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Cytotoxic Sesquiterpenes from *Nardostachys chinensis*

Hideji ITOKAWA,* Koichi MASUYAMA, Hiroshi MORITA and Koichi TAKEYA

Department of Pharmacognosy, Tokyo College of Pharmacy, Horinouchi 1432–1, Hachioji, Tokyo 192–03, Japan.
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Five cytostatic sesquiterpenes, desoxo-narchinol-A (1), nardosinone (2), debilon (3), nardosinonediol (4) and kanshone A (5), were isolated from the roots and rhizomes of *Nardostachys chinensis* (Valerianaceae). The steric structure of 1 was determined by nuclear Overhauser effects (NOEs) and the exciton chirality method. All five showed cytotoxic activity against P-388 cells and the structure–activity relationship of 1 was also discussed.

Keywords desoxo-narchinol-A; cytotoxic activity; *Nardostachys chinensis*; Valerianaceae; sesquiterpene

Introduction

The roots and rhizomes of *Nardostachys chinensis* have been used for stomach complaints and sedative purposes in Oriental medicine. During our survey of novel cytotoxic antitumour compounds from medicinal plants,[1] we found that the methanolic extract of *N. chinensis* showed cytotoxic activity (IC_{50} 21 μg/ml) against P-388 cells.

Chromatographic purification of the components exhibiting cytotoxicity led us to isolate some sesquiterpenes from *N. chinensis*. In this paper, the structural elucidation of these cytotoxic principles and their structure–activity relationships are reported.

Results and Discussion

The methanol extract of *N. chinensis* was successively partitioned between *n*-hexane, ethyl acetate, *n*-butanol and water. The cytotoxic activity of *N. chinensis* was concentrated in the ethyl acetate soluble fraction, which showed a cytotoxic activity of IC_{50} 12.5 μg/ml against P-388 cells. Repeated chromatographic purification, conducted in conjunction with bio-assay against P-388 cells, led to the isolation of five sesquiterpenes, desoxo-narchinol-A (**1**), nardosinone (**2**), debilon (**3**), nardosinonediol (**4**) and kanshone A (**5**).

Compound **1** was obtained as colourless needles, mp 105—109 °C, $[\alpha]_D$ $-186.0°$ ($c=0.21$, $CHCl_3$) with the molecular formula, $C_{12}H_{16}O_2$ (192.1128), suggesting a trinor-sesquiterpene. In the ^{1}H-nuclear magnetic resonance (NMR) spectrum ($CDCl_3$), two methyl groups at δ 0.95 and 0.96, an equally coupled ($J=10.0$ Hz) olefinic protons at δ 6.14 and 7.04 indicated the presence of an α, β-unsaturated carbonyl group and an olefinic proton at δ 7.04 indicated a β-proton on an α, β-unsaturated carbonyl group. Furthermore, a proton at δ 4.06 attached to the carbon bearing a hydroxyl group was coupled to the β-proton at δ 7.04. Infrared (IR) absorptions at 3650 and 1680 cm^{-1} revealed the presence of hydroxyl and conjugated carbonyl groups, respectively.

TABLE I. Cytotoxic Activities of Compounds **1**—**10** against P388 Cells

Compd.	IC_{50} (μg/ml)	Compd.	IC_{50} (μg/ml)
1	2.2	**6**	0.14
2	18.5	**7**	0.11
3	13.0	**8**	0.60
4	13.0	**9**	0.48
5	7.0	**10**	0.32

From these data, desoxo-narchinol-A derived from nardosinone[2] was deduced to have the structure **1**. ^{13}C-NMR data also corroborated this structure. However, as the configuration at C-6 was not clear, it was determined by NOEs and the allylic alcohol exciton chirality method[3] as follows. In the ^{1}H-NMR spectrum (pyridine-d_5), irradiation of a singlet methyl signal at δ 0.94 increased an integrated intensity of H-6 at δ 4.21. Then, the benzoate derivative (**6**) of **1** showed a negative Cotton effect at 241 nm. Therefore, the configuration at C-6 was determined to be *S*. This is the first example isolated from natural sources.

The structures of compounds **2**—**5** were found to be identical to nardosinone (**2**),[4] debilon (**3**),[5] nardosinonediol (**4**)[6] and kanshone A (**5**),[4] respectively, by comparing spectroscopic data.

All of the compounds obtained showed cytotoxic activities against P-388 cells (Table I). Compound **1**, which showed the most potent activity, was converted to the quinone-type compound **7** by oxidation with pyridinium chlorochromate (PPC) and to several esters; benzoate (**6**), acetate (**8**), propionate (**9**) and *n*-butyrate (**10**). As is clearly shown in Table I, the quinone-type compound **7** showed the most potent activity and increasing the ester chain length was considered to lead to increased cytotoxicity.

Experimental

All melting points were recorded on a Yanagimoto MP-3 micromelting

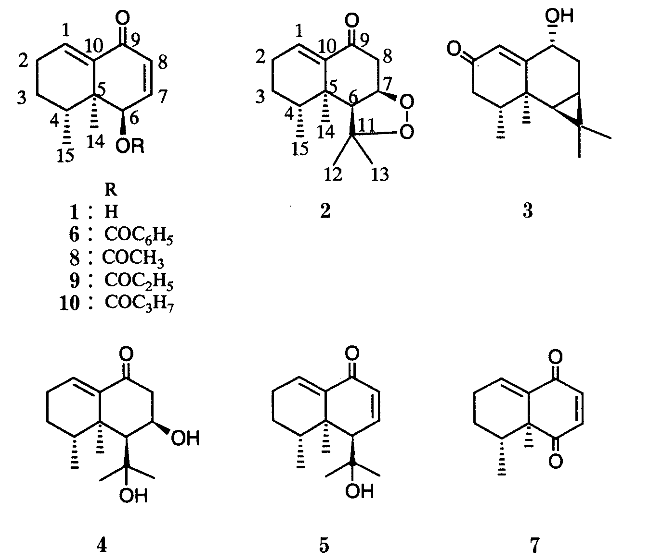

Fig. 1. Molecular Structures of Compounds **1**—**10**

point apparatus and are uncorrected. The spectral data were obtained on the following instruments: optical rotation on a JASCO DIP-4, IR spectra on a JASCO A-302, ultraviolet spectra (UV) on a Hitachi 557, NMR spectra on a Bruker AM400 and mass spectra (MS) on a Hitachi M-80. Medium-pressure liquid chromatography (MPLC) was carried out on a CIG column (Kusano Scientific Co., Tokyo) packed with 10 μm silica gel as the stationary phase. Reversed-phase high-pressure liquid chromatography (HPLC) was carried out on an Inertsil PREP-ODS column packed with 10 μm octadecyl silica (ODS).

Bioassay of Cytotoxic Activity against P388 Cells See previous paper.[7]

Extraction and Isolation The roots and rhizomes of *N. chinensis* (1.0 kg, purchased from Uchidawakanyaku, Tokyo, 1991) were extracted three times with hot methanol and concentrated to give a methanolic extract (204 g). This extract was successively partitioned between *n*-hexane, ethyl acetate, *n*-butanol and water. The cytotoxic activity was concentrated in the ethyl acetate soluble fraction (71 g), which was subjected to silica gel column chromatography using a methylene chloride–methanol gradient system. Further chromatographic purification of the active fraction by silica gel MPLC (*n*-hexane–ethyl acetate solvent system) and ODS-HPLC (methanol–water and acetonitrile–water solvent system) led to the isolation of desoxo-narchinol-A (**1**: 337 mg), nardosinone (**2**: 265 mg), debilon (**3**: 85 mg), nardosinonediol (**4**: 1225 mg) and kanshone A (**5**: 485 mg).

Desoxo-narchinol-A (**1**): Colorless needless, mp 105—109 °C, $[\alpha]_D$ −186° (c=0.21, $CHCl_3$). High Resolution-MS: Calcd for $C_{12}H_{16}O_2$: 192.1150. Found: 192.1128 (M^+). MS m/z (%): 192 (M^+, 52), 163 (100), 148 (80), 91 (85), 77 (75). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.95 (3H, s, H-14), 0.96 (3H, d, J=6.8 Hz, H-15), 4.06 (1H, d, J=6.0 Hz, H-6), 6.14 (1H, d, J=10.0 Hz, H-8), 7.04 (2H, m, H-1, H-7). ^{1}H-NMR (pyridine-d_5) δ ppm: 0.94 (3H, s, H-14), 0.97 (3H, d, J=6.8 Hz, H-15), 4.21 (1H, d, J=6.0 Hz, H-6), 6.32 (1H, d, J=10.0 Hz, H-8), 7.10 (1H, dd, J=4.9, 2.9 Hz, H-1), 7.19 (1H, dd, J=10.0, 6.0 Hz, H-7). ^{13}C-NMR ($CDCl_3$) δ ppm: 14.9 (C-15), 19.7 (C-14), 25.6 (C-3), 26.3 (C-2), 30.9 (C-4), 42.5 (C-5), 68.4 (C-6), 130.5 (C-1), 138.5 (C-10), 140.6 (C-7), 146.4 (C-8), 187.2 (C-9). IR ($CHCl_3$) cm^{-1}: 3650, 1680, 1620. UV λ_{max}^{MeOH} nm (log ε): 232 (5.21).

Nardosinone (**2**): $[\alpha]_D$ +27.5° (c=0.20, $CHCl_3$). MS m/z (%): 250 (M^+, 6), 192 (4), 93 (100), 79 (90). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.03 (3H, d, J=6.7 Hz, H-15), 1.14 (3H, s, H-14), 1.18, 1.38 (each 3H, s, H-12, H-13), 2.65 (1H,dd, J=18.8, 1.9 Hz, H-8a), 2.89 (1H, dd, J=18.8, 7.3 Hz, H-8b), 2.93 (1H, d, J=9.3 Hz, H-6), 4.91 (1H, ddd, J=9.3, 7.3, 1.9 Hz, H-7), 7.05 (1H, dd, J=5.1, 2.7 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ ppm: 16.1 (C-15), 22.1 (C-12), 23.8 (C-13), 25.8 (C-2, C-3), 26.9 (C-14), 33.0 (C-4), 38.5 (C-5), 39.9 (C-8), 59.7 (C-6), 78.0 (C-7), 85.0 (C-11), 137.6 (C-1), 140.1 (C-10), 196.4 (C-9). IR ($CHCl_3$) cm^{-1}: 2950, 1700, 1620. UV λ_{max}^{MeOH} nm (log ε): 248 (3.89).

Debilon (**3**): Colorless needles, mp 136 °C, $[\alpha]_D$ −5.8° (c=0.14, MeOH). MS m/z (%): 234 (M^+, 24), 216 (45), 192 (50), 104 (67), 105 (92), 91 (100), 77 (86). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.76 (1H, d, J=9.3 Hz, H-6), 0.92, 1.08, 1.44 (each 3H, s, H-12, H-13, H-14), 1.07 (3H, d, J=7.3 Hz, H-15), 4.21 (1H, dd, J=4.0, 2.2 Hz, H-9), 5.80 (1H, s, H-1). ^{13}C-NMR ($CDCl_3$) δ ppm: 15.0 (C-15), 16.4 (C-7), 17.5 (C-12), 19.1 (C-11), 23.9 (C-13), 28.0 (C-8), 29.2 (C-14), 31.9 (C-4), 37.1 (C-6), 37.4 (C-5), 42.8 (C-3), 72.6 (C-9), 126.4 (C-1), 170.4 (C-10), 200.0 (C-2). IR ($CHCl_3$) cm^{-1}: 3620, 3489, 1680, 1620. UV λ_{max}^{MeOH} nm (log ε): 231 (3.95).

Nardosinonediol (**4**): Colorless needles, mp 141—143 °C, $[\alpha]_D$ −1.7° (c=0.95, MeOH). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.93 (3H, d, J=6.6 Hz, H-15), 0.97 (3H, s, H-14), 1.30, 1.49 (each 3H, s, H-12, H-13), 2.43 (1H, d, J=3.8 Hz, H-8), 2.86 (1H, d, J=11.0 Hz, H-8), 4.53 (1H, ddd, J=11.0, 7.5, 3.8 Hz, H-7), 6.78 (1H, dd, J=5.2, 2.7 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ ppm: 16.1 (C-15), 25.4 (C-3), 25.9 (C-14), 26.2 (C-2), 29.2 (C-13), 32.6 (C-4), 37.4 (C-12), 40.2 (C-5), 46.0 (C-8), 52.5 (C-6), 68.1 (C-7), 77.5 (C-11), 136.8 (C-1), 142.0 (C-10), 200.7 (C-9). UV λ_{max}^{MeOH} nm (log ε): 249 (3.68).

Kanshone A (**5**): Colorless oil, $[\alpha]_D$ −150.6° (c=0.24, MeOH). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.00 (3H, d, J=6.5 Hz, H-15), 1.06, 1.15, 1.24 (each 3H, s, H-14, H-12, H-13), 2.25 (1H, m, H-4), 2.64 (1H, m, H-6), 6.18 (1H, d, J=10.0 Hz, H-8), 6.95 (1H, dd, J=10.3, 6.8 Hz, H-7), 7.00 (1H, dd, J=3.4 Hz, H-1). ^{13}C-NMR ($CDCl_3$) δ ppm: 16.7 (C-15), 23.8 (C-12), 24.5 (C-13), 26.0 (C-2, C-3), 32.3 (C-14), 33.2 (C-4), 42.0 (C-5), 54.4 (C-6), 76.2 (C-11), 129.1 (C-8), 137.1 (C-1), 141.5 (C-10), 150.7 (C-7), 187.7 (C-9). UV λ_{max}^{MeOH} nm (log ε): 249 (3.68).

Preparation of Benzoate (6), Acetate (8), Propionate (9) and Butyrate (10) A solution of **1** (13 mg) and benzoic anhydride in pyridine (1 ml) was stirred overnight, then poured into water, and extracted with CH_2Cl_2. The product was purified by silica gel MPLC eluting with an *n*-hexane–ethyl acetate solvent system to give **6** (7.2 mg). Compounds **8**, **9** and **10** were prepared in the same way as indicated above by using acetic anhydride, propionic anhydride and butyric anhydride, respectively.

Compound **6**: MS m/z (%): 296 (M^+, 5), 105 (100), 77 (46). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.91 (3H, d, J=6.9 Hz, H-15), 1.11 (3H, s, H-14), 5.49 (1H, d, J=6.0 Hz, H-6), 6.30 (1H, d, J=10.0 Hz, H-8), 7.10—7.14 (2H, m, H-1, H-7), 7.43 (2H, t, J=7.5 Hz), 7.56 (1H, t, J=7.5 Hz), 7.95 (2H, d, J=7.5 Hz). IR ($CHCl_3$) cm^{-1}: 3700, 1718, 1675, 1618, CD (MeOH): $\Delta\varepsilon_{240}$ = −49.83, $\Delta\varepsilon_{220}$ = +12.46.

Compound **8**: MS m/z (%): 234 (M^+, 5), 192 (38), 159 (49), 84 (100). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.88 (3H, d, J=6.9 Hz), 1.02 (3H, s), 2.02 (3H, s), 5.22 (1H, d, J=6.0 Hz), 6.23 (1H, d, J=10.0 Hz), 6.97 (1H, dd, J=10.0, 6.0 Hz), 7.03 (1H, t, J=4.0 Hz).

Compound **9**: CI-MS m/z (%): 249 (M^++1, 11), 175 (19), 84 (100). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.88 (3H, d, J=6.9 Hz), 1.03 (3H, s), 1.11 (3H, t, J=7.6 Hz), 5.22 (1H, d, J=6.0 Hz), 6.23 (1H, d, J=10.0 Hz), 6.99 (1H, dd, J=10.0, 6.0 Hz), 7.03 (1H, t, J=4.0 Hz).

Compound **10**: CI-MS m/z (%): 263 (M^++1, 6), 192 (28), 175 (60), 84 (100). ^{1}H-NMR ($CDCl_3$) δ ppm: 0.88 (3H, d, J=6.9 Hz), 0.94 (3H, t, J=8.4 Hz), 1.05 (3H, s), 5.22 (1H, d, J=6.0 Hz), 6.23 (1H, d, J=10.0 Hz), 6.99 (1H, dd, J=10.0, 6.0 Hz), 7.04 (1H, t, J=3.6 Hz).

Preparation of Compound 7 Compound **1** (20 mg) was stirred with PCC (30 mg) in CH_2Cl_2 (3 ml) for 6 h at room temperature. Dry ether was then added, the reaction mixture was filtered and the filtrate was subjected to silica gel MPLC eluting with an *n*-hexane–ethyl acetate (1 : 1) solvent system to give **7** (16 mg): MS m/z (%): 190 (M^+, 34), 175 (24), 148 (32), 84 (100). ^{1}H-NMR ($CDCl_3$) δ ppm: 1.15 (3H, d, J=6.6 Hz), 1.30 (3H, s), 6.65 (1H, d, J=10.3 Hz), 6.71 (1H, d, J=10.3 Hz), 6.85 (1H, m).

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