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Terpenoids of *Cupressus* species in California*1

Young-Kyoon Kim*2 · Eugene Zavarin*3

캘리포니아 産 삼나무 樹種의 Terpenoid 類*1

金 英 均*2 · Eugene Zavarin*3

要 約

북가주에 있는 삼나무 수종에서 cadinane과 acorane류의 sesquiterpenoids를 chromatography를 사용하여 분리한 후, 화학적인 방법과 분광학적인 방법을 이용하여 분리된 성분의 화학구조를 규명하였다. 그 sesquiterpenoids의 생합성 경로를 정량적인 상관성에 의하여 밝혔다. 또한, 여러 삼나무 수종에 존재하는 sesquiterpenoids의 분류학적 차이에 대하여 논하였다.

Keywords : Terpenoids, *Cupressus* species, California, column chromatography, silica gel, NMR

1. INTRODUCTION

The terpenes comprise an extraordinarily large number of volatile, nonpolar and often odorous compounds occurring in the volatile oil of wood, phloem, cortex or foliage of conifers, being best known as constituents of the essential oils of many plants[1]. Plant derived terpenes include hydrocarbons as well as alcohols, ketones, and other oxygenated compounds. It has been suggested that the term "terpenoid" be used for both hydrocarbon and oxygenated compounds while "terpene" should refer only to hydrocarbons[2]. The general biogenetic theory of terpenoids was established first by Ruzicka[3] and later by Hendrickson[4]. It is regarded that the earlier biosynthetic stages of terpenoids involve reductive processes whereas subsequent biomodifications mainly represent oxidative reactions[5]. Because of experimental difficulties[6], until now, there has been a lack of basic information on the later stages of the terpenoid biosynthesis.

The terpenoids are classified as secondary metabolites because they do not appear to have any explicit role in the basic processes of growth and development. Their functions in plants are still obscure, although, like other secondary metabolites, they certainly have ecological roles, serving as attractants to pollinators[7], allelopathic agents[8] or defenses against predators and pathogens[9]. These activities are mainly due to the content of mono- and sesquiterpenoids. Many reports dealing with isolation and structure elucidation of mono- and sesquiterpenoids as well as their biological significance have been appeared dur-

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*2 캘리포니아 주립대학 해양연구소 Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093-0236, U.S.A.
*3 캘리포니아 주립대학 임산물 연구소 Forest Products Laboratory, University of California, Berkeley Richmond, CA 94804, U. S. A.

ing the last decade[10].

Another interesting aspect of terpenoids is that mono- and sesquiterpenoids in conifers are responsible for the resistance to bark beetles[11]. In order to find such interesting compounds, we initially focused on the analysis and structural identification of the volatiles from *C. bakeri*, since our preliminary studies for terpenoids of *Cupressus* species suggested that *C. bakeri* not only contained most of terpenoids of other species but also seemed to produce many new oxygenated compounds[12]. Apart from the biological acitivities of mono- and sesquiterpenoids their structural relationship and distribution in the plants have been on many occasions used as a chemotaxonomic tool[13,14]. It is worthy to mention that there is still a handicap for chemosystematics where structural similarities commonly enter into the argument relating to the relationships of the taxa investigated[15]. All this demonstrates a great need of a grass roots approach to investigate terpenoids for *Cupressus* species, the taxonomic classification of which is still being debated[16].

In summary, since sesquiterpenoids are not only widely distributed in plants but also have important biological and ecological roles, we focused on sesquiterpenoids in *Cupressus* species, mainly for *C.bakeri* which possesses a wealth of both known and unidentified compounds. The present paper describes the isolation of sesquiterpenoids from *C. bakeri*, as well as their taxonomic significance and isolation of sesquiterpenoids from *C. bakeri*, as well as their taxonomic significance and biosynthetic pathways based on the quantitative method.

2. MATERIALS & METHOD

2. 1 General

Infrared spectra were recorded by Diffuse Reflectance IR(DRIFT) spectroscopy for neat substances in powdered KBr. All separations were carried out by flash column chromatography on Silica gel(Silica Gel 60,230−400 mesh ASTM, VWR Scientific Company). Final purification was carried out on Varian preparative thermal conductivity GC or an Isco isocratic HPLC instrument. Optical rotation was measured in a 0.1 ml cell, 5cm long, using a Rudolph Research Autopol ™ Ⅲ polarimeter. Spectral data are reported for compounds which were over 93 % pure measured by analytical gas chromatography. Nuclear Magnetic Resonance(NMR) spectra were recorded at room temperature on a Bruker−500 spectrometer operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C, interfaced with an Aspect 3000 computer using the DISR88 software package. Significant ^{1}H NMR data are given in the order of number of protons, multiplicity(s, singlet ; d, doublet ; dd, doublet of doublets ; t, triplet ; dt, doublet of triplets ; q, guartet ; sep, septet ; m, multiplet, *etc*), coupling constants in Hertz. Mass spectral data are listed as m/z and intensity expressed as percent of total ion current. Low resolution mass spectra(LRMS) were determined on an AEI−MS−12 mass spectrometer. High resolution mass spectra(HRMS) were determined on a Kratos MS−50 high resolution mass spectrometer in the Department of Chemistry, University of California, Berkeley.

2. 2 Separation and Isolation

Foliage of *C. bakeri*(ca 400 g) was ground in liquid nitrogen and hydrodistilled for 5 hours from saturated NaCl with addition of @ 2 g of $NaHCO_3$ with hexane in the receiver. The collected upper layer was decanted and dried with Na_2SO_4. The essential oil was stored at −10℃ until used for chromatography and then 1 g of oil was prefractionated on a Silica gel column chromatography(Merck No. 7729, 50 g) eluted with *n*-hexane/ethyl acetate(20:1, 9:1, 4:1) ; fractions were checked by capillary GC and similar fractions were combined. Repeated prep. GC of the relevant fractions on a 4 m×4 mm column packed with 10 % Carbowax 20 M on 60/100 mesh Chromosorb G, carrier He at 50 ml min^{-1}, isothermal 145℃, injector 190℃, TC detector 200℃, gave 10~12 mg

each of (-)-*cis*-calamenene([α]$_D$=−31°, $CDCl_3$, c 9.9), CB−1B, CB−2A, CB−4 and CB−15. Prep. GC of the relevant fractions on a 4 m×4 mm column packed with 4 % OV−17 on 60/100 mesh Chromosorb G, carrier He at 50 ml min^{-1}, isothermal 185℃, injector 190℃, TC detector 200℃, gave 12~15 mg each of CB−5, α- and β-acorenol, CB−9 and CB−11. CB−M182 did not separated from bornyl acetate on the above two columns but was resolved on a 4 m×4 mm column packed with SE−30 on 60/100 mesh Chromosorb G, carrier He at 50ml min^{-1}, isothermal 185℃, injector 190℃ TC detector 200℃. To obtain CB−6 and CB−7, repeated high pressure liquid chromatography was used with Rainin Microsorb−MV column(4.6 mm ID×15 cm, 5 μm Silica gel, 100 Å pore size) using 5 % EtOAc in *n*−hexane as eluent.

2. 2. 1 (-)-(2R,3S,6S)-2-Ethyl-3-mehtyl-6(1-mehtylethyl)-cyclohexan-1-one (CB-M182)

Colorless oil ; [α]$_D^{22}$ −98° (c 6.9, $CHCl_3$) ; IR(neat) V_{max} cm^{-1} : 2958, 2926, 2871, 1706, 1460, 1380, 1327, 1250, 1196, 1171, 1037, 952, 862 and 738 ; ^{1}H NMR (500.13 MHz, $CDCl_3$) : δ 0.82(3 H, d, *J*=6.28 Hz), 0.85(3 H, t, *J*=7.33), 0.90(3 H, d, *J*=6.28), 0.99(3 H, d, *J*=6.5), 1.48−1.56(2 H, m), 1.60−1.71 (1 H, m), 1.69−1.76(3 H, m), 1.82−1.87 (1 H, m), and 2.0−2.08 (3 H, m) ; ^{13}C NMR (125.73 MHz, $CDCl_3$) : δ 12.0(CH_3), 19.79(CH_3), 20.31(CH_3), 20.87(CH_3), 20.95(CH_2), 26.95(CH), 26.97(CH_2), 28.89(CH), 38.25(CH), 56.44(CH), 57.05(CH) and 216.19(C) ; MW=182.31 calc. for $C_{12}H_{22}O$; EI− MS *m/z* (relative intensity) : 182(21, M^+), 167(12), 153(5), 140(22), 125(7), 111(51), 97(52), 69(60), 55(86), 43(38) and 41(100).

2. 2. 2 Bakerol (-)-6, 12-oxido-5-nor-acoran-6-ol (CB-1B)

Colorless oil ; [α]$_D^{22}$ −10° (c 2.8, $CHCl_3$) ; IR(neat) V_{max} cm^{-1} : 3609, 3509, 2955, 2875, 1457, 1370, 1306, 1244, 1161, 1133, 1073, 994, and 877 ; ^{1}H NMR (500.13 MHz, $CDCl_3$) : δ 0.90(3 H, d, *J*=8.45 Hz), 0.99(3 H, d, *J*=7.9), 1.17 (3 H, s), 1.18−1.21(1 H, m), 1.20−1.29(1 H, m), 1.28(3 H, s), 1.43−1.47(1H, m), 1.46−1.52(1H, m), 1.54−1.57(1 H, m), 1.55−1.59(1H, m), 1.65−1.69(1H, m), 1.67−1.75(1 H, m), 1.94−2.01 (1 H, m), 2.19(1 H, dd, *J*=7.5, 12.0), 2.32(1H, sep, *J*=8.5) ; ^{13}C NMR (125.73 MHz, $CDCl_3$) : δ 11.69(CH_3), 15.35(CH_3), 24.35(CH_2), 26.68(CH_3), 28.99(CH_2), 31.18(CH_3), 37.75(CH_2), 38.08(CH), 46.0(CH), 64.91(CH), 66.90(C), 82.15(C), 112.49(C) ; HR−EIMS *m/z* $[M]^+$ 224.1773 for $C_{14}H_{24}O_2$ and 209.164 for $C_{13}H_{21}O_2$; EI−MS *m/z* (relative intensity) : 224(3, M^+), 209(12), 191(5), 181(100), 166(43), 149(7), 135(14), 123(78), 109(23), 95(18), 81(17), 81(17), 69(12), 59(5) and 43(2).

2. 2. 3 (-)-(1R,7S,10S)-Muurola-4(15), 5-diene(CB-2A)

Colorless oil ; [α]$_D^{22}$ +105° (c 1.7, $CHCl_3$) ; IR(neat) V_{max} cm^{-1} : 3076, 3010, 2951, 2938, 2926, 1639, 1454, 1375, 1280, 1130, 1062, 940 and 878 ; ^{1}H NMR (500.13 MHz, $CDCl_3$) : 0.76(3 H, d, *J*=6.5 Hz), 0.91(3 H, d, *J*=6.5Hz), 0.94(3 H, d, *J*=6.2 Hz), 1.18−1.23 (1H, m), 1.31−1.40(2H, m), 1.43−1.52(2H, m), 1.63−1.82(3H, m), 1.85−2.05(2H, m), 2.12(1 H, br, t, *J*=16.5), 2.28(1 H, dq, *J*=2.3, 12.4), 4.62(1 H, s), 4.67 (1 H, s) and 5.9(1 H, s) ; ^{13}C NMR (125.73 MHz, $CDCl_3$) : δ 20.27 (CH_3), 20.94(CH_3), 21.74(CH_3), 26.85(CH), 27.96(CH_2), 28.71(CH_3), 29.46(CHz_2), 30.27(CH_2), 39.64(CH), 40.54(CH), 51.59(CH), 107.74(CH_2), 124.40(CH), 144.41(C) and 147.36(C) ; EI−MS m/z (relative intensity) : 204(60, M^+), 189(26), 161(100), 133(12), 119(36), 105(48), 91(41), 81(24), 67(12), 55(14) and 41(38).

2. 2. 4 (-)-(1R,10S)-Muurola-4, 6-diene (CB-4)

Colorless oil ; [α]$_D^{22}$ −240° (c 2.1, $CHCl_3$) ; IR(neat) V_{max} cm^{-1} : 2950, 2900, 2870, 1640, 1590, 1460, 1370, 1340, 1260, 1130, 1005 and 861 ; ^{1}H NMR (400 MHz, $CDCl_3$) : 0.94(3 H, d, *J*=6.9 Hz), 0.95(3 H, d, *J*=6.9), 0.99(3 H, d, *J*=5.85 Hz), 1.15−1.24(2 H, m), 1.76(3 H, s), 1.95−2.15(5 H, m), 6.21(1 H, s) ; ^{13}C NMR (100.49 MHz, $CDCl_3$) : δ 20.37(CH_3), 20.51(CH_3), 20.98(CH_3), 23.88(CH_2), 24.12(CH_3), 28.10(CH), 28.14(CH_2), 31.16(CH_2), 31.79

(CH_2), 34.54(CH), 43.12(CH), 120.38(CH), 127.77(C), 135.35(C) and 151.96(C) ; EI−MS *m/z* (relative intensity) : 204(36, M^+), 189(6), 161(100), 133(12), 119(34), 105(43), 91(41), 81(24), 67(12), 55(12) and 41(34).

2. 2. 5 (-)-6,12-oxido-acor-4-ene(CB-5)

Colorless oil ; $[\alpha]_D^{22}$ −5.5° (c 3.2, $CHCl_3$) ; IR_{KBr} V_{max} cm^{-1} : 2881, 2886, 2839, 2818, 1458, 1380, 1360, 1230, 1141, 1023, 1002 and 899 ; ^1H NMR (500.13 MHz, $CDCl_3$) : δ 0.88(3 H, d, J=9.1 Hz), 1.18(3 H, s), 1.24(3 H, s), 1.35−1.42(1 H, m), 1.54−1.94(8 H, m), 1.68(3 H, s), 2.04(1 H, dd, J=4.3, 12.6), 3.76(1 H, d, J=1.62) and 5.78(1 H, br.d, J=1.62) ; ^{13}C NMR (125.73 MHz, $CDCl_3$) : δ 15.79(CH_3), 23.60(CH_3), 24.47(CH_3), 25.28(CH_2), 26.18(CH_2), 28.86(CH_2), 32.59(CH_3), 34.34(CH_2), 37.13(CH), 56.29(C), 58.66(CH), 77.32(CH), 120.36(CH) and 139.73(C) ; EI−MS *m/z* (relative intensity) : 220(17, M^+), 205(58), 177(14), 165(13), 147(31), 121(17), 105(39), 91(42), 81(36), 79(26), 67(21), 55(29) and 43(100).

2. 2. 6 (+)-(1R,4S,7S,10S)-4β-Hydroxy-muurol-5-ene(CB-6)

Colorless oil ; $[\alpha]_D^{22}$ +31.3° (c 7.3, $CHCl_3$) ; IR(neat) V_{max} cm^{-1} : 3280, 2950, 2900, 2870, 1460, 1370, 1205, 1130, 1005 and 940 ; ^1H NMR (500.13 MHz, $CDCl_3$) : 0.77(3H, d, J=6.5 Hz), 0.89(3 H, d, J=6.5), 0.92(3 H, d, J=6.2 Hz), 1.13−1.18(1 H, m), 1.25(3 H, s), 1.27−1.40(2 H, m), 1.42−1.53(2 H, m), 1.48(1 H, dt, J=2.6, 10.1) , 1.59−1.67(2 H, m), 1.72(1 H, qd, J=3.7, 12.6), 1.82−1.74(1 H, m), 1.88(1 H, dq, J=2.9, 12.6), 1.90−1.95(1 H, m) and 5.31(1 H, s) ; ^{13}C NMR (125.73 MHz, $CDCl_3$) : δ 20.05(CH_3), 20.86(CH_3), 21.62(CH_3), 24.58(CH_2), 26.29(CH), 28.62(CH_3), 28.97(CH_2), 30.43(CH_2), 36.57(CH_2), 39.26(CH), 39.92(CH), 51.59(CH), 69.27(C), 128.58(CH), and 143.62(C) ; EI−MS *m/z* (relative intensity) : 222(2, M^+), 207(82), 189(6), 179(4), 161(24), 137(12), 123(16), 105(18), 93(16), 81(18), 67(12), 55(24) and 43(100).

2. 2. 7 (+)-(1R,4R,7S,10S)-4 α-Hydroxy-muu-rol-5-ene(CB-7)

Colorless oil ; $[\alpha]_D^{22}$ +36° (c 5.7, $CHCl_3$) ; IR(neat) V_{max} cm^{-1} : 3360, 2920, 2900, 2880, 1460, 1370, 1140, 910 and 860 ; ^1H NMR (500.13 MHz, $CDCl_3$) : δ 0.70(3 H, d, J=6.5 Hz), 0.99(3 H, d, J=6.5), 0.95(3 H, J=6.30), 1.14−1.16(1 H, m), 1.27(3 H, s), 1.32−1.46(4 H, m), 1.52−1.57(2 H, m), 1.60(1H, dd, J=3.7, 10.6), 1.71−1.76(2 H, m), 1.85−1.94(2 H, m) and 5.34(1 H, s) ; ^{13}C NMR (125.73 MHz, $CDCl_3$) : δ 20.27(CH_3), 20.88(CH_3), 21.48(CH_3), 24.60(CH_2), 26.61(CH), 28.30(CH_2), 30.06(CH_2), 30.21(CH), 36.71(CH_2), 40.26(CH), 40.42(CH), 51.32(CH), 68.01(C), 128.15(CH), and 144.23(C) ; EI−MS *m/z* (relative intensity) : 222(4, M^+), 207(95), 189(6), 179(6), 161(28), 135(14), 123(12), 105(26), 91(29), 81(20), 67(12), 55(24) and 43(100).

2. 2. 8. (-)-(2R,3S,6S)-3-Methyl-6-(1-methylethyl-2-(3-oxobutyl)-cyclohexan-1-oen(CB-9)

Colorless oil ; $[\alpha]_D^{22}$ −39.5° (c 11.3, $CHCl_3$) ; IR(neat) V_{max} cm^{-1} : 2958, 2930, 2871, 2360, 1718, 1706, 1459, 1445, 1422, 1386, 1369 and 1163 ; ^1H NMR (500.13 MHz, $CDCl_3$) : δ 0.78(3 H, d, J=6.2 Hz), 0.90(3 H, d, J=6.20), 1.03(3 H, d, J=6.35), 1.50−1.56(1 H, m), 1.61−1.70(3 H, m), 1.77−1.81(2 H, m), 1.90(1 H, dt, J=4.6, 9.8), 1.99(1 H, dq, J=4.7, 11.6), 2.0−2.04(1 H, m), 2.11(3 H, s, Me−10), 2.07−2.12(1 H, m) 2.35(1 H, ddd, J=6.8, 8.1) and 17.45 Hz, H_β−C−8) and 2.5(1 H, ddd, J=6.1, 8.4 and 17.45 Hz, H_α−C−8) ; ^{13}C NMR (125.73 MHz, $CDCl_3$) : δ 19.92(CH_3), 20.41(CH_3), 20.79(CH_3), 21.30(CH_2), 27.10(CH), 27.30(CH_2), 29.14(CH_2), 29.91(CH), 39.14(CH), 41.35(CH_2), 53.98(CH), 57.17(CH), 208.84(C) and 216.12(C) ; EI−MS *m/z* (relative intensity) : 224(6, M^+), 209(8), 191(4), 166(6), 139(8), 124(8), 119(8), 111(9), 95(18), 81(13), 69(25), 55(33) and 43(100).

2. 2. 9. (+)−(1R,7S,10S)-15-Nor−muu-rol-5-en-4-one(CB−11)

Colorless oil ; $[\alpha]_D^{22}$ +47° (c 3.2, $CHCl_3$) ; UV λmax : 241(EtOH, 15,600) ; IR V_{max} cm^{-1} : 2960, 2920, 2980, 1680, 1620, 1460, 1250, 1210 and 870 ; ^1H NMR (500.13 MHz, $CDCl_3$) : δ 0.74(3 H, d, J=6.16 Hz), 0.93(3 H, d, J=6.16), 1.0(3 H, d, J=6.01), 1.33−1.44(2 H, m), 1.45−1.54(1 H, m), 1.61−1.72(1 H, m), 1.78−1.

88(2 H, m), 1.92−1.96(1 H, m), 1.96−2.0(1 H, m), 2.13−2.22(1 H, m), 2.14−2.26(1 H, m) 2.36(1 H, ddd, J=4.76, 7.62, 8.51), 5.78(1 H, d, J=1.69), ^{13}C NMR(125.73 MHz, $CDCl_3$) : δ 20.19(CH_3), 20.70(CH_3), 21.48(CH_3), 25.78(CH_2), 27.22(CH), 28.68(CH_2), 29.73(CH_2), 35.66(CH_2), 39.49(CH), 41.0(CH), 52.33(CH), 125.38(CH), 170.03(C), 199.97(C) ; EI−MS m/z (relative intensity) : 206(46, M^+), 191(8), 164(100), 149(39), 136(22), 122(36), 121(39), 107(33), 91(45), 89(38), 55(33) and 41(58).

2. 2. 10 (+)-Cedryl acetate(CB-15)

Colorless solid at 0℃ ; $[\alpha]_D^{22}$ +20° (c 4.6, $CHCl_3$) ; IR(neat) V_{max} cm^{-1} : 2957, 2951, 1732, 1467, 1455, 1374, 1366, 1261, 1244, 1118, 1108, 1019 and 927 ; ^{1}H NMR (500.13 MHz, $CDCl_3$) : δ 0.82(3 H, d, J=7.1), 0.97(3 H, s), 1.16(3 H, s), 1.25(1 H, ddt, J=1.2, 6.4, 13.5), 1.35(1 H, br. d, J=13.5), 1.38(1 H, dd, J=5.5, 13.0), 1.40−1.47(1 H, m), 1.49−1.55(1 H, m), 1.53(3 H, s), 1.61−1.65(2 H, m), 1.79(1 H, t, J=8.0), 1.85(1 H, q, J=6.0), 1.90−1.99(1 H, m), 1.94(3 H, s), 2.02(1 H, dd, J=5.6, 13.5), and 2.39(1 H, J=5.5) ; ^{13}C NMR(125.73 MHz, $CDCl_3$) : δ 15.48(CH_3), 22.76(CH_3), 25.26(CH_2), 25.83(CH_3), 26.93(CH_3), 28.45(CH_3), 31.20(CH_3), 33.18(CH_2), 36.94(CH_2), 40.94(CH_2), 41.30CH), 43.33(C), 53.95(C), 56.68(CH), 56.83(CH), 86.24(C) and 170.35(C) ; EI−MS m/z (relative intensity) : 204(46), 189(16), 175(42), 161(37), 147(18), 138(17), 122(28), 119(60), 105(29), 93(33), 91(31), 69(29) and 43(100).

3. RESULTS & DISCUSSION

3. 1 Isolation of Sespuiterpenoids

The foliage of *C.bakeri* was collected from the Cypress Camp population, Shasta County, California. The essential oil was obtained by steam distination of the crushed foliage. The crude essential oil was analyzed by gas chromatograpy (Figure 1). From our previous study[12], the names of compounds identified are given over the corresponding peak on the chromatogram. For the peaks not identified by GC\MS, arbitary names were assigned.

The collected total essential oil was subjected to silica gel column chromatography followed by preparative gas chromatography (see experimental). Separation of low-polarity fractions afforded CB−M182, CB−2A, CB−4 and CB−5, while mid-polarity fractions yielded CB−1B and CB−15. CB−6 and CB−7 were decomposed under our standard preparative GC conditions and, therefore, they were isolated by high pressure liquid chromatography. CB−9 and CB−11 were obtained from

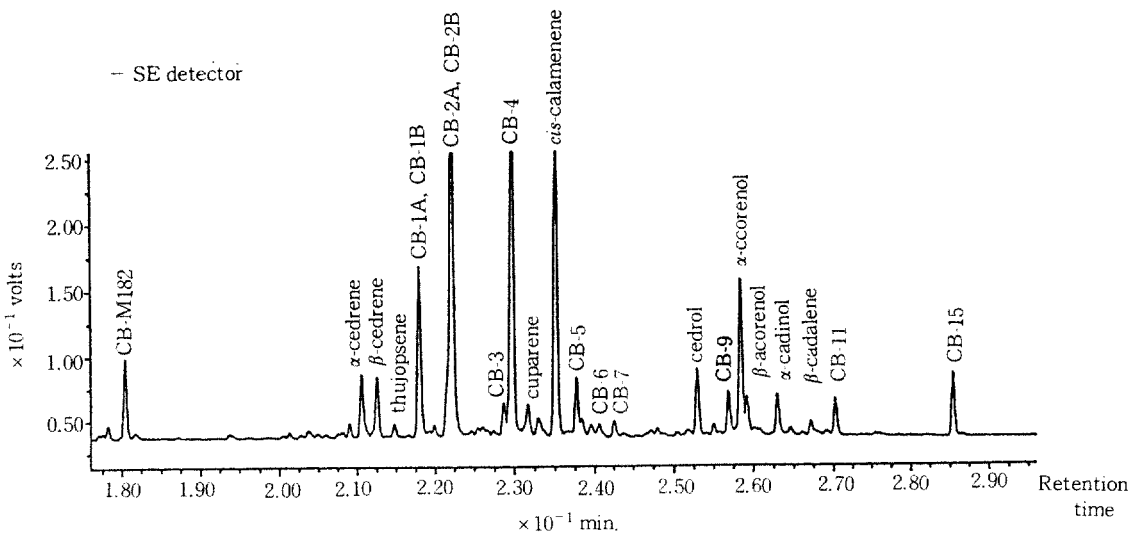

Fig. 1. GC chromatogram for the essential oil of *C. bakeri* foliage

most polar-fraction. The chemical structures of the isolated compounds(Figure 2) were identified by combined techniques such as IR, UV, MS, $^{1}H-$ and ^{13}C NMR spectroscopy. Details for the spectral analysis are appeared in the separate report[17]. The final structures of the identified compounds were supported by two dimensional Nuclear Overhauser Effect correlation spectroscopy(2D NOESY)[18], statistical correlation studies, and synthesis, if possible. Conformational information was obtained by molecular mechanics(MM2 and MNDO-PM3) calculated graphic models on the Cache computer system.

Table 1. Correlation matrix* for sesquiterpenoids from *Curpessus bakeri*

	calamenene	CB-7	CB-9	α-cadinol	CB-11
CB-M182	0.378				0.505
CB-4	0.502			0.412	
calamenene			0.660	0.747	0.761
CB-6		0.944			
CB-7				0.344	
CB-9				0.691	0.744
α-cadinol					0.711

* All correlations included were significant on<0.001 level.

3. 2 Statistical correlations of sesquiterpenoids

Statistical correlations between the amounts of natural products isolated from the series of

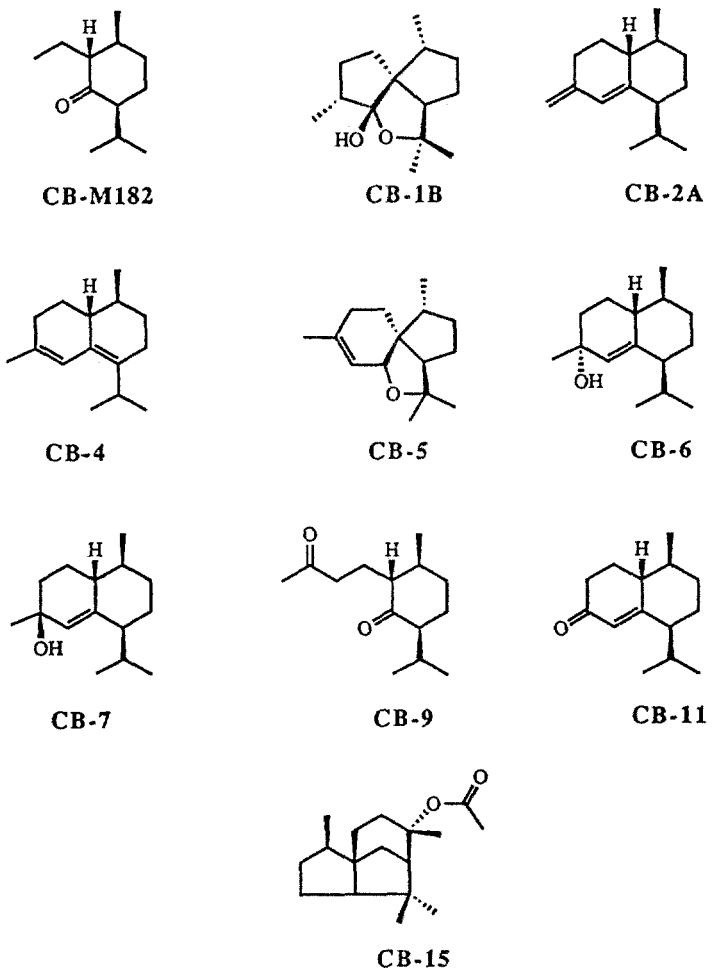

Fig. 2. Sesquiterpenoids identified from *Cupressus bakeri*

plants of the same species have been successfully used for the clarification of biosynthetic routes. The fundamental aspects of the method have been described in the previous report[19] and applied to mono- and sesquiterpenoids[20]. The essential feature of the method resides in finding of the statistically significant positive correlations between the amounts of the specific compounds, which are supposed to infer a biosynthetic closeness between such compounds. The method represents essentially a statistical extension of the commonly used biosynthetic arguments based on co-occurrence.

We examined such correlations for a series of mono- and sesquiterpenoids of *C. bakeri* using 63 trees from several locations. For poorly differentiated species like *C. bakeri*, using trees from many locations should not represent a major obstacle to the method. The

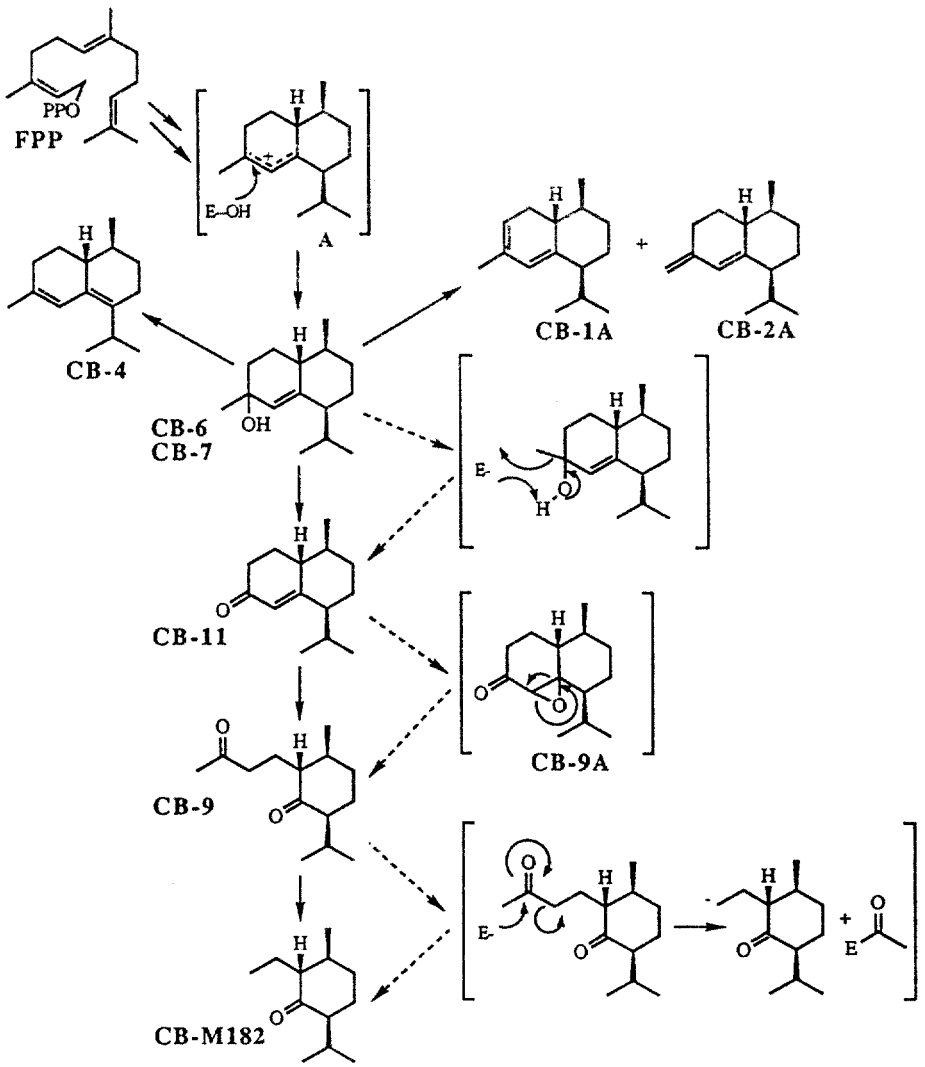

Fig. 3. Postulated biosynthetic pathways and suggested mechanisms for the formation of the cadinane related sesquiterpenoids from *Cupressus bakeri*. E-is a postulated nucleophilic enzyme site

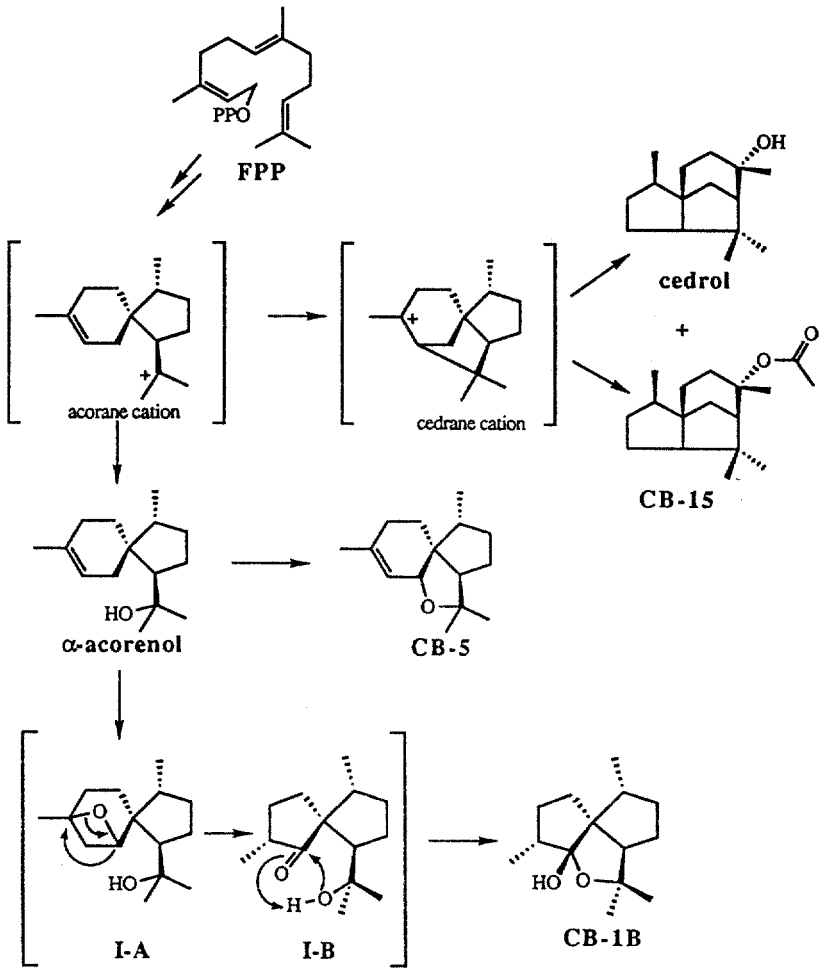

Fig. 4. Postulated biosynthetic pathways and suggested mechanisms for the formation of acorane related sesquiterpenoids

positive correlations obtained *C. bakeri* are given in Table.

All cadinane class sesquiterpenoids exhibited highly significant correlations *inter se*. Particularly significance was the highly significant correlation (+0.505) obtained between the compound CB−M182 and the cadinane ketone CB−11, indicating the derivation of the former compound from the cadinane precursors. Combining the correlative evidence obtained together with currently accepted chemical and biochemical theories on the biosynthesis of these compounds, we propose the following biosynthetic schemes for the cadinane and acorane classes encountered in our work(Fig. 3 and 4).

3. 3 Biogenetic Occurrence of Sesquiterpenoids

It has long regarded that cadinane-type sesquiterpenoids originate by appropriate cyclizations of farnesyl pyrophosphate[21]. CB−6 and CB−7 can be derived directly by hydroxylation at the C−4 position of active cation A(Fig. 3). The loss of a methyl group at C−15 of CB−6 and CB−7 activated by deprotonation and following ketonization will

produce CB−11. Alternatively, loss of water from CB−6 and 7 yield the dehydrated compounds CB−1A and CB−2A. The formation of CB−4 in this scheme can be visualized by double bond rearrangement, initiated by deprotonation at C−7. Since epoxidation of sesquiterpenoids is recognized in many plant sourcess[22], CB−9 can be derived from a postulated biological intermediate CB−9A followed by ring opening. Finally, enzymatic nucleophilic attack at C−13 of CB−9 will lead to the bond cleavage at C−12 and C−13 followed by protonation, resulting in the formation of CB−M182.

Unfortunately, no acceptable positive correlations were found among the acorane-type sesquiterpenoids and the biosynthetic routes were derived based on the current ideas on the biogenesis of these and similar compounds. The pertinent relationships for the acorane-type sesquiterpenoids[23] are given in Figure 4. Cedranes and acoranes were previously identified from *C. bakeri* in our laboratory[12]. Cedryl acetate CB−15 obviously can be derived by acetylation of the cedrane cation A. The acetyl group might even be available from the enzyme which reacted with CB−9 to form CB−M182.

Activation of the allylic position of α-acorenol followed by intra-molecular nucleophilic attack of the hydroxy group to the activated site will lead to the formation of CB−5. Although we found no intermediate which substantiated our proposed scheme from α-acorenol to CB−1B, the most plausible intermediates are *via* the oxidized compound I−A, leading by ring contraction to hydroxy ketone I−B which could be readily ketalized to form CB−1B.

3. 4 Taxonomic Significance of Sesquiterpenoids in Cupressus Species

The new and rare compounds discovered from *C. bakeri* are not generally restricted to this species. For instance, we also identified bakerol from *C. nevadensis* leaves by GC/MS. *C. nevadensis* is chemically most similar to *C. bakeri* and contains all the other *C. bakeri* acoranes and cadinanes. Similarly, *C. macnabiana* contains the cadinanes of *C. bakeri*, but bakerol is absent. Acoranes are minor compounds, and α−cadinol is the major sesquiterpenoid in *C. macnabiana*. *C. glabra* and *C. arizonica* also contain most of the sesquiterpenoids of *C. bakeri*, but differ in that CB−6 and CB−7 are usually the major compounds, while the acoranes and cedranes are much smaller. Other known compounds(α-acorenol, β-acorenol and (-)-calamenene) are recognized as common compounds among *Cupressus* species.

4. CONCLUSION

Cadinane and acorane related sesquiterpenoids were identified and isolated from *Cupressus* species in California. The chemical structures of the isolated compounds were determined by chemical and spectroscopic methods. For the isolated compounds their biosynthetic routes were clarified by statistical correlations among the identified components. The taxonomic significance of sesquiterpenoids in *Cupressus* species was also discussed.

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