2CO, 9:1). The most polar band gave 150 mg of **1a**.

Extraction of T. rotundifolia *from Coyuca de Benitez, Guerrero.* The air-dried plant material (1.04 kg) collected in July, 1981

(voucher MEXU 340474) was extracted with $CHCl_3$ (3 × 2 l.) and the resulting extract (135.6 g) was percolated on tonsyl with solvents of increasing polarity (hexane, $CHCl_3$, EtOAc). The $CHCl_3$ fraction (96.1 g) was percolated again on tonsyl eluting with hexane, $CHCl_3$, EtOAc and Me_2CO. The $CHCl_3$ fractions afforded a gum (38.4 g) which was chromatographed over silica gel eluting with $CHCl_3$ and increasing proportions of Me_2CO. The $CHCl_3$–Me_2CO (95:5) fractions gave **3a** (525 mg). Recrystallization from Me_2CO–hexane, mp 166–167°. $[\alpha]_D$ = −17.5° (MeOH, *c* 0.177). (Found C, 66.08; H, 7.18. Calc. for $C_{20}H_{26}O_6$: C, 66.28; 7.23%.) The ^1H NMR data were identical with those published [2].

The $CHCl_3$–Me_2CO (9:1) fractions gave a gum which was rechromatographed over Kieselgel G, eluting with $CHCl_3$–Me_2CO (9:1) to give 3.005 g of **4a** as white crystals from $CHCl_3$–hexane, mp 162–164°. $[\alpha]_D$ = +41.8° ($CHCl_3$, *c* 0.177). (Found: C, 65.91; H, 7.31. Calc. for $C_{20}H_{26}O_6$: C, 66.28; H, 7.23%.) The ^1H NMR data were identical with those published [2].

The $CHCl_3$–Me_2CO (9:1) fractions gave a gum which was purified in the same manner as described for **4a** to yield 7.6 g of **5a** from Me_2CO–hexane, mp 90–92°. $[\alpha]_D$ = +56.9° ($CHCl_3$, *c* 0.23); UV λ_{max}^{MeOH} nm: 205 (ε11255); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1765, 1750, 1675, 1655; CIMS (CH_4) 200 eV, *m/z* (rel. int.): 363 $[M+1]^+$ (58.2), 247 $[M+1-RCO_2H]^+$ (100), 229 $[M+1-RCO_2H-H_2O]^+$ (55.7).

The EtOAc fraction (16.9) obtained from the first percolation over tonsyl was chromatographed on a silica gel column eluting with $CHCl_3$ and increasing amounts of Me_2CO. The $CHCl_3$–Me_2CO (95:5) fractions gave **4a** (450 mg). The $CHCl_3$–Me_2CO (92:8) fractions gave 1.175 g of **5a**. $CHCl_3$–Me_2CO (85:15) fractions gave 703 mg of **4b**. Recrystallization from Me_2CO–hexane, mp 168–170°. $[\alpha]_D$ = +30.8° (MeOH, *c* 0.204); UV $\lambda_{max}^{CHCl_3}$ nm: 220 (ε6400), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380, 1780, 1715, 1680, 1630; EIMS 70 eV, *m/z* (rel. int.): 362 $[M]^+$ (7.9), 246 $[M-RCO_2H]^+$ (30.6), 228 $[M-RCO_2H-H_2O]^+$ (13.8), 99 $[C_5H_7O_2]^+$ (39.3), 81 $[C_5H_5O]^+$ (100). (Found: C, 65.91; H, 7.20. $C_{20}H_{26}O_6$ requires: C, 66.28; H, 7.23%.)

The mother liquor of **4b** purified in the same manner as described for **4a**, yielded 82.35 mg of **3b** crystallized from Me_2CO–hexane, mp 163–165°. $[\alpha]_D$ = −12.2° (MeOH, *c* 0.212); UV λ_{max}^{EtOH} nm: 220 (ε4850); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420, 1770, 1715, 1670, 1630; EIMS 70 eV, *m/z* (rel. int.): 362 $[M]^+$ (0.7), 347 $[M-CH_3]^+$ (0.7), 246 $[M-RCO_2H]^+$ (100), 228 $[M-RCO_2H-H_2O]^+$ (23.2), 99 $[C_5H_7O_2]^+$ (5), 81 $[C_5H_5O]^+$ (16.6). (Found: C, 65.84; H, 7.11. $C_{20}H_{26}O_6$ requires: C, 66.28; H, 7.23%.)

The $CHCl_3$–Me_2CO (85:15, 8:2) fractions gave **4c** (176.8 mg). Recrystallization from Me_2CO–hexane, mp 163–165°. UV λ_{max}^{MeOH} nm: 207 (ε21000); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 1770, 1725, 1670, 1650; EIMS 70 eV, *m/z* (rel. int.): 362 $[M]^+$ (11.2), 246 $[M-RCO_2H]^+$ (61.7), 228 $[M-RCO_2H-H_2O]^+$ (25.9), 99 $[C_5H_7O_2]^+$ (100), 81 $[C_5H_5O]^+$ (87.2), 53 $[C_4H_5]^+$ (60.49).

The Me_2CO fractions gave 70 mg of **5c**. Recrystallization from Me_2CO–hexane, mp 213–215°. $[\alpha]_D$ = +9.3° (MeOH, *c* 0.224); UV λ_{max}^{MeOH} nm: 202 (ε54400); IR ν_{max}^{nujol} cm^{-1}: 3525, 3440, 3380, 1775, 1734, 1680, 1650; CIMS (CH_4) 200 eV, *m/z* (rel. int.): 381 $[M+1]^+$ (100), 247 $[M+1-RCO_2H]^+$ (56.4), 229 $[M+1-RCO_2H-H_2O]^+$ (22.8); EIMS 70 eV, *m/z* (rel. int.): 117 $[C_5H_9O_3]^+$ (18.51), 89 $[C_4H_9O_2]^+$ (62.6), 45 $[C_2H_5O]^+$ (93.1), 43 $[C_2H_3O]^+$ (100).

Hydrogenation of **4a**. Compound **4a** (103.4 mg) in 10 ml EtOAc was hydrogenated for 3 hr with 23.9 mg 5% Pd–C at atm. pres. The usual work-up furnished a mixture which was purified by prep. TLC ($CHCl_3$–hexane–Me_2CO, 3:1:1). The most polar band gave 38.8 mg of non-crystalline **7**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3490, 1750; CIMS (CH_4) 200 eV, *m/z* (rel. int.): 365 $[M+1]^+$ (100), 249 $[M+1-RCO_2H]^+$ (60.1), 231 $[M+1-RCO_2H-H_2O]^+$ (51.7). The less polar band gave 28.4 mg of **8**. Recrystallization from Me_2CO–hexane, mp 161–163°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1770, 1735; EIMS 70 eV, *m/z* (rel. int.): 250 $[M+RCO_2H]^+$ (6.9), 71 $[C_4H_7O]^+$ (41.2), 43 $[C_2H_3O]^+$ (100). (Found: C, 64.82; H, 8.22. $C_{20}H_{30}O_6$ requires: C, 65.55; H, 8.25%.)

Acetylation of **5a**. Compound **5a** (214 mg) was acetylated with Ac_2O (2 ml) and pyridine (2 ml) for 30 min on a steam bath. Work-up in the usual manner afforded 76.2 mg of non-crystalline **5b**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1750, 1735, 1680, 1660; CIMS (CH_4) 200 eV, *m/z* (rel. int.): 405 $[M+1]^+$ (59.5), 345 $[M+1-HOAc]^+$ (39.3), 289 $[M+1-RCO_2H]^+$ (29.8), 229 $[M+1-RCO_2H-HOAc]^+$ (100); EIMS 70 eV, *m/z* (rel. int.): 43 $[C_2H_3O]^+$ (100).

Hydrogenation of **5a**. A soln of 112.5 mg **5a** in 5 ml EtOAc was hydrogenated for 1.5 hr over 29.5 mg 5% Pd–C under atm. pres. The usual work-up furnished a mixture which was purified by prep. TLC ($CHCl_3$–hexane–Me_2CO, 3:1:1). The band containing the major constituent gave 8 mg of **8**.

Extraction of T. rotundifolia *from Agua de Obispo, Guerrero.* The air-dried plant material (1.420 kg) collected in July, 1981 (voucher ARV 0050 MEXU 340473), was extracted with $CHCl_3$ (3 × 2 l.) and the resulting extract (135.6 g) was worked up as previously described for the other populations to yield 815.7 mg of **3a**, 841.4 mg of **4a**, 1.447 g of **5a**, a mixture of **3b**, **4b** and **4c** (1.5 g) and 371.4 mg of **5c**.

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