TERPENOIDS FROM LEAVES OF CALOCEDRUS FORMOSANA

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Abstract—Besides six labdane-type diterpenes, three new compounds of geranyl trans-coumarate, epitorusolyl ciscoumarate and diterpenoid γ -hydroxybutenolide, were isolated from leaves of Calocedrus formosana.

INTRODUCTION

The constituents of the heartwood of *Calocedrus formos*ana, a member of Cupressaceae indigenous to Taiwan [1], have been extensively investigated in this laboratory [2–5]. In addition to a large quantity of terpenoid acids, such as shonanic, thujic and chaminic acids, the wood also contains tropolones, monoterpenes, naphthalenetype sesquiterpenes, diterpenoid phenols, and lignans of hinokinin, hibalactone, and calocedrin We herein report the terpenoidal components isolated from leaves of this economically important tree

RESULTS AND DISCUSSION

By means of repetitive chromatography and crystallization, one monoterpenoid derivative and eight diterpenes (Table 1) were isolated from C formosana. Six known diterpenes were identified as phytol (2) [6, 7], transcommunic acid (3) [8, 9], isocupressic acid (5) [10], the acetate of isocupressic acid (6) [11], agatholal (7) [12, 13], and pinosolide acid (8) [14-16] from their physical and spectroscopic properties (mp, $[\alpha]$, IR, $\dot{M}S$, ¹H and ¹³CNMR) Their pertinent ¹³CNMR data are listed in Table 2. The novel monoterpenoid derivative (M^+ at m/z300) was assigned as geranyl trans-coumarate (1) The IR absorptions at 1678 and 1629 cm⁻¹, the UV absorption at 308 nm, as well as the base peak at m/z 147, revealed that 1 contained a p-hydroxycinnamoyl molety The trans-configuration was inferred from a large coupling constant of 160 Hz between two vinyl protons [17] The geranyl portion was determined by comparing the ¹H and ¹³C NMR spectra with those of geraniol and geranyl acetate [18] The chemical shifts of C-4 (δ 39.45) and C-10 $(\delta 16 48)$ for structure 1 are close to those of geranyl acetate (δ 39 65 and 16 05), but different from corresponding signals of neryl acetate (δ 31 34 and 23.56) Saponification of compound 1 afforded geraniol and trans-coumaric acid, thus confirming the structural assignment of 1.

Compound 4 (M⁺ at m/z 452) contained a coumaroyl molety as inferred from λ_{max} at 308 nm and the base peak at m/z 147 However, the double bond was in the *cis*configuration as two vinyl protons had a relatively small coupling constant of 12.6 Hz, compared with 160 Hz in compound 1 [17]. This assignment was supported by saponification of 4 to give *cis*-coumaric acid as a predominant product The other saponification product was found to be 13-epitorusolol (19-hydroxy-13-epimanool), mp 108-100°, $[\alpha]_D^{25} + 422°$ (ltt. [12, 19, 20] 111-112°, $[\alpha]_D^{25} + 423°$). The methylene protons at C-19 (δ 3.42 and 3 65, AB quartet) in 13-epitorusolol shifted to lower fields (δ 3 88 and 4 15) in ester 4 Thus, the ester linkage in 4 must be formed by the 19-hydroxyl group and *cis*-coumaric acid

The new diterpenoid γ -hydroxybutenolide **9**, mp 175–176°, was determined as 16-hydroxy-labda-8(17),13diene-15,19-dioic acid butenolide from its spectroscopic properties Compound **9** actually comprised two C-16 epimers (11–14) as shown by two H-16 resonances in the ¹H NMR spectrum at $\delta 5$ 94 (br s) and 5 97 (br s) as well as by two C-16 resonances at δ 98.8 (d) and 99.3 (d) The appropriate IR absorptions for carboxylic acid and conjugated γ -lactone appeared at 3000–2500, 1685 and 1728 cm⁻¹ On treatment with a small amount of sodium methoxide in CD₃OD, compound **9** underwent a ringopening reaction to exhibit a red shift of UV absorptions

Table 1 R_f values and percentage contents of terpenoids 1–9 obtained from *Calocedrus formosana*

Compound	R_f^*	Content (%)
1	0 54	1 17
2	0 69	0 83
3	0 47	23 52
4	0 37	3 33
5	0 47	0 39
6	0 49	1 94
7	043	1 49
8	0 34	12 54
9	0 10	1 90

* The samples were run on a silica gel plate developed with ethyl acetatehexane (3 7)



and to give a new signal in the ¹H NMR spectrum at $\delta 8$ 54 attributable to the resonance of an aldehyde proton [21]

In summary, three novel terpenoids 1, 4 and 9 were isolated from C formosana The major diterpenoidal components in leaves of this plant are trans-communic acid (3) and pinusolide acid (8)

EXPERIMENTAL

Plant material Calocedrus formosana (Florin) Florin was collected in the Taichung county Leaves (1 34 kg) were air-dried and exhaustively extracted with Me_2CO After removal of solvent, the residue was extracted several times with hexane, followed by extraction with EtOAc The extractive of EtOAc was coned to give 188 g of dark brown viscous oil The oily sample was absorbed on 48 g of silica gel and subjected to CC (packed with 250 g of silica gel) with elution using a hexane-EtOAc gradient. Isolated compounds were further purified by crystallization or by HPLC on a Waters μ -Porasil column

Geranyl trans-p-hydroxycinnamate (1) An oily sample, UV $\lambda_{max}^{CHCl_1}$ nm (c) 238 (11 750), 308 (30 200) IR v_{max}^{neat} cm⁻¹ 3347, 1678, 1629, 1604, 1585, 1512, 982, 832 MS m/z (rel int.) 300 [M]⁺ (6), 231 (10), 164 (90), 147 (100), 136 (85), 121 (80), 93 (75), 92 (60), 80 (80), 69 (40), 41 (50) ¹H NMR (CDCl₃, 300 MHz) δ1 58 (3H, s, Me-9), 1 66 (3H, s, Me-8), 1 71 (3H, s, Me-10), 2 02-2 11 (4H, m), 4 72 (2H, d, J = 7 2 Hz), 5 07 (1H, br s, H-6), 5 40 (1H, t, J =7 2 Hz), 6 28 (1H, d, J = 16 0 Hz), 6 85 (2H, d, J = 8 6 Hz), 7 35 $(2H, d, J = 8.6 \text{ Hz}), 7.62 (1H, d, J = 16.0 \text{ Hz})^{-13} \text{C NMR} (\text{CDC})_3,$ 75 MHz) &16 5 (q, C-10), 17 6 (q, C-8), 25 6 (q, C-9), 26 2 (t, C-5), 39 5 (t, C-4), 61 6 (t, C-1), 115 0 (d, C-3'), 115 9 (d, C-6', C-8'), 118 1 (d, C-6), 123 7 (d, C-2), 126 7 (s, C-4'), 130 0 (d, C-5', C-9'), 131 8 (s, C-7), 142 5 (s, C-3), 145 0 (d, C-2'), 158 3 (s, C-7'), 168 2 (s, C-1') Treatment of compound 1 with KOH by a similar procedure for saponification of 4 gave products of geraniol and trans-phydroxycinnamic acid

13S-Labda-8(17),14-diene-13-(cis-p-hydroxycinnamoxy)-19-ol-(4) An oily sample, $[\alpha]_{D}^{25} + 78$ (CHCl₃, c 1 0) UV $\lambda_{max}^{CHCl_3}$ nm (ε) 240 (10 000), 308 (13 490) IR ν_{max}^{neat} cm ⁻¹ 3391, 1709, 1601, 990, 907 MS m/z (rel int) 452 [M]⁺ (1) 434 (5), 288 (14), 270 (25), 257

С	3	4	5	6	7	8	9
1	39 2	39 4	39 1	38 9	38 2	39 1	39 2
2	198	178	20 9	197	190	198	210
3	38 4	34 0	37 8	377	34 1	38 6	38 6
4	44 1	44 2	44 1	44 0	48 4	44 2	44 5
5	56 3	56 0	56 2	56 2	557	56 2	56.6
6	258	24 4	260	259	238	25 9	26.7
7	377	36 0	38 3	38.2	34.1	378	37 8
8	1478	1471	1478	147 6	1470	147 3	147 2
9	56 2	56 0	554	55 2	54 5	556	56 3
10	40 3	39 4	40 3	40.2	39 7	50.4	41 1
11	23 3	20 5	22 7	21.6	217	21 8	21 1
12	141 5	38 7	38 6	38 5	38 1	28 9	26 8
13	133 4	74 6	140 2	142.6	1393	143 9	170.2
14	1338	1436	1228	1179	123 2	134.8	1171
15	109 9	1156	59 1	613	58 8	70 1	1702
16	118	27 2	163	163	161	1744	99 3*
17	1076	1133	106 4	106 3	107 0	106 8	106 8
18	29 0	23 2	28 9	28 8	24 1	28 9	28 9
19	183 7	66 7	183 3	1839	205 6	183 8	1831
20	128	177	127	126	13 3	127	12 7

Table 2 ¹³C NMR spectral data of compounds 3–9 (CDCl₃, 75 MHz, δ)

*Signal for the major isomer The corresponding signal of the minor isomer appeared at δ 98.8

(17), 189 (44), 164 (6), 147 (100), 121 (7). ¹H NMR (CDCl₃). δ 0 74. (3H, s, Me-10), 0 87 (3H, s, Me-4), 1.08 (3H, s, Me-13), 3.88 (1H, d, J = 110 Hz), 4 15 (1H, d, J = 110 Hz), 4 93 (1H, br s, H-17), 4.98 (1H, br s, H-17), 5 01 (1H, br d, J = 110 Hz), 5 24 (1H, br d, J = 176 Hz), 5 74 (1H, d, J = 126 Hz), 6 29 (1H, dd, J = 176, 110 Hz), 6 75 (2H, d, J = 80 Hz), 6 77 (1H, d, J = 126 Hz), 7.52 (2H, d, J = 80 Hz) Compound 4 (52 mg) was treated with KOH (1 N in 4 ml of 95% EtOH) After reflux for 5 hr, the mixture was diluted with H₂O and extracted with Et₂O The ethereal phase was concentrated to afford 34 mg of colourless solids identical to 13-epitorulosol The aq phase was acidified with HCl and extracted with Et₂O to give 18 mg of *p*-hydroxy-cinnamic acid as a mixture of *cis*- and *trans*-isomers in a ratio of 3 1

16-Hydroxy-labda-8(17), 13-diene-15, 19-dioic acid butenolide (9) UV $\lambda_{\text{max}}^{\text{EOH}}$ nm (e) 208 (2372); in the presence of KOH, 204 (5472), 228 (3257), 254 (1612) IR $\nu_{\text{Max}}^{\text{KBr}}$ cm⁻¹ 3373, 3000–2500, 1728, 1685, 1207, 1139, 890 MS m/z (rel int) 348 [M]⁺ (6), 330 (22), 284 (10), 235 (28), 189 (38), 167 (46), 121 (100), 105 (18), 81 (26). ¹H NMB (CDCI₅), $\delta 0.60.(3H, s, Me-10)$, 1 22 (3H, s, Me-4), 448 (1H, br s), 488 (1H, br s), 5.83 (1H, br s), 5.94/5.97 (br s, OCHO) ¹³C NMR (acetone-d₆) $\delta 13$ 1, 20.7, 21 7, 27 0, 27.3, 29 0, 38 9, 39 3, 39 8, 41 4, 44 5, 56.4, 56.5, 99.6/100.0, 106.8, 117 2, 148 8, 171 3, 178 8.

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