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First formal synthesis of (+)-nimbidiol. Synthesis, X-ray structure and anticancer activity of a novel ring C aromatic diterpene: dimethyl (+)-podocarpa-8,11,13-triene-12,13-dicarboxylate

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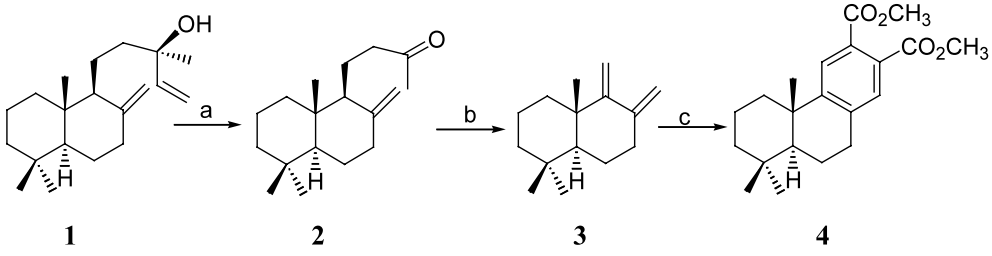
Abstract—A novel ring C aromatic diterpene (**4**) has been prepared in three steps from natural (+)-manool (**1**). The structure and anticancer activity data for **4** has been investigated. This key intermediate (**4**) was easily transformed into 7-deoxo nimbidiol dimethyl ether (**8**). The present work represents the first formal synthesis of (+)-nimbidiol (**10**).

Abietane and biosynthetically related polycyclic diterpenes are a major group of ring C aromatic diterpenes.[1] Abietane diterpenes show a wide range of biological activities, e.g. antibiotic,[2] antivirus,[3] antioxidant,[4] antimalarial[5] and cytotoxic[6] activity. This enormous biological data has motivated numerous synthetic investigations of racemic abietanes via polyene cyclization.[7] While enantioselective syntheses of abietane diterpenes are known, they are scarce.[8]

Commercially available (+)-manool (**1**)[9] has been used as a key intermediate for the efficient syntheses of drimane[10] and abietane-type[11] terpenes. Common to all these endeavors was the use of naturally occurring (+)-manool as starting material. In this study, two cleavage reactions (one oxidative and one photochemical) were used sequentially to transform (+)-manool to the exocyclic diene **3** in 52% overall yield. The utility of diene **3** is demonstrated for the synthesis of diterpene **4**. We report herein the synthesis, structural and complete anticancer activity data for diester **4** and its synthetic application in the formal synthesis of (+)-nimbidiol (**10**).

Synthesis of compound **4**: Our synthesis started with diene **3**,[15b] which was readily prepared from (+)-manool (**1**) in two steps and 52% overall yield (Scheme 1).[12] To construct the C-ring of abietane we envisioned using a Diels–Alder reaction between diene **3** and dimethyl acetylenedicarboxylate (DMAD) with a concomitant aerobic oxidation.[13] In practice, the transformation worked as planned after significant optimization. The



1 **2** **3** **4**

Scheme 1. (a) $KMnO_4$, acetone, rt; (b) *hv*, pentane, −30°C, 52% overall yield; (c) DMAD, 110–220°C, xylene, 3 days, 48%.

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Diels–Alder adduct was obtained by heating a mixture of diene and DMAD (1:3 ratio) in a sealed tube at 110°C for 24 h. To induce aromatization, the temperature was rapidly increased to 220°C for additional 48 h. The yield was strongly dependent on the reaction temperature (e.g. >220°C gave decomposition, <220°C showed incomplete conversion) and stoichiometry (e.g. threefold excess of alkyne was required to compensate its lost by thermal polymerization).[14] Under these optimized conditions for aromatization[15a] the desired product (**4**) was obtained in 48% isolated yield.[15c]

X-Ray determination of compound **4**:[18b] While the spectral data (^{1}H and ^{13}C NMR), elemental analyses and LR/HRMS for compound **4** were completely consistent with its structure, further confirmation was sought by means of single crystal X-ray analyses. X-Ray quality crystals of **4** were grown by slow evaporation of a hexane–ethyl acetate solution. An ORTEP depiction of **4** is shown in Figure 1. It is interesting to notice that two independent molecules were found in the asymmetric unit and have different conformations and geometric parameters. The molecular dimensions for both structures in the rings A and B are slightly different compared to the previously reported values for abietane compounds,[16] with mean bond distances being C(sp3)–C(sp3) 1.492 Å for structure **I** and 1.491 Å for structure **II**. The longest C–C distance in structure **I** [1.564 (5) Å] was found at the ring A/B junction according to previous reports.[17] However, structure **II** showed its longest C–C bond in ring A [C31–C36 = 1.561 (5) Å]. In both structures the carbonyl esters are located *anti* to each other in order to minimize unfavorable dipole–dipole interactions. Neither carbonyl groups are coplanar with the aromatic ring in structures **I** and **II**, according to their dihedral angles C17–C16–C18–O1 = 31.6° and C47–C46–C48–O10 = 46.8° respectively. The aromatic ring is slightly twisted near the decalin system (C17–C10–C11–C14 = 3.8° for **I** and C47–C40–C41–C44 = 1.2° for **II**).[18a]

Antitumor testing of compound **4**: Evaluation of antitumor activity was performed on compound **4** at the National Cancer Institute (NCI), following the known in vitro oriented antitumor screening program against a panel of 62 tumor cell lines derived from nine cancer types (leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast) according to a standard protocol.[19] In each test, dose–response curves for each cell line were measured with five different drug concentrations. The concentration causing 50% cell growth inhibition (GI_{50}) was calculated. Table 1 shows the biological data for selected cell lines. Compound **4** was active against all cell lines with mean log GI_{50} values ranging from −4.51 (ovarian cancer/OVCAR-8) to −6.29 (breast cancer/T-47D). The cytotoxicity activity against human leukemia cell (HL-60) of **4** (Table 1, log $GI_{50} = -5.06$) showed a strong similarity with the value reported for incanone, a structurally related natural abietane diterpene (−5.22).[20] As previously mentioned, compound **4** was particularly active against breast cancer cell T-47D. However, labdane diterpenoids isolated from natural sources have shown non-specific cytotoxicities against several tumor cell lines.[21]

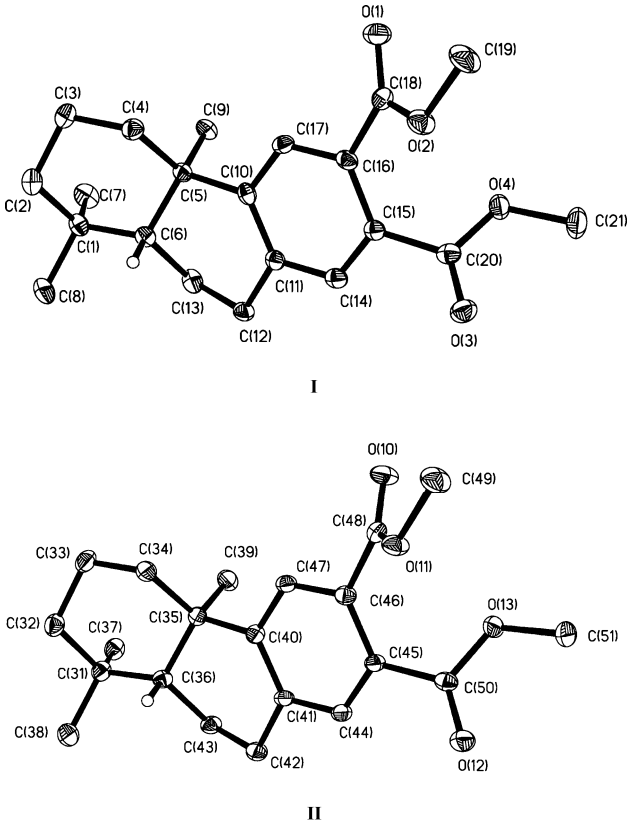


Figure 1. ORTEP plot of **4** (structures **I** and **II**). Displacement ellipsoids are drawn at 50% probability level and H atoms are omitted for clarity

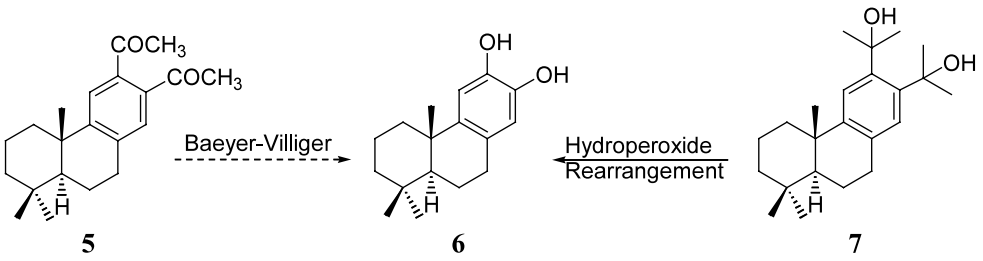
Table 1. In vitro selected antitumor activity data for **4**

Cell line	Cytotoxicity log GI_{50} (M)[a,b]
Leukemia (HL-60 TB)	−5.06
Non-small cell lung (HOP-92)	−5.00
Colon (KM12)	−4.80
CNS (SF-268)	−4.80
Melanoma (MALME-3M)	−5.91
Ovarian (IGROV1)	−4.98
Renal (RXF 393)	−4.99
Prostate (DU-145)	−4.84
Breast (T-47D)	−6.29
Mean value	−5.19

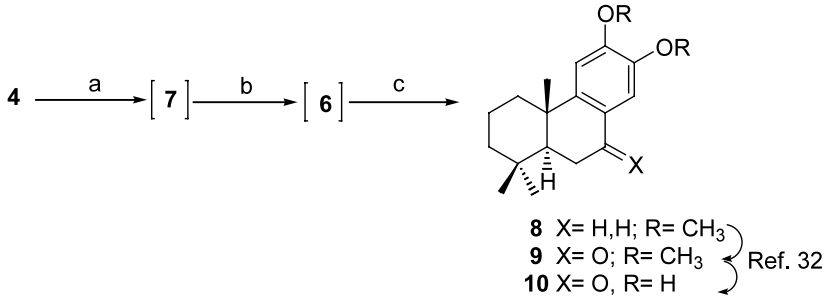
[a] Data obtained from NCIs in vitro tumor cells screen.
[b] Mean value over all 62 cell lines tested.

Formal synthesis of (+)-nimbidiol (10):

To further demonstrate the utility of this methodology, we investigated the use of diester **4** as a chiral building block for the synthesis of (+)-nimbidiol (**10**).[22] (+)-Nimbidiol (**10**) is a diterpene isolated from the root bark of *Azadirachta indica*.[23] Thus, we investigated the conversion of the two aromatic ester groups of **4** into the 1,2-*bis*-phenols of nimbidiol. We initially attempted this



Scheme 2.



Scheme 3. (a) MeLi (8 equiv.), $CeCl_3$, THF, −78°C; (b) 30% H_2O_2, *p*-TsOH cat., THF, 0°C to rt; (c) CH_2N_2, ether, 45% overall yield.

transformation by means of a Baeyer–Villiger reaction on the corresponding dimethyl ketone **5** with the hope of selectively forming **6** (Scheme 2). Unfortunately the formation of dimethyl ketone **5** using MeLi/Me_3SiCl[24] or MeMgCl[25] gave a complex crude mixture from which the desired dimethyl ketone could not be isolated.[26] Next we investigated the selective oxidation of the two carbon–carbon bonds of the bis-tertiary alcohol **7** by a hydroperoxide rearrangement.[27] Exhaustive methylation of diester **4** with excess MeLi (8 equiv.)[28] gave the diol **7**, which due to its instability to silica gel chromatography was used as is (Scheme 3). Exposure of the crude diol **7** to a solution of hydrogen peroxide in acidic THF[29] yielded 7-deoxo nimbidiol **6**[30] which was immediately dimethylated to give the known 7-deoxo nimbidiol dimethyl ether **8** in 45% overall yield (Scheme 3).[31] The two steps conversion of **8** to nimbidiol **10** has previously been reported by Majetich and co-workers.[32]

This work provides a short formal synthesis of natural (+)-nimbidiol (**10**) from (+)-manool (**1**). The key intermediate for such preparation protocol is diterpene **4**, which was easily accessible from diene **3**. The first X-ray structure of a (+)-manool-derived diterpene with ring C aromatic was also investigated. Biological in vitro testing of diterpene **4** against 62 cell human tumor cell lines showed particularly strong activity for breast cancer (T-47D, $\log GI_{50} = -6.29$).

Supplementary material

Experimental procedure and characterization data for compounds **2**, **3** and **8**, crystallographic data for compound **4**.

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15. (a) Mori, K.; Watanabe, H. *Tetrahedron* **1986**, *42*, 273–281; (b) Compound **2**:[12] IR (KBr) 2939, 1717, 1643, 1363, 1160, 888 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 4.79 (s, 1H), 4.41 (s, 1H), 2.58–2.52 (m, 2H), 2.37–2.33 (m, 1H), 2.31–2.24 (m, 1H), 2.07 (s, 3H), 1.95–1.89 (m, 1H), 1.84–1.75 (m, 2H), 1.71–1.67 (m, 1H), 1.55–1.44 (m, 4H), 1.38–1.35 (m, 1H), 1.31–1.23 (m, 1H), 1.18–1.14 (m, 1H), 1.07–1.01 (m, 2H), 0.83 (s, 3H), 0.77 (s, 3H), 0.66 (s, 3H). ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 209.4, 148.3, 106.3, 56.3, 55.5, 42.9, 42.1, 39.8, 39.0, 38.3, 33.6, 33.5, 30.0, 24.4, 21.7, 19.3, 17.5, 14.3. HRMS (EI) m/z calcd for $C_{18}H_{30}O$ 262.2297, found 262.2298. Compound **3**:[12] IR (KBr) 2927, 1459, 1261, 892 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 4.78 (dd, $J=2.29$, 2.75 Hz, 1H), 4.74 (d, $J=1.83$ Hz, 1H), 4.63 (dd, $J=2.29$, 2.75 Hz, 1H), 4.52 (d, $J=1.83$ Hz, 1H), 2.45 (ddd, $J=2.29$, 4.58, 13.75 Hz, 1H), 2.11 (tdt, $J=2.29$, 5.5, 13.29 Hz, 1H), 1.74–1.69 (m, 1H), 1.65–1.57 (m, 2H), 1.55–1.39 (m, 4H), 1.18 (td, $J=4.13$, 9.17 Hz, 1H), 1.11 (dd, $J=2.75$, 12.83 Hz, 1H), 0.94 (s, 3H), 0.87 (s, 3H), 0.85 (s, 3H). ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 161.8, 150.1, 108.9, 103.1, 52.5, 42.3, 40.2, 37.6, 35.9, 33.8, 33.4, 22.7, 22.0, 20.7, 19.2. HRMS (EI) m/z calcd for $C_{15}H_{24}$ 204.1878, found 204.1877; (c) Compound **4**: A mixture of diene **3** (0.15 g, 0.78 mmol), DMAD (0.33 g, 2.32 mmol) and xylene (1 mL) was heated in a sealed tube under vacuo at 110°C for 24 h and then at 220°C for an additional 48 h. After cooling, the solvent was evaporated and the crude mixture was directly chromatographed over silica gel (1%–5% diethyl ether/hexane) to give **4** as a white solid (0.13 g, 48%). Mp 84–86°C, $[\alpha]_D$ +50.4 (*c* 0.8, $CHCl_3$). IR (KBr) 3008, 2961, 2920, 2842, 1725, 1609, 1438, 1308, 1135, 979 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.61 (s, 1H), 7.35 (s, 1H), 3.85 (s, 6H), 2.99–2.94 (dd, $J=6.41$, 17.4 Hz, 1H), 2.89–2.81 (m, 1H), 2.31–2.28 (m, 1H), 1.91–1.87 (m, 1H), 1.78–1.58 (m, 3H), 1.49–1.46 (m, 1H), 1.37 (dt, $J=3.67$, 13.29 Hz, 1H), 1.26 (dd, $J=2.29$, 12.83 Hz, 1H), 1.21 (dd, $J=4.14$, 13.29 Hz, 1H), 1.15 (s, 3H), 0.94 (s, 3H), 0.91 (s, 3H).^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 168.5, 168.4, 153.4, 139.3, 129.6, 128.9, 128.9, 125.6, 52.4, 52.4, 49.9, 41.5, 38.5, 38.1, 33.5, 33.2, 30.2, 24.6, 21.6, 19.1, 18.6. LRMS (EI): 297 (100), 329 (68), 344 (24), 247 (24), 229 (34). HRMS (EI) m/z calcd for $C_{21}H_{28}O_4$ 344.1987, found 344.1989. Anal. calcd for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.09; H, 7.98.
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30. 7-Deoxo nimbidiol was rapidly derivatized due to its instability.
31. Compound **8**: IR (KBr) 3070–2820, 1605, 1515, 1450, 1228, 1070 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz) δ 6.80 (s, 1H), 6.50 (s, 1H), 3.85 (s, 3H), 3.81 (s, 3H), 2.78–2.88 (m, 2H), 2.18–2.45 (m, 2H), 1.19–1.95 (m, 7H), 1.18 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H). ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 146.9, 146.6, 142.1, 127.3, 111.3, 107.9, 55.9, 55.6, 50.6, 41.5, 39.0, 37.4, 33.3, 33.2, 30.1, 24.8, 21.6, 19.2, 19.1. LRMS (EI, $[M^+]$ for $C_{19}H_{28}O_2=288.2$): 288 (35), 245 (100), 273 (32), 191 (33).
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