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QUINONES FROM ARDISIA CORNUDENTATA

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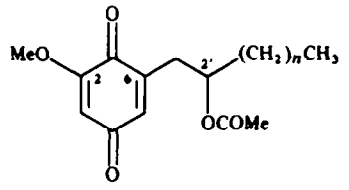
Key Word Index—*Ardisia cornudentata*; Myrsinaceae; benzoquinone; ardisianone; cornudentanone; leukotriene binding assay; inhibition.

Abstract—The structure of a new benzoquinone, cornudentanone, isolated from *Ardisia cornudentata M.* was determined.

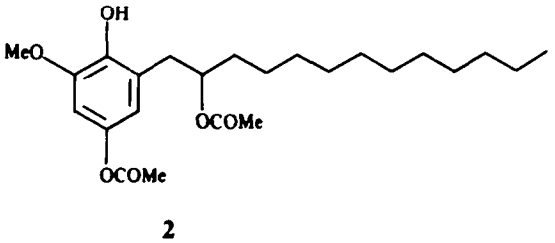
INTRODUCTION

Ardisia cornudentata Mez. is one of the many Ardisia species used in folk medicine in the south east part of China. It is used as an anti-inflammatory/analgesic medication and to improve general blood circulation. It is also used as an antidote for snake and insect bites. Like many other Myrsinaceae species, *Ardisia* is known to contain many benzoquinone pigments [1]. In a study to identify the active components, different extracts of the root of *A. cornudentata* were evaluated in several *in vitro* receptor and enzymatic assays in our laboratory. The methylene chloride extract was found to inhibit the binding of leukotriene, D_4(LTD_4); to a receptor preparation from guinea pig lung tissue. Using the LTD_4 binding assay as a guide, two benzoquinones (**1a** and **1b**) were obtained as active components and were identified. Ardisianone (**1a**) is a known compound [2]. The structure assignment of the new benzoquinone cornudentanone (**1b**) and the binding data for both **1a** and **1b** are reported.



	n
1a	12
1b	10

1b



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*Author to whom correspondence should be addressed.
*Assignments may be interchanged.

RESULTS AND DISCUSSION

Ardisianone (**1a**) and cornudentanone (**1b**) accounted for all the LTD_4 receptor antagonistic activity of the crude methylene chloride extract.

Cornudentanone (**1b**), $C_{22}H_{34}O_5$, is a yellow amorphous solid. It accounts for 0.05% by weight of dry roots. Cornudentanone (**1b**) is optically active, $[\alpha]_D^{22} = -31.5°$ ($CHCl_3$; *c* 1.60). Its UV spectra (methanol solution) displayed λ_{max} ($\log\varepsilon$); 363 (2.97), 264 (4.02). Its IR (CH_2Cl_2 solution) absorption bands at 2920, 2850, 1734, 1680, 1627, 1600, 1452, 1370, 1320, 1230 and 1050 cm^{-1} are characteristic of a 2,6-di-substituted benzoquinone [6]. Its EI mass spectrum showed fragments at (*m/z*): 380 $[M+2H]^+$, 378 $[M]^+$, 336, 318, 194, 153 and 152. Elemental analysis (calc. for $C_{22}H_{34}O_5$: C, 69.81% H, 9.05%. Found: C, 69.43% H, 9.37%, confirmed the empirical formula assigned. The ^1H NMR spectrum showed resonances at ($CDCl_3$); δ0.91 (*t*, 3H), 1.28 (*m*, 18H), 1.58 (*m*, 2H), 1.95 (*s*, 3H), 2.43 (*ddd*, HCH, J = 14.0, 8.8, 1.0 Hz), 2.83 (*ddd*, HCH, J = 14.0, 3.5, 1.3 Hz), 3.80 (s, 3H) 5.00 (*m*, 1H), 5.86 (*d* 1H, J = 2.4 Hz) and 6.43 (*ddd*, 1H, J = 2.4, 1.3, 1.0 Hz). ^{13}C NMR ($CDCl_3$); δ14.1 (Me), 21.1 (MeCO), 22.7 (C-12'), 25.4 (C-4'), 29.3, 29.4, 29.5, 29.57, 29.6 (C_2) (C-5'–C-10'), 31.9 (C-11'), 34.2 (C-3')*, 34.5 (C-1')*, 56.4 (MeO) 72.3 (HCO), 107.3 (C-3), 134.5 (C-5), 143.5 (C-6), 158.9 (C-2), 171.0 (COO), 181.6 (C-1) and 187.4 (C-4).

Hydrogenation of **1b** over platinum oxide in acetic anhydride yielded a colourless oily product, **2**. Examination of its ^1H NMR spectrum revealed one additional acetyl group and the compound was identified as 4-*O*-acetyldihydrocornudentanone (**2**). The spectral data agreed for the most part with the data reported in ref. [5] for 4-*O*-acetyldihydroardisianone.

Compound **1a** was identified as ardisianone. It accounted for 0.084% by weight of dry roots. Its UV, IR, EIMS and ^1H NMR spectral data were identical to those reported in the literature [2]. Elemental analysis (Calc. for $C_{24}H_{38}O_5$: C, 70 90% H, 9.42%. Found. C, 70.28%, H, 9.50%) provided further support for the assignment. Compound **1a** is optically active, $[\alpha]_D^{22} = -40°$ ($CHCl_3$; *c* 1.75).

Cornudentanone (**1b**) and ardisianone were evaluated in the ^3H-LTD_4, ^3H-LTC_4 and ^3H-LTB_4 receptor binding assays. Cornudentanone inhibited ^3H-LTB_4 receptor binding in a dose-dependent manner with an IC_{50} of 1.8

$\times 10^{-5}$ M. Cornudentanone is also active in inhibiting the ^{3}H-LTD$_4$ binding with an IC_{50} of 1.58×10^{-5} M. Ardisianone also showed inhibition in the ^{3}H-LTB$_4$ binding assay in a dose-dependent manner with an IC_{50} of 2.7×10^{-5} M.

EXPERIMENTAL

^{1}H and ^{13}C NMR: 400 MHz, TMS as an int. standard.

Isolation of cornudentanone (**1b**) *and ardisianone* (**1a**). The dried plant material was collected from Taiwan. Twenty grams of the roots of *A. cornudentata* was ground and macerated in 500 ml CH_2Cl_2 for 24 hr at ambient temp. The extract was filtered and concentrated under red. pres. to yield 0.25 g of a brown semi-solid. This crude extract was found active in the LTD$_4$ receptor binding assay with IC_{50} of 3.5×10^{-5} M. A flash column (2 × 30 cm) packed with 30 g of silica gel (Kieselgel 60, 230–400 mesh) was equilibrated with hexane–EtOAc (20:1). The crude mixture from the CH_2Cl_2 extract was dissolved in 0.5 ml CH_2Cl_2 was loaded onto the column and eluted at 6 psi of N_2 with 150 ml hexane–EtOAc (20:1), 150 ml hexane–EtOAc (10:1), 150 ml hexane–EtOAc (5:1), 150 ml hexane–EtOAc (1:1) and finally 150 ml EtOAc–MeOH (1:1). Fractions of 30 ml were collected and concentrated. Samples from each of the 25 fractions collected were tested in the LTD$_4$ receptor binding assay. Active fractions were combined to give 34 mg of a bright yellow semi-solid. This fraction was further purified by semi-prep. HPLC using a normal phase silica column (Zorbax SIL, 0.46 × 25 cm) and eluted with hexane–EtOAc (19:3). Two compounds active in LTD$_4$ binding assay were obtained: cornudentanone (**1b**) and ardisianone (**1a**).

Reduction of cornudentanone (**1b**). Cornudentanone (5 mg, 0.013 mmol.) was dissolved in 0.5 ml HOAc and 0.1 ml Ac_2O. Five milligrams of PtO_2 was added and the mixture was hydrogenated under 40 psi of H_2 on a PAR shaker for 15 min. Ten ml of EtOAc was added and the catalyst filtered off. The resulting soln was concentrated and residual solvent removed under red. pres. A colourless oily product (5.2 mg, 90% yield) was recovered and was identified as 4-acetyldihydrocornudentanone (**2**).

LTB$_4$, LTC$_4$ and LTD$_4$ binding assays. Guinea pig lung membranes were prepared by the procedure of ref. [4]. LTC$_4$ and LTD$_4$ binding assays were performed by the procedure of ref. [5] with a minor modification. Routinely, 0.80 nM [^{3}H] LTD$_4$ or 0.40 nM [^{3}H] LTC$_4$ was used as the ligand. After incubation at room temp. for 1 h, bound radioactivity was harvested with GF/B filters using the Skatron cell harvester. The amount of radioactivity remaining on the filters was determined in Scintin Verse 1 Cocktail.

[^{3}H] LTB$_4$ receptor binding assay was run with human PMN membranes. Human PMN leukocytes were isolated from human leukocyte concentrate [6, 7]. Membranes were prepared by the sonification and centrifugation of human PMN leukocytes. Routinely, 70 pM of [^{3}H]-LTB$_4$ was used as the ligand. Specific binding and nonspecific binding was defined as the difference between total binding and binding in the presence of 0.65 μM LTB$_4$ [8]. All assays were run in triplicate and experiments were repeated twice.

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