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THUJOPSENE- AND *CIS*-MUUROLANE-RELATED SESQUITERPENOIDS FROM *CUPRESSUS BAKERI*

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Key Word Index — *Cupressus bakeri*; Cupressaceae; sesquiterpenoids; mayurone epoxide; thujopsenol epoxide; indipone; 2-alkylmenthones.

Abstract — Several thujopsene-related sesquiterpenoids from the foliage of *Cupressus bakeri* were isolated and identified: the known thujopsadiene, thujopsan-2α-ol and mayurone; and two compounds, mayurone epoxide and thujopsenol epoxide, which have been reported only as products of oxidation reactions of thujopsene. Two new *cis*-muurolane-related terpenoids were also isolated and identified as (+)-2-ethylmenthone and (+)-indipone [(1*R*, 6*R*, 9*S*)-6-(1-oxo-2-methylpropyl)-4,9-dimethylbicyclo[4.0.3]non-4-ene]. The latter, with a novel carbon skeleton, is probably derived from the co-occurring (−)-epizonarene.

INTRODUCTION

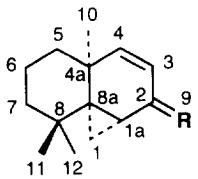
We have reported on the geographic variability of *Cupressus bakeri* Jeps. foliar terpenoids [1] and the identification of a number of new sesquiterpenoids, several of them oxidatively degraded, from that species [2, 3]. In the original study [1] we noted that the percentage of thujopsene, which was a major component in some trees, correlated positively with that of six other compounds, only one of which (cuparene) could be identified by GC-mass spectrometry. The five unknowns were presumably biosynthetically close to thujopsene or cuparene. Likewise, there was a group of unknown compounds biosynthetically related to (−)-*cis*-calamenene, most of which were subsequently identified [2]. In this paper we report the identification of several new sesquiterpenoids from *C. bakeri* foliar essential oil belonging to the thujopsene or *cis*-muurolane biosynthetic groups.

RESULTS AND DISCUSSION

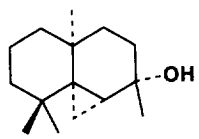
Three of the unknown thujopsene-related components of *C. bakeri* essential oil reported in ref. [1] (there designated CuBkF-Ug, -222c and -222d) were identified as the known natural products thujopsadiene (**1**), (−)-thujopsan-2α-ol (**2**) and (+)-mayurone (**3**). Compounds **1** and **2** were identified by comparison of their GC retention times and mass spectra with data on the authentic substances (Adams, R.P., personal communication). Sufficient **2** was also isolated for comparison of its IR data with that reported for the material isolated from *Cryptomeria japonica* D.Don (Cupressaceae) [4], and for determination of its optical rotation. This substance had been originally identified from the foliage of another member of the Cupressaceae, *Microbiota decussata* Kom. [5]. (+)-Mayurone (**3**), first reported from *Platycladus orientalis* (L.) Franco (= *Thuja orientalis* L.) [6, 7], was isolated and identified by comparison of its GC-mass spectrum, IR spectrum and optical rotation with the authentic substance.

Thujopsenol α-epoxide (**4**) (CuBkF-222e in ref. [1]) was identified by 1D and 2D ^{1}H and ^{13}C NMR data (Table 1) and proved to be identical (IR, ^{1}H NMR) to one of the substances produced by the oxidation of (−)-thujopsene by long exposure to air [8]. The ^{1}H NMR and IR spectral data of another *C. bakeri* unknown (CuBkF-224c in [1]) were identical with those reported [9] for mayurone α-epoxide (**5**), which was found by these authors as a very minor (0.1%) component of the thujopsene oxidation mixture but was prepared in high yield by H_2O_2 oxidation of (+)-mayurone. The absolute stereochemistry of **4** and **5** from *C. bakeri* was determined by comparison of their optical rotation with that of the authentic substances isolated in the thujopsene and mayurone oxidation studies (see Experimental). We consider it unlikely that the *C. bakeri* substances **3**–**5** are produced by simple air oxidation of thujopsene present in the resin canals, since the other oxidation products reported in ref. [8] were not detected in *C. bakeri* foliage oil and the proportions of natural **3**–**5** were quite different from what was found in ref. [8].

Another unknown component of *C. bakeri* essential oil, designated CuBkF-Uh in [1], correlated positively with (−)-*cis*-calamenene and its biosynthetic congeners [2]. Its mass spectrum was unlike that of any known sesquiterpenoid, having a base peak of *m/z* 149; a very small parent ion at *m/z* 220 only became visible when a large injection of the purified material was made. A 1D



1 (R = CH_2)
3 (R = O)



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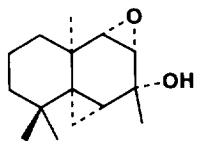

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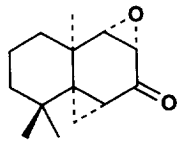

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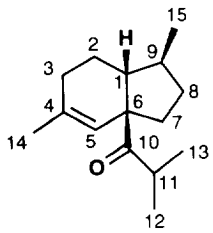

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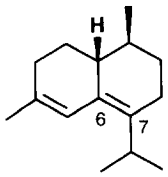

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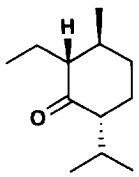

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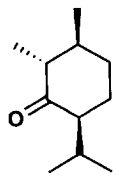

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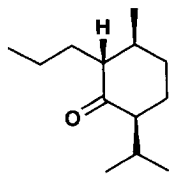

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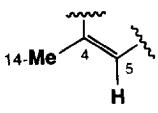
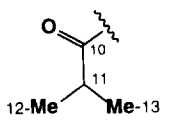


A



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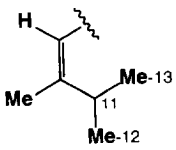

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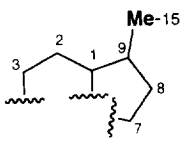

D

and 2D NMR study of this unknown led to the structure **6**, a dimethylhexahydroindenyl isopropyl ketone for which we propose the trivial name indipone.

The ^{13}C NMR and DEPT data (Table 2) showed the presence of four methyl groups, four methylenes, four methines and three quaternary carbons. One of the last was a carbonyl (δ 217.3) and there was the expected strong absorption at 1704 cm^{-1} in the IR spectrum. No other carbon atoms had chemical shifts indicating attached oxygen. These data led to the formula $C_{15}H_{24}O$ (M_r 220) and suggested that **6** was a bicyclic sesquiterpene ketone. A methine at δ 124.2 and a quaternary signal at δ 135.4 were attributed to a trisubstituted double bond.

The ^1H NMR spectrum (Table 2) confirmed the presence of the vinyl proton (*br s*, 1H, δ 5.34) and Me (*br s*, 3H, δ 1.68), giving fragment **A**. The other three methyl groups were doublets at δ 0.96, 1.02 and 1.04. The appearance of a sharp septet at δ 3.00 (1H, J = 6.7) with the same coupling constant as the methyl doublets at δ 1.02 and 1.04 indicated fragment **B**. The other possible, though biosynthetically less probable, fragment which could give a septet near δ 3.00 is **C**. The correct carbon skeleton was determined with a ^1H–^1H COSY experiment (correlations in Table 2). The data gave fragment **D**, a continuous chain of six proton-bearing carbons with a methyl substituent (doublet at δ 0.96). Long-range correlations between the protons of the vinyl methyl and methine with two protons of fragment **D** (H-3 and H-1, respectively; letters in parentheses in Table 2) eliminated **C** as a candidate fragment. The COSY data necessitated a bicyclo[4.0.3]nonane skeleton, and the positions of fragments **A**, **B** and Me-15 in the final structure were all unambiguous. Proton–carbon assignments in Table 2 were determined in a one-bond ^1H–^{13}C multiple quantum coherence experiment.

The relative stereochemistry at C-1, C-6 and C-9 was determined by a NOESY experiment (correlations in Table 2). A cross-peak between H-1 and Me-15 proved their *cis*-relationship, and weak cross-peaks from H-11, Me-12 and Me-13 to H-1 verified the expected *cis*-ring junction.

An attempt to determine the absolute stereochemistry of indipone by measurement of its CD spectrum gave inconclusive results: although there were several unresolved positive maxima between 320 and 290 nm their significance (especially given the freedom of rotation of the carbonyl group) could not be interpreted. However, we were able to synthesize a small amount of **6** by BF_3 reduction and rearrangement of the 6,7-epoxide of (−)-epizonarene (**7**) which has known absolute stereochemistry. The sign of the optical rotation of the synthetic material was the same as natural indipone. We assume that indipone is biosynthesized from epizonarene by just such an epoxidation/ring-contraction mechanism, ana-

Table 1. ^{13}C and ^{1}H NMR spectral data (δ, ppm from TMS) of thujopsenol α-epoxide (**4** and mayurone α-epoxide (**5**) (^{13}C: 125.73 MHz, ^{1}H: 500.13 MHz, $CDCl_3$)

	4		5	
C	^{13}C	^{1}H* (*J*, Hz)	^{13}C†	^{1}H* (*J*, Hz)
1	9.4	H_α 1.06 *dd* (6.0, 4.5)	17.5	H_α 2.10 *dd* (5.0, 4.9)
		H_β 0.17 *dd* (9.9, 4.5)		H_β 0.97 *dd* (10.0, 4.9)
1a	30.3	1.31 *ddd* (9.9, 6.0, 1.8)	31.6	1.96 *ddd* (10.0, 5.0, 1.8)
2	67.9		203.5	
3	60.5	3.05 *dd* (4.2, 1.9)	56.3	3.27 *dd* (4.0, 1.9)
4	67.9	2.76 *d* (4.2)	71.3	3.05 *d* (4.0)
4a	31.8		33.2	
5	36.7	H_α 1.53–1.57	34.5	1.53–1.58
		H_β 1.26 *td* (13.3, 4.1)		1.32 *td* (13.5, 4.2)
6	17.9	H_α 1.76 *qt* (13.7, 3.3)	18.0	1.78 *qt* (13.8, 3.3)
		H_β 1.55–1.60		1.57–1.62
7	40.4	H_α 1.48–1.53	39.5	1.43–1.50
		H_β 1.18 *td* (13.4, 3.6)		1.25 *td* (13.6, 3.5)
8	33.7		34.5	
8a	34.6		43.5	
9	27.4	3H, 1.41 *s*		
10	24.7	3H, 1.33 *s*	24.2	3H, 1.50 *s*
11	26.8	3H, 0.99 *s*	26.7	3H, 1.07 *s*
12	28.4	3H, 0.52 *s*	27.6	3H, 0.56 *s*

* For unresolved multiplets, the range in ppm is given.

† Carbon multiplicities of **5** by DEPT; assignments by analogy and therefore some are tentative.

logous to the formation of oplopanone [10] from α-cadinol and cyperolone [11] from a selina-4,11-diene precursor.

A minor component in the oil proved to be (+)-2-ethylmenthone (**8**), the isopropyl epimer of the previously reported (−)-2-ethylisomenthone [2]. The IR spectrum of **8** was superimposable upon that of synthetic (−)-2-ethylmenthone [2], while its $[\alpha]_D$ value (+22°) showed it to be the enantiomer of the material synthesized from (−)-menthone.

GC-mass spectrometric analysis of the ketone fraction of *C. bakeri* oil also showed the presence of small amounts of what are almost certainly two homologues of ethylisomenthone: 2-methylisomenthone (**9**) and 2-propylisomenthone (**10**). The similarity of their distinctive mass spectra with that of ethylisomenthone [2], and their co-occurrence with that compound and 2-(3-oxobutyl)-isomenthone, support these identifications.

We have detected (by GC-mass spectrometry) **6** and **8** in the same *Cupressus* species as the rest of the *cis*-muurolane biosynthetic group [2]. The thujopsene-related compounds **1**–**5** also occur in individuals of *C. macnabiana* A. Murr. and *C. nevadensis* Abrams.

EXPERIMENTAL

Collection of foliage (Cypress Camp population, Shasta Co., CA), hydrodistillation (from satd NaCl soln with addition of $NaHCO_3$), flash LC of the oil (silica gel; hexane–EtOAc eluants) and FTIR of purified compounds were described previously [2]. In the case of **1**–**5**, only foliage from high-thujopsene trees was used. Because of the poor yield of **2**–**5** under direct hydrodistillation of the foliage, it was chopped and extracted × 3 with *n*-hexane, the combined extracts concd, and the extracted oil hydrodistilled over satd NaCl soln with addition of $NaHCO_3$.

Final purification of **2** by silica HPLC (where the stationary phase is presumably drier than that in flash LC) failed owing to the facile dehydration of **2** to thujopsene or rearrangement to widdrol. Compound **2** also dehydrated completely in prep. GC. Reverse-phase HPLC (15 cm × 4.7 mm, 5μ ODS column; 4:1 MeOH–H_2O eluant) did give a small amount of 88% pure **2**. Final purification of **3** and **5** was by repeated prep. HPLC of the relevant LC frs on a 25 cm × 10 mm, 5μ silica column, with hexane–EtOAc mixtures as eluants: 24:1 for **5**; 93:7 for **3**. Purification of **4**, **6** and **8** was by prep. GC: a 4 m × 4 mm column packed with 15% Carbowax 20M/Chromosorb W, isothermal 190° for **4**; a 4 m × 4 mm column packed with 10% SE-30/Chromosorb W, isothermal 185° followed by the Carbowax column, isothermal 165° for **6**; and the SE-30 column, isothermal 170° for **8**; carrier He at 50 ml min^{-1}, injector 190°, detector 200°. GC-MS was on a 30 m × 0.25 mm SE-54 WCOT column, carrier He, 30 cm sec^{-1}; temp. 35°–250° at 6° min^{-1}; detection EI at

Table 2. ^{13}C and ^{1}H NMR spectral data (δ, ppm from TMS) and 2D correlations* of indipone (**6**) (^{13}C: 125.73 MHz, ^{1}H: 500.13 MHz, $CDCl_3$)

C	^{13}C	^{1}H† (*J*, Hz)	^{1}H–^{1}H	NOESY‡
1	45.6	1.91–1.95	de(i)	abcd
2	21.6	2H, 1.60–1.64	cd	de
3	25.7	2H, 1.75–1.88	c(h)	e
4	135.4			
5	124.2	5.34 *br s*	(i)	i
6	60.3			
7	35.4	H_α 1.49 *dt* (13.0, 8.0)	g	
		H_β 2.03 *ddd* (13.0, 9.3, 4.2)	g	fg
8	32.6	H_α 1.75–1.83	g	
		H_β 1.16 *ddt* (12.3, 9.1, 8.0)	fg	bg
9	35.4	1.70–1.78	efk	h
10	217.3			
11	36.3	3.00 *sept* (6.7)	ab	c
12	20.3	3H, 1.02 *d* (6.7)	a	cf
13	19.9	3H, 1.04 *d* (6.7)	b	cf
14	24.1	3H, 1.68 *br s*	(h)	i
15	18.9	3H, 0.96 *d* (6.5)	k	ah

* Atoms with the same letter show a cross-peak; parentheses indicate weak (i.e. long-range) coupling.

† For unresolved multiplets, the range in ppm is given.

‡ Geminal cross-peaks not listed.

70 eV. Final purity of isolated compounds, except **2**, was > 94%.

(−)-*Thujopsan-2α-ol* (**2**). $[\alpha]_D^{22} \approx -30°$ (hexane; *c* 3.9), corrected to 100% **2** and assuming negligible error from the *ca* 20 small (< 1%) impurities.

(+)-*Mayurone* (**3**). $[\alpha]_D^{22}$ + 236° (hexane; *c* 0.54); GC-MS 70 eV, *m/z* (rel.int.): 204 $[M]^+$ (19), 189 $[M-Me]^+$ (39), 176 (18), 148 (22), 147 (27), 136 (21), 135 (39), 134 (34), 133 (36), 123 (24), 122 (53), 121 (37), 120 (24), 119 (33), 117 (15), 115 (14), 109 (11), 108 (20), 107 (63), 106 (25), 105 (60), 103 (10), 96 (10), 95 (19), 94 (19), 93 (30), 92 (20), 91 (100), 83 (15), 82 (14), 81 (21), 80 (11), 79 (43), 78 (16), 77 (45), 69 (24), 67 (26), 65 (28), 55 (64), 53 (32), 51 (20), 41 (99).

(+)-*Thujopsenol-α-epoxide* (**4**). $[\alpha]_D^{22}$ + 12° (hexane; *c* 0.58); GC-MS 70 eV, *m/z* (rel.int.): 221 $[M - Me]^+$ (1), 207 (12), 151 (10), 150 (12), 137 (11), 135 (49), 133 (11), 123 (24), 121 (22), 119 (17), 109 (24), 107 (31), 105 (20), 95 (31), 94 (11), 93 (27), 91 (26), 83 (11), 81 (27), 79 (25), 77 (20), 71 (13), 69 (39), 67 (18), 57 (10), 55 (42), 53 (19), 43 (100), 41 (69).

(−)-*Mayurone-α-epoxide* (**5**). $[\alpha]_D^{22}$ − 11° (hexane; *c* 2.4); GC-MS 70 eV, *m/z* (rel.int.): 220 $[M]^+$ (0.1), 205 $[M-Me]^+$ (7), 177 (10), 163 (18), 149 (14), 137 (26), 136 (12), 135 (41), 123 (33), 122 (22), 121 (35), 109 (18), 107 (53), 105 (26), 95 (29), 93 (99), 91 (45), 81 (33), 79 (45), 77 (39), 69 (37), 67 (25), 65 (19), 55 (68), 53 (31), 51 (13), 43 (39), 41 (100).

(+)-*Indipone*, (1R, 6R, 9S)-6-(1-*oxo*-2-*methylpropyl*)-4,9-*dimethylbicyclo*[4.0.3]*non*-4-*ene* (**6**). Oil; $[\alpha]_D^{22}$ + 81° (hexane; *c* 1.2); IR ν_{max}^{KBr} cm^{-1}: 2953, 2931, 2868, 2836, 1704 (> C = O), 1451, 1379, 1061, 945. GC-MS 70 eV, *m/z* (rel.int.): 220 $[M]^+$ (0.2), 177 $[M - isoPr]^+$ (0.3), 149 $[M - C_3H_7CO]^+$ (100), 133 (2), 121 (8), 107 (20), 93 (35), 81 (16), 69 (8), 55 (10), 43 (22), 41 (20).

Synthesis of (+)-*indipone*. (−)-Epizonarene (50 mg) (isolated from the hydrocarbon fr of *C. bakeri* oil by LC on 15% $AgNO_3$–SiO_2 with *n*-hexane–EtOAc gradients) was treated with 43 mg of 3-chloroperoxybenzoic acid for 1 hr at 40°. The resulting mix of monoepoxides (2 major, with some diepoxide and several hydrocarbons) was purified by repeated prep. HPLC (10 mm × 25 cm, SiO_2, 3% EtOAc in *n*-hexane). During HPLC of the monoepoxides a build-up of indipone in various frs was noted (by GC-MS) which indicated rearrangement of monoepoxide on SiO_2. Each of the two major monoepoxide frs was treated with BF_3–etherate in CH_2Cl_2 at 20° for 10 min; both yielded additional indipone. Prep. GC (15% Carbowax 20M on Chromosorb W, 4 m × 4 mm, 160°) of the combined indipone-containing frs gave a small amount of 90% pure indipone. $[\alpha]_D^{22} \approx +60°$ (hexane; *c* ≈ 0.3), corrected to 100% indipone; no correction made for optical rotation of an unknown hydrocarbon impurity.

(+)-2-*Ethylmenthone*, (2R, 3S, 6R)-6-*isopropyl*-2-*ethyl*-3-*methylcyclohexan*-1-*one* (**8**). Oil; $[\alpha]_D^{22}$ + 22° (hexane; *c* 1.0).

2-*Methylisomenthone*, (2*R*, 3*S*, 6*S*)-6-*isopropyl*-2,3-*dimethylcyclohexan*-1-*one* (**9**, tentative). GC-MS 70 eV, *m/z* (rel.int.): 168 $[M]^+$ (26), 153 $[M-Me]^+$ (17), 126 $[M - isoPr + H]^+$ (50), 111 $[126 - Me]^+$ (46), 95 (21), 83 (47), 69 (53), 55 (100), 41 (93).

2-*Propylisomenthone*, (2R, 3S, 6S)-6-*isopropyl*-2-*propyl*-3-*methylcyclohexan*-1-*one* (**10**, tentative). GC-MS 70 eV, *m/z* (rel.int.): 196 $[M]^+$ (5), 181 $[M-Me]^+$ (4), 154 $[M - isoPr + H]^+$ (34), 139 $[154 - Me]^+$ (72), 111 $[154 - Pr]^+$ (42), 97 (28), 84 (34), 69 (49), 55 (100), 41 (93).

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