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*Investigación*

# 5a,7b,9a,10b,13a-Pentaacetoxy-4(20),11-Taxadiene (7b-Acetoxy-Taxusin) and Other Constituents from the Bark of the Mexican Yew, *Taxus globosa* (Taxaceae)

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*Dedicated to the memory of Dr. Jacobo Gómez-Lara*

**Abstract.** Chemical investigation of the bark of the Mexican yew (*Taxus globosa*) led to the isolation of sterols, vanillic acid and a peracetyl taxane identified as 5α, 7β, 9α, 10β, 13α-pentaacetoxy-4(20), 11-taxadiene (7β-acetoxy-taxusin, **2**).
**Key Words.** 5α, 7β, 9α, 10β, 13α-pentaacetoxy-4(20), 11-taxadiene, 7β-acetoxy-taxusin, taxane, Mexican yew, *Taxus globosa*, Taxaceae.

**Resumen.** La investigación química de la corteza del tejo mexicano (*Taxus globosa*) condujo al aislamiento de esteroles, ácido vainíllico y un peracetil taxano identificado como 5α, 7β, 9α, 10β, 13α-pentaacetoxi-4(20), 11-taxadieno (7β-acetoxi-taxusina, **2**).
**Palabras clave.** 5α, 7β, 9α, 10β, 13α-pentaacetoxi-4(20), 11-taxadieno, 7β-acetoxi-taxusina, taxano, tejo mexicano, *Taxus globosa*, Taxaceae.

## Introduction

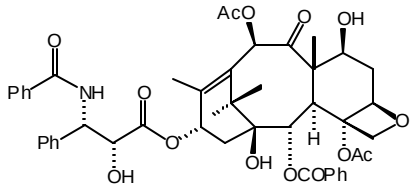
The taxane diterpenoids are biosynthesized mainly by the members of the *Taxus* genus (Taxaceae) and represent one of the most interesting groups of bioactive natural products due to the discovery and development of taxol® (**1**, paclitaxel) [1,2], which has become an efficient therapeutic agent and the best selling anticancer drug in history [3]. Although the chemical study of *Taxus* species began in the middle of the last century [4], only few taxanes were known around 1971, at the time of the discovery of taxol, and the interest for this substance increased during the late seventies derived from its novel model of antitumoral action [5] and successful clinical trials [6]. Recent reviews have included over 350 taxoids [7], and the investigation on this group of natural products has emerged as an important field in chemistry and biology [8]. Taxoids are relatively scarce in Nature and different strategies to increase the supply of these substances, and in particular of taxol, include total synthesis [9], relay synthesis [10], cultivation of *Taxus* plants [11], biosynthesis and cell culture production [12,13]. Most of the *Taxus* species has been chemically investigated, however, the Mexican yew, *T. globosa*, is perhaps the less studied [14]. From the bark of this plant we have isolated β-sitosterol, β-sitosteryl-β-D-glucoside, vanillic acid and 5α, 7β, 9α, 10β, 13α-pentaacetoxy-4(20),11-taxadiene (7β-acetoxy-taxusin, **2**).

## Results and Discussion

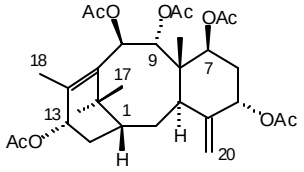
The geographical distribution of *T. globosa* is quite sporadic and ranges from the Mexican states of Nuevo León, Tamaulipas, San Luis Potosí, Veracruz, Querétaro, Hidalgo, Oaxaca, and Chiapas to Guatemala, Honduras y El Salvador. It is a small tree up to 15-20 m and its populations are relatively scarce and located in difficult terrains. It is commonly known as granadillo, palmira, romerillo and tlatscal, and it is used locally for fence posts, as ornamental, and for tanning. The continuous harvesting of the trunks represents a threat for the populations [15,16].

The methanolic extract of the bark of the Mexican yew (collected in the State of Chiapas) was partitioned between dichloromethane and water. The dichloromethane fraction was subjected to a silica gel column followed by subsequent chromatographies of selected fractions. This procedure allowed to isolate a white solid, mp 205-207 °C, whose molecular formula $C_{30}H_{42}O_{10}$ was determined by HRMS. IR absorptions at 1735 and 1247 $cm^{-1}$ suggested the presence of ester groups.

The fragment peaks at *m/z* 502 [$M^+$ - 60], 442 [$M^+$ - 120], 382 [$M^+$ - 180], 322 [$M^+$ - 240] and 262 [$M^+$ - 300] in the EIMS, as well as the five methyl singlets at $\delta_H$ 2.19, 2.09, 2.08. 2.03 and 1.98 in the $^1$H NMR spectrum, established that the structure included five acetates. Additional methyl groups were observed at $\delta_H$ 2.20, 2.02, 1.17 and 0.85 ($\delta_C$ 14.94, 20.80, 31.16 and 13.29) and the hydrogens of an exomethylene were observed at $\delta_H$ 5.28 and 4.95 ($\delta_C$ 146.78 and 115.66). $^1$H - $^1$H COSY spectrum of **2** established connectivities of C(9) to C(10), C(13) to C(14), C(14) to C(1), C(1) to C(2) and C(2) to C(3). HMBC correlations [17] of H(10) with C(11), C(12) and C(15) located the tetrasubstituted double bond at C(11)-C(12) and the C(11)-C(15)-C(1) connectivity, characteristic for the taxane skeleton. This last connectivity was confirmed by the HMBC correlations of H(13) with C(11), C(12) and C(14). The cyclohexane ring was evident from the observed cross-peaks of H(7) with C(6), C(8) and C(19), and from the cross-peaks of the H-20 hydrogens with C(3) and C(5) in the HMBC spectrum. Therefore, the structure corresponded to a taxane with the acetoxy groups attached at C(5), C(7), C(9), C(10) and C(13). The stereochemistry for the tetrasubstituted olefin and the configurations at C(1), C(3), C(8) were defined by the taxane skeleton [7], and the orientation of the acetoxy groups were established by the observed NOESY interactions and comparison with the data informed in the literature. Therefore, the substance isolated from the bark of *T. globosa* was 5α,7β,9α,10β,13α-pentaacetoxy-4(20), 11-taxadiene (7β-acetoxy-taxusin, **2**), previously isolated from *T. baccata* [18] and *T. mairei* [19]. β-Sitosterol, β-sitosteryl-β-D-glucopyranoside and vanillic acid were also isolated from the bark. The chemical and comparative analyses of additional populations of the Mexican yew are currently underway.




**1** Taxol (Paclitaxel)




**2** 5α,7β,9α,10β,13α-Pentaacetoxy-4(20),11-taxadiene

## Experimental Section

**Collection and Extraction.** The bark of one tree of *T. globosa* Schlecht. (*T. baccata* ssp *globosa* (Schlecht.) Pilger) was collected at Jocosic, State of Chiapas, México, in July 1995, by. J. C., and plant samples were deposited at the National Herbarium, Instituto de Biología de la UNAM (voucher MEXU 669748; JC 726). The identity of the plant material was verified by comparison with authentic material. The air-dried bark (2.2 Kg) was powdered and extracted three times with ethanol. The combined extracts were concentrated at reduced pressure to obtain a dark brown gum. This residue was partitioned three times between dichloromethane and water, and the aqueous fractions were further extracted with dichloromethane. The combined organic fractions were dried ($Na_2SO_4$) and concentrated, to yield 22 g residue.

**Isolation and Characterization.** Part of the dichloromethane extract (20 g) was separated over Si-gel by vacuum liquid chromatography (vlc) [20] packed with 60 g of silica (mesh 230-400) using a gradient of *n*-hexane-EtOAc, and collecting a total of 102 fractions (400 ml each one). Eluates were pooled based on TLC profiles, resulting in a total of 24 sub-fractions, designated A-W. Subfraction D (2 g, eluted with *n*-hexane-EtOAc 49:1) was separated over Si-gel column using an open column chromatography to afford 390 mg of β-sitosterol, identified by direct comparison with an authentic sample. Fraction K (1 g, eluted with *n*-hexane-EtOAc 4:1) was purified over Si gel using vacuum liquid chromatography (*n*-hexane-EtOAc gradient) to obtain a fraction (622 mg) which was rechromatographed by column chromatography using *n*-hexane-acetone as gradient elution system. This procedure yielded a residue which was further purified by Si gel TLC plates (2 mm, using chloroform-acetone 20:1 as elution system, one development) to afford a total of 40 mg of **2** after several runs ($1.8 \times 10^{-3}$ % yield). Subfraction L (267 mg) was rechromatographed using vacuum liquid chromatography (*n*-hexane-EtOAc gradient) to obtain a residue which was further purified by prep Si gel TLC (2 mm, using dichloromethane-acetone-acetic acid 9:1:0.1 as eluent, two developments). Usual procedure allowed to isolate 12 mg of vanillic acid (*p*-hydroxy-*m*-methoxy-benzoic acid). Mp: 210-212 °C. From subfraction U (eluted with EtOAc from the original column), was isolated β-sitosteryl-β-D-glucopyranoside (30 mg) by sequential crystallizations, and its identity was confirmed by direct comparison with authentic samples.

**5a,7b,9a,10b,13a-pentaacetoxy-4(20),11-taxadiene (7b-acetoxy-taxusin, 2).** Mp: 205-207 °C (lit: 205-207 °C [18, 19]; IR ($CHCl_3$) $\nu_{max}$ 3691, 3604, 2956, 1735, 1247 cm$^{-1}$; $^1$H NMR ($CDCl_3$, 500 MHz, assignments by $^1$H - $^1$H COSY and HMQC experiments) $\delta_H$ 1.88 (1H, m, H-1), 1.72 and 1.86 (1H each, m, H-2a, H-2b), 2.91 (1H, br d, J = 5.1 Hz), 5.39 (1H, t, J = 2.5 Hz), 1.91 and 1.78 (1H each, m, H-6a, H-6b), 5.55 (1H, dd, J = 5, 10.7, H-7), 5.91 (1H, d, J = 10.5, H-9), 6.25 (1H, d, J = 10.5, H-10), 5.94 (1H, m, H-13), 2.66 and 1.03 (1H each, m, H-14a, H-14b), 1.63 (3H, s, H-16), 1.17 (3H, s, H-17), 2.20 (3H, br s, $w_{1/2}$ 1.0 Hz, H-18), 0.84 (3H, s, H-19), 5.95, 5.28 (1H each, d, J = 1.2, H-20a, H-20b). 2.18, 2.07, 2.06, 2.02, 1.98 (3H each, 5 acetates); $^{13}$C NMR ($CDCl_3$, 125 MHz, assignments by APT and HMQC) $\delta_C$ 4.40 (C-1), 27.55 (C-2), 37.60 (C-3), 146.78 (C-4), 74.77 (C-5), 34.13 (C-6),

70.11 (C-7), 46.18 (C-8), 76.84 (C-9), 71.67 (C-10), 134.54 (C-11), 137.40 (C-12), 71.76 (C-13), 31.41 (C-14), 39.45 (C-15), 27.36 (C-16), 31.16 (C-17), 14.94 (C-18), 13.29 (C-19), 115.66 (C-20), 169.82 (OAc at C-5), 169.80 (OAc at C-7), 170.41 (OAc at C-9), 169.2 (OAc at C-10), 170.25 (OAc at C-13), 21.73, 21.58, 21.37, 20.95 and 20.80 (methyls of the acetate groups); HRMS: $M^+$ 562.6416, Calcd for $C_{30}H_{42}O_{10}$: 562.6558; EIMS *m/z* (rel. int.): 562 $[M]^+$ (3), 502 (30), 460 (14), 442 (10), 400 (24), 382 (10), 340 (32), 322 (15), 280 (49), 262 (14), 149 (28), 119 (31), 43 (100).

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