

HEXACARBOCYCLIC TRITERPENES FROM LEAVES OF *CRYPTOMERIA JAPONICA*

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Key Word Index—*Cryptomeria japonica*; Taxodiaceae; leaves, hexacarboyclic triterpenes; chamaecyadin; 6-hydroxychamaecydins; 10'-hydroxycryptoquinones.

Abstract—Five hexacarboyclic triterpenes, namely chamaecyadin, 6 α -hydroxychamaecyadin, 6 β -hydroxychamaecyadin, 10' α -hydroxycryptoquinone and 10' β -hydroxycryptoquinone, whose skeleton comprises spiro-annulation of abietane-type diterpenes and thujane-type monoterpenes, were isolated from the leaves of *Cryptomeria japonica*.

INTRODUCTION

The Japanese cedar, *Cryptomeria japonica* D. Don., is a widely distributed conifer called 'sugi' in Japanese [1]. Its constituents have been extensively studied. The leaves are reported to contain monoterpenes, diterpenes and flavones [2–9]. In this paper, we report five uncommon triterpenes 1–5, isolated from the ethyl acetate-soluble part of the leaves of *C. japonica*.

RESULTS AND DISCUSSION

The molecular formula $C_{30}H_{40}O_3$ of 1 was deduced from its exact mass $[M]^+$ at m/z 448.298. The ^{13}C NMR spectrum (Table 1) showed two carbonyl groups (δ 205.7 and 182.6) and three double bonds (δ 123.6, 136.3, 144.6, 145.1, 149.1 and 151.7). These analyses indicated that 1, mp 197–198°, $[\alpha]_D^{25} + 33^\circ$ ($CHCl_3$; c 2.5), is an uncommon hexacarboyclic triterpene, chamaecyadin, previously isolated from the seed of *Chamaecyparis obtusa* (Cupressaceae) [10]. Compound 1 contains two subunits, an abietane-type diterpene and a thujane-type monoterpene, which are spiro-annulated. Assignment of the proton and carbon resonances (Tables 1 and 2) was further supported by a C–H COLOC spectrum and the NOE experiments (Fig. A). For example, the quaternary C-4' at δ 57.9 was shown to correlate with H-2' and H-6'. Irradiation of the H-15 resonance at δ 3.16 caused NOEs on the two cyclopropane methylene protons. In the CD spectrum, compound 1 exhibited a distinct positive Cotton effect with the maximum at 455 nm in addition to others.

Compound 2 ($C_{30}H_{40}O_4$), mp 220–222°, $[\alpha]_D^{25} - 51^\circ$ ($CHCl_3$; c 0.8), was similarly determined to be 6 α -hydroxychamaecyadin [10], (6 α ,11-dihydroxyabieta-7,9,13-trien-12-one)-7,10':4',14-(10'-oxothujane). Since the res-

onance of H-6 at δ 5.02 showed a large coupling constant ($J_{5,6} = 10$ Hz), the H-6 should be on the axial position (the β -face). The IR absorptions at 1693 and 1608 cm^{-1} as well as the UV absorption at 350 nm (ϵ 25 000) were characteristics of the quinone methide moiety of 2.

By analyses of the spectral properties, compound 3 ($C_{30}H_{40}O_4$) was readily recognized as 6 β -hydroxychamaecyadin, an epimer of 2. The equatorial 6 α -proton occurred at δ 5.10 with a small coupling constant, $J_{5,6} = 3.5$ Hz. The new triterpene 3 exhibited a positive Cotton effect with the maximum at 453 nm, whereas 2 showed a negative Cotton effect with the minimum at 457 nm.

Compound 4 showed the exact mass $[M]^+$ at m/z 464.292, indicating its molecular formula $C_{30}H_{40}O_4$. The presence of intramolecular hydrogen-bonded hydroxyl group was inferred from the IR absorption at 3298 cm^{-1} (sharp). The hydroxyl resonance at δ 7.66 in the NMR spectrum was not shifted by changing to a different concentration of sample. The IR spectrum also displayed strong absorptions at 1650 and 1637 cm^{-1} attributable to a conjugated carbonyl group and a quinone moiety, respectively. Analysis of the 1H NMR spectrum (Table 2) revealed two isopropyl groups and three methyl groups positioned on tertiary carbons. Two methylene protons of a cyclopropane ring occurred at δ 0.57 and 0.83, but no olefinic protons were present. The ^{13}C NMR spectrum exhibited a resonance at δ 191.8 for a ketone, a resonance at δ 183.1 for a quinone and the signals for six olefinic carbons bearing no proton. Judging from the above spectral properties, we suggest that 4 is a hexacyclic triterpene (11-hydroxyabieta-7,9,13-triene-6,12-dione)-7,10':4',14-(10' β -hydroxythujane), constructed by two subunits of the abietane-type diterpene and the thujane-type monoterpene. These two subunits were spiro-annulated (7,10':14,4') to form an extra carbocyclic ring. The λ_{max} at 354 nm (ϵ 29 800) was in agreement with the calculated value of a vinylogous quinone moiety. Irradiation of H-10' (δ 4.70) geminal to the hydroxyl group

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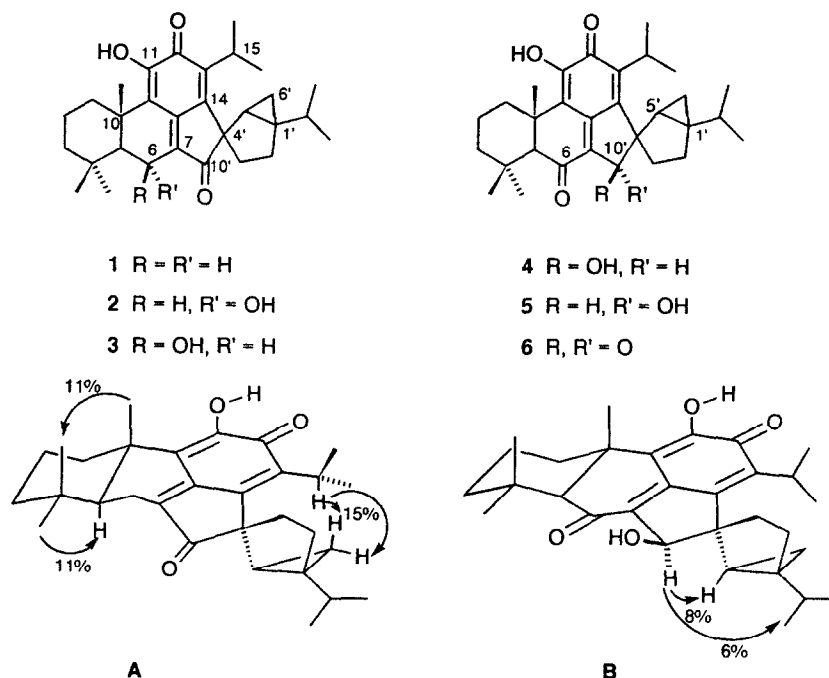


Table 1. ^{13}C NMR spectral data of compounds 1–4 and 6 (CDCl₃ solution, δ values in ppm)*

C	1	2	3	4	6
1	36.5	37.2	37.9	36.9	36.9
2	18.6	18.6	18.9	18.4	18.4
3	41.6	43.2	43.9	42.7	42.7
4	33.8	34.2	34.7	33.6	35.3
5	50.8	56.1	53.3	63.7	64.4
6	19.4	67.5	60.6	191.8	195.6
7	145.1	144.5	145.7	144.5	146.3
8	151.7	150.9	151.7	156.8	158.1
9	123.6	122.8	123.9	121.8	122.3
10	38.6	40.2	38.7	43.6	43.3
11	144.6	142.9	142.1	143.5	140.4
12	182.6	182.8	182.4	183.1	181.8
13	136.3	137.7	137.9	140.0	129.2
14	149.4	148.9	149.8	144.8	150.9
15	28.7	28.9	28.9	28.5	29.1
16	20.0	20.1	20.1	20.3	20.4
17	20.4	20.4	20.5	20.5	20.6
18	33.4	36.4	33.4	33.5	33.3
19	22.0	22.7	24.1	22.5	22.2
20	19.1	21.3	21.9	21.9	21.8
1'	34.7	34.8	34.8	32.9	33.1
2'	30.7	30.7	30.3	32.1	31.1
3'	29.8	29.7	29.7	26.3	30.0
4'	57.9	58.4	58.0	59.8	58.7
5'	30.9	31.0	31.4	35.0	31.3
6'	13.2	13.4	13.6	14.2	13.0
7'	31.6	31.6	31.8	28.6	30.7
8'	19.6	19.7	19.8	20.0	19.6
9'	20.2	20.2	20.2	20.1	20.2
10'	205.7	207.4	206.1	80.2	200.7

*Owing to small amount of **5** available, its ^{13}C signals were not assigned.

caused NOEs of H-5' and the resonances of C-1' isopropyl groups (Fig. B). Thus, H-10' is α -orientated. The absolute configuration is assigned (5*S*, 10*S*, 1'*R*, 5'*S*) by analogy to that of **1**. The novel compound **4** is named as 10' β -hydroxycryptoquinone, of which 'crypto' implies the plant source of *Cryptomeria* genus and the structural feature of the hidden (crypt) quinone.

The C-10' epimer **5**, 10' α -hydroxycryptoquinone, was isolated in minute amount. Its structure was deduced from spectral analyses and confirmed by a chemical correlation. Thus, oxidation of **4** and **5** with MnO₂, respectively, gave the same ketone **6**, 10'-oxocryptoquinone. Compound **5** is more polar than **4**.

In summary, we isolated chamaecyadin (**1**), 6 α -hydroxycamaecyadin (**2**) and three new analogues, 6 β -hydroxycamaecyadin (**3**), 10' β -hydroxycryptoquinone (**4**) and 10' α -hydroxycryptoquinone (**5**) from the leaves of *Cryptomeria japonica*. These uncommon hexacarbocyclic triterpenes are constructed by the spiro-annulation of abietane-type diterpenes and thujane-type monoterpenes. The biological significance and possible pharmacological effects related to their extended vinylogous quinone moieties await further investigation.

EXPERIMENTAL

General. Merck silica gel 60F sheets were used for analyt. TLC. HPLC was carried out on a Hibar Lichrosorb Si 60 (7 μm) column (25 cm \times 1 cm).

Plant material. The plant used in this study was introduced from Japan and cultivated in suburban Taipei. A voucher specimen is deposited in our laboratory. The leaves (1.4 kg) of *C. japonica* D. Don. were exhaustively extracted with Me₂CO. The extract was passed through a

Table 2. ¹H NMR spectral data of compounds 1–6 (CDCl₃ solution, δ values in ppm, J values in Hz)

H	1	2	3	4	5	6
1α	1.56 (ddd, J 13, 12, 3.5)	1.55 (ddd, J 12, 12, 3)	1.45 (ddd, J 13, 13, 4)	1.73 (ddd, J 12, 11, 3)	1.69 (m)	1.70 (m)
1β	2.82 (ddd, J 13, 4, 1.5)	2.73 (ddd, J 12, 4, 1.5)	2.83 (ddd, J 13, 4, 1)	2.79 (ddd, J 11, 4, 1)	2.72 (ddd, J 12, 4, 1)	2.80 (ddd, J 11, 4, 1)
2α	1.60 (m)	1.56 (m)	1.57 (m)	1.60 (m)	1.58 (m)	1.60 (m)
2β	1.64 (m)	1.61 (m)	1.82 (m)	1.80 (m)	1.79 (m)	1.80 (m)
3α	1.21 (ddd, J 12, 12, 2)	1.40 (ddd, J 12, 12, 2)	1.23 (ddd, J 12, 12, 3)	1.21 (ddd, J 12, 12, 3)	1.21 (ddd, J 12, 12, 3)	1.71 (ddd, J 12, 12, 2)
3β	1.46 (ddd, J 12, 4.5, 2.5)	1.54 (ddd, J 12, 4.5, 2.5)	1.40 (ddd, J 12, 4, 2)	1.41 (ddd, J 12, 4, 1)	1.40 (ddd, J 12, 4, 1)	1.40 (ddd, J 12, 4, 2)
5	1.49 (dd, J 12, 3.5)	1.69 (d, J 10)	1.35 (d, J 3.5)	2.64 (s)	2.62 (s)	2.58 (s)
6α	2.69 (dd, J 19, 3.5)		5.10 (d, J 3.5)			
6β	2.18 (dd, J 19, 12)	5.02 (d, J 10)				
15	3.16 (sept, J 7)	3.16 (sept, J 7)	3.17 (sept, J 7)	3.32 (sept, J 7)	3.56 (sept, J 7)	3.26 (sept, J 7)
16	1.26 (d, J 7)	1.26 (d, J 7)	1.27 (d, J 7)	1.27 (d, J 7)	1.24 (d, J 7)	1.25 (d, J 7)
17	1.28 (d, J 7)	1.28 (d, J 7)	1.29 (d, J 7)	1.28 (d, J 7)	1.27 (d, J 7)	1.30 (d, J 7)
18	0.94 (s)	1.13 (s)	1.10 (s)	1.14 (s)	1.14 (s)	1.11 (s)
19	0.97 (s)	1.16 (s)	1.33 (s)	1.26 (s)	1.24 (s)	1.28 (s)
20	1.14 (s)	1.20 (s)	1.52 (s)	1.26 (s)	1.25 (s)	1.30 (s)
2'α	1.70 (dddd, J 12, 8, 2, 1)	1.67 (dddd, J 12, 8, 2, 1)	1.80 (dddd, J 12, 8, 1, 1)	1.91 (dddd, J 12, 7.5, 2.5, 1)	1.82 (dddd, J 12, 7.5, 2.5, 1)	1.73 (ddd, J 12, 8, 1.5)
2'β	1.67 (ddd, J 12, 7, 1.5)	1.66 (ddd, J 12, 7, 1.5)	1.70 (ddd, J 12, 7, 1.5)	1.52 (ddd, J 12, 7.5, 1)	1.43 (ddd, J 12, 7.5, 1)	1.68 (ddd, J 12, 7, 2)
3'α	2.24 (ddd, J 12, 8, 1.5)	2.20 (ddd, J 12, 8, 1.5)	2.27 (ddd, J 12, 8, 1.5)	2.18 (ddd, J 12, 7.5, 1)	2.02 (ddd, J 12, 7.5, 1)	2.25 (ddd, J 12, 8, 2)
3'β	1.81 (ddd, J 12, 7, 2)	1.83 (ddd, J 12, 7, 2)	1.83 (ddd, J 12, 7, 1)	1.66 (ddd, J 12, 7.5, 2.5)	1.64 (ddd, J 12, 7.5, 2.5)	1.83 (ddd, J 12, 7, 1.5)
5'	1.02 (dd, J 8, 4)	1.08 (dd, J 8, 4)	1.05 (dd, J 8, 4)	0.96 (dd, J 8, 4)	0.84 (dd, J 8, 4)	1.10 (dd, J 8, 4)
6'α	0.69 (ddd, J 8, 6, 1)	0.65 (ddd, J 8, 6, 1)	0.70 (ddd, J 8, 6, 1)	0.57 (ddd, J 8, 6, 1)	0.74 (ddd, J 8, 6, 1)	0.84 (dd, J 8, 6)
6'β	0.75 (dd, J 6, 4)	0.81 (dd, J 6, 4)	0.78 (dd, J 6, 4)	0.83 (dd, J 6, 4)	0.76 (dd, J 6, 4)	0.89 (dd, J 6, 4)
7'	1.78 (sept, J 7)	1.77 (sept, J 7)	1.71 (sept, J 7)	1.70 (sept, J 7)	1.70 (sept, J 7)	1.81 (sept, J 7)
8'	0.78 (d, J 7)	0.78 (d, J 7)	0.80 (d, J 7)	0.86 (d, J 7)	0.85 (d, J 7)	0.75 (d, J 7)
9'	1.03 (d, J 7)	1.01 (d, J 7)	1.03 (d, J 7)	0.99 (d, J 7)	1.00 (d, J 7)	1.02 (d, J 7)
10'				4.70 (s)	4.60 (s)	
11-OH	7.71 (s)	7.75 (s)	7.75 (s)	7.66 (s)	7.63 (s)	7.93 (s)

pad of charcoal, concd and re-extracted with EtOAc. The EtOAc-soluble portion (45 g) was chromatographed on a silica gel column by elution with gradients of hexane and EtOAc. The appropriate frs were combined and purified by HPLC to give **1** (30 mg), **2** (8 mg), **3** (8 mg), **4** (8 mg) and **5** (4 mg).

Chamaecyadin (1). Orange crystals. Mp 197–198° (from hexane). $[\alpha]_D^{25} + 33^\circ$ (CHCl₃; c 2.5). TLC (0.7% EtOAc in hexane) R_f 0.16. IR ν_{\max}^{KBr} cm⁻¹: 3273, 1693, 1611, 1389, 1370. UV $\lambda_{\max}^{\text{CHCl}_3}$ nm (ε): 352 (32 600), 342 (25 400), 336 (27 800), 282 (2130), 240 (12 300). CD (CHCl₃): $[\theta]_{474} + 1010$, $[\theta]_{455} + 1480$, $[\theta]_{430} - 1050$, $[\theta]_{410} + 630$, $[\theta]_{393} - 620$, $[\theta]_{375} + 710$, $[\theta]_{357} - 600$, $[\theta]_{337} + 960$, $[\theta]_{316}$

–460, $[\theta]_{290} + 420$, $[\theta]_{267} - 190$, $[\theta]_{253} + 80$, $[\theta]_{231} - 1170$. EIMS (70 eV) m/z (rel. int.) 448 [M]⁺ (100), 433 (10), 405 (64), 377 (9), 366 (16), 352 (13), 340 (18), 281 (14). HRMS for C₃₀H₄₀O₃ requires 448.2979. Found 448.2976.

6α-Hydroxychamaecyadin (2). Orange crystals. Mp 220–222° (from hexane). $[\alpha]_D^{25} - 51^\circ$ (CHCl₃; c 0.8). TLC (0.7% EtOAc in hexane) R_f 0.13. IR ν_{\max}^{KBr} cm⁻¹: 3529, 3303, 1693, 1608, 1380, 1372. UV $\lambda_{\max}^{\text{CHCl}_3}$ nm (ε): 350 (25 000), 341 (21 600), 335 (22 000), 274 (2300), 241 (9300). CD (CHCl₃): $[\theta]_{457} - 990$, $[\theta]_{434} + 80$, $[\theta]_{411} - 720$, $[\theta]_{381} + 60$, $[\theta]_{362} - 110$, $[\theta]_{345} + 50$, $[\theta]_{323} - 480$, $[\theta]_{301} + 280$, $[\theta]_{278} - 540$, $[\theta]_{265} - 370$, $[\theta]_{256} - 400$,

$[\theta]_{232} + 770$. EIMS (70 eV) (m/z) (rel. int.): 464 $[M]^+$ (86), 446 (100), 431 (14), 418 (25), 403 (30), 377 (20), 363 (35), 350 (24), 335 (30), 281 (30). HRMS for $C_{30}H_{40}O_4$ requires 464.2927. Found 464.2887.

6 β -Hydroxychamaecydin (3). Orange crystals. Mp 206–208°. $[\alpha]_D^{25} + 100^\circ$ ($CHCl_3$; c 0.8). TLC (6% EtOAc in hexane) R_f 0.42. IR $\nu_{max}^{KBr} cm^{-1}$: 3510, 3305, 1694, 1610. UV $\lambda_{max}^{CHCl_3} nm$ (ϵ): 348 (31 000), 339 (25 500), 333 (27 200), 270 (2100), 241 (11 600). CD ($CHCl_3$): $[\theta]_{453} + 1040$, $[\theta]_{426} + 40$, $[\theta]_{416} + 100$, $[\theta]_{396} - 260$, $[\theta]_{378} + 105$, $[\theta]_{360} - 80$, $[\theta]_{342} + 40$, $[\theta]_{332} + 30$, $[\theta]_{301} - 240$, $[\theta]_{274} + 700$, $[\theta]_{249} - 150$. EIMS (70 eV) (m/z) (rel. int.): 464 $[M]^+$ (65), 446 (100), 431 (8), 418 (27), 403 (25), 375 (22), 363 (35), 350 (22), 335 (32), 281 (15). HRMS for $C_{30}H_{40}O_4$ requires 464.2927. Found 464.2945.

10 β -Hydroxycryptoquinone (4). Orange crystals. Mp 210–212°. $[\alpha]_D^{25} + 40^\circ$ ($CHCl_3$; c 0.5). TLC ($CHCl_3$ –hexane, 1:1) R_f 0.30. IR $\nu_{max}^{KBr} cm^{-1}$: 3480, 3298, 1650, 1637. UV $\lambda_{max}^{CHCl_3} nm$ (ϵ): 354 (29 800), 344 (22 800), 340 (26 500), 270 (3200), 240 (500). EIMS (70 eV) (m/z) (rel. int.): 464 $[M]^+$ (30), 446 (100), 402 (18), 364 (8), 306 (40), 288 (36). HRMS for $C_{30}H_{40}O_4$ requires 464.2927. Found 464.2916.

10 α -Hydroxycryptoquinone (5). Orange crystals. Mp 202–203°. TLC ($CHCl_3$ –hexane, 1:1) R_f 0.25. IR $\nu_{max}^{KBr} cm^{-1}$: 3500, 3295, 1650, 1637. UV $\lambda_{max}^{CHCl_3} nm$ (ϵ): 352 (29 600), 342 (22 300), 338 (26 500), 274 (2200), 240 (510). EIMS (70 eV) (m/z) (rel. int.): 464 $[M]^+$ (35), 446 (100), 402 (20), 364 (8), 306 (45), 288 (38). HRMS for $C_{30}H_{40}O_4$ requires 464.2927. Found 464.2916.

10'-Oxocryptoquinone (6). A soln of the alcohol 4 (6 mg) in CH_2Cl_2 (3 ml) was treated with MnO_2 (5 mg). The mixt. was stirred at room temp. (27°) for 3 hr, filtered, and the filtrate was concd *in vacuo* to give the ketone 6 (6 mg). A sample of 5 (3 mg) was oxidized by a similar procedure

to give 6 (3 mg). Orange crystals. Mp 182–183°. $[\alpha]_D^{25} + 25^\circ$ ($CHCl_3$; c 0.6). IR $\nu_{max}^{KBr} cm^{-1}$: 3300, 1670, 1660, 1640. UV $\lambda_{max}^{CHCl_3} nm$ (ϵ): 356 (29 400), 345 (22 600), 340 (26 800), 274 (2100), 240 (900). EIMS (70 eV) (m/z) (rel. int.): 462 $[M]^+$ (100), 447 (18), 419 (72), 401 (13), 391 (15), 380 (25), 366 (58), 354 (72), 295 (30). HRMS for $C_{30}H_{38}O_4$ requires 462.2771. Found 462.2750.

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Note added in proof:

At the stage of proof, we are aware of a recent report on hexacarbocyclic triterpene quinone methides. Shibuya, T. (1992) *Phytochemistry* **31**, 4289.