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Original article

Antiviral stereoisomers of 3,5-bis(2-hydroxybut-3-en-1-yl)-1,2,4-thiadiazole from the roots of *Isatis indigotica*

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ABSTRACT

Four stereoisomers of 3,5-bis(2-hydroxybut-3-en-1-yl)-1,2,4-thiadiazole, named insatindigothiadiazoles A–D (**1a**–**1d**), were isolated from the roots of *Isatis indigotica*. Their structures were determined by spectroscopic analysis; specifically, the absolute configurations were assigned by using the MPA determination rule based on $\Delta\delta^{RS}$ values of MPA esters, and supported by electronic CD (ECD) calculations. Proposed biosynthetic pathways and preliminary investigations of the biological activities of **1a**–**1d** against influenza virus A (H3N2), Coxsackie virus B3, and/or HSV-1 are also discussed.

1. Introduction

Isatis indigotica Fort., a biennial herbaceous plant of the Cruciferae family, is widely cultivated to meet demands of medicinal utilization in China. Its dried roots and leaves, named "ban lan gen" and "da qing ye" in Chinese, respectively, are commonly used in traditional Chinese medicine for the treatment of influenza, cold, fever, and other infections [1]. Many formulations containing "ban lan gen" and/or "da qing ye" are marketed and recorded in Chinese Pharmacopoeia [2], which play an important role to treat and prevent influenza during influenza pandemics in China. Clinical efficacy, together with diverse structures and biological activities from extracts of these herbal medicines, has long attracted interest from chemists and pharmacologists. Pharmacological studies showed that extracts of these medicines displayed antiviral, antiendotoxic, antinociceptive, antiinflammatory, and antipyretic effects and cytotoxicity against leukemia cells [3–7]. Meanwhile, bioactive chemical components, such as alkaloids, lignans, ceramides, flavonoids, and sulfur containing metabolites [8–19], were isolated from the extracts. However, previous chemical studies were mainly performed on the ethanol and methanol extracts of the drug materials, which is inconsistent with their practical application by decocting with water. Therefore, as part of a program to assess the chemical and biological diversity of traditional Chinese medicines, focusing on the minor components [20–30], we carried out on detailed chemical analysis of an aqueous extract of the *I. indigotica* roots 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, and 12 glycosidic indole and bisindole alkaloids, as well as 54 known compounds including 33 constituents isolated from *I. indigotica* for the first time [31–36]. Biological assays showed that some of these compounds showed antiviral activity against influenza virus A/Hanfang/359/95 (H3N2) or Coxsackie virus B3 and protective activity against dl-galactosamine-induced hepatocyte (WB-F344 cell) damage. Further examination of the same extract led to separation of unusual stereoisomers of 3,5-bis(2-hydroxybut-3-en-1-yl)-1,2,4-thiadiazole (Fig. 1), named insatindigothiadiazoles A–D (**1a**–**1d**). Herein, we report details of the isolation, structure elucidation, postulated biogenetic pathway, and biological activity of the stereoisomers.

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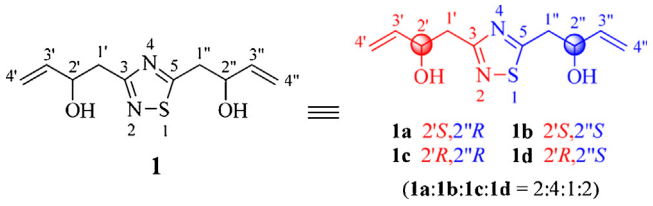

Fig. 1. Structures of **1a–1d**.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a P-2000 polarimeter. UV spectrum was recorded on a V-650 spectrometer. CD spectra were measured on a JASCO J-815 CD spectrometer. IR spectrum was recorded on a Nicolet 5700 FT-IR Microscope spectrometer (FT-IR Microscope Transmission). 1D- and 2D-NMR spectra were obtained at 500 or 600 for ^{1}H and 125 or 150 MHz for ^{13}C, respectively, on a Varian 600 MHz spectrometer or a Bruker 500 MHz spectrometer with solvent peaks as references. ESIMS and HR-ESIMS data were obtained on an AccuToFCS JMS-T100CS spectrometer. Column chromatography (CC) was performed with HPD-110 (Cangzhou Bon Absorber Technology Co. Ltd, Cangzhou, China), MCI resin (120 μm, Mitsubishi Chemical Inc., Tokyo, Japan), silica gel (200–300 mesh, Qingdao Marine Chemical Inc., China), or RP silica gel (Grace Davison Discovery Science, Deerfield, USA). HPLC separation was performed on a system consisting of a Waters 600 controller, a Waters 600 pump, and a Waters 2487 dual λ absorbance detector with an Alltima (250 × 10 mm) preparative column packed with C_{18} (5 μm), an analytical chiral column (Chiralpak AD-H, 250 mm × 4.6 mm), or a semi-preparative chiral column (Chiralpak AD-H, 250 mm × 10 mm) packed with amylose tris-(3,5-dimethylphenylphenylcarbamate) coated on 5 μm silica-gel. TLC was conducted on the precoated silica gel GF_{254} plates. Spots were visualized under UV light (254 or 365 nm) or by spraying with 10% H_2SO_4 in 95% EtOH followed by heating.

2.2. Plant material

The roots of *I. indigotica* were purchased in Anhui province, China, in December 2009. The plant was identified by Mr. Lin Ma (Institute of Materia Medica, Beijing 100050, China). A voucher specimen (No. ID-S-2385) was deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Beijing, China.

2.3. Extraction and isolation

The air-dried and pulvarized plant material (50 kg) was decocated with H_2O (150 L; 3 × 1 h). The aqueous extracts were combined and evaporated under reduced pressure to yield a dark-brown residue (32 kg). The residue was dissolved in H_2O (122 L), loaded on a macroporous adsorbent resin (HPD-110, 19 kg) column (20 × 200 cm), and eluted successively with H_2O (50 L), 50% EtOH (125 L), and 95% EtOH (100 L) to yield three corresponding fractions A, B and C. After removing the solvent under reduced pressure, fraction B (0.9 kg) was separated by CC over MCI resin (5 L), with successive elution using H_2O (10 L), 30% EtOH (30 L), 50% EtOH (20 L), 95% EtOH (10 L), and Me_2CO (8 L), to give fractions B1–B5. Fraction B2 (547 g) was subjected to CC over silica gel, with elution by a gradient of increasing MeOH concentration (0–100%) in EtOAc and then with 30% EtOH, to yield subfractions B2-1–B2-5 based on TLC analysis. Subfraction B2-1 (16.3 g) was chromatographed over Sephadex LH-20 with elution by a petroleum ether–chloroform–methanol (5:5:1, *v/v/v*) mixture to yield B2-1-1–B2-1-10, of which B2-1-2 (600 mg) was further fractionated by RP flash CC with a gradient of increasing MeOH concentration (0–100%) in H_2O to yield B2-1-2-1–B2-1-2-4. Fraction B2-1-2-1 (125.5 mg) was purified by RP HPLC (63% MeOH in H_2O, flow rate 2.0 mL/min) to give **1** (20.1 mg, t_R = 15.4 min). Subsequent separation of **1** by HPLC, using a Chiralpak AD-H column (250 × 10 mm) packed with amylose tris-(3,5-dimethylphenylphenylcarbamate) coated on 5 μm silica-gel and iPrOH-n-hexane mixture (20:80, flow rate 2 mL/min) as the mobile phase, yielded **1a** (4.1 mg, t_R = 37.0 min), **1b** (8.2 mg, t_R = 20.9 min), **1c** (2.0 mg, t_R = 17.4 min), and **1d** (4.2 mg, t_R = 16.3 min).

3,5-Bis(2-hydroxybut-3-en-1-yl)-1,2,4-thiadiazole (**1**): Colorless gum; $[\alpha]^{20}_{D}$ − 7.7 (*c* 1.2, MeOH); UV (MeOH) λ_{max} (log ε): 204 (4.38), 235 (4.37) nm; IR (cm^{-1}): ν_{max} 3325, 2962, 2923, 1643, 1491, 1414, 1201, 1095, 1022, 923, 799; The data of ^{1}H NMR (acetone-d_6, 600 MHz; DMSO-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 150 MHz; DMSO-d_6, 125 MHz) were detailed in Table S3 in Supporting information; (+)-ESIMS *m/z* 249 $[M + Na]^+$; (+)-HR-ESIMS *m/z* 227.0857 $[M + H]^+$ (calcd. for $C_{10}H_{15}N_2O_2S$, 227.0849).

(+)-(2′*S*,2″*R*)-3,5-Bis(2-hydroxybut-3-en-1-yl)-1,2,4-thiadiazole (insatindigothiadiazole A, **1a**): Colorless gum; $[\alpha]^{20}_{D}$ + 21.3 (*c* 0.50, MeOH); CD (MeOH) 210 (Δε + 1.09), 234 (Δε + 0.67) nm. ^{1}H NMR (acetone-d_6, 600 MHz) data and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 1; (+)-HR-ESIMS *m/z* 227.0849 $[M + H]^+$ (calcd. for $C_{10}H_{15}N_2O_2S$, 227.0849), 249.0670 $[M + Na]^+$ (calcd. for $C_{10}H_{14}N_2O_2SNa$, 249.0668).

(−)-(2′*S*,2″*S*)-3,5-Bis(2-hydroxybut-3-en-1-yl)-1,2,4-thiadiazole (insatindigothiadiazole B, **1b**): Colorless gum; $[\alpha]^{20}_{D}$ − 25.1 (*c* 1.10, MeOH); CD (MeOH) 213 (Δε − 2.69), 236 (Δε − 1.44) nm. ^{1}H NMR (acetone-d_6, 600 MHz) data and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 1; (+)-HR-ESIMS *m/z* 227.0847 $[M + H]^+$ (calcd. for $C_{10}H_{15}N_2O_2S$, 227.0849), 249.0668 $[M + Na]^+$ (calcd. for $C_{10}H_{14}N_2O_2SNa$, 249.0668).

(+)-(2′*R*,2″*R*)-3,5-Bis(2-hydroxybut-3-en-1-yl)-1,2,4-thiadiazole (insatindigothiadiazole C, **1c**): Colorless gum; $[\alpha]^{20}_{D}$ + 24.3 (*c* 0.30, MeOH); CD (MeOH) 214 (Δε + 2.39), 236 (Δε + 1.54) nm. ^{1}H NMR (acetone-d_6, 600 MHz) data and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 1; (+)-HR-ESIMS *m/z* 227.0849 $[M + H]^+$ (calcd. for $C_{10}H_{15}N_2O_2S$, 227.0849), 249.0667 $[M + Na]^+$ (calcd. for $C_{10}H_{14}N_2O_2SNa$, 249.0668).

(−)-(2′*R*,2″*S*)-3,5-Bis(2-hydroxybut-3-en-1-yl)-1,2,4-thiadiazole (insatindigothiadiazole D, **1d**): Colorless gum; $[\alpha]^{20}_{D}$ − 21.1 (*c* 0.60, MeOH); CD (MeOH) 211 (Δε − 1.62), 238 (Δε − 0.97) nm. ^{1}H NMR (acetone-d_6, 600 MHz) data and ^{13}C NMR (acetone-d_6, 150 MHz) data, see Table 1; (+)-HR-ESIMS *m/z* 227.0851 $[M + H]^+$ (calcd. for $C_{10}H_{15}N_2O_2S$, 227.0849), 249.0672 $[M + Na]^+$ (calcd. for $C_{10}H_{14}N_2O_2SNa$, 249.0668).

2.4. Synthesis of MPA esters of *1a–1d*

To a solution of **1a** (1.5 mg), **1b** (4.0 mg), **1c** (1.0 mg), or **1d** (1.5 mg) in freshly distilled methylene chloride (2 mL), was added (*R*)-(−)- or (*S*)-(+)-α-methoxyphenylacetic acid [(*R*)- or (*S*)-MPA, 3.0 mg), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 4.5 mg), and 4-dimethylaminopyridine (DMAP, 4.5 mg). The solution was kept at room temperature overnight then evaporated under reduced pressure to yield a residue. The residue was isolated by preparative TLC (mobile phase: petroleum ether-Me_2CO, 2:1) to yield **1a**-bis-(*R*)-MPA (1.2 mg) or **1a**-bis-(*S*)-MPA (1.3 mg) from **1a**; **1b**-2″-(*R*)-MPA (1.1 mg) and **1b**-bis-(*R*)-MPA (1.8 mg) or **1b**-2″-(*S*)-MPA (1.2 mg) and **1b**-bis-(*S*)-MPA (2.0 mg) from **1b**; **1c**-bis-(*R*)-MPA (0.8 mg) or **1c**-bis-(*S*)-MPA (0.7 mg) from **1c**; and **1d**-bis-(*R*)-MPA (1.1 mg)or **1d**-bis-(*S*)-MPA (1.2 mg) from **1d**. ^{1}H NMR (acetone-d_6,