A New Tetranortriterpenoid from *Trichilia havanensis*

Benjamín Rodríguez,*,† Cristina Caballero,‡ Félix Ortego,‡ and Pedro Castañera‡

Instituto de Química Orgánica, Consejo Superior de Investigaciones Científicas (CSIC), Juan de la Cierva 3, E-28006 Madrid, Spain, and Departamento de Biología de Plantas, Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas (CSIC), Velázquez 144, E-28006 Madrid, Spain

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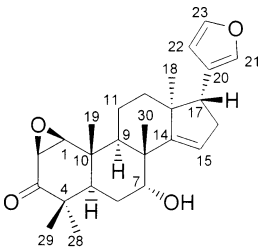
A new triterpenoid, 1β,2β;21,23-diepoxy-7α-hydroxy-24,25,26,27-tetranor-apotirucalla-14,20,22-trien-3-one (**1**), has been isolated from an acetone extract of seeds of *Trichilia havanensis*, along with other known limonoids. The structure of **1** was established by spectroscopic methods, particularly by 1D and 2D NMR studies. Compound **1** was tested as an antifeedant agent against *Leptinotarsa decemlineata* larvae, showing a significant antifeedant activity at 300 ppm.

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

Trichilia havanensis Jacq. (Meliaceae) is a tree widely distributed in Central America, and its limonoid constituents have previously been studied by other authors.[3−6] Recently,[7] an extraction of the seeds of *T. havanensis* 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) and *Spodoptera exigua* (Hübner) larvae. The acetone extract of the seeds contained several previously known compounds,[3−6] including large quantities (1.96%, of dry plant material) of azadirone (7α-acetoxy-21,23-epoxy-24,-25,26,27-tetranor-apotirucalla-1,14,20,22-tetraen-3-one). The azadirone fraction contained minute amounts of another substance (**1**), which was isolated by column chromatography (see Experimental Section).



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Elemental analysis and low-resolution mass spectrometry established the molecular formula $C_{26}H_{34}O_4$ for **1**, and its IR spectrum showed hydroxyl (3545 sharp and 3435 broad cm^{-1}), ketone (1693 cm^{-1}), and furan (3150, 3110, 1504, 875 cm^{-1}) absorptions. The ^{1}H and ^{13}C NMR spectra of **1** displayed typical signals for a β-substituted furan ring [δ_H 7.26, 6.28, and 7.38; δ_C 124.3 (s, C-20), 139.7 (d, C-21), 111.0 (d, C-22), and 142.7 (d, C-23)], a ketone [δ_C 212.6 (s, C-3)], a 1,2-disubstituted oxirane [δ_H 3.54 and 3.36, 1H each, both d, J_{vic} = 4.7 Hz; δ_C 63.3 (d, C-1) and 56.6 (d, C-2)], a trisubstituted olefinic double bond [δ_H 5.59 (1H, dd, J = 3.5, 1.4 Hz, H-15); δ_C 160.8 (s, C-14) and 120.1 (d, C-15)], a secondary hydroxyl group in an axial configuration and placed between a methylene group and a fully substituted sp^3 carbon [δ_H 3.98, 1H, t, J = 2.7 Hz, H-7β; δ_C 71.4 (d, C-7)], and finally four quaternary sp^3 carbons, four methylene groups, three methine carbons, and five methyl groups attached to fully substituted sp^3 carbons (see Experimental Section). All these data can be accommodated[4−6] in a structure such as **1** for this compund.

The HMBC spectrum of **1** showed connectivities between the carbonyl carbon at δ 212.6 (s, C-3) and the H-2, Me-28, and Me-29 protons (δ 3.36 d, 1.11 s, and 1.00 s, respectively), whereas the H-1 proton (δ 3.54 d) was connected with the C-2, C-5, C-9, C-10, and C-19 carbons (δ 56.6 d, 37.4 d, 35.7 d, 39.0 s, and 14.8 q, respectively), thus establishing that **1** possessed in ring A an 1,2-epoxy-3-keto substitution pattern. The H-7β proton (δ 3.98 t) showed HMBC cross-peaks with the C-5, C-6, C-8, C-9, C-14, and C-30 carbons (δ 37.4 d, 24.1 t, 44.4 s, 35.7 d, 160.8 s, and 27.3 q, respectively), and the H-15 olefinic proton (δ 5.59 dd) was connected with the C-8, C-13, C-14, C-16, and C-17 carbons (δ 44.4 s, 47.4 s, 160.8 s, 34.3 t, and 51.5 d, respectively). Moreover, the HMBC spectrum of **1** also displayed connectivities between the C-17 methine proton [δ 2.84 ddd, allylic coupled with the H-21 furan proton (J = 0.8 Hz)] and the C-12, C-13, C-16, and C-18 carbons (δ 32.1 t, 47.4 s, 34.3 t, and 20.1 q, respectively), as well as with the C-20, C-21, and C-22 furanic carbons (δ 124.3 s, 139.7 d, 111.0 d, respectively). Finally, C-9 of **1** (δ 35.7 d) was connected with the H-1, H-5, H-7, H$_2$-11, H-12α, Me-19, and Me-30 protons. All these correlations firmly established structure **1** for this new meliacin, except for its stereochemistry.

Irradiation at δ 3.98 (H-7β proton of **1**) under NOE experimental conditions caused, among others, NOE enhancement in the signals of the H-15 (+9.3%) and Me-30 (δ 1.15 s, +4.5%) protons, thus confirming that the hydoxyl group was at the C-7 position and in an axial 7α-configuration. On the other hand, when the signal of the H-1 proton (δ 3.54) was irradiated, NOE enhancements were observed in the signals of the H-2, H-5α, H-9α, H-11α, H-11β, and Me-19 protons (δ 3.36, 2.63, 2.47, 2.00, 1.76, and 0.98, respectively, NOEs +5.6, +0.6, +1.2, +10.0, +1.3, and +2.3%, respectively). This behavior established that

* To whom correspondence should be addressed. Tel: +34 91 5622900. Fax: +34 91 5644853. E-mail: iqor107@iqog.csic.es.
† Instituto de Química Orgánica, CSIC.
‡ Departamento de Biología de Plantas, Centro de Investigaciones Biológicas, CSIC.

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Table 1. Effect of Azadirone and Compound **1** on the Ingestion of Fourth Instar Larvae of *L. decemlineata*

	AI[a]		
compound	500 ppm	300 ppm	100 ppm
azadirone	26.9 ± 5.1[b]	22.4 ± 7.4[b]	11.6 ± 6.3
1	24.9 ± 3.7[b]	21.4 ± 2.6[b]	10.8 ± 4.5

[a] Antifeedant index $[(C - T)/C] \times 100\% \pm$ standard error ($n = 20$). [b] Significant differences between the consumption of control (C) and treated (T) potato leaf disks (Dunnett two-tailed test $p \leq 0.05$).

H-1 was on the same side of the plane of the molecule as H-5α, H-9α, and H-11α and, hence, that the oxirane of **1** possessed an 1*β*,2*β*-configuration.[9] This conclusion was also in agreement with the chemical shifts of the C-5, C-10, and C-19 carbon atoms of **1** (*δ* 37.4 d, 39.0 s, and 14.8 q, respectively), which were almost identical to those reported for other structurally related meliacins possessing an 1*β*,2*β*-epoxy-3-oxo arrangement, such as in 1*β*,2*β*-epoxyazadiradione[9] [7α-acetoxy-1*β*,2*β*;14*β*,15*β*;21,23-triepoxy-24,25,26,-27-tetranor-apotirucalla-20,22-diene-3,16-dione: *δ* 38.5 (d, C-5), 38.7 (s, C-10), and 15.9 (q, C-19)], and very different from those of the 1α,2α-epoxy stereoisomers, like in 1α,2α-epoxy-17*β*-hydroxyazadiradione[10] [*δ* 39.9 (d, C-5), 41.4 (s, C-10), and 21.3 (q, C-19)]. Moreover, irradiation at the signal of the H-17*β* proton (*δ* 2.84) caused NOE enhancement in the signals of the H-21 and H-22 furanic protons (+1.3 and +1.1%, respectively), in those of the H-12*β* (*δ* 1.59, +6.9%), H-15 (+0.4%), H-16α (*δ* 2.59, −3.8%), and H-16*β* (*δ* 2.41, +4.1%) protons, as well as in the Me-18 (*δ* 0.84, +1.0%) and Me-30 (*δ* 1.15, +1.6%) signals. This result confirmed the apotirucallane stereochemistry of **1**[9,10] and allowed the unambiguous assignment of both C-16 methylene hydrogens, because the H-16α proton showed a negative NOE enhancement when the *trans* H-17*β* proton was irradiated, whereas the signal of the *cis* H-16*β* proton displayed a positive NOE.

From all the above data, structure **1** was assigned to the minor constituent of *T. havanensis*, and it differs from that of deacetylazadirone,[11] a limonoid previously isolated from *Teclea grandifolia* Engl. (Rutaceae), only in the presence in the former of a 1*β*,2*β*-oxirane instead of the C-1, C-2 olefinic double bond of the latter.

The antifeedant activity of **1** was tested against larvae of *L. decemlineata* (Say) (Colorado potato beetle) and compared with that of azadirone, whose structure[5,8] differs from **1** in the acetylation of the 7α-hydroxyl group and in a C-1, C-2 olefinic double bond that replaces the 1,2-oxirane. Both compounds significantly reduced feeding at 500 and 300 ppm (Table 1). The activity observed with Colorado potato beetle larvae (500 ppm equivalent to 20.8 μg/disk or 11.7 μg/cm^2) was higher than that reported[12] for azadirone on another coleopteran species, *Epilachna varivestis* (AI_{50} = 5500 ppm), but less than the effect caused on Colorado potato beetle by other limonoids isolated from *Citrus* species, such as limonin (AI = 64% at 10 μg/disk)[13] and epilimonol (AI_{50} = 10 μg/cm^2).[14]

Experimental Section

General Experimental Procedures. The melting point was determined on a Kofler block and is uncorrected. Optical rotation was measured on a Perkin-Elmer 241 MC polarimeter. The IR spectrum was obtained on a Perkin-Elmer Spectrum One spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Varian INOVA 400 apparatus at 400 and 100 MHz, respectively, and chemical shifts are reported with respect to residual $CHCl_3$ (*δ* 7.25) for protons and to the solvent signals (δ_{CDCl3} 77.00) for carbons. All the assignments for protons and carbons were in agreement with 2D COSY, gHSQC, gHMBC, and 1D NOESY spectra. The mass spectrum was registered in the positive EI mode on a Hewlett-Packard 5973 instrument (70 eV). Elemental analysis was made with a Carlo Erba EA 1108 apparatus. Merck Si gel 60 (230−400 mesh) was used for column chromatography. Merck 5554 Kieselgel 60 F254 sheets were used for TLC analysis. Petroleum ether bp 50−70 °C was used for column chromatography.

Plant Material. Seeds of *Trichilia havanensis* Jacq. (Meliaceae) were collected in June 1994 near Puebla, Mexico, and voucher specimens were deposited in the Herbarium of the "Departamento de Biología de Plantas del Centro de Investigaciones Biológicas", CSIC, Madrid (ref. J.L-O. s/n).

Extraction and Isolation. Dried and powdered seeds of *T. havanensis* (83 g) were extracted with Me_2CO in a Soxhlet for 12 h. The extract (18 g) was subjected to column chromatography as previously described,[5,7] yielding 1.63 g of impure azadirone[8] (1.96% on dry plant material) together with other limonoids.[5,7] The ^{1}H NMR spectrum of a sample of azadirone showed signals of a minor compound (~3%) which was isolated subjecting impure azadirone (800 mg) to column chromatography [Si gel, 200 g, CH_2Cl_2−EtOAc (9:1) as eluent], obtaining pure azadirone (760 mg, less polar compound, 1.87% on dry plant material) and **1** (19 mg, 0.047%).

Compound 1 (1*β*,1*β*;21,23-Diepoxy-7α-hydroxy-24,25,-26,27-tetranor-apotirucalla-14,20,22-trien-3-one): colorless plates (EtOAc−*n*-hexane), mp 234−236 °C; $[\alpha]^{18}_D$ +63.6° (*c* 0.341, $CHCl_3$); IR (KBr) ν_{max} 3545, 3150, 3110, 3030, 2940, 1693, 1504, 1474, 1390, 1160, 1049, 1025, 875 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ* 7.38 (1H, t, *J* = 1.6 Hz, H-23), 7.26 (1H, dt, *J* = 1.6, 0.8 Hz, H-21), 6.28 (1H, dd, *J* = 1.6, 0.8 Hz, H-22), 5.59 (1H, dd, *J* = 3.5, 1.4 Hz, H-15), 3.98 (1H, t, *J* = 2.7 Hz, H-7*β*), 3.54 (1H, d, *J* = 4.7 Hz, H-1α), 3.36 (1H, d, *J* = 4.7 Hz, H-2α), 2.84 (1H, ddd, *J* = 11.0, 7.4, 0.8 Hz, H-17*β*), 2.63 (1H, dd, *J* = 11.9, 4.7 Hz, H-5α), 2.59 (1H, ddd, *J* = 15.3, 11.0, 1.4 Hz, H-16α), 2.47 (1H, dd, *J* = 11.9, 7.4 Hz, H-9α), 2.41 (1H, ddd, *J* = 15.3, 7.4, 3.5 Hz, H-16*β*), 2.12 (1H, br s, disappeared after addition of D_2O, OH-7α), 2.00 (1H, m*, H-11α), 1.91 (1H, m*, H-12α), 1.76 (3H, m*, H-6α, H-6*β*, and H-11*β*), 1.59 (1H, dt, *J* = 12.8, 9.2 Hz, H-12*β*), 1.15 (3H, s, Me-30), 1.11 (3H, s, Me-28), 1.00 (3H, s, Me-29), 0.98 (3H, s, Me-19), 0.84 (3H, s, Me-18), signals marked with asterisk appeared as overlapped multiplets and their assignments were in agreement with the HSQC spectrum; ^{13}C NMR ($CDCl_3$, 100 MHz) *δ* 212.6 (s, C-3), 160.8 (s, C-14), 142.7 (d, C-23), 139.7 (d, C-21), 124.3 (s, C-20), 120.1 (d, C-15), 111.0 (d, C-22), 71.4 (d, C-7), 63.6 (d, C-1), 56.6 (d, C-2), 51.5 (d, C-17), 47.4 (s, C-13), 44.4 (s, C-8), 44.4 (s, C-4), 39.0 (s, C-10), 37.4 (d, C-5), 35.7 (d, C-9), 34.3 (t, C-16), 32.1 (t, C-12), 27.6 (q, C-28), 27.3 (q, C-30), 24.1 (t, C-6), 20.9 (q, C-29), 20.1 (q, C-18), 16.6 (t, C-11), 14.8 (q, C-19); EIMS *m*/*z* (rel int) 410 $[M]^+$ (57), 395 (15), 392 (12), 377 (46), 349 (4), 328 (32), 315 (100), 267 (6), 227 (9), 211 (10), 159 (23), 145 (24), 131 (21), 105 (28), 95 (31), 91 (39), 81 (44), 69 (21), 55 (27), 43 (27), 41 (29); *anal.* C 75.87%, H 8.41%, calcd for $C_{26}H_{34}O_4$, C 76.06%, H 8.34%.

Biological Assays. Larvae and adults of the Colorado potato beetle, *L. decemlineata* (Say), were reared on potato, *Solanum tuberosum* cv. Kennebec, at 25 ± 2 °C, 90 ± 10% rh and 16:8 h (L:D) photoperiod in an environmental chamber. This laboratory colony is renewed annually with wild adults collected from Spanish potato fields.

The arena for the antifeedant no-chioce assays consisted of plastic Petri dishes (15 × 90 mm), coated on their bottom half with about 20 mL of a 2.5% agar solution.[15] Potato leaf disks (1.77 cm^2) were cut with a cork borer No. 15 and fit into holes punched in the agar layer. The disks were treated on the upper surface with 20 μL of an acetone solution containing 500, 300, or 100 ppm of the test compound or the solvent carrier alone. After complete evaporation of the solvent, fourth mid-instars were starved for 6 h and placed in each dish in a growth chamber at 26 ± 0.5 °C and 85 ± 10% rh, where they were allowed to feed for 20 h. Eight treated or control disks were

used in each arena, and 20 replications per treatment were performed in all assays.

The antifeedant index (AI) was calculated on a dry weight basis by the equation $[(C - T)/C] \times 100\%$, where C and T represent the consumption of control and treated disks, respectively.[16]

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