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LIMONOIDS FROM *TRICHILIA HAVANENSIS*

CARLOS ARENAS and LYDIA RODRÍGUEZ-HAHN*

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán, 04510 México, D.F.

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Key Word Index—*Trichilia havanensis*; Meliaceae; limonoids.

Abstract—The limonoids of the seeds of *Trichilia havanensis* were studied in two ripening stages. From the unripened seeds azadirone, havanensin triacetate and three new limonoids were isolated. The mature seeds contained havanesin triacetate and its hydroxy lactone derivative. The structures of the new limonoids were deduced by spectroscopic and chemical means.

INTRODUCTION

Trichilia havanensis is a tree widely distributed in Mexico. Different parts of the plant (popular name 'xopiltetl' or 'tinajillo') have been used in popular medicine by the Totonac community of the Sierra of Puebla [1]. The seed paste is used for corn protection. The limonoid constituents of the ripe fruit of *Trichilia havanensis* collected in Jamaica was described in 1973 by Chan *et al.* [2].

We have examined the limonoid contents of the seeds in two ripening stages and found that it varies dramatically on maturation. The limonoids isolated from the unripened seeds revealed a lower degree of oxidation of the tetranortriterpenoid skeleton and could be considered the biogenetic precursors of the limonoids isolated from the mature seeds, as has been proposed [3].

RESULTS AND DISCUSSION

The hexane extract of the unripened seeds was partitioned between hexane and aqueous methanol [4]. Chromatography of the ethyl acetate soluble fraction gave azadirone (**1**) [5] havanensin triacetate (**2**) previously isolated from the fruit of *Trichilia havanensis* collected in Jamaica [2] and three new limonoids whose structures **3**–**5** were deduced on the following evidence.

The triacetate of 14,15-deoxyhavanensin (**3**) $C_{32}H_{44}O_7$, showed in the IR spectrum ester absorption at 1722 cm^{-1} and furan bands at 1502 and 873 cm^{-1}. The ^1H NMR spectrum of **3** (Table 1) confirmed the presence of a β-substituted furan ring and five tertiary methyl groups. A singlet at δ2.0 (9H) was due to three acetate methyl groups whose geminal protons appeared as triplets at δ4.60, 4.68 and 5.17 (1H each, $J = 3$ Hz) indicating their secondary nature, and were attributed to equatorial H-3, H-1 and H-7 by comparison with the spectra of related products [5]. A triplet ($J = 3$ Hz) at δ5.35 was assigned to the vinylic H-15. The chemical shift of H-7β is greatly influenced by the C-15 substituent [5].

The second limonoid isolated **4**, $C_{30}H_{42}O_6$, showed hydroxyl absorption in the IR spectrum at 3550 cm^{-1}, a carbonyl ester band at 1715 cm^{-1} and the furan absorption at 1500 and 870 cm^{-1}. The ^1H NMR spectrum of **4** differed from that of **3** in the presence of only two acetate methyl groups (2*s*, 3H each, δ2.00 and 2.05), their geminal protons appeared as triplets at δ4.82 and 5.20 ($J = 3$ Hz) and were assigned to the equatorial H-1 and H-7, respectively. A triplet at δ3.35 ($J = 3$ Hz) was ascribed to the equatorial proton geminal to an hydroxyl group and was assigned to H-3. The vinylic H-15 appeared as a triplet at δ5.35 ($J = 3$ Hz).

The identification of **4** as the new limonoid 14,15-deoxyhavanensin-1,7-diacetate was possible by comparison with the spectral data described for 14,15-deoxyhavanensin-3,7-diacetate previously isolated from *Khaya anthotheca* [6]. Oxidation of **4** with Jones' reagent gave the 3-oxo derivative **5**, whose ^1H NMR spectrum (Table 1) showed H-1 shifted downfield to δ5.00 (*t*, $J = 4$ Hz), and the two acetyl methyl groups at δ1.95 and 2.00; the axial H-2 appeared as part of an ABX system at δ3.00 ($J = 4$ and 16 Hz). The oxidation of **4** with PDC followed by silica gel chromatography, yielded azadirone (**1**), thus proving that the hydroxyl group in **4** is at C-3. Acetylation of **4** gave 14,15-deoxyhavanensin triacetate (**3**).

The structure of the third new compound **6** was deduced from its spectral data and comparison with related structures. It had molecular formula $C_{32}H_{44}O_9$ and showed in the IR spectrum a hydroxyl band at 3590 cm^{-1}, an ester carbonyl band at 1721 cm^{-1} and absorption at 1760 cm^{-1} which was attributed to an α,β-unsaturated γ-lactone function. Its ^1H NMR spectrum showed three acetyl methyl groups (Table 1) whose germinal protons signals suggested that they were α-axially bound to C-1, C-3 and C-7, the vinylic H-15 appeared as a triplet ($J = 3$ Hz) at δ5.35. The signals due to the β-substituted furan ring present in compounds **1**–**5** were absent in the ^1H NMR spectrum of **6**. Instead two broad singlets observed at δ5.87 and 5.98 were assigned to H-21 and the vinylic H-22, respectively. Their chemical shifts were in agreement with the presence of a γ-hydroxybutenolide function as shown in **6**. This type of derivative

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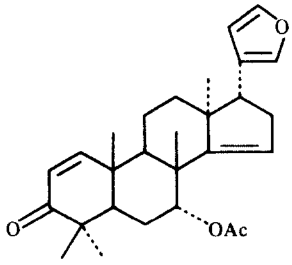
*Author to whom correspondence should be addressed.



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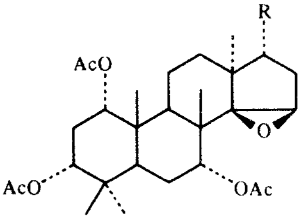

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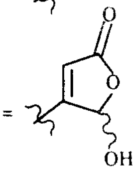

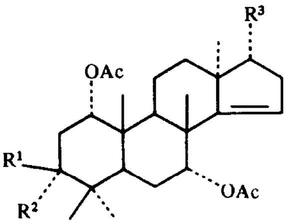

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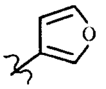

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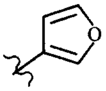

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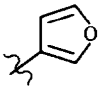

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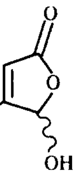

Table 1. ^{1}H NMR spectra of compounds **1**–**7** ($CDCl_3$, TMS as int. standard)

H	**1**	**2***	**3**	**4**	**5**	**6**	**7***
1	7.18 *d* (10)	4.6 *m*	4.65 *t* (3)	4.82 *t* (3)	5.00 *t* (3)	4.68 *t* (3)	4.63 *m*
2	5.83 *d* (10)						
3	—	4.6 *m*	4.55 *t* (3)	3.35 *t* (3)	—	4.63 *t* (3)	4.63 *m*
7	5.35 *t* (3)	4.6 *m*	5.17 *t* (3)	5.2 *t* (3)	5.2 *t* (3)	5.15 *t* (3)	4.63 *m*
15	5.24 *t* (3)	3.27 *br s*	5.35 *t* (3)	5.34 *t* (3)	5.35 *t* (3)	5.35 *t* (3)	3.19 *s*
21	7.25 *m*	6.97 *m*	7.2 *m*	7.22 *m*	7.20 *m*	5.82 *br s*	5.84 *br s*
22	6.30 *m*	6.05 *m*	6.25 *m*	6.24 *m*	6.22 *m*	5.98 *s*	5.65 *s*
23	7.35 *m*	7.22 *m*	7.35 *m*	7.33 *m*	7.35 *m*	—	—
MeCO	1.97 *s*	1.95 *s*	2.0 *s* (9H)	2.04 *s*	2.00 *s*	2.16 *s*	1.91 *s*
		1.87 *s*		1.98 *s*	1.95 *s*	2.04 *s*	1.82 *s*
		1.85 *s*				2.00 *s*	1.75 *s*
Me	1.23 *s*	0.96 *s*	1.17 *s*	1.22 *s*	1.22 *s*	1.17 *s*	1.00 *s*
	1.20 *s*	0.95 *s*	0.99 *s*	0.97 *s*	1.16 *s*	0.98 *s*	0.90 *s*
	1.10 *s* (6H)	0.85 *s*	0.92 *s*	0.93 *s*	1.05 *s* (6H)	0.95 *s* (6H)	0.75 *s*
	0.81 *s*	0.80 *s*	0.81 *s* (6H)	0.85 *s*		0.82 *s*	0.67 *s*
		0.75 *s*		0.77 *s*	0.75 *s*		0.57 *s*

*Benzene-d_6–carbon tetrachloride (1 : 1).
Coupling constants in Hz are in parentheses.
Chemical shifts are in δ values.

Table 2. ^{13}C NMR of compounds **2**–**4** [20 MHz, benzene-d_6–CCl_4 (1:1)]

C	**2**	**3**	**4**
1	72.44 *d*	72.27 *d*	75.44 *d*
2	25.70 *t*	25.57 *t*	28.04 *t*
3	75.92 *d*	75.27 *d*	74.19 *d*
4	36.15 *s*	36.04 *s*	37.26 *s*
5	37.19 *d*	37.29 *d*	36.41 *d*
6	23.61 *t*	23.01 *t*	23.15 *t*
7	74.32 *d*	74.88 *d*	74.86 *d*
8	42.41 *s*	42.34 *s*	42.40 *s*
9	39.97 *d*	35.65 *d*	35.87 *d*
10	40.61 *s*	40.32 *s*	40.52 *s*
11	15.90 *t*	15.83 *t*	15.81 *t*
12	32.44 *t*	33.42 *t*	33.37 *t*
13	42.01 *s*	47.28 *s*	47.19 *s*
14	71.31 *s*	150.61 *s*	159.54 *s*
15	56.26 *d*	119.07 *d*	119.07 *d*
16	30.26 *t*	34.55 *t*	34.51 *t*
17	37.65 *d*	51.74 *d*	51.71 *d*
18	19.16 *q*	20.79 *q*	20.85 *q*
19	16.81 *q*	15.83 *q*	15.81 *q*
20	124.14 *s*	124.70 *s*	124.67 *s*
21	139.50 *d*	139.71 *d*	139.69 *d*
22	111.12 *d*	111.17 *d*	111.16 *d*
23	142.74 *d*	142.44 *d*	142.44 *d*
28	27.41 *q*	27.42 *q*	28.29 *q*
29	21.37 *q*	21.72 *q*	21.92 *q*
30	22.08 *q*	27.07 *q*	27.08 *q*
$COCH_3$	168.63 *s* 20.91 *q*	168.71 *s* 20.79 *q*	168.84 *s* 20.76 *q*
$COCH_3$	168.51 *s* 20.62 *q*	168.60(2) *s* 20.65(2) *q*	168.67 *s* 20.71 *q*
$COCH_3$	168.43 *s* 20.91 *q*		

has been isolated from several Meliaceae plants [7, 8] and could be considered an intermediate stage in the formation of the furan ring [7] or a singlet oxygen oxidation product of the furan present in most of the limonoids isolated.

Mild treatment of the 14,15-deoxyhavanensin triacetate (**3**) with *m*-chloroperbenzoic acid [9] gave compound **6** and a small yield of the havanensin triacetate derivative **7**, $C_{32}H_{44}O_{10}$, which showed hydroxyl (3400 cm^{-1}) and γ-lactone absorption (1764 cm^{-1}) in the IR spectrum. In the ^{1}H NMR spectrum (Table 1) a broad singlet at δ3.2 suggested the presence of an epoxide group and was ascribed to H-15 by comparison with the ^{1}H NMR spectrum of **2**. The three protons geminal to the acetate groups axially bound to C-1, C-3 and C-7, appeared as a multiplet at δ4.65. Two broad singlets at δ5.65 and 5.45 were ascribed to the hydroxybutenolide protons H-22 and H-21. From a mature crop of seeds of *Trichilia havanensis*, havanensin triacetate (**2**) was obtained in high yield together with small amounts of the hydroxy butenolide derivative **7** and a new limonoid whose structure is under study.

EXPERIMENTAL

Mps: uncorr. MS were obtained at 70 eV by direct inlet. ^{1}H and ^{13}C NMR were performed at 80 and 20 MHz, respectively, using TMS as int. standard. The ^{13}C NMR assignments were made by SFORD and APT experiments and comparison with data of similar structures. The plant material (seeds) was collected in the Sierra Norte, Xochitlan Puebla (Mexico) and a voucher is deposited at the Herbarium of Colegio Posgraduados, Chapingo.

Isolation of limonoids from the (unripened) seeds of Trichilia havanensis. The dried and ground unripened seed kernels (1.75 kg) were extracted at room temp. with hexane (3 × 10 l) and MeOH. The residue obtained from the hexane extract (636 g) was partitioned between hexane and MeOH–H_2O (19:1) [4]. The aq. methanolic soln was concentrated under vacuum and extracted with EtOAc to yield 102 g of a gummy residue *A*. The methanolic extract of the seeds was treated in the same manner to yield 52 g of crude residue *B*.

The residue *A* was subjected to vacuum liquid chromatography (VLC) [10] over silica gel (deactivated with 5% H_2O). Elution with petrol–EtOAc (19:1) yielded a solid product mp 123–125°, 0.230 g (0.013% yield), identified as azadirone (**1**), by comparison with the physical and spectral data described in the literature [5].

From the fractions eluted with petrol–EtOAc (9:1) the triacetate of 14,15-deoxy-havanensin (**3**) was isolated as a crystalline product, mp 168–169° from Me_2CO–petrol (1.5 g, 0.08% yield), $[\alpha]_D^{20}-130$ (MeOH; *c* 0.4). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1502, 873, 1377, 1265, 1048. ^{1}H NMR see Table 1 ^{13}C NMR see Table 2. MS *m/z* (rel. int.): 540 $[M]^+$ (3.3), 525 (0.1), 360 (19), 345 (27), 81 (12), 43 (100). $C_{32}H_{44}O_7$ requires $[M]^+$ at *m/z* 540.

Elution with petrol–EtOAc (17:13) yielded havanensin triacetate **2**, (0.27 g) identified by comparison with the data described in the literature [2]. From the fractions eluted with petrol-EtOAc (4:1) the havanensin derivative **4** was obtained as a crystalline product, mp 175–176° (0.67 g; 0.04%), $[\alpha]_D^{20}-85$

(MeOH; *c* 2.0); IR $\nu_{\max}^{\text{nujol}}$ cm^{-1}: 3550, 1715, 1500, 870. ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2. MS *m/z* (rel. int.): 498 $[M]^+$ (9.3), 483 (0.5), 360 (21.5), 345 (61.4), 81 (33), 43 (100). $C_{30}H_{42}O_6$ requires $[M]^+$ at *m/z* 498.

The polar fraction *B* (51.6 g) was also subjected to VLC over silica gel (deactivated with 5% H_2O). Elution with petrol–EtOAc (3:2) yielded the hydroxylactone derivative **6** as a crystalline product mp 205–209° (0.3 g, 0.02%). IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 3585, 1759, 1722, 1644, 1376, 1048. ^{1}H NMR: see Table 1. MS *m/z* (rel. int.): 572 $[M]^+$ (0.7), 555 (0.2), 392 (10.4) 377 (14.5), 91 (7.8), 43 (100). $C_{32}H_{44}O_9$ requires $[M]^+$ at *m/z* 572.

Isolation of limonoids from ripened seeds of Trichilia havanensis. The dried and ground ripened seed kernels (227 g) were exhaustively extracted with hexane in a Soxhlet. The residue obtained was partitioned [4] between hexane and MeOH–H_2O (19:1). The aq. methanolic soln was concd *in vacuo* and the aq. residue extracted with EtOAc. The organic layer was dried over. Na_2SO_4 and the solvent removed *in vacuo* to yield 23 g of crude extract which was submitted to dry CC over silica gel (deactivated with 10% H_2O). Repeated chromatography of the fractions eluted with petrol–EtOAc (3:1) yielded havanensin triacetate (**2**) (1.57 g, 0.7%) identified by comparison with an authentic sample. Purification of the fractions obtained by elution with EtOAc gave limonoid **7** as a crystalline product, mp 155–156° (33 mg, 0.015%). $[\alpha]_D^{20} -62°$ (MeOH; *c* 2.0). IR $\nu_{\max}^{\text{nujol}}$ cm^{-1}: 3400, 1764, 1726, 1642, 1258, 1050. ^{1}H NMR: see Table 1. CIMS *m/z* (rel. int.): 572.5 $[M-16]^+$ (4.2), 571.4 (6.9), 573.4 (4.2), 409.4 (100), 393.3 (64.8), 111 (55.6), 101 (52). $C_{32}H_{44}O_{10}$ requires $[M]^+$ at *m/z* 588.

Acetylation of **4**. Product **4** (100 mg) in dry pyridine (1 ml) was treated with Ac_2O (1 ml) at room temp. for 24 hr. The crystalline product obtained after the usual work-up, (78 mg) was identical to 14,15-deoxyhavanensin triacetate (**3**).

Oxidation of **4** *with Jones reagent*. Product **4** (100 mg) in Me_2CO (5 ml) was treated with Jones reagent at 5° for 5 min. After the usual work-up the product obtained, **5**, (90 mg) showed mp 145–148°. IR $\nu_{\max}^{\text{CHCl}_3}$ cm^{-1}: 1723, 1500, 1250, 1026, 874. ^{1}H NMR: see Table 1. MS *m/z* (rel. int.): 496 $[M]^+$ (11), 436 (5), 376 (27), 361 (33), 291 (15), 209 (4), 105 (12), 81 (20), 43 (100); $C_{30}H_{40}O_6$ requires $[M]^+$ at *m/z* 496.

Oxidation of **4** *with pyridinium dichromate* (PDC). Product **4** (100 mg) in CH_2Cl_2 (10 ml) was treated with PDC (100 mg) and Na trifluoroacetate (0.4 eq, 15.4 mg) at room temp. for 24 hr. The reaction mixture was filtered through silica gel. The crude product obtained was purified by flash chromatography using silica gel (230–400). Elution with *n*-hexane–EtOAc (7:3) gave azadirone (**1**) identified by comparison with a sample of azadirone obtained as a natural product.

Treatment of **3** *with* meta-*chloroperbenzoic acid*. The triacetate **3** (200 mg) in CH_2Cl_2 (10 ml) was treated with MCPA (3 eq, 230 mg) in the presence of aq. Na bicarbonate soln (1.0% 5 ml). The reaction mixture was stirred for 10 hr, EtOAc was added and the organic layer washed with brine, dried and the solvent removed *in vacuo*. The crude product obtained was purified by flash chromatography on silica gel (230–400) using *n*-hexane–EtOAc mixtures of increasing polarity. The major product obtained was identified as the hydroxylactone derivative **6**. A small proportion of the 14,15-epoxy-derivative **7** was also isolated.

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