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A LIGNAN FROM ROOTS OF *TAXUS MAIREI*

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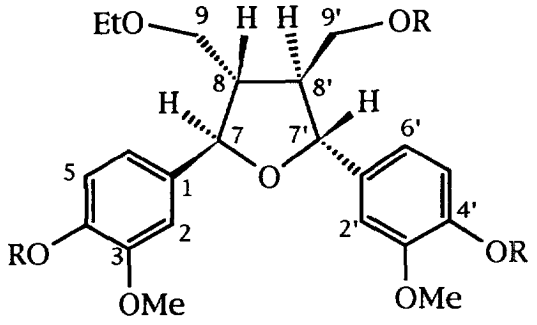
Key Word Index—*Taxus mairei*; Taxaceae; roots; lignan; taxumairin.

Abstract—A new lignan, taxumairin was isolated from the roots of Formosan *Taxus mairei*, along with known lignans. The structure of taxumairin has been characterized as (+)-7,8-*trans*-8,8′-*trans*-7′,8′-*trans*-7-(3-methoxy, 4-hydroxy)phenyl-7′-(3′-methoxy, 4′-hydroxy)-phenyl-8-hydroxymethyl-8′-ethoxymetyltetrahydrofuran, on the basis of spectral analyses. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

A great number of lignans have been isolated from plant families such as the Rutaceae [1], Umbelliferae [2, 3], Araucariaceae [4], Piperaceae [5, 6], Euphorbiaceae [7] and Cupressaceae [8]. In addition, some yew trees were also rich in aryltetralin lignans [9–11]. In our study of the roots of *Taxus mairei*, new taxane diterpenes have been isolated and characterized [12–14]. During this investigation, a new lignan, named taxumairin (**1**), was also found in the roots, together with (−)-α-conidendrin, (−) secoiso-lariciresinol and isotaxiresinol. Herein, we wish to report the isolation and structural elucidation of the new compound (**1**).



1, R = H
2, R = Ac

RESULTS AND DISCUSSION

The ethanolic extract of *T. mairei* roots gave, after extensive chromatography, compound **1** and known lignans, which were identified by comparison of their spectral data (UV, IR, ^{1}H-, ^{13}C NMR, EI-mass spectra and [α]) with those in the literature, as (−)-α-conidendrin [15], (−) secoisolariciresinol [16] and isotaxiresinol [17].

Taxumairin (**1**) was isolated as an amorphous solid. The EI mass spectrum showed a $[M]^+$ at *m/z* 404, consistent with the formula $C_{22}H_{28}O_7$. The UV and IR spectra revealed the presence of hydroxyl (3404 cm^{-1}) and phenolic (232 and 280 nm, 1608 and 1516 cm^{-1}) groups. Analysis of the ^{1}H NMR spectrum (Table 1) suggested that compound **1** is a lignan, exhibiting two aromatic methoxyl groups at δ 3.91 and δ 3.92, and an ethoxyl function at δ 1.61 and δ 3.59. In addition, a pair of multiplets at δ 2.24 and δ 2.60, two methine protons at δ 4.05 and δ 4.36 and two pairs of methylene protons at δ 3.63 and δ 3.58 were also observed. The remaining six aromatic protons indicated the presence of two sets of 3-methoxy-4-hydroxyphenyl systems [δ 6.86 (2H, *d*, *J* = 8.4 Hz, H-5, 5′), δ 6.94 (2H, *d*, *J* = 8.4 Hz, H-6, 6′) and 7.0 (2H, *br s*, H-2, 2′). A COSY spectrum was performed to determine the relationship of each proton in **1**. Supported by ^{13}C NMR and DEPT spectral data, compound **1** displayed three oxymethylene carbons (δ 70.1, 63.8 and 63.6), two oxymethine carbons at rather low field (δ 84.5 and 84.6) and two sets of methoxyl carbons (δ 56.0 and 56.1) , as well as carbon signals of two aromatic rings. Taken together, these signals reflected the presence of two non-equivalent 3-methoxy-4-hydroxyphenyl moieties attached to the tetrahydrofuran skeleton, probably at the C-7 and C-7′ positions. An HMBC experiment provided correlations between δ 4.04 (H-7) and two carbons (δ

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Table 1. ^1H, ^{13}C NMR, COSY and HMBC spectral data for taxumairin (1)

Position	^{13}C‡	Carbon type*	^1H†	COSY	HMBC
1	132.6	S			H5
2	108.8	D	6.88 (*s*)		H7
3	147.0	S			H5, OMe
4	145.8	S			H2
5	114.2[c]	D	6.81 (*d*, 8.4)	H6	
6	120.8	D	6.87 (*d*, 8.4)	H5	H2, H7
7	84.5[d]	D	4.05 (*d*, 9.9)	H8	H2, H6, H8, H9
8	56.0	D	2.60 (*m*)	H7, H9, H8′	H7, H9
9	70.1	T	3.63 (*m*)	H8	H8
1′	132.0	S			H5′
2′	108.8	D	6.88 (*s*)		H7′
3′	146.7	S			H5′, OMe
4′	145.5	S			H2′
5′	114.1[c]	D	6.81 (*d*, 8.4)	H6′	
6′	119.5	D	6.87 (*d*, 8.4)	H5′	H2′, H7′
7′	84.6[d]	D	4.36 (*d*, 9.3)	H8′	H2′, H6′, H8′, H9′
8′	52.9	D	2.24 (*m*)	H9′, H7′, H8	H7′, H9′
9′	63.8	T	3.58 (*m*)	H8′	H8, H7′, H8′
OH			5.74 (*br s*)		
OMe	56.0	Q	3.91 (*s*)		
	5.61	Q	3.92 (*s*)		
$OCH_2\underline{C}H_3$	14.9	Q	1.16 (*t*, 7.5)	$OC\underline{H}_2CH_3$	$OC\underline{H}_2CH_3$
$O\underline{C}H_2CH_3$	63.8	T	3.34 (*q*, 7.5)	$OCH_2C\underline{H}_3$	$OCH_2C\underline{H}_3$

* S = C, D = CH, T = CH_2, Q = CH_3. Multiplicities and assignments made by DEPT and HMBC techniques.
† Multiplicities and coupling constants in Hz in parentheses.
‡ Data (c, d) interchangeable.

108.8 and 120.8) assignable to C-2 and C-6, and correlations between H-7′ (δ 4.36), C-2′ and C-7′. Moreover, correlations between C-7 and C-8′ and the methylene H-9, and correlations between C-7′, C-8′ and H-9′ also confirmed the structural features of **1**. Although there was no HMBC correlation found between H-7, C-7′ and C-7, H-7′, it required a tetrahydrofuran ring for compound **1** from calculations of the degrees of unsaturation in the molecular formula. Furthermore, comparison of the ^{13}C NMR data of **1** with that of neo-olivil 9′-*O*-β-D-glucoside revealed that they were very similar, except for the side-chain at C-9 [18].

To prove the above presumption, acetylation of **1** gave a triacetate (**2**), $C_{28}H_{34}O_{10}$ (*m/z* [M]$^+$ 530) which showed two aromatic acetyl groups (δ 2.32), in addition to one aliphatic acetyl group (δ 1.97). EI mass spectral fragmentation studies of both taxumairin and its triacetate were also in agreement with the structures **1** and **2**. The relative stereochemistry of **1** was determined on the basis of comparison between the observed coupling constants with those of neo-olivil 9′-*O*-β-D-glucoside and extensive NOE studies of **1**. The *trans*-configurations between the methine protons at C-7 and C-8, and C-7′ and C-8′, were established by the coupling constants as H-7 (δ 4.05, *d*, J = 9.9 Hz) and H-7′ (δ 4.36, *d* J = 9.3 Hz), respectively [18]. On irradiation of the oxymethine signal at δ 4.05 (H-7), peak enhancement was observed at δ 2.24 and δ 3.63, suggesting α-disposition of H-7, H-8′ and H-9. Furthermore, irradiation of H-7′ at δ 4.36 caused NOE effects at δ 2.60 and at δ 3.58, which also suggested that the configuration of H-7′, H-8 and H-9′ are all β. In addition, no NOE effect was observed between H-7 and H-7′, between H-7 and H-8, and between H-8 and H-8′, as well as between H-7′ and H-8′. These findings indicated the *trans*-configuration between the above pairs of protons. On the basis of the spectral and chemical evidence, taxumairin (**1**) was thus determined to be (+)-7,8-*trans*-8,8′-*trans*-7′,8′-*trans*-7-(3-methoxy, 4-hydroxy)phenyl-7′-(3′-methoxy, 4′-hydroxy)-phenyl-8-hydroxymethyl-8′-ethoxymetyltetrahydrofuran, which belongs to the type of (+) 7*R*, 8*S*, 7′*R*, 8′*S*-7, 7′-epoxylignans.

Natural products with a 2,5-diaryl-3, 4-*bis* (hydroxymethyl)-tetrahydrofuran skeleton have been reported by Sugiyama *et al.* [18], Matsushita *et al.* [19] and Hernandez *et al.* [20]. Because neolignans having an ethoxyl moiety at the C-9 position, such as taxumairin (**1**) are extremely rare in nature, compound **1** might possibly be an artifact produced during extraction.

EXPERIMENTAL

Plant material. Roots of *Taxus mairei* (Lemee and Levi.) S.Y. Hu were purchased in Kaohsiung, Taiwan, 1993. A voucher specimen is deposited in the Institute

of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan, Republic of China.

Extraction and isolation. Dried roots (60 kg) were ground and repeatedly extracted with EtOH (200 l) at room temp. The combined extracts were concd to a brown tar, which was added to a mixt. of MeOH (10 l) and H_2O (10 l) and stirred overnight. The MeOH–H_2O soluble fr. was extracted exhaustively with $CHCl_3$ (20 l). The lower layer ($CHCl_3$–MeOH) was concd under vacuum to give a residue (1.5 kg). Part of the residue (200 g) was applied to a Sephadex LH-20 column and eluted with MeOH to afford a residue (85 g). This was chromatographed on a silica gel column (850 g) and eluted with a solvent mixt. of $CHCl_3$–Me_2CO of increasing polarity to provide 7 frs. Fr. D (3 g) was rechromatographed on a silica gel column and eluted with $CHCl_3$–*n*-hexane–MeOH according to the following ratios and vols (10:10:1, 9:9:1, 8:8:1, 7:7:1, 6:6:1 and 5:5:1, each 350 ml) to yield (−)-α-conidendrin (120 mg). Fr. E (11.8 g) was rechromatographed on a silica gel column and eluted with the aforementioned solvent systems (each 1 l) to yield a residue (4.4 g). Separation of this residue on a reverse-phase C-18 column (100 g) using solvent mixtures of MeOH–H_2O of decreasing polarity (7:3, 7:4, 7:5, 7:6 and 1:1, each 300 ml) gave 7 frs, a (235 mg), b (175 mg), c (85 mg), d (420 mg), e (165 mg), f (500 mg) and g (210 mg). Fr. d was chromatographed on a LH-20 column (*n*-hexane–$CHCl_3$–MeOH, 1:1:2) to give fr. d2 (90 mg), which was purified by prep. TLC developed with *n*-hexane–$CHCl_3$–MeOH (5:5:1) to yield compound **1** (18 mg).

From another batch of plant material, roots (315 g) were ground into powder and extracted with EtOH (21 × 3). The alcoholic extracts were comb. and concd *in vacuo* to give a residue (100 g). Extraction of the residue with EtOAc (200 ml × 3) afforded an EtOAc-soluble fr. (60 g). Part of this (60 mg) was applied to a prep. TLC plate (silica gel) and developed with $CHCl_3$–MeOH (10:1) to yield secoisolariciresinol (3 mg) and isotaxiresinol (14 mg).

Taxumairin (**1**). Amorphous solid. $[\alpha]_D^{25}$ + 2° (MeOH, *c* 0.16). UV λ_{max}^{MeOH} nm (log ε.): 232 (3.56), 280 (3.17). IR ν_{max}^{KBr} cm^{-1}: 3404 (OH), 2928, 1608, 1516, 1462, 1374, 1274, 1034, 756. ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$: Table 1. EIMS *m/z* rel. int.: 404 ($[M]^+$, 16), 182 (34), 181 (100), 163 (14), 153 (60), 150 (16), 137 (43), 125 (21), 103 (9), 93 (47), 77 (10), 65 (30).

Taxumairin triacetate (**2**). Acetylation (Ac_2O—pyridine 1:1; room temp.) of **1** (7 mg) furnished, after work-up, **2** (7 mg). $[\alpha]_D^{25}$ + 4.2° (MeOH, *c* 0.085). UV λ_{max}^{MeOH} nm (log ε: 227 (3.52), 278 (3.13). IR ν_{max}^{KBr} cm^{-1}: 2928, 1766, 1742, 1608, 1468, 1426, 1372, 1198, 1034. ^1H NMR ($CDCl_3$): δ 7.00 (2H, *s*, H-2, 2′), 6.87 (2H, *d*, *J* = 8.4 Hz, H-5, 5′), 6.96 (2H, *d*, *J* = 8.4 Hz, H-6, 6′), 4.11 (1H, *d*, *J* = 8.7 Hz, H-7), 4.62 (1H, *d*, *J* = 7.8 Hz, H-7′), 2.54 (1H, *m*, H-8), 2.45 (1H, *m*, H-8′), 4.20 (1H, *dd*, *J* = 10.8, 7.5 Hz, H-9a), *dd*, *J* = 10.8, 3.5 Hz, H-9b), 3.74 (2H, *m*, H-9′), 3.86 (6H, *s*, OMe), 1.12 (3H *t*, *J* = 7.2 Hz, $OCH_2\underline{CH_3}$), 3.30 (2 H, *m*, $OC\underline{H_2}CH_3$), 1.97 (3H, *s*, OAc), 2.32 (6H, *s*, OAc). EIMS *m/z* rel. int.: 530 ($[M]^+$, 5), 488 (5), 471 (2), 424 (2), 411 (5), 382 (2), 245 (5), 223 (16), 205 (14), 181 (100), 163 (10), 153 (16), 151 (30), 149 (24), 137 (21), 125 (10), 97 (14), 93 (22), 87 (20), 69 (23), 57 (31), 55 (22). FABMS: *m/z* 553 ($[M+Na]^+$).

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