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Terpenoids from *Juniperus polycarpus* var. *seravschanica*

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Abstract

Two eudesmanes, two abietanes, two podocarpanes and other nine known compounds were isolated from the dried fruits of *Juniperus polycarpus* var. *seravschanica*. Their structures were established on the basis of analysis of spectroscopic evidence. Some of the isolated terpenoids showed antimalarial activity.

Keywords: *Juniperus polycarpus* var. *seravschanica*; Cupressaceae; Sesquiterpenoid; Diterpenoid; Antimalarial activity

1. Introduction

Juniperus polycarpus var. *seravschanica* (=*J. seravschanica*) is an evergreen tree indigenous to the mountains of Central Asia and belongs to the family Cupressaceae, with about 70 species distributed over the Northern Hemisphere. Previously, from the genus *Juniperus* some terpenoids (De Pascual Teresa et al., 1980; Fang et al., 1992; Fang et al., 1996; Barrero et al., 2000; Lee and Cheng, 2001; Nakanishi et al., 2005; Martin et al., 2006), neolignans (Nakanishi et al., 2004) and flavonoids (Yuldashev and Rasulova, 2001; Inatomi et al., 2005) have been isolated. The seed decoction of *J. polycarpus* var. *seravschanica* is used as folk medicine for kidney diseases, and as a diuretic and abortive in Uzbekistan (Shalyt, 1951; Karriev, 1967). Additionally, the isolation and anti-inflammatory activity of some diterpenoids of *J. polycarpus* (El-Sayed, 1998) and several studies about the essential oil of *J. seravschanica* have been published (Adams, 1999; Basher et al., 1999). In the present study, the isolation and structure elucidation of two new sesquiterpenoids, four new diterpenoids and nine known compounds is described. Some of the isolated compounds showed moderate antimalarial activity.

2. Result and discussion

The air-dried fruits of *J. polycarpus* var. *seravschanica* (1.5 kg) were extracted with hot methanol. The methanol extract was successively partitioned with hexane, EtOAc, *n*-BuOH, and H_2O. The hexane and EtOAc extracts were fractionated by column chromatography to obtain 15 compounds, including six new compounds (compounds **1**–**6**) and nine known compounds [cedrol (Joseph-Nathan et al., 1984), 12,15-dihydroxylabda-8(17),13-dien-19-oic acid (Fujimoto et al., 1990), sugiol (Ying and Kubo, 1991), hinokiflavone (Markham et al., 1987), (−)-2,

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3-dihydro-7-hydroxy-3-hydroxymethyl-2-(4′-hydroxy-3′-methoxyphenyl)-5-benzofuranpropanol-4′-*O*-α-L-rhamnopyranoside (Gonźalez-Laredo and Karchesy, 1996), 2,3-dihydrobenzofuran-2-(4′-hydroxy-3′-methoxyphenyl)-3-α-L-rhamnopyranosyloxy-methyl-7-methoxy-5-propanol (De Leo et al., 2004), icaride E_4 (Nakanishi et al., 2004), (7*S*,8*S*)-3-methoxy-3′,7-epoxy-8,4′-oxyneoligna-4,9,9′-triol (Fang et al., 1992), cedrusin (Agrawal et al., 1983)]. The known compounds exhibited physical and spectroscopic data identical to values reported in the literature.

Compound **1** was assigned the molecular formula of $C_{15}H_{26}O_3$, as established from its HREIMS (*m/z* 254.1863 $[M]^+$). Its IR spectrum showed absorbance for hydroxyl (3397 cm^{-1}) and olefinic (1646 cm^{-1}) groups. The ^1H NMR spectrum of **1** showed the presence of two vinylic protons [δ_H 4.77, 4.48 (each 1H, *d*, $J = 1.4$ Hz, H-15a,b)], one oxygenated methine [δ_H 3.52 (1H, *dd*, $J = 4.6$, 11.6 Hz, H-1)], and three singlet methyls [δ_H 1.29, 1.28, 0.68 (each 3H, *s*, Me-12, 13 and 14)]. The ^{13}C NMR spectrum of **1** showed 15 carbon signals, including two olefinic carbons and three oxygenated carbons (Table 1). On the basis of its ^1H–^1H COSY, HMBC spectrum, together with the chemical shifts and coupling constants, **1** was assumed to be a eudesmane sesquiterpene. The ^1H–^1H COSY spectrum of **1** showed the following correlations: H-2a with H-1 and H-3a,b; H-5 with H-6b; H-8 with H-9. The HMBC spectrum of **1** showed the following correlations: H-6b with C-8; H-8a with C-7 and 10; Me-12 and 13 with C-7 and 11; Me-14 with C-1, 5, 9 and 10; H-15a,b with C-3, 4 and 5. The relative stereochemistry of **1** was determined by the following NOE correlations in its NOESY spectrum: H-1 with H-5; H-6b with Me-14 and Me-12. Thus, the structure of **1** was elucidated as shown (Fig. 1).

Table 1
^{13}C NMR spectroscopic data of **1**–**6** (δ_C ppm, 100 MHz)

Position	1[a]	2[a]	3[a]	4[a]	5[b]	6[b]
1	78.9	75.9	38.7	38.5	40.4	40.6
2	31.5	32.2	19.7	18.8	19.6	20.8
3	34.2	119.8	37.1	35.2	36.6	39.2
4	148.7	135.2	43.3	38.1	39.3	44.6
5	41.9	40.9	45.4	45.0	49.5[c]	49.5[c]
6	28.8	28.6	29.5	28.5	30.7	32.5
7	75.6	75.6	68.4	68.0	72.1	72.4
8	26.3	26.4	135.6	135.5	166.7	166.7
9	32.1	30.6	146.5	148.1	49.5[c]	47.1
10	39.8	37.1	38.3	37.8	40.7	41.8
11	75.5	75.4	124.7	124.4	21.4	21.4
12	24.8	24.6	125.3	124.6	36.6	37.4
13	24.8	24.7	146.7	146.6	203.3	203.3
14	9.1	8.4	125.8	125.8	127.9	127.2
15	106.7	20.8	72.3	72.0	27.5	29.3
16			31.5	31.3	65.1	181.1
17			31.5	31.5	16.2	14.2
18			28.4	26.5		
19			182.7	65.1		
20			21.9	24.5		

[a] $CDCl_3$.
[b] CD_3OD.
[c] Overlapped with solvent.

The positive-ion HRFABMS of **2** gave a pseudomolecular ion at *m/z* 277.1780 ($[M + Na]^+$, calcd 277.1780), suggesting the molecular formula of $C_{15}H_{26}O_3$. The ^1H NMR spectrum of **2** revealed the presence of one vinylic proton [δ_H 5.28 (1H, *brs*, H-3)], one oxygenated methine [δ_H 3.60 (1H, *dd*, $J = 6.5$, 10.2 Hz, H-1)], and four singlet methyls [δ_H 1.55 (3H, *s*, Me-15), 1.26 (6H, *s*, Me-12 and 13), 0.76 (3H, *s*, Me-14)]. The ^{13}C NMR spectrum of **2** showed 15 carbon signals similar to that of 3-eudesmene-1β,11-diol (Su et al., 1995). The ^1H–^1H COSY spectrum of **2** displayed the following correlations: H-2a,b with H-1 and H-3; H-5 with H-6a,b; H-8a,b with H-9a,b. The HMBC spectrum of **2** showed the following correlations: H-6a,b with C-7; H-8a,b with C-7 and 10; Me-12 and 13 with carbons at C-7 and 11; Me-14 with C-1, 5, 9 and 10; Me-15 with C-3, 4 and 5. In the NOESY spectrum, the following NOE correlations were observed: H-1 with H-5; H-6a with Me-14 and Me-12. From these data, the structure of **2** was established as shown (Fig. 1). The absolute configurations of compound **1** and **2** were not determined.

The molecular formula, $C_{20}H_{28}O_4$, of **3** was inferred from its HREIMS (*m/z* 332.2001 $[M]^+$). The IR spectrum of **3** showed hydroxyl and carbonyl bands at 3380 and 1697 cm^{-1}, respectively. The ^1H NMR spectrum of **3** showed the presence of three aromatic protons [δ_H 7.44 (1H, *d*, $J = 2.0$ Hz, H-14), 7.38 (1H, *dd*, $J = 2.0$, 8.4 Hz, H-12), 7.31 (1H, *d*, $J = 8.4$ Hz, H-11)], one oxygenated methine [δ_H 4.80 (1H, *brt*, $J = 3.0$ Hz, H-7)], and four singlet methyls [δ_H 1.55 × 2, 1.34, 1.11 (each 3H, *s*, Me-16, 17, 18 and 20)]. The ^{13}C NMR spectrum of **3** showed 20 carbon signals, including one carboxylic group, six olefinic carbons and two oxygenated carbons (Table 1). The ^{13}C NMR spectroscopic data showed the structural resemblance of **3** and 7,15-dihydroxydehydroabietic acid (Prinz et al., 2002) except for the chemical shifts of C-4, 5, 10, 18, 19 and 20. In the HMBC spectrum of **3**, the following long-range correlations were observed: H-6a,b with C-5 and 10; Me-18 with C-3, 4, 5 and 19; Me-20 with C-1, 5,

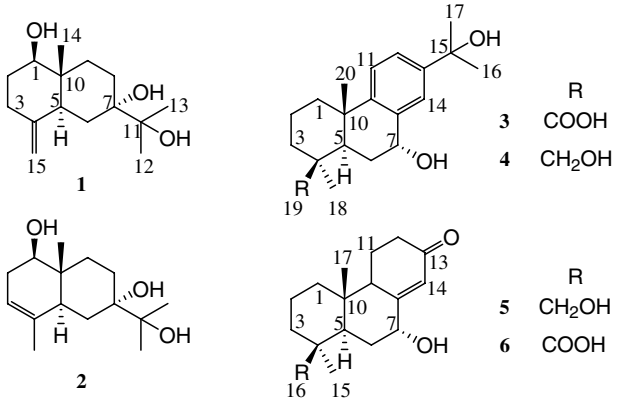

Fig. 1. Structure of sesquiterpenoids and diterpenoids from *J. polycarpus* var. *seravschanica*.

9 and 10. In the NOESY spectrum, the following NOE correlations were observed: Me-18 with H-5. The α-orientation of C-7 hydroxyl group was assigned by its small coupling constant value. Accordingly, the structure of **3** was concluded as shown in Fig. 1. The stereochemistry at C-7 in **3** was determined by CD exciton chirality method with 7-benzoyl-19-methylester of **3** (**3a**). The CD spectrum of **3a** showed a negative Cotton effect at 233 nm ($[\theta] - 769.27$) and 225 nm ($[\theta] + 1634.44$). Thus, the configuration of **3** was decided as *R* and therefore, the absolute configuration of **3** was assigned as shown in Fig. 1.

Compound **4** was assigned the molecular formula of $C_{20}H_{30}O_3$, as established from its HREIMS (*m/z* 318.2206 $[M]^+$). The ^1H NMR spectrum of **4** showed the presence of three aromatic protons [δ_H 7.44 (1H, *d*, $J = 2.0$ Hz, H-14), 7.35 (1H, *dd*, $J = 2.0$, 8.7 Hz, H-12), 7.25 (1H, *d*, $J = 8.7$ Hz, H-11)], one oxygenated methine [δ_H 4.81 (1H, *brs*, H-7)], one oxygenated methylene [δ_H 3.84, 3.56 (each 1H, *d*, $J = 10.9$ Hz, H-19a,b)], and four singlet methyls [δ_H 1.57 (6H, *s*, Me-16 and 17), 1.13, 1.08 (each 3H, *s*, Me-20 and 18)]. Comparison of the ^{13}C NMR chemical shifts of **4** and those of abieta-8,11,13-triene-7α,15,18-triol (Ohtsu et al., 2001) showed a good agreement, except for the chemical shifts of C-5, 18 and 19 (Table 1). The HMBC spectrum of **4** showed the following correlations: Me-18 with C-3, 4, 5 and 19; Me-20 with C-1, 5, 9 and 10. The NOESY of **4** showed cross peaks between Me-18 and H-5, H-19a,b and Me-20. Therefore, the structure of **4** assigned as shown in Fig. 1. Final structural confirmation was obtained by the reduction of **3a** with LiAlH4 which gave a product identical with **4**.

The HREIMS of compound **5** (pseudomolecular ion peak at *m/z* 260.1774 $[M\text{-}H_2O]^+$) indicated the molecular formula of $C_{17}H_{26}O_3$. Its IR spectrum showed absorbance of a hydroxyl group (3326 cm^{-1}) and a carbonyl group (1650 cm^{-1}), respectively. Analysis of the ^1H NMR spectrum of **5** indicated the presence of one olefinic proton [δ_H 5.98 (1H, *d*, $J = 2.1$ Hz, H-14)], one oxygenated methine [δ_H 4.33 (1H, *brt*, $J = 2.7$ Hz, H-7)], one oxygenated methylene [δ_H 3.79, 3.41 (each 1H, *d*, $J = 11.2$ Hz, H-16a,b)], and two singlet methyls [δ_H 1.04, 0.86 (each 3H, *s*, Me-15 and 17)]. The ^{13}C NMR spectrum of **5** showed 17 carbon signals, including one carbonyl group, two olefinic carbons and two oxygenated carbons (Table 1). On the basis of these data, **5** was assumed to be a podocarpane diterpene. The ^{13}C NMR spectrum of **5** was similar to that of 7α,15-dihydroxypodocarp-8(14)-en-13-one (Ohtsu et al., 2000) except for the chemical shifts of C-5, 15 and 16. In the HMBC spectrum of **5**, the following long-range correlations were observed: Me-15 with C-3, 4, 5 and 16; Me-17 with C-1, 5, 9 and 10. This relative configuration was supported by the NOESY spectrum that following NOE correlations between Me-15 and H-5, H-16a,b and Me-17. Thus, the structure of **5** was elucidated as 4-epi-7α,15-dihydroxypodocarp-8(14)-en-13-one (Fig. 1). The CD spectra of **5** and 7α,15-dihydroxypodocarp-8(14)-en-13-one (Ohtsu et al., 2000) showed a similar negative Cotton effect at 375 nm ($[\theta] + 0.74$), 327 nm ($[\theta] - 1974.48$) and 275 nm ($[\theta] - 18.94$). Therefore, the absolute configuration of **5** was concluded as illustrated in Fig. 1.

Compound **6** has the molecular formula of $C_{17}H_{24}O_4$ by HREIMS (*m/z* 292.1675 $[M]^+$). The ^1H and ^{13}C NMR spectrum of **6** were compared with those of **5**, and they showed a good agreement, except for the chemical shift of position 16 (δ_H 3.79, 3.41, δ_C 65.1 for **5**; δ_C 181.1 for **6**). The HMBC spectrum of **6** showed the following correlations: Me-15 with C-3, 4, 5 and 16; Me-17 with C-1, 5, 9 and 10. The NOE correlation between Me-15 and H-5 was observed in its NOESY spectrum. The structure of **6** was elucidated as 7α-hydroxypodocarp-8(14)-en-13-one-16-oic acid (Fig. 1). Since the optical rotation of **6** was similar to that of **5**, the stereostructure of **6** was assumed to be the same as that of **5**.

The isolated compounds (**3**, cedrol, sugiol, hinokiflavone, 12,15-dihydroxylabda-8(17),13-dien-19-oic acid, (−)-2,3-dihydro-7-hydroxy-3-hydroxymethyl-2-(4′-hydroxy-3′-methoxyphenyl)-5-benzofuranpropanol 4′-*O*-α-L-rhamnopyranoside, (7*S*,8*S*)-3-methoxy-3′,7-epoxy-8,4′-oxyneoligna-4,9,9′-triol) were evaluated for antimalarial and anti-HIV activity. None of the tested compounds showed anti-HIV activity. Regarding antimalarial activity, these compounds were tested against three *Plasmodium falciparum* clones, D6, TM91C235 and W2 (Guan et al., 2002). As result, three compounds showed weak antimalarial activity. Cedrol showed activity against the D6 (IC_{50} 58.9 ng/ml), TM91C235 (IC_{50} 301.3 ng/ml) and W2 (224.0 ng/ml) clones; Sugiol showed activity against the D6 (IC_{50} 472.4 ng/ml), TM91C235 (IC_{50} 1009.8 ng/ml) and W2 (409.4 ng/ml) clones; 12,15-dihydroxylabda-8(17),13-dien-19-oic acid showed activity against the D6 (IC_{50} 742.4 ng/ml), TM91C235 (IC_{50} 1367.3 ng/ml) and W2 (1562.3 ng/ml) clones. The other tested compounds did not show significant antimalarial activity.

3. Experimental

3.1. General

NMR (^1H NMR: 400 MHz, ^{13}C NMR: 100 MHz, using TMS as int. stand.) spectra were measured on an AVANCE 400 Fourier transform spectrometer (Bruker). MS were obtained on a JMSD-300 mass spectrometer (JEOL) and LCT PREMIER (Waters). Optical rotations were measured with a DIP-370 digital polarimeter (JASCO). CD spectra were recorded on a J-600 spectrometer (JASCO). IR spectra were acquired on a FT-IR 420 Fourier transform infrared spectrometer (JASCO). Column chromatography: silica gel 60 N (63–210 nm; Kanto Kagaku), Sephadex LH-20 (Pharmacia). HPLC columns: gel permeation (GS-310 2G; Asahipak), ODS (Mightysil RP-18 GP 250-20; Kanto Kagaku).

3.2. Plant material

The fruits of *J. polycarpus* var. *seravschanica* were collected from Karjantau, Uzbekistan, in September 2003. Herbarium specimens were deposited in the botanical garden of the University of Tokushima (specimen number: UTP031001).

3.3. Extraction and isolation

The fruits of *J. polycarpus* var. *seravschanica* (1.5 kg) were extracted with hot methanol. The methanol extract was successively partitioned with hexane, EtOAc, *n*-BuOH, and H_2O. The hexane layer (82.6 g) was subjected to silica gel column chromatography (CC) with different solvents of increasing polarity (hexane–EtOAc; EtOAc–MeOH) to give fraction 1–11. Fraction 3 (12.4 g) was separated by silica gel CC ($CHCl_3$–MeOH) to give cedrol (16 mg). Fraction 11 (55 mg) was washed with $CHCl_3$ and MeOH to obtain sugiol (32 mg) as an insoluble material.

The EtOAc layer (43.7 g) was subjected to silica gel CC ($CHCl_3$–MeOH) to give fraction 1–10. Fraction 5 was separated by Sephadex LH-20 (MeOH), silica gel CC ($CHCl_3$–MeOH), and ODS (MeOH–H_2O, 3:2) to give **6** (5 mg). Fraction 6 (1.0 g) was separated by Sephadex LH-20 (MeOH), silica gel CC ($CHCl_3$–MeOH), and gel permeation CC (MeOH) to give **5** (12 mg). Fraction 7 (8.8 g) was applied to Sephadex LH-20 (MeOH) to give fraction 7.1–7.4. Fraction 7.2 was separated by silica gel CC ($CHCl_3$–MeOH) to give fraction 7.2.1–7.2.7. Fraction 7.2.6 was purified by ODS (MeOH–H_2O, 3:2) to give **3** (10 mg) and 12,15-dihydroxylabda-8(17),13-dien-19-oic acid (12 mg). Fraction 7.2.7 was separated by silica gel CC ($CHCl_3$–MeOH), followed by Sephadex LH-20 (MeOH), silica gel CC ($CHCl_3$–MeOH), and GPC (MeOH) to give fraction 7.2.7.1–7.2.7.3. Fraction 7.2.7.2 was purified by ODS (MeOH–H_2O, 7:3) to give **4** (6 mg). Fraction 7.2.7.3 was purified by ODS (MeOH–H_2O, 1:1) to give **1** (2 mg) and **2** (2 mg). Fraction 7.4 was separated by silica gel CC ($CHCl_3$–MeOH) to give hinokiflavone (112 mg). Fraction 8 (1.3 g) was separated by Sephadex LH-20 (MeOH) to give fraction 8.1–8.5. Fraction 8.4 was purified by ODS (MeOH–H_2O, 1:1) to give 2,3-dihydrobenzofuran-2-(4′-hydroxy-3′-methoxyphenyl)-3-α-L-rhamnopyranosyloxy-methyl-7-methoxy-5-propanol (5 mg). Fraction 8.5 was separated by ODS (MeOH–H_2O, 1:1) to give 6 fractions and (7*S*,8*S*)-3-methoxy-3′,7-epoxy-8,4′-oxyneoligna-4,9, 9′-triol (24 mg). Fraction 8.5.1 was purified by GPC (MeOH) to give cedrusin (2 mg). Fraction 9 (3.4 g) was separated by Sephadex LH-20 (MeOH) followed by silica gel CC ($CHCl_3$–MeOH) to give fraction 9.1–9.5. Fraction 9.3 was purified by silica gel CC ($CHCl_3$–MeOH) and ODS (MeOH–H_2O, 1:1) to give icaride E_4 (5 mg). Fraction 9.5 was purified by GPC (MeOH) to give (−)-2,3-dihydro-7-hydroxy-3-hydroxymethyl-2-(4′-hydroxy-3′-methoxyphenyl)-5-benzofuranpropanol 4′-*O*-α-L-rhamnopyranoside (54 mg).

3.4. Antimalarial assay

Antimalarial assays were performed as described previously (Guan et al., 2002; Milhous et al., 1985; Desjardins et al., 1979).

3.5. Anti-HIV assay

Anti-HIV assays were performed as described previously (Fujioka et al., 1994; Kilkuskie et al., 1992; Lee et al., 1992)

3.6. 4(15)-Eudesmene-1β,7,11-triol (*1*)

Colorless oil; $[\alpha]_D$ + 27.4 (MeOH, *c* 0.1); EIMS *m/z* (rel. int.): 254 $[M]^+$ (7), 236 (25), 195 (100), 178 (11), 159 (16), 93 (12), 59 (18), 43(15); HREIMS *m/z* 254.1863 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882); IR (KBr) υ_{max} 3397, 2938, 2867, 1646, 1045, 1018 cm^{-1}; ^1H NMR spectral data (400 MHz, $CDCl_3$): δ 4.77 (1H, *d*, *J* = 1.4, H-15a), 4.48 (1H, *d*, *J* = 1.4, H-15b), 3.52 (1H, *dd*, *J* = 4.6, 11.6, H-1), 2.32 (1H, *m*, H-3a), 2.20 (1H, *m*, H-5), 2.18 (1H, *m*, H-3b), 1.88 (1H, *m*, H-2a), 1.79 (1H, *m*, H-9a), 1.61 (1H, *m*, H-8a), 1.60 (3H, *m*, H-2b, 6a and 8b), 1.55 (2H, *m*, H-6b and 9b), 1.29 (3H, *s*, Me-12), 1.28 (3H, *s*, Me-13), 0.68 (3H, *s*, Me-14); for ^{13}C NMR spectroscopic assignments, see Table 1.

3.7. 3-Eudesmene-1β,7,11-triol (*2*)

Colorless oil; $[\alpha]_D$ − 15.4 (MeOH, *c* 0.1); HRFABMS *m/z* 277.1780 $[M + Na]^+$ (calcd for $C_{15}H_{26}O_3Na$, 277.1780); IR (KBr) υ_{max} 3397, 2971, 1666, 1037 cm^{-1}; ^1H NMR spectral data (400 MHz, $CDCl_3$): δ 5.28 (1H, *brs*, H-3), 3.60 (1H, *dd*, *J* = 6.5, 10.2, H-1), 2.35 (1H, *m*, H-5), 2.31 (1H, *m*, H-2a), 1.93 (1H, *m*, H-2b), 1.76 (1H, *m*, H-9a), 1.72 (1H, *m*, H-6a), 1.64 (1H, *m*, H-8a), 1.56 (1H, *m*, H-8b), 1.55 (3H, *s*, Me-15), 1.46 (1H, *m*, H-9b), 1.42 (1H, *m*, H-6b), 1.26 (6H, *s*, Me-12 and 13), 0.76 (3H, *s*, Me-14); for ^{13}C NMR spectroscopic assignments, see Table 1.

3.8. 4-Epi-7,15-dihydroxydehydroabietic acid (*3*)

White amorphous powder; $[\alpha]_D$ + 63.9 (MeOH, *c* 0.4); EIMS *m/z* (rel. int.): 332 $[M]^+$ (20), 317 (100), 253 (24), 235 (10), 195 (29), 59 (25), 43 (60); HREIMS *m/z* 332.2001 $[M]^+$ (calcd for $C_{20}H_{28}O_4$, 332.1988); IR (KBr) υ_{max} 3380, 2969, 1697, 1498, 1045 cm^{-1}; ^1H NMR spectral data (400 MHz, CD_3OD): δ 7.44 (1H, *d*, *J* = 2.0, H-14), 7.38 (1H, *dd*, *J* = 2.0, 8.4, H-12), 7.31 (1H, *d*, *J* = 8.4, H-11), 4.80 (1H, *brt*, *J* = 3.0, H-7), 2.37 (1H, *m*, H-1a), 2.29 (1H, *m*, H-3a), 2.09 (1H, *m*, H-2a), 2.07 (1H, *m*, H-5), 2.04 (2H, *m*, H-6a,b), 1.66 (1H, *m*, H-2b), 1.55 (6H, *s*, Me-16 and 17), 1.44 (1H, *m*, Hb-1), 1.34 (3H, *s*, Me-18), 1.20 (1H, *m*, H-3b), 1.11 (3H, *s*, Me-20); for ^{13}C NMR spectroscopic assignments, see Table 1.

3.9. Derivaization of **3** to obtain 4-epi-7-benzoyl-15-hydroxydehydroabietic acid methyl ester (**3a**)

Trimethylsilildiazomethane (200 μl) was added to **3** (4 mg) dissolved in a mixture of $CHCl_3$–MeOH (1:1) and stirred for 1 h. Then, the solvent was evaporated and the residual was dissolved in CH_2Cl_2. Pyridine and excess of benzoyl chloride were added to the CH_2Cl_2 solution, and kept stirring overnight. 3 mg of **3a** was purified by PTLC ($CHCl_3$–MeOH, 9:1). HRESIMS *m/z* 473.2292 $[M + Na]^+$ (calcd for $C_{28}H_{34}O_5Na$, 473.2304); CD (EtOH, *c* 0.00021 M):$[\theta]_{248} + 130.15$, $[\theta]_{233} - 769.27$, $[\theta]_{225} + 1634.44$, $[\theta]_{218} - 2468.06$; ^{1}H NMR spectral data (400 MHz, $CDCl_3$): δ 8.04 (2 H, *d*, $J = 7.5$), 7.54 (1H, *t*, $J = 7.5$), 7.43 (4H, *m*), 7.33 (1H, *d*, $J = 9.0$), 6.29 (1H, *brs*), 3.67 (3H, *s*), 2.50 (1H, *d*, $J = 15.4$), 2.36 (3H, *m*), 2.06 (2H, *m*), 1.70 (1H, *m*), 1.55 (3H, *s*), 1.54 (3H, *s*), 1.51 (1H, *m*), 1.20 (3H, *s*), 1.16 (1H, *m*), 1.04 (3H, *s*).

3.10. 4-Epi-abieta-8,11,13-triene-7α,15,18-triol (**4**)

White amorphous powder; $[\alpha]_D + 8.0$ (MeOH, *c* 0.2); EIMS *m/z* (rel. int.): 318 $[M]^+$ (40), 303 (98), 300 (61), 289 (11), 267 (100), 49 (10), 59 (59); HREIMS *m/z* 318.2206 $[M]^+$ (calcd for $C_{20}H_{30}O_3$, 318.2195); IR (KBr) υ_{max} 3328, 2929,1698, 1496, 1027 cm^{-1}; ^{1}H NMR spectral data (400 MHz, $CDCl_3$): δ 7.44 (1H, *d*, $J = 2.0$, H-14), 7.35 (1H, *dd*, $J = 2.0$, 8.7, H-12), 7.25 (1H, *d*, $J = 8.7$, H-11), 4.81 (1H, *brs*, H-7), 3.84 (1H, *d*, $J = 10.9$, H-19a), 3.56 (1H, *d*, $J = 10.9$, H-19b), 2.32 (1H, *m*, H-1a), 2.10 (1H, *m*, H-6a), 1.99 (1H, *m*, H-6b), 1.92 (1H, *m*, H-3a), 1.87 (1H, *m*, H-5), 1.74 (1H, *m*, H-2a), 1.66 (1H, *m*, H-2b), 1.57 (6H, *s*, H-16 and 17), 1.43 (1H, *m*, H-1b), 1.13 (3H, *s*, Me-20), 1.08 (3H, *s*, Me-18), 1.05 (1H, *m*, H-3b); for ^{13}C NMR spectroscopic assignments, see Table 1.

3.11. 4-Epi-7α,15-dihydroxypodocarp-8(14)-en-13-one (**5**)

Colorless crystals; $[\alpha]_D - 41.9$ (MeOH, *c* 0.1); CD (EtOH, *c* 0.00036 M): $[\theta]_{375} + 0.74$, $[\theta]_{327} - 1974.48$, $[\theta]_{275} - 18.94$; EIMS *m/z* (rel. int.): 278 $[M]^+$ (31), 260 (57), 229 (50), 151 (60), 123 (100), 81 (73), 69 (82), 55 (87); HREIMS *m/z* 260.1774 $[M–H_2O]^+$ (calcd for $C_{17}H_{24}O_2$, 260.1776); IR (KBr) υ_{max} 3326, 2940, 2883, 1650 cm^{-1}; ^{1}H NMR spectral data (400 MHz, CD_3OD): δ 5.98 (1H, *d*, $J = 2.1$, H-14), 4.33 (1H, *brt*, $J = 2.7$, H-7), 3.79 (1H, *d*, $J = 11.2$, H-16a), 3.41 (1H, *d*, $J = 11.2$, H-16b), 2.59 (1H, *m*, H-9), 2.41 (1H, *m*, H-12a), 2.35 (1H, *m*, H-12b), 2.09 (1H, *m*, H-11a), 2.01 (1H, *m*, H-6a), 1.96 (1H, *m*, H-3a), 1.89 (1H, *m*, H-5), 1.87 (1H, *m*, H-1a), 1.81 (1H, *m*, H-11b), 1.76 (1H, *m*, H-6b), 1.59 (1H, *m*, H-2a), 1.55 (1H, *m*, H-2b), 1.27 (1H, *m*, H-1b), 1.07 (1H, *m*, H-3b), 1.04 (3H, *s*, Me-15), 0.86 (3H, *s*, Me-17); for ^{13}C NMR spectroscopic assignments, see Table 1.

3.12. 7α-Hydroxypodocarp-8(14)-en-13-one-16-oic acid (**6**)

White amorphous powder; $[\alpha]_D - 35.3$ (MeOH, *c* 0.1); EIMS *m/z* (rel. int.): 292 $[M]^+$ (16), 274 (20), 229 (13), 120 (100), 109 (28), 91 (18), 67 (20), 45 (69); HREIMS *m/z* 292.1680 $[M]^+$ (calcd for $C_{17}H_{24}O_4$, 292.1675); IR (KBr) υ_{max} 3262, 2938, 1695, 1039 cm^{-1}; ^{1}H NMR spectral data (400 MHz, CD_3OD): δ 5.99 (1H, *d*, $J = 2.1$, H-14), 4.36 (1H, *brt*, $J = 2.8$, H-7), 2.62 (1H, *m*, H-9), 2.41 (1H, *m*, H-12a), 2.32 (1H, *m*, H-12b), 2.24 (1H, *m*, H-3a), 2.22 (2H, *m*, H-6a,b), 2.08 (1H, *m*, H-11a), 1.93 (2H, *m*, H-1a and 5), 1.90 (1H, *m*, H-11b), 1.57 (1H, *m*, H-2a), 1.54 (1H, *m*, H-2b), 1.30 (1H, *m*, H-1b), 1.28 (3H, *s*, Me-15), 1.16 (1H, *m*, H-3b), 0.81 (3H, *s*, Me-17); for ^{13}C NMR spectroscopic assignments, see Table 1.

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