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Spectral Assignments and Reference Data

Complete assignments of the ^{1}H and ^{13}C NMR spectra of 15 limonoids†

Benjamín Rodríguez*

Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, E-28006 Madrid, Spain

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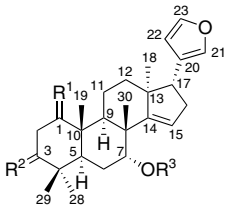
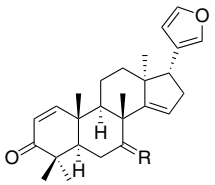
Unambiguous and complete assignments of ^{1}H and ^{13}C NMR chemical shifts for 15 limonoids, eight of them found in natural sources and seven other synthetic derivatives, are presented. The assignments are based on 2D shift-correlated [$^{1}H,^{1}H$-COSY, $^{1}H,^{13}C$-gHSQC-^{1}J(C,H), $^{1}H,^{13}C$-gHMBC-^{n}J(C,H) (n = 2 and 3)] and NOE experiments.

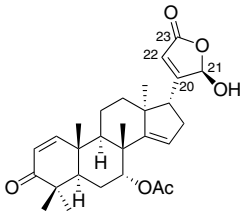
KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; triterpenoids; limonoids; meliacins; 24,25,26,27-tetranor-apotirucallane derivatives



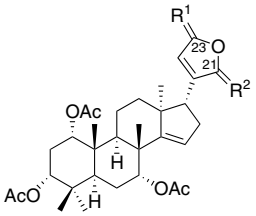
1 R^1 = R^2 = α-OAc, β-H; R^3 = Ac
2 R^1 = α-OH, β-H; R^2 = α-OAc, β-H; R^3 = Ac
3 R^1 = α-OAc, β-H; R^2 = α-OH, β-H; R^3 = Ac
4 R^1 = R^2 = α-OH, β-H; R^3 = Ac
5 R^1 = R^2 = α-OH, β-H; R^3 = H
6 R^1 = O; R^2 = α-OAc, β-H; R^3 = Ac
7 R^1 = α-OAc, β-H; R^2 = O; R^3 = Ac



8 R = α-OAc, β-H
9 R = α-OH, β-H
10 R = O



11



12 R^1 = O; R^2 = α-H, β-OH
13 R^1 = H, OH; R^2 = O (both C-23 epimers)
14 R^1 = O, R^2 = H_2

INTRODUCTION

The limonoids (24,25,26,27-tetranor-apotirucallanes) are a large group of naturally occurring triterpenoids isolated from several plants and particularly from those belonging to the Meliaceae and Rutaceae families.[1,2] The limonoids occurring in Meliaceae are also known as meliacins. These compounds display a vast array of biological activities such as antimalarial, antidiabetic, anti-inflammatory, insecticidal, bactericidal, spermicidal, vaginal contraceptive, hypoglycaemic, antifeedant, nematicidal, piscicidal, amoebicidal, larvicidal and several others.[3]

Recently,[4] an extraction of the seed of *Trichilia havanensis* Jacq. (Meliaceae) was carried out in our laboratories in order to isolate its meliacin constituents and to test these compounds as antifeedant agents against *Leptinotarsa decemlineata* (Say) (Colorado potato beetle) and *Spodoptera exigua* (Hübner) larvae. The acetone extract of that seed contained several previously known limonoids,[5–8] including large quantities of 3,7-di-*O*-acetyl-14,15-deoxyhavanensin[9] (**2**), 1,7-di-*O*-acetyl-14,15-deoxyhavanensin[5] (**3**) and azadirone[5,10] (**8**). Starting from these available compounds, the naturally occurring limonoids 1,3,7-tri-*O*-acetyl-14,15-deoxyhavanensin[5] (**1**), 7-deacetylazadirone[11,12] (**9**), 7-deacetoxy-7-oxoazadirone[13] (**10**), isoazadironolide[14] (**11**) and 1α,3α, 7α-triacetoxy-21ζ-hydroxy-24,25,26,27-tetranor-apotirucalla-14,20(22)-dien-23,21-olide[5] (**12**) were synthesized for this work, as well as compounds **4**–**7, 13** (as a mixture of the C-23 epimers **13a** and **13b**) and **14**, all of them not hitherto found in natural sources, although **6** and **7** are previously known as synthetic derivatives.[5,9] Only partial ^{1}H NMR spectral data have been published for these limonoids, except for **6**[9] and the new compounds **4, 5, 13** and **14**, and the ^{13}C NMR spectra of **2, 4**–**9** and **12**–**14** have not been reported previously. The previous assignments of the ^{13}C NMR spectra of **1**,[5] **3**,[5] **10**[13] and **11**[14] were performed only on the basis of general chemical shift arguments and by comparison with the data reported for other structurally related triterpenoids. Thus, previous ^{13}C NMR assignments contain several ambiguities and are not unequivocal. These facts suggested attempting the complete assignment of the ^{1}H and ^{13}C NMR spectral data of **1**–**14** in the hope they could be useful for future assignments of compounds belonging to this biologically and chemically interesting kind of triterpenoids.

*Correspondence to: Benjamín Rodríguez, Instituto de Química Orgánica, CSIC, Juan de la Cierva 3, E-28006 Madrid, Spain. E-mail: iqor107@iqog.csic.es
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RESULTS AND DISCUSSION

Treatment of **3** with acetic anhydride–pyridine gave **1**, another constituent of *T. havanensis*,[5] whereas oxidation of **3** with chromium trioxide–pyridine[15] yielded **7**, previously obtained[5] as a synthetic derivative. Oxidation[15] of **2** provided the 1-ketolimonoid **6**, a substance that had already been obtained[9] as an intermediate for the synthesis of isoazadirone, although its physical and spectroscopic data have not been reported. Alkaline hydrolysis of **1** yielded the new limonoids **4** and **5** (see Experimental). Deacetylation of azadirone (**8**) gave **9**, a natural limonoid found in *Teclea* species[11,12] (Rutaceae), and **9** was transformed into **10** by oxidation with Jones' reagent.[16] 7-Deacetoxy-7-oxoazadirone (**10**) has previously been found in *Teclea ouabanguiensis*.[13] Oxidation of **8** with Jones' reagent[16] for a long time (see Experimental) yielded **11** as an unique 21*R* isomer, whereas both C-21 epimers of **11** have been found in *Azadirachta indica* as a mixture named isoazadironolide.[14] The 21*R* absolute configuration of **11** was now established by application of the Horeau's method[17] (see Experimental). Oxidation of **1** with magnesium monoperoxyphthalate (MMPP) predominantly yielded the γ-hydroxybutenolide **12**, previously known[5] as a constituent of *Trichilia havanensis*, together with minute amounts of the regioisomer **13** as a 1.3 : 1 unseparable mixture of the C-23 epimers **13a** and **13b**. Finally, reduction of **12** with sodium borohydride[18] gave the new limonoid **14**.

For the complete assignment of the ^{1}H and ^{13}C NMR spectra of **1**–**14**, a combination of two-dimensional COSY, HSQC and HMBC experiments was carried out, together with NOE experiments[19] for distinguishing the C-2 methylene protons in **1**–**7** and **12**–**14**, and the C-12 and C-16 methylene protons in all the compounds. Tables 1–3 show the complete and unambiguous assignments of the ^{1}H and ^{13}C NMR spectra of these limonoids.

The assignment of both C-6 and C-11 methylene protons in **1**–**14** was evident from the vicinal coupling constant values shown by each of these methylene protons with the H-5α and H-9α axial methine protons, respectively (Tables 1 and 2). On the other hand, in the case of **1**–**5** and **12**–**14**, the C-2 methylene protons appeared as a doublet of triplets with identical coupling constants with the H-1β and H-3β vicinal equatorial protons, whereas in **6** and **7** the C-2 protons showed double doublet signals, in which the vicinal coupling values are identical [**6**: $J(2\alpha, 3\beta) = J(2\beta, 3\beta) = 4.2$ Hz] or very close [**7**: $J(2\alpha, 1\beta) = 3.0$ Hz, $J(2\beta, 1\beta) = 4.0$ Hz], thus precluding

an assignment of the C-2 protons based on these data. Moreover, the assignment of the C-16 methylene protons of **1**–**14** is not unambiguous from the vicinal *J* values because this methylene is involved in a cyclopentene ring. Furthermore, in these limonoids (**1**–**14**), the C-12 methylene protons are eclipsed with those of the C-11 methylene group, as a consequence of a boat conformation ($B_{9,13}$) in ring C, and consequently the vicinal *J*(H − 12, H − 11) coupling values are not reliable data for establishing their configurations.

The assignment of both C-2 methylene protons in **1**–**7** and **12**–**14** (Tables 1 and 2) was supported by NOE experiments. On irradiation at the H-2 proton signal appearing at δ 2.18–3.26 (dt in **1**–**5** and **12**–**14** and dd in **6** and **7**) strong NOE enhancement in the signals of the H-1β, H-3β, Me-19 and Me-29 axial protons were always observed, thus establishing that all these hydrogens, including the irradiated H-2 proton, are on the same side of the plane of the molecule. Consequently, the other C-2 methylene proton (δ1.91–2.44, dt in **1**–**5** and **12**–**14** and dd in **6** and **7**) is the 2α-equatorial hydrogen, which appeared always upfield shifted with respect to the H-2β proton. Similarly, when the Me-18 protons signal (δ 0.74–0.92 s) was irradiated NOE was observed in the *cis* H-16α proton signal (δ 2.38–2.57 ddd), which appeared downfield shifted with respect to its geminal proton (δ 2.29–2.40 ddd) in all the compounds studied (**1**–**14**). In addition, irradiation at the H-17β proton signal (δ 2.65–2.87 ddd) caused, among others, NOE enhancement in the *cis* H-16β

Table 1. ^{1}H NMR chemical shifts, δ (ppm), and coupling constants, *J*(H,H) (Hz), for 1–7[a]

Proton	1	2	3	4	5	6	7
1β	4.61 t	3.48 t	4.84 t	3.55 t	3.49 t	—	5.03 dd
2α	1.99 dt	1.83 m[b]	1.93 dt	1.91 dt	1.92 dt	2.24 dd	2.44 dd
2β	2.16 dt	2.29 dt	2.26 dt	2.20 dt	2.19 dt	3.26 dd	3.01 dd
3β	4.69 t	4.92 t	3.36 t	3.51 t	3.54 t	5.03 t	—
5α	2.26 dd	2.00 dd	2.11 dd	2.13 dd	2.31 dd	2.10 dd	2.38 dd
6α	1.78 m[b]	1.74 dt	1.78 m[b]	1.72 ddd	1.76 m[b]	1.73 ddd	1.78 m[b]
6β	1.78 m[b]	1.82 m[b]	1.84 ddd	1.83 ddd	1.79 m[b]	1.96 ddd	1.94 ddd
7β	5.19 t	5.20 dd	5.21 t	5.19 dd	3.94 t	5.16 dd	5.22 dd
9α	2.58 dd	2.67 dd	2.47 dd	2.67 dd	2.65 dd	2.50 dd	2.50 dd
11α	1.28 dddd	1.79 m[b]	1.27 m[b]	1.72 m[b]	1.70 m[b]	2.23 dddd	1.41 dddd
11β	1.52 m[b]	1.49 m[b]	1.52 dddd	1.50 m[b]	1.51 m[b]	1.42 dddd	1.58 m[b]
12α	1.77 m[b]	1.85 m[b]	1.74 m[b]	1.83 m[b]	1.80 m[b]	1.88 ddd	1.79 m[b]
12β	1.53 m[b]	1.49 m[b]	1.55 m[b]	1.51 m[b]	1.51 m[b]	1.61 ddd	1.58 m[b]
15	5.36 dd	5.31 dd	5.35 dd	5.30 dd	5.55 dd	5.35 dd	5.36 dd
16α	2.41 ddd	2.41 ddd	2.39 ddd	2.40 ddd	2.56 ddd	2.39 ddd	2.38 ddd
16β	2.32 ddd	2.30 ddd	2.31 ddd	2.29 ddd	2.39 ddd	2.31 ddd	2.31 ddd
17β	2.77 ddd	2.75 ddd	2.76 ddd	2.74 ddd	2.80 ddd	2.78 ddd	2.77 ddd
Me-18	0.79 s	0.82 s	0.76 s	0.79 s	0.83 s	0.88 s	0.74 s
Me-19	0.97 s	0.91 s	0.96 s	0.88 s	0.88 s	1.30 s	1.16 s
21	7.22 dt	7.22 dt	7.21 ddd	7.21 dt	7.23 dt	7.22 dt	7.21 dt
22	6.26 dd	6.27 dd	6.25 dd	6.26 dd	6.27 dd	6.28 dd	6.24 dd
23	7.36 t	7.35 t	7.36 t	7.34 t	7.36 t	7.36 t	7.35 t
Me-28	0.80 s	0.78 s	0.93 s	0.89 s	1.00 s	0.81 s	1.03 s
Me-29	0.91 s	0.90 s	0.85 s	0.82 s	0.85 s	1.12 s	1.05 s
Me-30	1.16 s	1.15 s	1.15 s	1.14 s	1.11 s	1.18 s	1.20 s
1α-OAc	2.00 s	—	2.06 s	—	—	—	1.99 s
3α-OAc	2.00 s	2.09 s	—	—	—	2.03 s	—
7α-OAc	1.99 s	1.98 s	1.99 s	1.97 s	—	1.95 s	1.96 s
J(H,H)							
1β,2α	3.0	2.9	3.1	2.7	2.8	—	3.0
1β,2β	3.0	2.9	3.1	2.7	2.8	—	4.0
2α,2β	16.4	16.0	16.2	15.1	15.3	14.4	17.0
2α,3β	3.0	2.9	3.1	2.7	2.8	4.2	—
2β,3β	3.0	2.9	3.1	2.7	2.8	4.2	—
5α,6α	4.8	3.2	3.4	2.7	6.3	2.4	1.7
5α,6β	10.8	12.8	12.2	12.9	9.2	13.2	10.7
6α,6β	[b]	14.4	14.8	14.8	[b]	14.4	14.3
6α,7β	2.8	3.2	2.8	3.4	2.9	3.6	3.6
6β,7β	2.8	2.4	2.8	2.4	2.9	2.0	2.4
9α,11α	6.6	6.8	6.4	7.2	7.5	5.4	5.5
9α11β	11.8	11.6	11.6	11.2	11.4	11.8	11.7

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Spectral Assignments and Reference Data

Table 1. (*Continued*)

Proton	1	2	3	4	5	6	7
11α,11β	13.1	[b]	13.2	[b]	[b]	14.0	14.1
11α,12α	3.6	[b]	[b]	[b]	[b]	4.6	5.1
11α,12β	5.6	[b]	[b]	[b]	[b]	9.6	9.4
11β,12α	[b]	[b]	9.6	[b]	[b]	9.4	[b]
11β,12β	[b]	[b]	2.8	[b]	[b]	5.4	[b]
12α,12β	[b]	[b]	[b]	[b]	[b]	13.6	[b]
15,16α	1.9	1.6	1.9	1.6	1.4	1.7	1.6
15,16β	3.4	3.6	3.3	3.6	3.5	3.4	3.6
16α,16β	15.2	15.2	15.2	15.1	15.4	15.2	15.2
16α,17β	10.8	11.2	10.8	10.9	11.2	10.8	10.9
16β,17β	7.6	7.2	7.6	7.4	7.3	7.5	7.6
17β,21	0.8	0.8	0.7	0.8	0.8	0.8	0.8
21,22	0.8	0.8	0.8	0.8	0.8	0.8	0.8
21,23	1.6	1.6	1.7	1.7	1.7	1.6	1.6
22,23	1.6	1.6	1.7	1.7	1.7	1.6	1.6

[a] In $CDCl_3$ solution. All these assignments were in agreement with COSY, HSQC and HMBC spectra, and NOE experiments.
[b] Overlapped or partially overlapped signal; δ values were measured from the HSQC spectra.

Table 2. ^{1}H NMR chemical shifts, δ (ppm), and coupling constants, *J*(H,H) (Hz), for **8**–**14**[a]

Proton	8	9	10	11	12	13a[b]	13b[b]	14
1β	7.14 d	7.11 d	7.12 d	7.13 d	4.64 t	4.59 t	4.57 t	4.60 t
2α	5.83 d	5.80 d	5.89 d	5.86 d	1.98 dt	[c]	[c]	1.98 dt
2β	—	—	—	—	2.20 dt	[c]	[c]	2.18 dt
3β	—	—	—	—	4.69 t	4.68 t	4.68 t	4.68 t
5α	2.18 dd	2.39 dd	2.09 dd	2.18 m[c]	2.24 dd	[c]	[c]	2.23 dd
6α	1.77 ddd	1.85 m[c]	2.39 dd	1.80 m[c]	1.78 m[c]	[c]	[c]	1.78 m[c]
6β	1.90 ddd	1.88 m[c]	2.88 t	1.95 m[c]	1.78 m[c]	[c]	[c]	1.78 m[c]
7β	5.26 dd	4.01 t	—	5.25 dd	5.17 t	5.17 t	5.17 t	5.17 t
9α	2.21 dd	2.20 dd	2.12 dd	2.18 m[c]	2.51 dd	[c]	[c]	2.52 dd
11α	1.88 m[c]	1.93 m[c]	1.94 m[c]	1.97 m[c]	1.31 m[c]	[c]	[c]	1.31 dddd
11β	1.68 m[c]	1.75 dddd	1.88 m[c]	1.78 m[c]	1.54 m[c]	[c]	[c]	1.51 m[c]
12α	1.84 m[c]	1.89 m[c]	1.85 ddd	1.93 m[c]	1.76 m[c]	[c]	[c]	1.70 m[c]
12β	1.63 m[c]	1.60 ddd	1.69 ddd	1.81 m[c]	1.72 m[c]	[c]	[c]	1.52 m[c]
15	5.36 dd	5.58 dd	6.06 dd	5.39 dd	5.37 dd	5.32 dd	5.31 dd	5.35 dd
16α	2.41 ddd	2.56 ddd	2.57 ddd	2.48 ddd	2.45 ddd	[c]	[c]	2.44 ddd
16β	2.31 ddd	2.40 ddd	2.40 ddd	2.35 ddd	2.33 ddd	[c]	[c]	2.34 ddd
17β	2.79 ddd	2.83 ddd	2.82 ddd	2.87 ddd	2.84 ddd	2.77 m[c]	2.77 m[c]	2.65 dddd
Me-18	0.77 s	0.78 s	0.77 s	0.92 s	0.92 s	0.87 s	0.90 s	0.90 s
Me-19	1.18 s	1.16 s	1.36 s	1.18 s	0.97 s	0.97 s	0.96 s	0.95 s
21A	7.22 ddd	7.24 dt	7.24 dt	6.01 dd[d]	6.00 dd[d]	—	—	4.66 ddd
21B	—	—	—	—	—	—	—	4.77 dd
22	6.26 dd	6.27 dd	6.27 dd	5.91 dd	5.88 dd	6.92 t	6.90 t	5.86 td
23	7.36 t	7.36 t	7.36 t	—	—	6.07 d	6.12 d	—
Me-28	1.06 s	1.14 s	1.14 s	1.07 s	0.80 s	0.79 s	0.79 s	0.79 s
Me-29	1.06 s	1.08 s	1.11 s	1.07 s	0.91 s	0.91 s	0.91 s	0.91 s
Me-30	1.21 s	1.16 s	1.42 s	1.22 s	1.16 s	1.15 s	1.15 s	1.14 s
1α-OAc	—	—	—	—	2.01 s	[c]	[c]	2.02 s
3α-OAc	—	—	—	—	2.04 s	[c]	[c]	2.00 s
7α-OAc	1.94 s	—	—	1.95 s	1.99 s	[c]	[c]	1.99 s

(*continued overleaf*)

Table 2. (*Continued*)

Proton	**8**	**9**	**10**	**11**	**12**	**13a**[b]	**13b**[b]	**14**
21-OH	—	—	—	4.22 d[e]	4.92 d[e]	—	—	—
J(H,H)								
1,2	10.2	10.2	10.2	10.4	—	—	—	—
1β,2α	—	—	—	—	3.0	3.0	3.0	2.9
1β,2β	—	—	—	—	3.0	3.0	3.0	2.9
2α,2β	—	—	—	—	16.5	[c]	[c]	16.6
2α,3β	—	—	—	—	3.0	3.0	3.0	2.9
2β,3β	—	—	—	—	3.0	3.0	3.0	2.9
5α,6α	2.4	3.4	2.6	[c]	4.7	[c]	[c]	6.0
5α,6β	13.2	11.9	14.5	[c]	11.0	[c]	[c]	10.4
6α,6β	14.8	[c]	14.5	[c]	[c]	[c]	[c]	[c]
6α,7β	3.3	2.9	—	3.2	2.8	2.8	2.8	2.8
6β,7β	2.2	2.9	—	2.0	2.8	2.8	2.8	2.8
9α,11α	6.0	7.1	5.8	[c]	6.0	[c]	[c]	6.6
9α,11β	12.0	11.9	11.8	[c]	12.5	[c]	[c]	11.8
11α,11β	[c]	14.0	[c]	[c]	[c]	[c]	[c]	13.6
11α,12α	[c]	[c]	3.6	[c]	[c]	[c]	[c]	2.8
11α,12β	[c]	4.0	7.4	[c]	[c]	[c]	[c]	6.4
11β,12α	[c]	10.0	9.6	[c]	[c]	[c]	[c]	[c]
11β,12β	[c]	2.0	3.4	[c]	[c]	[c]	[c]	[c]
12α,12β	[c]	13.2	13.2	[c]	[c]	[c]	[c]	[c]
15,16α	1.6	1.6	1.6	1.8	1.8	[c]	[c]	1.5
15,16β	3.5	3.5	3.6	3.4	3.5	[c]	[c]	3.4
16α,16β	15.4	15.6	15.4	15.6	15.5	[c]	[c]	15.2
16α,17β	11.2	11.0	11.2	10.5	10.5	[c]	[c]	10.4
16β,17β	7.4	7.3	7.1	7.2	7.0	[c]	[c]	7.2
17β,21A	0.8	0.8	0.8	0	0	—	—	1.0
17β,22	0	0	0	1.6	1.5	1.2	1.2	0.4
21A,21B	—	—	—	—	—	—	—	17.4
21,21(OH)	—	—	—	8.2[e]	7.5[e]	—	—	—
21A,22	—	—	—	—	—	—	—	1.8
21B,22	—	—	—	—	—	—	—	1.8
21,22	1.0	0.8	0.8	1.0	1.0	—	—	—
21,23	1.7	1.7	1.7	—	—	—	—	—
22,23	1.7	1.7	1.7	—	—	1.2	1.2	—

[a] In $CDCl_3$ solution. All these assignments were in agreement with COSY, HSQC and HMBC spectra, and NOE experiments.

[b] It was not possible to assign all the proton signals for **13a** and **13b** owing to the complexity of the spectra of the mixture of the two C-23 epimers.

[c] Overlapped signal; δ values were measured from the HSQC spectra.

[d] Collapsed into a d ($J = 1.0$ Hz) after addition of D_2O.

[e] Disappeared after addition of D_2O.

proton signal always greater than that exhibited for the *trans* H-16α proton. Moreover, both C-12 methylene protons were easily distinguished (Tables 1 and 2) because on irradiation at the H-17β proton signal, NOE was observed in the signal of the *cis* H-12β proton and not in that of its geminal H-12α proton.

The ^{1}H NMR assignments for **1**–**14** (Tables 1 and 2) are in agreement with the partial ^{1}H NMR data published before[5,9,10–14] for the previously known compounds (**1**–**3** and **7**–**12**), although in **2** and **8** the previous assignment[9,10] for the olefinic H-15 proton and the H-7β sp^3 proton must be reversed, as well as for the assignment of the H-1β and H-3β protons in **1** and **12**.[5] These reassignments were strongly supported by HSQC and HMBC experiments, because the H-15 olefinic proton of **2** and **8** (δ 5.31 dd and 5.36 dd, respectively) showed HSQC correlation with the olefinic C-15 carbon (δ 118.21 d and 119.01 d, respectively), whereas the H-7β proton (δ 5.20 in **2** and 5.26 in **8**, both dd) was connected with the C-7 carbon (δ 75.03 d and 74.42 d, respectively). In the case of **1** and **12**, the HMBC connectivities observed between the H-1β proton (δ 4.61 t and 4.64 t, respectively) and the C-9, C-10 and C-19 carbons (δ_{C-1} 35.50 d and 35.40 d, δ_{C-10} 40.19 s and 40.16 s, and δ_{C-19} 15.88 q and 15.90 q, respectively), and between the H-3β proton (δ 4.69 t in both **1** and **12**) and the C-4 and C-29 carbons (δ_{C-4} 35.96 s and 35.97 s, δ_{C-29} 21.68 q and 21.64 q, respectively) were in agreement with this reassignment.

The previous ^{13}C NMR assignments for **1**,[5] **3**,[5] **10**[13] and **11**[14] are, in general, in good agreement with those shown in Table 3. However, the use of 2D experiments (HSQC and HMBC) in the

Table 3. ^{13}C NMR chemical shifts, δ (ppm), for **1**–**14**[a]

Carbon	1	2	3	4	5	6	7	8	9	10	11	12	13a[b]	13b[b]	14
1	72.50 d	71.21 d	74.70 d	72.23 d	72.21 d	211.41 s	75.31 d	158.18 d	158.20 d	156.41 d	158.22 d	72.64 d	72.71 d	72.63 d	72.35 d
2	25.54 t	28.24 t	27.91 t	29.46 t	29.35 t	39.26 t	39.31 t	125.41 d	125.44 d	125.96 d	125.47 d	25.63 t	25.57 t	25.47 t	25.47 t
3	76.17 d	78.66 d	76.07 d	77.77 d	78.12 d	80.49 d	213.27 s	204.58 s	205.11 s	203.52 s	204.95 s	76.17 d	76.24 d	76.61 d	75.98 d
4	35.96 s	36.19 s	37.26 s	37.23 s	37.33 s	36.71 s	47.17 s	44.07 s	44.17 s	44.66 s[d]	44.09 s	35.97 s	35.96 s	35.96 s	35.24 s
5	37.15 d	36.38 d	36.45 d	35.31 d	33.97 d	44.84 d	41.79 d	46.05 d	44.43 d	52.40 d	46.01 d	37.12 d	37.14 d	37.16 d	37.08 d
6	22.89 t	22.94 t	23.05 t	23.11 t	23.63 t	23.24 t	23.83 t	23.73 t	24.21 t	36.15 t	23.68 t	22.85 t	22.84 t	22.84 t	22.79 t
7	75.20 d	75.03 d	75.09 d	75.29 d	72.17 d	74.68 d	74.69 d	74.42 d	71.50 d	209.56 s	74.46 d	75.20 d	75.21 d	75.25 d	74.93 d
8	42.16 s	42.00 s	42.29 s	42.09 s	44.29 s	42.12 s	42.03 s	42.73 s	44.82 s	52.48 s	42.92 s	42.39 s	42.32 s	42.32 s	42.29 s
9	35.50 d	35.57 d	35.84 d	35.47 d	34.33 d	36.67 d	35.19 d	38.55 d	36.89 d	44.89 d	38.42 d	35.40 d	35.18 d	35.18 d	35.90 d
10	40.19 s	41.58 s	40.38 s	41.67 s	42.02 s	52.34 s	40.25 s	39.87 s	40.16 s	39.64 s[d]	39.82 s	40.16 s	40.17 s	40.16 s	40.07 s
11	15.72 t	15.63 t	15.76 t	15.71 t	15.59 t	18.23 t	15.90 t	16.43 t	16.22 t	17.22 t	16.42 t	15.78 t	15.84 t	15.87 t	15.65 t
12	33.17 t	32.85 t	33.11 t	32.94 t	32.41 t	33.76 t	32.85 t	32.89 t	32.20 t	33.28 t	33.34 t	33.63 t	34.08 t	33.75 t	33.59 t
13	47.12 s	47.31 s	47.05 s	47.32 s	47.56 s	47.02 s	47.08 s	47.10 s	47.29 s	47.73 s	47.39 s	47.36 s	47.32 s	47.53 s	47.66 s
14	159.18 s	159.85 s	159.00 s	159.94 s	162.32 s	158.99 s	158.77 s	158.78 s	161.16 s	152.55 s	158.18 s	158.50 s	159.00 s	158.85 s	158.49 s
15	119.06 d	118.21 d	119.17 d	118.18 d	119.54 d	119.26 d	119.26 d	119.01 d	120.05 d	126.46 d	118.93 d	119.09 d	118.41 d	118.55 d	118.78 d
16	34.28 t	34.24 t	34.24 t	34.28 t	34.10 t	34.27 t	34.26 t	34.30 t	34.29 t	34.84 t	33.27 t	33.22 t	32.66 t	32.93 t	32.99 t
17	51.39 d	51.32 d	51.35 d	51.35 d	51.40 d	51.73 d	51.40 d	51.53 d	51.48 d	51.67 d	52.77 d	52.63 d	50.21 d	50.45 d	54.31 d
18	20.44 q	19.84 q	20.66 q	19.95 q	19.90 q	21.88 q	20.34 q	21.26 q	20.22 q	21.65 q[c]	21.34 q	21.29 q	21.25 q	21.28 q	21.62 q
19	15.88 q	16.30 q	15.88 q	16.16 q	16.07 q	15.18 q	15.16 q	19.02 q	18.92 q	18.44 q[c]	18.98 q	15.90 q	15.65 q	15.65 q	15.88 q
20	124.68 s	124.85 s	124.60 s	124.89 s	124.61 s	124.77 s	124.55 s	124.52 s	124.24 s	124.48 s	169.36 s	169.29 s	137.68 s	137.99 s	169.70 s
21	139.61 d	139.55 d	139.60 d	139.56 d	139.65 d	139.60 d	139.63 d	139.63 d	139.70 d	139.78 d[e]	98.95 d	98.71 d	171.54 s	170.43 s	73.28 t
22	111.03 d	111.10 d	111.00 d	111.11 d	111.10 d	111.06 d	110.99 d	111.00 d	110.97 d	111.04 d	119.09 d	119.64 d	145.60 d	145.74 d	116.92 d
23	142.50 d	142.39 d	142.51 d	142.40 d	142.53 d	142.50 d	142.57 d	142.52 d	142.65 d	142.58 d[e]	171.45 s	171.26 s	96.49 d	96.62 d	173.74 s
28	27.42 q	27.24 q	28.32 q	27.96 q	27.89 q	26.70 q	24.84 q	26.99 q	27.10 q	26.64 q[c]	26.98 q[d]	27.40 q	27.40 q	27.40 q	27.38 q
29	21.68 q	21.36 q	21.82 q	21.33 q	21.64 q	21.83 q	21.36 q	20.56 q	21.48 q	20.98 q[c]	21.22 q[d]	21.64 q	21.68 q	21.68 q	20.39 q
30	26.98 q	27.50 q	27.06 q	27.45 q	27.72 q	27.26 q	26.99 q	27.28 q	27.54 q	28.04 q[c]	27.27 q	26.96 q	27.12 q	27.14 q	27.06 q
1α-$\underline{C}OCH_3$	169.94 s[c]	—	169.51 s[c]	—	—	—	169.92 s	—	—	—	—	170.05 s	170.05 s	170.18 s	169.86 s
1α-CO$\underline{C}H_3$	21.08 q[c]	—	21.35 q[c]	—	—	—	21.00 q	—	—	—	—	21.09 q	21.24 q[f]	21.20 q[f]	21.10 q
3α-$\underline{C}OCH_3$	170.07 s[c]	169.51 s	—	—	—	170.32 s	—	—	—	—	—	170.22 s	170.24 s	170.24 s[f]	170.07 s
3α-CO$\underline{C}H_3$	21.14 q[c]	21.16 q	—	—	—	21.07 q	—	—	—	—	—	21.14 q	21.15 q[f]	21.11 q[f]	21.16 q
7α-$\underline{C}OCH_3$	170.07 s[c]	170.37 s	170.29 s[c]	170.61 s	—	170.18 s	170.06 s	170.11 s	—	—	170.13 s	170.34 s	170.43 s	170.25 s[f]	169.93 s
7α-CO$\underline{C}H_3$	21.14 q[c]	21.24 q	21.30 q[c]	21.45 q	—	21.13 q	21.13 q	21.13 q	—	—	21.10 q	21.22 q	20.65 q[f]	20.60 q[f]	21.19 q

[a] In $CDCl_3$ solution. All these assignments were in agreement with HSQC and HMBC spectra.

[b] These ^{13}C NMR spectra were recorded with a sample containing an 1.3 : 1 mixture of both C-23 epimers: **13a** major epimer, **13b** minor epimer.

[c] Assignments for these carbons have not been reported previously.[5,13]

[d,e] Within each column, assignments with the same superscript are reversed with respect to those reported previously.[13,14]

[f] Interchangeable assignments due to overlapping of the signals in the HMBC spectrum of **13a** + **13b**.

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present work allowed the assignment of the acetyl groups in **1** and **3** and the C-Me groups in **10**, not achieved previously,[5,13,14] and also the unambiguous assignment for several carbons of **10**[13] and the correct reassignment for the C-28 and C-29 carbons of **11**.[14]

EXPERIMENTAL

General experimental procedures

Melting-points were determined on a Kofler block and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. IR spectra were obtained on a Perkin-Elmer Spectrum One spectrophotometer. UV spectra were recorded on a Perkin-Elmer Lambda 2 UV/VIS spectrophotometer. Mass spectra were registered in the positive electron ionization (EI) mode on a Hewlett-Packard model 5973 instrument (70 eV). Elemental analyses were made with a Carlo Erba EA 1108 apparatus. Merck silica gel No. 7734 (70–230 mesh) was used for column chromatography. Merck 5554 Kieselgel 60 F_{254} sheets were used for thin-layer chromatographic (TLC) analysis.

NMR spectroscopy

All experiments were performed on a Varian INOVA-400 spectrometer equipped with 5 mm inverse detection *z*-gradient probe. ^{1}H and ^{13}C NMR spectra (at 400 and 100 MHz, respectively) were measured at room temperature (22–23 °C) using $CDCl_3$ as solvent. Chemical shifts are given on the δ scale and were referenced to residual $CHCl_3$ at 7.25 ppm for proton and to the solvent at 77.00 ppm for carbons. One-dimensional ^{1}H and ^{13}C NMR spectra were acquired with standard conditions. The pulse programs of the gHSQC and gHMBC experiments were taken from the Varian software library. The data for the HSQC spectra were collected in a 1024 × 256 matrix with a spectral width of 2485 Hz in the proton domain and 10 000 Hz in the carbon domain and processed in a 1024 × 512 matrix. The null time following the BIRD pulse was 400 ms. The HMBC experiments were optimized for long-range coupling constants of 8 Hz and the data were processed using parameters very similar to those used in the HSQC experiments. The NOE data were recorded using the double pulsed field gradient spin-echo (DPFGSE)-NOE experiment[19] with a mixing time of 600 ms, a recycle delay of 2 s and 128–256 transients per spectrum.

Samples of limonoids

Large quantities of **2**,[9] **3**[5] and **8**[5,10] were available from a previous work.[4] Compound **1** (1,3,7-tri-*O*-acetyl-14,15-deoxyhavanensin)[5] was obtained from **3** by treatment with acetic anhydride–pyridine in the usual manner.

Alkaline hydrolysis of 1 to give 4 and 5

To a solution of **1** (2 g) in EtOH (30 ml) was added an ethanolic solution of KOH (10%, v/w, 20 ml) and the reaction mixture was heated at 60 °C for 3 h. Then, water (150 ml) was added and the reaction mixture was extracted with CH_2Cl_2 (4 × 50 ml). The extracts were dried (Na_2SO_4), filtered and the solvents removed, giving a residue which was subjected to column chromatography [silica gel, 300 g, CH_2Cl_2–EtOAc (9 : 1) as eluent], collecting fractions of 30 ml. The residue obtained from fractions 12–24 was crystallized from EtOAc–n-hexane yielding 850 mg of **4** (50.3%). Fractions 37–53 gave, after evaporation of the solvents and crystallization from EtOAc–n-hexane, pure **5** (680 mg, 44.3%).

Compound **4** (7-*O*-acetyl-14-15-deoxyhavanensin): colourless prims, m.p. 195–196 °C; $[\alpha]_D^{16}$ −19.5° (*c* 0.709, $CHCl_3$); IR (KBr), ν_{max} 3480 (OH), 3133, 1503, 874 (furan), 1705, 1269 (acetate), 2956, 1629, 1458, 1377, 1075, 1028, 957, 806, 600 cm^{-1}; EI-MS, *m/z* (rel. int., %) 456 $[M]^+$ (31), 441 (3), 396 (20), 378 (42), 360 (51), 345 (100), 279 (22), 171 (12), 159 (13), 145 (17), 131 (15), 105 (17), 91 (19), 81 (27), 43 (51). Found: C 73.74, H 9.01. $C_{28}H_{40}O_5$ requires C 73.65, H 8.83%. For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 3, respectively.

Compound **5** (14,15-deoxyhavanensin): colourless fine needles, m.p. 292–294 °C; $[\alpha]_D^{16}$ −32.0° (*c* 0.441, $CHCl_3$–MeOH, 3 : 2); IR (KBr), ν_{max} 3550, 3400 (OH), 3120, 1500, 875 (furan), 2930, 1630, 1442, 1386, 1089, 1074, 1041, 1030, 788, 599 cm^{-1}; EI-MS, *m/z* (rel. int., %) 414 $[M]^+$ (47), 399 (17), 396 (6), 378 (13), 363 (23), 360 (15), 345 (67), 332 (24), 319 (100), 283 (22), 159 (14), 145 (14), 105 (15), 91 (19), 81 (23), 69 (13), 55 (17), 43 (24). Found: C 75.39, H 9.07. $C_{26}H_{38}O_4$ requires C 75.32, H 9.24%. For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 3, respectively.

Chromium trioxide–pyridine oxidation of 2 to give 6

To a solution of **2** (320 mg) in pyridine (17 ml) was added a mixture of CrO_3 (1.7 g) and pyridine (17 ml) and the reaction mixture was left at room temperature for 24 h (Sarett oxidation procedure[15]). Then, the reaction mixture was poured into water (100 ml) and extracted with Et_2O (5 × 25 ml). The extracts were dried (Na_2SO_4) and evaporated to dryness, and the residue (260 mg) was chromatographed [silica gel column, 50 g, EtOAc–petroleum ether (1 : 1) as eluent], giving 210 mg (66%) of **6** (3α,7α-diacetoxy-21,23-epoxy-24,25,26,27-tetranor-apotirucalla-14,20,22-trien-1-one): colourless needles, m.p. 218–219 °C (EtOAc–n-hexane); $[\alpha]_D^{22}$ −44.6° (*c* 0.399, $CHCl_3$); IR (KBr), ν_{max} 3160, 1504, 873 (furan), 1727, 1249 (acetate), 1708 (ketone), 2967, 1435, 1378, 1031, 950, 787, 600 cm^{-1}; EI-MS, *m/z* (rel. int., %) 496 $[M]^+$ (49), 481 (4), 436 (31), 421 (32), 376 (30), 361 (34), 343 (77), 279 (45), 225 (24), 197 (23), 137 (30), 81 (28), 43 (100). Found: C 72.56, H 8.39. $C_{30}H_{40}O_6$ requires C 72.55, H 8.12%. For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 3, respectively.

This compound (**6**) had previously been obtained[9] as an intermediate for the preparation of isoazadirone, although its physical and spectroscopic data were not determined.[9]

Chromium trioxide–pyridine oxidation of 3 to give 7

Compound **3** (400 mg) was treated with CrO_3–pyridine, as described above for **2**, yielding **7** (350 mg, 87.8%), identical in all respects (m.p. $[\alpha]_D$, IR and mass spectra) with the synthetic product[5] described previously. For ^{1}H and ^{13}C NMR spectra, see Tables 1 and 3, respectively.

Preparation of 7-deacetoxyazadirone (9) from azadirone (8)

To a solution of **8** (890 mg) in EtOH (35 ml) was added an ethanolic solution of KOH (10%, v/w, 50 ml) and the reaction mixture was heated at 60 °C for 6 h. Water (200 ml) was added to the reaction and the mixture extracted with CH_2Cl_2 (4 × 60 ml). The extracts were dried (Na_2SO_4), filtered and the solvents removed *in vacuo*, yielding a residue of **9** (760 mg, after crystallization from EtOAc–n-hexane, 94.5% yield): m.p., $[\alpha]_D$, UV and IR spectra identical with those reported[11,12] for the natural compound. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3, respectively.

Preparation of 7-deacetoxy-7oxoazadirone (10) from 9

A solution of **9** (180 mg) in Me_2CO (10 ml) was treated with an excess of Jones' reagent[16] at 0 °C for 10 min. Work-up in the usual manner yielded **10** (153 mg, after crystallization from EtOAc–n-hexane, 85.4% yield): m.p., $[\alpha]_D$, IR and UV spectra identical with those reported[13] for the natural product. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3, respectively.

Oxidation of azadirone (8) to give 11

A solution of **8** (680 mg) in Me_2CO (30 ml) was treated with an excess of Jones' reagent[16] at room temperature for 3 h. Then water (150 ml) was added and the mixture was extracted with CH_2Cl_2 (4 × 40 ml). The extracts were dried (Na_2SO_4), filtered and the solvents removed *in vacuo* giving a residue (600 mg). This residue was subjected to column chromatography [silica gel 60 g, EtOAc–petroleum ether (1 : 1) as eluent] yielding **11** (305 mg, after crystallization from EtOAc–n-hexane, 41.8% yield). Compound **11** [7α-acetoxy-21*R*-hydroxy-3-oxo-24,25,26,27-tetranor-apotirucalla-1,14,20(22)-trien-23,21-olide]: colourless prisms, m.p. 274–276 °C; $[\alpha]_D^{22}$ + 55.6° (*c* 0.792, $CHCl_3$); UV (MeOH), λ_{max} 211 nm (log ε 4.21); IR (KBr), ν_{max} 3434 (OH), 1766 (γ-lactol), 1734, 1249 (acetate), 1650 (α,β-unsaturated ketone), 2950, 1451, 1380, 1125, 1030, 959, 894, 822 cm^{-1}; EI-MS, *m/z* (rel. int., %) 468 $[M]^+$ (20), 450 (24), 435 (2), 408 (42), 390 (45), 375 (35), 339 (16), 259 (48), 241 (35), 150 (47), 137 (49), 121 (96), 105 (33), 93 (49), 91 (48), 79 (29), 69 (28), 55 (19), 43 (100). Found: C 71.75, H 7.99. $C_{28}H_{36}O_6$ requires C 71.77, H 7.74%. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3, respectively.

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Spectral Assignments and Reference Data

Compound **11** had previously been isolated from a natural source as a mixture of the C-21 epimers.[14]

Application of Horeau's method[17] to 11
Compound **11** (54 mg, 0.115 mmol) and (±)-α-phenylbutyric anhydride (92.2 mg, 0.297 mmol) in pyridine solution (2.0 ml) for 18 h at room temperature: $\alpha_1 = +1.959$, $\alpha_2 = +1.650$, $\alpha_1 - 1.1\alpha_2 = +0.144$; configuration 21*R*.

Oxidation of 1 to give 12 and 13
To a solution of **1** (600 mg) in MeOH (50 ml) was added magnesium monoperoxyphthalate (MMPP, 2.2 g) at 0 °C, then the reaction mixture was stirred at room temperature for 7 days. After partial elimination of the solvent *in vacuo*, the reaction mixture was diluted with CH_2Cl_2 (100 ml) and successively washed with an aqueous saturated solution of $NaHCO_3$ and water. The organic extracts were dried (Na_2SO_4), filtered and the solvents removed, giving a residue (410 mg) which was chromatographed [silica gel column, 60 g, petroleum ether–EtOAc (3 : 2) as eluent], yielding **12** (210 mg, 33%, less polar compound) and minute amounts (2.3 mg, 0.36%) of the mixture of the C-23 epimers **13**.

Compound **12** [1α,3α, 7α-triacetoxy-21ζ-hydroxy-24,25,26,27-tetranor-apotirucalla-14,20(22)-dien-23,21-olide] showed physical (m.p.) and spectroscopic (IR and mass spectra) data identical with those reported[5] for a constituent of *Trichilia havanensis*. For ^{1}H and ^{13}C NMR spectra, see Tables 2 and 3, respectively

The epimeric mixture **13** [in a 1.3 : 1 ratio: 1α,3α, 7α-triacetoxy-23ζ-hydroxy-24,25,26,27-tetranor-apotirucalla-14,20(22)-dien-21, 23-olide] showed only one spot on TLC with several eluents. For ^{1}H and ^{13}C NMR spectra of the two epimers (**13a** and **13b**), see Tables 2 and 3, respectively.

Conversion of 12 into compound 14
A stirred solution of **12** (190 mg) in MeOH (15 ml) was treated with an excess of $NaBH_4$ at room temperature for 2 h. Work-up in the usual manner yielded 142 mg (76.8%, after crystallization from EtOAc–n-hexane) of **14** [1α,3α, 7α-triacetoxy-24,25,26,27-tetranor-apotirucalla-14,20(22)-dien-23,21-olide]: colourless needles, m.p. 227–228 °C; $[\alpha]_D^{22}$ −40.8° (*c* 0.887, $CHCl_3$); UV (MeOH), λ_{max} 209 nm (log ε 4.37); IR (KBr), ν_{max} 1781, 1760, 1627 (α,β-unsaturated γ-lactone), 1729, 1259 (acetates), 2953, 1377, 1171, 1051, 890 cm^{-1}; EI-MS, *m/z* (rel. int., %) 556 $[M]^+$ (3), 496 (3), 436 (10), 421 (2), 394 (3), 376 (100), 361 (84), 334 (16), 279 (13), 209 (9), 157 (11), 145 (11), 119 (13), 105 (13), 91 (11), 43 (86). Found: C, 68.94, H 8.10. $C_{32}H_{44}O_8$ requires C 69.04, H 7.97%. For ^{1}H and ^{13}C NMR spectra, see Tables 3 and 4, respectively.

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