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Isolation, synthesis, and anti-tumor activities of a novel class of podocarpic diterpenes

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Abstract—A novel unusual 17-carbon diterpenoid, named (+)-7-deoxynimbidiol, was isolated from the stalks of *Celastrus hypoleucus* (*Oliv.*) Warb. Its racemate and derivatives were synthesized, and the inhibitory activities of these compounds against four cultured human-tumor cell lines were evaluated. The structure–activity relationship was discussed.

Cancer remains the second leading cause of death in most of the countries and as a result there is a need for effective compounds. To totally synthesize and modify structure of bioactive natural products is an effective approach to find some potential compounds with anti-tumor activity. The natural tri-ring diterpene is a significant kind of potential anti-cancer agent.[1–5] Some tri-ring diterpenes from natural plants have been synthesized or modified, and their anti-tumor activities have been investigated.[6–11] During our chemical studies on the bioactive compounds from the plants of Celastraceae family, a novel podocarpic diterpene (+)-7-deoxynimbidiol (Fig. 1)[12], which attracts our intensive interest for its good anti-tumor activity, was isolated from *Celastrus hypoleucus.* We wish to find some potent anti-tumor agents by modifying the structure of (+)-7-deoxynimbidiol.

In this paper, we report a convenient and high-yield route to totally synthesize the (±)-7-deoxynimbidiol (**1**) and its diastereoisomer named *cis*-(±)-7-deoxynimbidiol (**2**)[13] along with isolation of (+)-7-deoxynimbidiol. In modifying the structure of **1**, two different approaches were designed to find out the pharmacophore including etherification of the phenolic hydroxyl with the substituted benzyl bromide and oxidation of the C-6 or C-7. The diastereoisomer **2** was treated in a similar manner. A class of analogs of the (+)-7-deoxynimbidiol (all the compounds are racemic) were synthesized, and their anti-tumor activities against four cultured human-tumor cell lines (HeLa, A549, CNE, and MCF) have been investigated.

The shade-dried stalks (10 kg) were extracted with methanol, and 514 g of extract was obtained, which was partitioned with petroleum ether, EtOAc, and *n*-BuOH successively. The petroleum ether extract (103 g) was subjected to column chromatography (CC) over silica gel (200–300 mesh, 2 kg) eluting with petroleum ether/EtOAc (10:0–0:10, gradients) to afford 5 fractions. First fraction was separated on silica gel CC (300–400 mesh, 100 g) repeatedly, using *n*-hexane/acetone (20:1) as eluent to yield pure (+)-7-deoxynimbidiol (20.1 mg).

Synthesis of the (±)-7-deoxynimbidiol was from the cyclization reaction of geranic acid **3**. The cyclocitric acid **4** was methylated by dimethyl sulfate in acetone with the presence of potassium carbonate. Methyl ester cyclocitric acid **5** was reduced by $LiAlH_4$ in absolute diethyl ether, and then the obtained cyclocitric alcohol

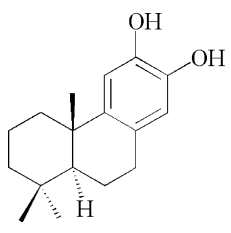

Figure 1. The structure of (+)-7-deoxynimbidiol.

Keywords: Diterpene; Isolation; Synthesis; Anti-tumor activity.
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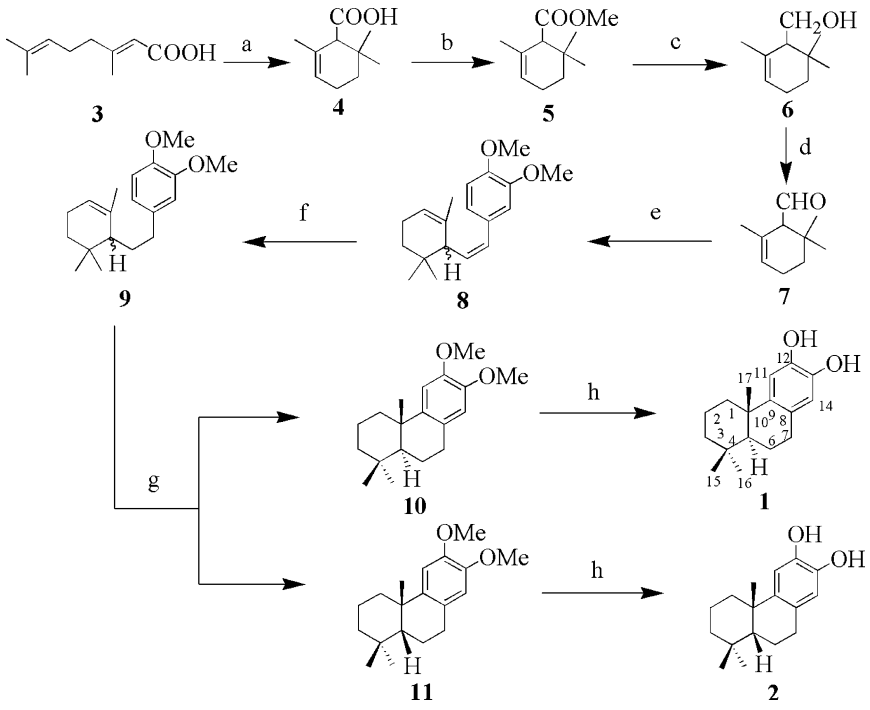
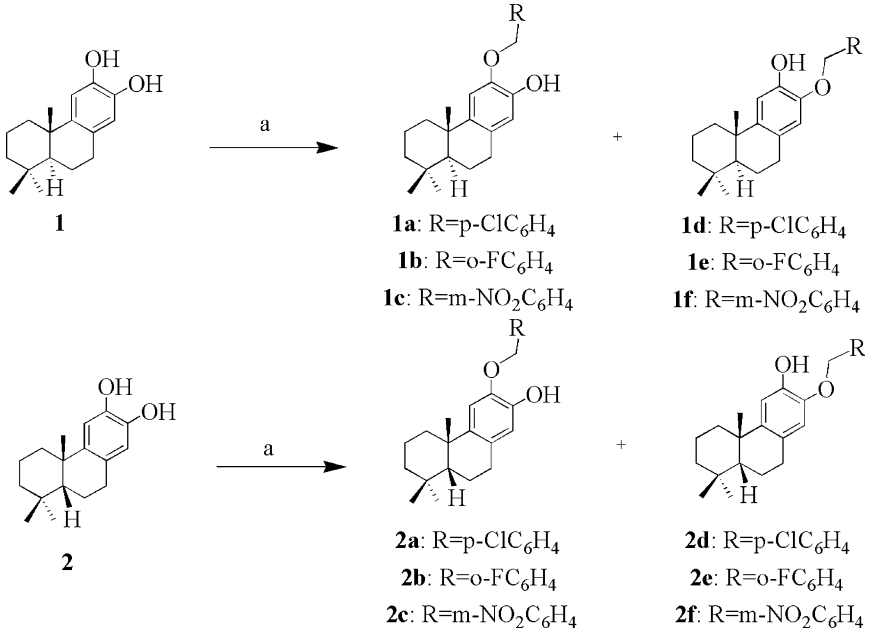
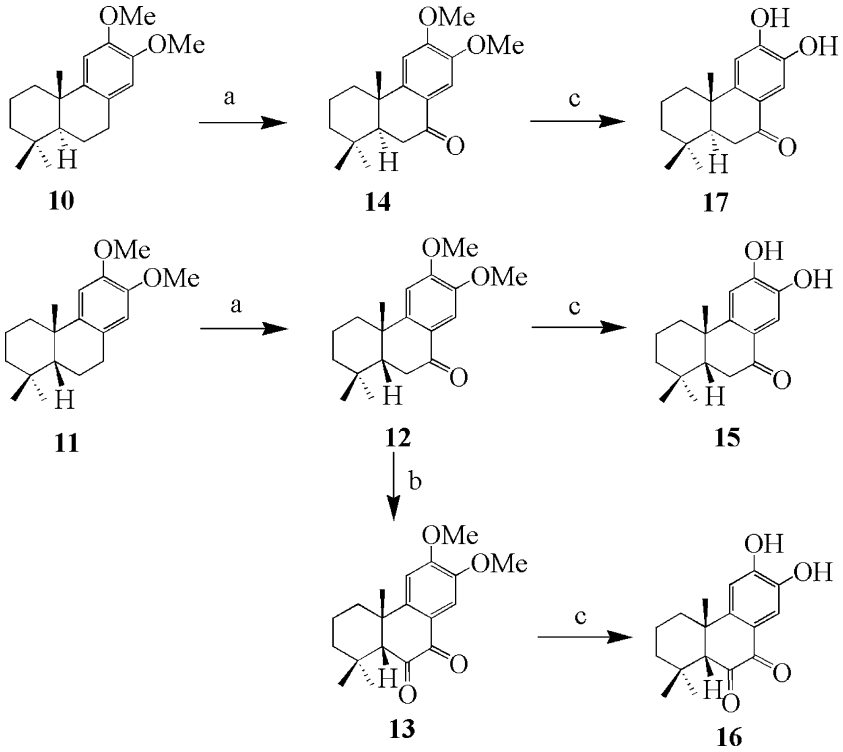


Scheme 1. Reagents and conditions: (a) 85% phosphate acid, toluene, 100 °C, 2 h, 82%; (b) Me_2SO_4, K_2CO_3, acetone, rt, 5 h, 91%; (c) Ar, $LiAlH_4$, absolute diethyl ether, rt, 4 h, 85%; (d) PCC, CH_2Cl_2, rt, 4 h, 55%; (e) Ar, *n*-BuLi, 3,4-dimethyl-benzyl triphenyl phosphonium chloride, absolute THF, rt, 6 h, 74.5%; (f) H_2, 10% Pd/C, diethyl ether, 30 min, quantitative yield; (g) $BF_3{\cdot}(C_2H_5)O$, CH_2Cl_2, 24 h, 83.5%; (h) BBr_3, CH_2Cl_2, 0–5 °C, 2 h.



Scheme 2. Reagents and conditions: (a) substituted benzyl bromide, NaI, K_2CO_3, acetone, reflux, 5 h.

6 was dissolved in methylene chloride and oxidized by the pyridine chromium trioxide chloride (PCC) to get cyclocitric aldehyde **7** (35% yields of total four steps). After **7** was added to the clear solution of *n*-BuLi and 3,4-dimethyl-benzyl triphenyl phosphonium chloride in absolute THF at room temperature, the mixture was stirred for 3 h to yield the olefin **8** (74.5%). The double bond at C6-C7 of **8** was hydrogenated with H_2 and 10% Pd/C in diethyl ether to yield **9** (quantitative yield). **9** was treated with the boron trifluoride etherate ($BF_3{\cdot}(C_2H_5)_2O$) in methylene chloride at room temperature and the mixture stood for 24 h, given the product **10** and its diastereoisomer **11** (isomer ratio = 2:1). Treatment of the methyl ether **10** with boron tribromide in methylene chloride at 0–5 °C for 2 h yielded the target molecular (±)-7-deoxynimbidiol (76.3%). The diastereoisomer **2** was obtained from **11** in the same manner as described above (74.5%) (Scheme 1). Compound **1** was



Scheme 3. Reagents and conditions: (a) CrO_3, CH_2Cl_2, rt, 2 h; (b) CrO_3, CH_2Cl_2, 50 °C, 2 h; (c) BBr_3, CH_2Cl_2, 0–5 °C, 2 h.

Table 1. Biological activities of the synthesized compounds

Compound	HeLa[a] IC_{50} (μg/ml)[b]	A549 IC_{50} (μg/ml)	CNE IC_{50} (μg/ml)	MCF IC_{50} (μg/ml)
1	24.3 (±0.98)	19.6 (±0.22)	25.1 (±0.17)	41.2 (±0.11)
2	10.9 (±0.48)	5.7 (±0.24)	3.6 (±0.22)	9.6 (±0. 51)
10	41.5 (±0.23)	41.7 (±0.09)	25.1 (±0.25)	39.5 (±0.07)
11	13.2 (±0.04)	9.6 (±0.07)	4.5 (±0.09)	10.9 (±0.11)
1a	>50	>50	>50	>50
1b	>50	>50	>50	>50
1c	>50	>50	>50	>50
1d	>50	>50	>50	>50
1e	>50	>50	>50	>50
1f	>50	>50	>50	>50
2a	42.2 (±0.07)	46.9 (±0.15)	27.2 (±0.04)	>50
2b	15.3 (±0.97)	13.7 (±0.27)	7.2 (±0.16)	12.4 (±0.09)
2c	>50	36.4 (±0.18)	32.9 (±0.34)	>50
2d	>50	>50	>50	>50
2e	13.4 (±0.25)	35.0 (±0.33)	17.8 (±0.78)	42.9 (±0.05)
2f	18.6 (±0.08)	>50	>50	>50
12	12.7 (±0.13)	26.3 (±0.08)	3.1 (±0.05)	7.8 (±0.22)
13	16.1 (±0.09)	33.8 (±0.06)	5.6 (±0.54)	16.5 (±0.07)
14	25.7 (±0.06)	>50	>50	>50
15	17.6 (±0.45)	>50	13.5 (±0.02)	36.2 (±0.14)
16	18.0 (±0.09)	25.8 (±0.11)	9.1 (±0.12)	24.8 (±0.12)
17	27.7 (±0.02)	>50	22.1 (±0.07)	>50
(+)-7-Deoxynimbidiol	42.0 (±1.25)	36.1 (±0.03)	12.1 (±0.33)	36.5 (±0.04)
CDDP[c]	5.89 (±0.15)	3.58 (±0.14)	2.34 (±0.04)	6.53 (±0.25)

[a] Cultured human-tumor cell lines: HeLa, cervical carcinoma; A549, lung adenocarcinoma; CNE, nasopharyngeal carcinoma; MCF, breast adenocarcinoma.

[b] Values are means of three experiments, standard deviation is given in parentheses.

[c] CDDP was used as positive control.

added to the mixture of sodium iodide, potassium carbonate, and substituted benzyl bromide in acetone, and then the mixture was stirred at 50–60 °C for 5 h to give the products **1a**–**1f** in which 12-OH or 13-OH was etherified solely. In the same method, we obtained the compounds **2a**–**2f** from compound **2**. The ratio of 12-substituted products and 13-substituted products was 1:1 (Scheme 2).

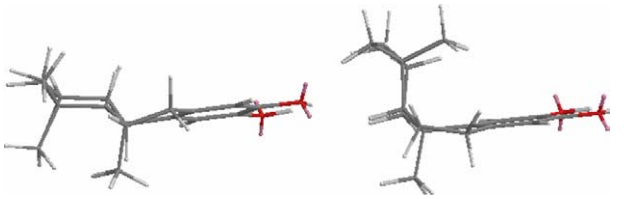


Figure 2. Comparison of the backbone structures of compounds **1** and **2**.

Treatment of the compound **11** with chromium trioxide in acetic acid at room temperature for 2 h yielded the C-7 oxidized compound **12** (81.3%). When the temperature was increased to 50 °C, compound **13** (66.5%) in which both C-7 and C-6 were oxidized appeared. But for the compound **10**, we didnot find the further oxidized product besides compound **14** (85.6%) obtained by oxidizing C-7. The compounds **12**, **13**, and **14** were demethylated with boron tribromide in methylene chloride at 0–5 °C for 2 h to yield **15** (58.2%), **16** (44.7%), and the known nature product (±)-nimbidiol **17** (62.4%), respectively (Scheme 3).

The biologic activities of (+)-7-deoxynimbidiol and all synthesized compounds were assessed against cultured human-tumor cell lines using the MTT assay[14] (Table 1). Compounds **2**, **11**, **2b**, **2e**, and **12** showed potential activities against HeLa; compounds **2**, **11**, and **2b** showed potential activities against A549; compounds **2**, **11**, **2b**, **12**, **13**, and **16** showed potential activities against CNE; compounds **2**, **11**, **2b**, and **12** showed potential activities against MCF. As shown in Table 1, the racemate **1** showed better activity than the (+)-enantiomer against HeLa and A549. The compounds in which 13-OH was etherified (**2e**, **2d**, and **2f**) had as much potent activity as those in which 12-OH was etherified (**2a**, **2b**, and **2c**). Comparing the methylated compounds with those demethylated, the anti-cancer activity had no obvious contrast. We obtained an attractive matter from Table 1 that the bioactivities of the compounds with 5-β-H were prominently better than those of the analogs with 5-α-H. With the purpose of developing a preliminary SAR concept, the major conformations of compounds **1** and **2** were investigated with MM2 energy minimization by Chem3D program (Fig. 2). The skeleton of the tricycle of the compound **1** was found close to planar configuration, but that of the compound **2** showed distinctly a hooked configuration. Further research of the relation between this structural diversity and the bioactivity is in progress.

In summary, a novel podocarpic diterpene (+)-7-deoxynimbidiol was isolated and synthesized conveniently, whose structure was modified as a lead compound. **2**, **11**, and **2b** were identified to have potent anti-cancer activity. Further SAR studies to find more potent antagonists are being pursued.

Acknowledgment

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12. Physical characters and spectral data for the (+)-7-deoxynimbidiol. White powder; mp (petroleum ether/EtOAc (5:1)) 90–92°; $[\alpha]_D^{20}$ = +49.44° (*c* 0.10, MeOH); UV (MeOH) λ_{max}: 288, 212 nm; IR (KBr) cm^{-1}: 3362, 1607, 1515; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.90 (s, 3 H, H-C(15)), 0.93 (s, 3H, H-C(16)), 1.14 (s, 3H, H-C(17)), 1.17–1.75 (m, 7H, H-C(1,2,3,5,6)), 1.85 (m, 1H, H-C(6)), 2.15 (m, 1H, H-C(1)), 2.78 (m, 2H, H-C(7)), 6.52 (s, 1H, H-C(14)), 6.75 (s, 1H, H-C(11)); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.33 (t, C-2), 19.58 (t, C-6), 21.81 (q, C-16), 25.04 (q, C-17), 30.02 (t, C-7), 33.52 (q, C-15), 33.62 (s, C-4), 37.59 (s, C-10), 39.31 (t, C-1), 41.89 (t, C-3), 50.81 (d, C-5), 111.70 (d, C-11), 115.47 (d, C-14), 128.31 (s, C-8), 141.19 (s, C-13), 141.52 (s, C-12), 143.58 (s, C-9); HRMS (ESI) calcd for $C_{17}H_{24}O_2Na$ $[M+Na]^+$ 283.1669 found 283.1667.
13. Spectral data for the key compounds **1** and **2**. Compound **1**, ^1H NMR ($CDCl_3$, 500 MHz) δ 0.92 (s, 3 H), 0.95 (s, 3H), 1.15 (s, 3H), 1.19–1.76 (m, 7H), 1.86 (m, 1H), 2.16 (m, 1H), 2.79 (m, 2H), 5.08 (b, 2H), 6.53 (s, 1H), 6.77 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 19.31, 19.56, 21.82, 25.13, 30.06, 33.54, 33.62, 37.60, 39.29, 41.89, 50.73, 111.71, 115.41, 128.25, 141.19, 141.54, 143.55; HRMS (ESI) calcd for $C_{17}H_{24}O_2Na$ $[M+Na]^+$ 283.1669 found 283.1673. Compound **2**, ^1H NMR ($CDCl_3$, 500 MHz) δ 0.41 (s, 3H), 0.91 (s, 3H), 1.11 (s, 3H), 1.25–1.47 (m, 6H), 1.92 (m, 1H), 2.14 (m, 1H), 2.28 (m, 1H), 2.76 (m, 2H), 4.92 (b, 2H), 6.53 (s, 1H), 6.79 (s, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 18.28, 19.53, 22.95, 25.85, 32.98, 34.65, 34.75, 36.99, 38.51, 43.41, 50.21, 111.59, 115.71, 130.41, 137.01, 141.00, 141.39; HRMS (ESI) calcd for $C_{17}H_{24}O_2Na$ $[M+Na]^+$ 283.1669 found 283.1671.
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