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A SECOAMORPHANE TRI-ETHER FROM *CUPRESSUS MACNABIANA* FOLIAGE

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Key Word Index—*Cupressus macnabiana*; Cupressaceae; foliage; sesquiterpenoids; macnabin; (4,5),(4,6),(5,10)-trioxido-4,5-secoamorphane.

Abstract—A novel sesquiterpenoid tri-ether with a 4,5-secoamorphane carbon skeleton was isolated from the foliage of *Cupresss macnabiana* and characterized by 1D and 2D NMR. It appears to be biosynthetically related to τ-cadinol.

INTRODUCTION

Cupressus macnabiana Jeps., one of the more common Californian species of the genus, is found on serpentine or volcanic soils in the arid foothills of the interior Coast and Sierran ranges of northern California. It is a small tree with very resinous glandular foliage, and the only report on its foliar terpenoid chemistry was restricted to monoterpenes [1].

Previous research on the foliar terpene chemistry and chemotaxonomy of another glandular northern Californian cypress, *C. bakeri* Jeps. [2–5], has shown it to be a rich source of new sesquiterpenoids. *Cupressus macnabiana* foliage contains a very complex essential oil with most of the same terpenoids as *C. bakeri* plus many additional mono- and sesqui-terpenoids. Here, we report the identification of macnabin, a novel secoamorphane tri-ether from *C. macnabiana* foliar essential oil.

RESULTS AND DISCUSSION

Gas chromatography–mass spectrometric (GC-MS) analysis of extracts of *C. macnabiana* foliage showed the presence of an unknown component with an apparent molecular ion at $m/z = 252$. In GC analysis of 142 trees from 10 populations of the species, the amount of this unknown correlated positively with the major components α-cadinol and *cis*-calamenene, suggesting a common biosynthesis [6] with this group of sesquiterpenoids. A similar correlation among sesquiterpenoids related to (−)-*cis*-calamenene was found for *C. bakeri* [2, 3, 5] and they all proved to have the same absolute stereochemistry at the isopropyl group.

The IR spectrum of the purified unknown showed no hydroxyl or carbonyl absorbances. High-resolution FAB-MS confirmed the M_r of 252 and the molecular formula was $C_{15}H_{24}O_3$. One- and two-dimensional NMR led to the structure **1**, a tetracyclic secoamorphane tri-ether for which we propose the trivial name macnabin. The 1D experiments were first performed in $CDCl_3$ (see Experimental), but overlaps of a number of proton signals required a solvent change to benzene-d_6 for the 2D work. The chemical shift data below and in Table 1 are in the latter solvent.

Carbon-13 NMR and DEPT data (Table 1) showed the presence of four methyls, four methylenes, four methines and three quaternary carbons. Downfield signals for one of the methines (δ104.4) and one quaternary (δ110.5) were attributable to carbon atoms bearing two oxygens each, while quaternary signals at δ88.2 and 92.8 were interpreted as carbons with single oxygen atoms attached. The ^{1}H NMR spectrum showed two methyl doublets (3H each, δ0.85 and 0.95, $J = 6.8$ Hz) attributable to an isopropyl group. Three-proton singlets at δ1.15 and 1.42 indicated methyl groups attached to quaternary carbons bearing one and two oxygen atoms, respectively. A sharp singlet at δ5.50 was obviously the proton attached to the methine carbon at δ104.4.

The correlations for the 2D NMR of **1** are shown in Table 1. Proton–proton COSY gave two fragments **A** and **B**, confirming the presence and location of the isopropyl group. Proton-carbon assignments were made by a one-bond ^{1}H–^{13}C heteronuclear (HMQC) NMR experiment. The connections of fragments **A** and **B** and the oxygen atoms to the quaternary carbons were determined in a long-range ^{1}H–^{13}C (HMBC) experiment. Three-bond ^{1}H–^{13}C couplings through the oxygen atoms made it possible to deduce their attachments unambiguously. Examination of a molecular model of the resulting structure showed that the relative stereochemistry at carbons 1, 4, 5, 6 and 10 could only be as depicted for **1**; thus, the only questions remaining were the orientation of the isopropyl group and the absolute stereochemistry of **1**. An equatorial (i.e. β) orientation of the isopropyl was implied by the strong ($J = 12.6$ Hz) diaxial coupling between H-7 and H-8$_\beta$; this was confirmed by a NOESY experiment, which showed cross-peaks from

Table 1. ^{13}C and ^{1}H NMR chemical shifts (δ, ppm from TMS) and 2D correlations* of macnabin (1) (^{13}C: 100.602 MHz, ^{1}H: 400.13 MHz, benzene-d_6)

C	^{13}C	^{1}H† (*J*, Hz)	$^{1}H-^{13}C$‡	$^{1}H-^{1}H$	NOESY§
1	46.0	1.19, *dd* (9.0, 6.7)	2?, 5, 6, 7, 9, 15?	g	cd
2	19.0	H_α 1.39–1.47	1, 3, 6, 10	gh	i
		H_β 2.06–2.17		gh	ai
3	32.8	2H, 1.61–1.67	1, 2, 4	h	hi
4	110.5				
5	104.4	5.50, *s*	1/7, 4, 6, 10		—‖
6	92.8				
7	46.0	1.70, *dt* (12.6, 5.0)	1, 5, 6, 8, 11, 12, 13	cd	dg
8	23.5	H_α 1.33–1.40	6, 7, 9, 10	de	e
		H_β 1.28, *ddt* (12.6, 12.1, 6.0)		de	f
9	39.5	H_α 1.02, *ddd* (13.9, 12.1, 6.6)	1/7, 8, 10, 15	ef	bc
		H_β 1.52 *ddd* (13.9, 6.0, 1.5)		ef	b
10	88.2				
11	28.8	2.04–2.10	6, 7, 8, 12, 13	abc	g
12	19.8	3H, 0.95, *d* (6.8)	7, 11, 13	b	fg
13	23.3	3H, 0.85, *d* (6.8)	7, 11, 12	a	eg
14	27.0	3H, 1.42, *s*	3, 4		h
15	22.2	3H, 1.15, *s*	1, 9, 10		ab

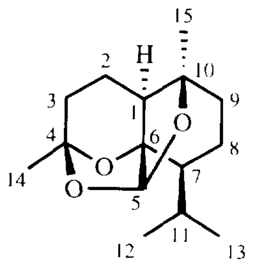
* Atoms with the same letter show a cross-peak.

† For multiplets, the range in ppm is given.

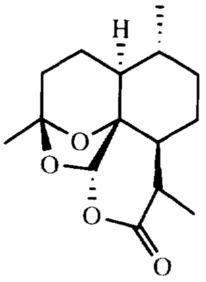
‡ 2- and 3-bond couplings. The number given is that of the carbon atom to which the protons in the row show a cross-peak. Uncertain (weak) cross-peaks marked by query.

§ Geminal cross-peaks not listed.

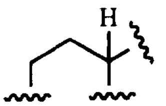
‖ Not examined.



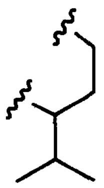
1



2



A



B

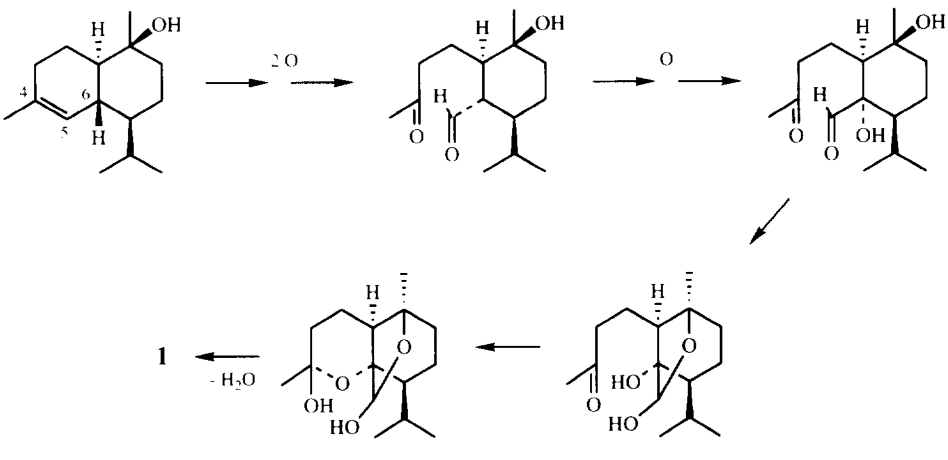
H-1 to H-7 and H-9 (all α). The absence of an H-7/H-9 cross peak was at first puzzling, but force-field (MM2) calculation of the minimum-energy conformation of **1** showed that the cyclohexane ring has a deformed chair conformation which spreads these two protons apart (2.9 Å between H-7/H-9 vs 2.2 Å between H-1/H-7 and 2.4 Å between H-1/H-9). The other NOESY correlations were consistent with the structure and calculated conformation of **1**.

The absolute stereochemistry of **1** is expected to be as depicted, based on its likely common biogenesis with the other cadinane-type compounds in *C. macnabiana*. The major compounds in this group (α-cadinol, τ-cadinol and *cis*-calamenene) were isolated and were all found to have the same 7*S* absolute stereochemistry. A biosynthetic pathway from (+)-τ-cadinol to **1** can be envisioned (Scheme 1), which would involve: (a) oxidative scission of the 4,5 double bond, giving carbonyl groups at C-4 and C-5; (b) inversion of C-6 stereochemistry (e.g. via aldehyde–enol isomerization); (c) oxidative hydroxylation at C-6; (d) 5,10 hemiacetal and 4,6 hemiketal formation; and (e) ultimate dehydration between C-4 and C-5 hydroxyls, leading to **1**.

It is perhaps worth noting that the structure of **1** bears a marked similarity to desoxyartemisinin **2**, one of the polyoxygenated secoamorphane lactones found in *Artemisia annua* L. [7, 8].

EXPERIMENTAL

Foliage of *C. macnabiana* was collected on 30 August 1993 from 20 trees in the Lake County (CA) population



Scheme 1. Suggested biosynthetic route from (+)-τ-cadinol to macnabin (**1**).

(lat. 38°56.3′ N, long. 122°55.6′ W). Quantitative analysis of extracts from individual trees was by capillary GC on two columns (SE-54 and Carbowax 20M) using the conditions given in ref. [9], except that splitless injection and a starting column temperature of 35° were used. Hydrodistillation of the *n*-hexane foliage extract (over satd NaCl soln with addition of $NaHCO_3$), column LC of the oil (silica gel; hexane–EtOAc eluents), GC-MS of the frs and FTIR of purified **1** were described before [3]. Final purification of **1** was by prep. GC, first on a 4 m × 4 mm column packed with 10% SE-30/Chromosorb W, isothermal 190°; then on 15% Carbowax 20M/Chromosorb W, isothermal 185°; carrier He at 50 ml min^{-1}, injector 190°, detector 200°. The latter column was used for final purification of *cis*-calamenene, α-cadinol and τ-cadinol from the relevant LC frs. (−)-cis-*Calamenene*. $[\alpha]_D^{22}$ − 27° (hexane; *c* 0.7). (−)-α-*Cadinol*, $[\alpha]_D^{22}$ − 22° (hexane; *c* 1.4). (+)-τ-*Cadinol*, $[\alpha]_D^{22}$ + 5° (hexane; *c* 1.4). (−)-*Macnabin*, 1S-(1α, 3β, 5aβ, 6α, 9α, 9aβ)-3,6-*Dimethyl*-9-(1-*methylethyl*)-*hexahydro*-1H,5H-1,6:3,9a-*diepoxy*-2-*benzoxepin* (**1**). Viscous oil; $[\alpha]_D^{22}$ − 22° (hexane; *c* 0.85); IR ν_{max}^{KBr} cm^{-1}: 2938, 2874, 1668, 1386, 1268, 1170, 1153, 1086, 1044, 1002, 974, 944, 877, 863, 844, 831; HR-FAB MS: 253.18036 $[MH]^+$, calc. for $C_{15}H_{25}O_3$: 253.18037; GC-MS 70 eV, *m/z* (rel. int.): 252 $[M]^+$ (0.2), 237 $[M - Me]^+$ (0.2), 210 (2), 209 (3), 195 (5), 192 (21), 163 (10), 153 (9), 149 (14), 139 (6), 121 (9), 111 (8), 95 (11), 81 (9), 69 (9), 55 (13), 43 (100); ^{1}H NMR (400.13 MHz, $CDCl_3$, δ from TMS): δ0.89 (3H, *d*, H-13), 0.96 (3H, *d*, H-12); 1.29 (3H, *s*, H-15); 1.43 (3H, *s*, H-14); 5.35 (1H, *s*, H-5); ^{13}C NMR (100.602 MHz, $CDCl_3$, δ from TMS): 110.0 (C-4), 102.5 (C-5), 91.6 (C-6), 87.7 (C-10), 45.6, 45.5 (C-1, C-7), 38.2 (C-9), 32.0 (C-3), 27.2 (C-11), 25.5 (C-14), 21.8 (C-13), 21.7 (C-8), 20.9 (C-15), 18.4 (C-2), 18.2 (C-12).

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