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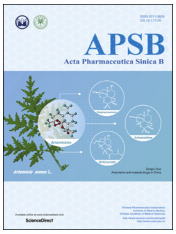

ORIGINAL ARTICLE

Three pairs of alkaloid enantiomers from the root of *Isatis indigotica*

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KEY WORDS

Cruciferae;
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Chiral separation;
2-Oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid;
Indolo[2,1-*b*]quinazolinone;
3-Thioxohexahydro-1-*H*-pyrrolo[1,2-c]imidazol-1-one

Abstract Three pairs of enantiomerically pure alkaloids with diverse structure features, named isatindigoticoic acid A and epiisatindigoticoic acid A [(−)-**1** and (+)-**1**], phaitanthrin A and epiphaitanthrin A [(−)-**2** and (+)-**2**], and isatindopyrromizol A and epiisatindopyrromizol A [(−)-**3** and (+)-**3**], respectively, were isolated from an aqueous extract of the roots of *Isatis indigotica*. Racemic and scalemic mixtures of these enantiomers were separated by HPLC on a chiral semi-preparative column. Their structures including absolute configurations were determined by extensive spectroscopic analysis in conjunction with the calculation of electronic circular dichroism (ECD) spectra. The enantiomer pairs possess parent structures of 2-oxo-1,2,3,4-tetrahydroquinoline-4-carboxylic acid, indolo[2,1-*b*]quinazolinone, and 3-thioxohexahydro-1*H*-pyrrolo[1,2-*c*]imidazol-1-one, respectively. Except for phaitanthrin A [(−)-**2**] which the configuration was previously undetermined, these compounds are new enantiomeric natural products.

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1. Introduction

"Ban lan gen" (Radix Isatidis) is one of the most important traditional Chinese medicines used for the treatment of influenza and infection diseases. This medicine is derived from the dry roots of cultivated plant *Isatis indigotica* Fort. (Cruciferae)[1]. A literature survey shows that previous pharmacological and chemical investigations associated with this herbal medicine were mainly focused on methanol or ethanol extracts[2–9]. This differs from practical application of water decoctions of "ban lan gen", as well as formulations containing "ban lan gen". Therefore, as part of a program to assess the chemical and biological diversity of traditional Chinese medicines[10–22], we investigated a water decoction of "ban lan gen" and have reported characterization of 28 new alkaloids, including a pair of indole alkaloid enantiomers containing dihydrothiopyran and 1,2,4-thiadiazole rings, a pair of bisindole alkaloid enantiomers, seven glycosidic bisindole alkaloids, and 54 known compounds, as well as their antiviral and hepatocyte-protective activities[23–27]. A further in-depth investigation on the same extract led to the chiral separation of three pairs of alkaloid enantiomers (−)/(+)-**1**−(−)/(+)-**3** having diverse structural features (Fig. 1). This paper describes the details of isolation and structural elucidation of these enantiomers.

2. Results and discussion

An enantiomeric mixture **1** was isolated as a white amorphous powder ($[\alpha]_D^{20}$ −35.3, *c* 0.25, MeOH), which is homogeneous as indicated by normal thin layer chromatography (TLC) and reversed phase high performance liquid chromatography (RP-HPLC) analyses. The IR spectrum of **1** showed the presence of hydroxy and amino (3233 cm^{-1}), conjugated carbonyl (1684 cm^{-1}), and aromatic ring (1586 and 1516 cm^{-1}) functional groups. The molecular formula of **1** was determined as $C_{17}H_{14}N_2O_5$ based on HR-ESI-MS (*m/z* 349.0803 $[M+Na]^+$) and NMR spectral data (Table 1). The ^{1}H NMR spectrum of **1** in DMSO-d_6 showed signals attributed to two *ortho*-disubstituted phenyl rings at δ_H 8.53 (brd, $J=8.4$ Hz, H-3), 7.47 (brdd, $J=8.4$ and 7.2 Hz, H-4), 7.10 (brt, $J=7.2$ Hz, H-5), and 8.01 (brd, $J=7.2$ Hz, H-6) and 7.42 (brd, $J=7.2$ Hz, H-5′), 6.96 (brt, $J=7.2$ Hz, H-6′), 7.21 (brt, $J=7.6$ Hz, H-7′), and 6.88 (brd, $J=7.2$ Hz, H-8′); an isolated methylene at δ_H 2.94 (1 H, d, $J=16.2$ Hz, H-3′a) and 2.72 (1 H, d, $J=16.2$ Hz, H-3′b); as well as signals due to a carboxylic proton at δ_H 13.63 (brs, COO*H*), two nitrogen-bearing protons at δ_H 12.32 (brs, N*H*-2), 10.12 (s, N*H*-1′), and a hydroxyl proton at δ_H 6.83 (brs, O*H*-4′). The ^{13}C NMR and DEPT spectra of **1** displayed 17 carbon resonances corresponding to the above structural units and two additional carbonyl carbons at δ_C 172.5 (C-1″) and 167.6 (C-2′). As compared with those of the previously reported compounds from *I. indigotica*[23–27], these spectroscopic data indicated that **1** is an uncommon alkaloid containing two aromatic rings, two amide carbonyls, one carboxylic group and an isolated methylene unit. To construct the final structure of **1**, connections among these units were further established by 2D NMR data analysis. Comprehensive analysis of the ^{1}H−^{1}H COSY and HSQC spectra of **1** confirmed the presence of the above structural units and unambiguously assigned the proton and proton-bearing carbon resonances in the NMR spectra. The HMBC spectrum of **1** showed two- and three-bond hetereonuclear correlations from H-3 to C-1 and C-5; from H-4 to C-2 and C-6; from H-5 to C-1 and C-3; and from H-6 to C-2, C-4 and C-7 (Fig. 2). These correlations, combined with their chemical shifts and the broadened and diminished resonance of C-7 (typical for carboxylic acid carbon with a dissociation property), indicated that there was an *N*-substituted 2′-aminobenzoic acid (anthranilic acid) moiety in **1**. Meanwhile, the HMBC spectrum exhibited the long-range correlations from N*H*-1′ to C-2′, C-3′, C-4′a, C-8′, and C-8′a; from H_2-3′ to C-2′, C-4′, and C-4′a; from H-5′ to C-4′, C-7′ and C-8′a; from

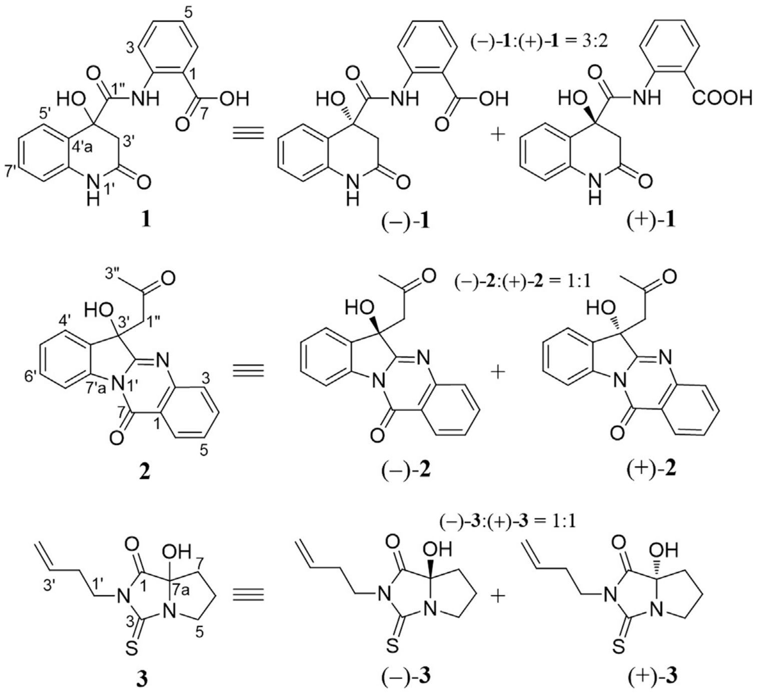

Figure 1 The structures of enantiomer mixtures **1**−**3** and compounds (−)-/(+)-**1**−(−)-/(+)-**3**.