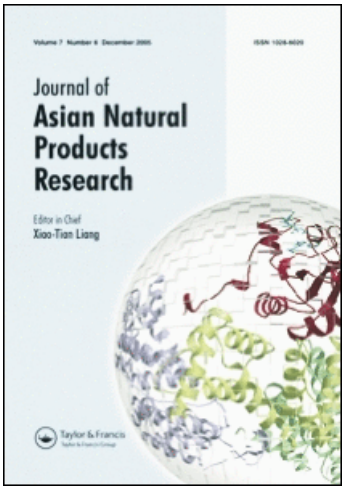

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The structure and absolute configuration of Shuangkangsu: a novel natural cyclic peroxide from *Lonicera japonica* (Thunb.)

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A novel cyclic peroxide, shuangkangsu (**1**), has been obtained from the water extract of the buds of *Lonicera japonica* (Thunb.). The structure was elucidated as 5,8-divinyl-1,4-dihydro-1,4-di-*O*-β-D-glucopyranosyl-2,3-dioxane (**1**) on the basis of the spectral data. Its absolute stereochemistry was determined to be (1S, 4S) by comparison of its CD curves with those of its optically pure analogs **2** and **3**, which were synthesized from the phthalaldehyde. The absolute configurations of **2** and **3** were determined to be (1R, 4R) and (1S, 4S) by X-ray analysis and CD spectra, respectively. Compound **1** showed significant antiviral activities against influenza virus in chicken embryo and respiratory syncytial virus in cells.

Keywords: shuangkangsu; honeysuckle; cyclic peroxide; antiviral activity

1. Introduction

Honeysuckle, the fresh or dried buds of *Lonicera japonica* (Thunb.), is a famous Chinese traditional medicine that possesses antipyretic and detoxic activities, and was used for the treatment of influenza and pneumonia [1]. Previous studies on the chemical constituents of *Lonicera genis* plants led to the isolation of flavonoids [2,3], saponins [4,5], iridoids [6,7], and caffeoylquinic acid derivatives [8]. In the course of our search for new antiviral agents from the Chinese herb medicine, the H_2O extract of the buds of the title plant was found to show obvious antiviral activity. Subsequently, bioactivity-guided fractionation and repeated chromatography on macroporous resin and Sephadex LH-20 columns led to the isolation of shuangkangsu (**1**) (Figure 1) [9], a new cyclic peroxide with novel structure, as an antiviral principle. Here, we report the isolation, structural elucidation, absolute configuration, and the antiviral activity of the novel peroxide.

2. Results and discussion

The EtOH-soluble fraction of the H_2O extract of buds of *L. japonica* (Thunb.) was chromatographed on macroporous resin and Sephadex LH-20 columns and HPLC, with antiviral activity-guided fractionation, leading to the isolation of the bioactive cyclic peroxide shuangkangsu (**1**).

Shuangkangsu (**1**), $[\alpha]_D^{19}$ − 23.6 (*c* 1.0, H_2O), was obtained as white amorphous powder. The molecular formula was determined as $C_{20}H_{30}O_{14}$ by FAB-MS at *m/z* 495 $[M + H]^+$ and 517 $[M + Na]^+$, and

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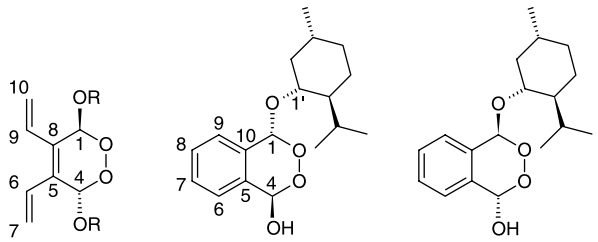


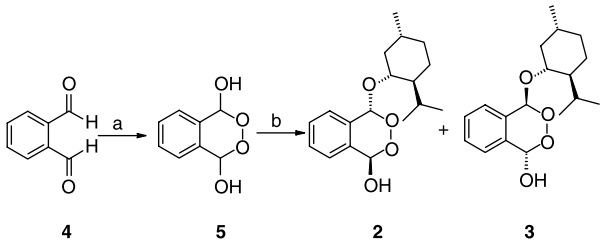
Figure 1. Structures of compounds **1**–**3**.

elemental analysis. The IR spectrum of **1** revealed the presence of hydroxyl group (3495 cm^{-1}). The UV spectrum of **1** showed characteristic absorption maximum at 240 nm, indicating the presence of a hexatriene chromophore (calculated value, 247 nm) in the molecule. The ^1H NMR spectrum of **1** displayed 11 proton signals, including 3 olefinic protons at δ_H 5.36 (1H, dd, $J = 17.0$, 1.5 Hz), 5.31 (1H, dd, $J = 10.0$, 1.5 Hz), and 5.55 (1H, dd, $J = 17.0$, 10.0 Hz) for a mono-substituted double bond; 1 hemiacetal proton singlet at δ_H 5.60 (1H, s); and 7 oxymethine and oxymethylene protons at δ_H 4.85 (1H, d, $J = 8.0$ Hz), 3.92 (1H, dd, $J = 12.5$, 2.0 Hz), 3.72 (1H, dd, $J = 12.5$, 6.0 Hz), and 3.51–3.29 (4H, m) due to sugar moiety. The sugar moiety was confirmed as glucose by the hydrolysis of **1** followed by TLC comparison with authentic sample. The ^{13}C NMR and DEPT spectra exhibited 10 carbon signals, and the carbon resonances at δ_C 101.3 (CH), 79.7 (CH), 79.2 (CH), 75.6 (CH), 72.5 (CH), and 63.7 (CH_2) in the ^{13}C NMR and DEPT spectra also confirmed the sugar moiety as glucose; the carbon resonance at δ_C 100.4 (CH) suggested the presence of a dioxygenated methine group, which can be confirmed by a singlet at δ_H 5.60 (1H, s) in the ^1H NMR spectrum. The other three olefinic carbon resonances at δ_C 123.8 (CH_2), 134.4 (CH), and 156.1 (C) in the ^{13}C NMR and DEPT spectra indicated that there may be three double bonds corresponding to the hexatriene group in the molecule, and the whole molecule should be symmetric.

Aforementioned spectroscopic data and the molecular formula $C_{20}H_{30}O_{14}$ indicated that the molecule must possess six degrees of unsaturation. The three double bonds and two hexapyranose moieties accounted for five degrees of unsaturation, and the remaining one degree of unsaturation was attributed to the existence of one peroxide ring in shuangkangsu (**1**). Therefore, the structure of **1** was formulated, as shown in Figure 1.

Substituents on C-1 and C-4 of shuangkangsu (**1**) existed possible *cis* or *trans* relative stereochemistry. The conformation of *trans* isomer with both pseudo-equatorial substituents should be more stable than the *cis* isomer's conformation with one pseudo-axial substituent and one pseudo-equatorial substituent. The *cis* isomer is achiral due to symmetric plane, which is easily detectable. The *trans* isomer exists as two possible diastereomers. According to its obvious Cotton effect in the CD spectrum (Figure 3) and optical rotation $[\alpha]_D^{19}$ − 23.6, shuangkangsu (**1**) could be identified as an optically pure compound and its relative stereochemistry could be determined as *trans*.

Shuangkangsu (**1**) does not lend itself to X-ray crystallographic diffraction analysis due to the lack of a suitable crystal, so it is difficult to determine the absolute configuration of C-1 and C-4. In order to confirm the absolute stereochemistry of **1**, its optically pure analogs **2** and **3** (Figure 1) were synthesized from phthalaldehyde **4**, as shown in Scheme 1. Compound **4** reacted with 30% H_2O_2 in CH_3OH to afford cyclic peroxide **5**,



Scheme 1. (a) 30% H_2O_2, MeOH, room temperature, 12 h; (b) (−)-menthol, $BF_3{\cdot}Et_2O$, Et_2O, room temperature, 24 h.

the diastereoisomers **2** and **3** (the ratio was 1:1 according to the LC–MS analysis) were obtained by reaction of **5** with (−)-menthol catalyzed by $BF_3{\cdot}Et_2O$.

The absolute configuration of **2** was determined to be (1R, 4R) by X-ray analysis (Figure 2) based on the known configuration of the optically pure (−)-menthol (one of the starting materials for the synthesis). According to the reaction mechanism of acetylation and the symmetry of the CD spectra of compounds **2** ($\lambda = 224$ nm, $\Delta\varepsilon = -0.92$; $\lambda = 215$ nm, $\Delta\varepsilon = +0.33$) and **3** ($\lambda = 224$ nm, $\Delta\varepsilon = +0.91$; $\lambda = 215$ nm, $\Delta\varepsilon = -0.32$), the absolute configuration of **3** was determined to be (1S, 4S). The absolute configuration of **1** ($\lambda = 240$ nm, $\Delta\varepsilon = +19$; $\lambda = 210$ nm, $\Delta\varepsilon = -35$) was determined to be (1S, 4S) by comparison of the CD spectra with those of **2** and **3** (Figure 3).

Although, numerous cyclic peroxides have been known so far, to our knowledge, the shuangkangsu (**1**) represents the first natural occurrence of 1,4-dihydro-1,4-di-*O*-β-D-glucopyranosyl-2,3-dioxane skeleton.

Compound **1** exhibited significant antiviral activities against influenza virus in chicken embryo and respiratory syncytial virus in cells.

3. Experimental

3.1 General experimental procedures

Melting points were determined on XT_4-100_X micromelting apparatus and are uncorrected. IR spectra were run on Nicolet Impact-400 spectrometer. Optical rotations were measured on PE-241 digital polarimeter. CD spectra were taken on Jasco J-725 spectrophotometer. NMR spectra were recorded on Varian Mercury 400 or Varian Inova-500 spectrometer (400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C). Mass spectra were obtained on a ZAB-2F spectrometer. Elemental analysis was carried out with a Carlo-Erba 1112 apparatus.

3.2 Plant material

Buds of *L. japonica* (Thunb.) used in this investigation were collected from Pingyi County, Shandong province, China, and identified by Mr Lin Ma of the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College. A voucher specimen (ID-S-2237) has been deposited in the Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College.

3.3 Extraction and isolation

Air-dried buds of *L. japonica* (Thunb.) (1 kg) were extracted with hot water (3 × 5 l). The extraction was concentrated under reduced pressure to yield a brown extract (about 1.2 kg) and the EtOH was added. After removing the deposits by centrifugation, the EtOH-soluble fraction was concentrated *in vacuo* to give a residue (225 g) that was chromatographed on macroporous resin column and successively

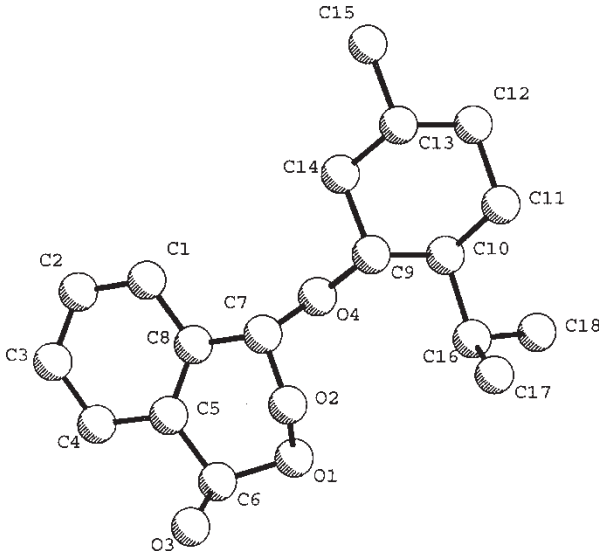


Figure 2. X-ray crystal structure of **2**.

eluted with H_2O and EtOH (10, 30, and 95%) to give four fractions. The 30% EtOH eluate was concentrated, and it showed significant antiviral activity. It was subjected to chromatography over NKA-9 macroporous resin column eluted with H_2O. The concentrated H_2O eluate was chromatographed on Sephadex LH-20 column and HPLC to afford compound **1** (Shuangkangsu).

3.4 Compound 1

White amorphous powder, $[\alpha]_D^{19}$ − 23.6 (*c* 1.0, H_2O); IR (KBr, cm^{-1}) ν_{max}: 3495; UV (H_2O) λ_{max} (nm) (ε): 240 (15,000); ^1H NMR (500 MHz, D_2O): δ(ppm) 5.60 (s, 2H, H-1 and H-4), 5.55 (dd, 2H, $J = 17.0$, 10.0 Hz, H-6 and H-9), 5.36 (dd, 2H, $J = 17.0$, 1.5 Hz, H-7 and H-10), 5.31 (dd, 2H, $J = 10.0$, 1.5 Hz, H-7 and H-10), 4.85 (d, 2H, $J = 8.0$ Hz,

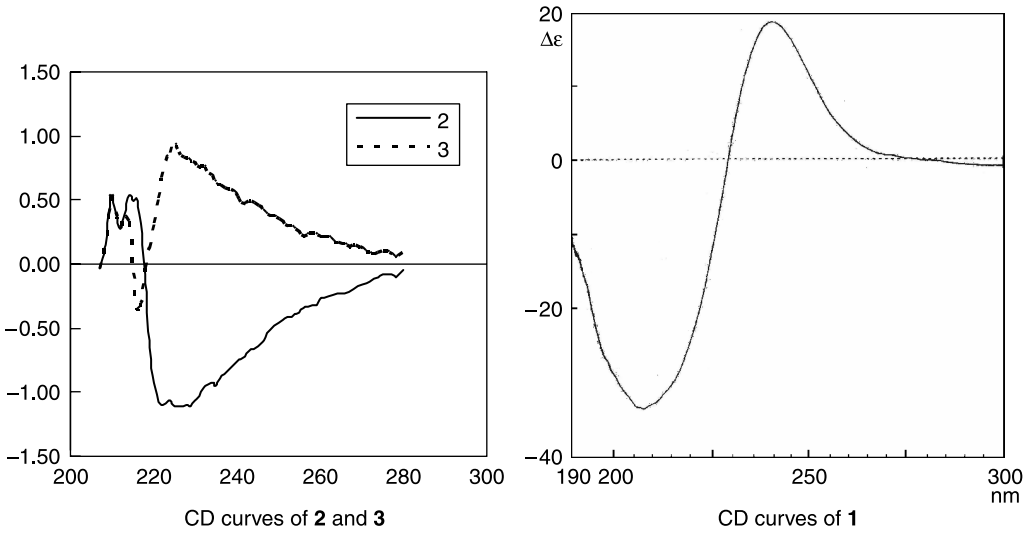


Figure 3. The CD spectra of **1**–**3**.

anomeric H), 3.92 (dd, 2H, $J = 12.5$, 2.0 Hz, H-6′), 3.72 (dd, 2H, $J = 12.5$, 6.0 Hz, H-6′), 3.51–3.29 (m, 8H, H-2′, H-3′, H-4′, and H-5′); ^{13}C NMR (125 MHz, D_2O): δ(ppm) 156.1 (C-5 and C-8), 134.4 (C-6 and C-9), 123.8 (C-7 and C-10), 101.3 (C-1′), 100.4 (C-1 and C-4), 79.7, 79.2, 75.6, 72.5, 63.7; FAB-MS *m/z*: 495 $[M + H]^+$, 517 $[M + Na]^+$; elemental analysis (%): C 48.40, H 6.23 (calcd for $C_{20}H_{30}O_{14}$: C 48.5, H 6.12); CD (H_2O): $\Delta\varepsilon$ (λ nm) + 19 (240), − 35 (210).

3.5 Compound 5

To a solution of phthalaldehyde (1 g, 7.4 mmol) in 40 ml CH_3OH was added 30% H_2O_2 (0.83 g, 7.4 mmol). The reaction mixture was stirred at room temperature for 12 h and then concentrated under reduced pressure. The residue was washed with EtOAc–PE (1:5) and compound **5** was obtained in 84% yield, mp 110–115°C; ^{1}H NMR (300 MHz, DMSO-d_6): δ(ppm) 7.35–7.31 (m, 2H), 7.30–7.25 (m, 2H), 5.89 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6): δ(ppm) 132.9 (2 × C), 128.1 (2 × CH), 126.6 (2 × CH), 92.7 (2 × CH); FAB-MS *m/z* (%): 191 ($[M + Na]^+$, 20), 173 (30), 151 (50), 133 (90), 115 (100); IR (KBr, cm^{-1}) ν_{max}: 3340, 1028.

3.6 Compounds 2 and 3

To a solution of **5** (1 g, 6 mmol) and (−)-menthol (9.3 g, 60 mmol) in 30 ml anhydrous Et_2O was added four drops $BF_3{\cdot}Et_2O$. The reaction mixture was stirred at room temperature for 24 h. Water (10 ml) was added and the organic phase was washed with water (2 × 10 ml), dried over Mg_2SO_4, and evaporated to give the crude product that was purified by column chromatography (PE–EtOAc 20:1). Compounds **2** (0.7 g) and **3** (0.7 g) were obtained as colorless needles. Compound **2**: mp 130–134°C, $[\alpha]_D^{25}$ − 196.6 (*c* 0.75, acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 7.40–7.37 (m, 3H, Ar-H), 7.27–7.25 (m, 1H, Ar—H), 5.99 (d, 1H, $J = 7.6$ Hz), 5.73 (s, 1H), 3.74 (t × d, 1H, $J = 10.4$, 4.0 Hz), 3.56 (d, 1H. $J = 7.6$ Hz, —OH), 2.43–2.36 (m, 1H), 2.23 (d, 1H, $J = 6.0$ Hz), 1.71–0.83 (m, 16H, for menthol); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 131.9 (C═C), 130.6 (C═C), 129.1 (C═C), 128.9 (C═C), 126.9(C═C, 2 × C), 96.0 (CH), 93.7 (CH), 77.1 (CH),47.8, 40.5, 34.4, 31.5, 24.9, 22.9, 22.3, 21.0, 15.4; FAB-MS *m/z* (%): 329 ($[M + Na]^+$, 100), 151 (30), 133 (60), 115 (45); CD spectra (*c* 0.0023, THF): $\Delta\varepsilon$ (λ nm) − 0.92 (224), + 0.33 (215). Compound **3**: mp 135–139°C, $[\alpha]_D^{25}$ + 126.0 (*c* 0.16, acetone); ^{1}H NMR (400 MHz, $CDCl_3$): δ(ppm) 7.43–7.36 (m, 3H, Ar-H), 7.31–7.27 (m, 1H, Ar-H), 5.98 (s, 1H), 5.70 (s, 1H), 3.64 (t × d, 1H, $J = 14.8$, 6.0 Hz), 3.56 (br s, 1H, —OH), 2.46–2.33 (m, 2H), 1.70–0.81 (m, 16H, for menthol); ^{13}C NMR (100 MHz, $CDCl_3$): δ(ppm) 131.7 (C═C), 130.7 (C═C), 129.2 (C═C), 129.1 (C═C), 127.0 (C═C), 126.8 (C═C), 100.3 (C-1), 93.9 (C-4), 81.5 (C-1′), 48.4, 42.9, 34.3, 31.8, 25.5, 23.0, 22.2, 21.1, 16.0; FAB-MS *m/z* (%): 329 ($[M + Na]^+$, 30), 115 (100); CD spectra (*c* 0.0023, THF): $\Delta\varepsilon$ (λ nm) + 0.91 (224), − 0.32 (215).

3.7 X-ray crystallographic analysis of 2

Single-crystal X-ray diffraction data on compound **2** were collected at 295 K using Mo Kα radiation and MAC DIP-2030K area detector. A colorless sheet of **2** (0.10 × 0.30 × 0.50 mm) was prepared for data collection. The crystal was monoclinic in space group $P2_1$ with unit cell dimensions as $a = 8.540(1)$ Å, $b = 6.178(1)$ Å, $c = 16.687(4)$ Å, and $\beta = 79.10(1)°$. The structure was solved by direct methods and refined by full-matrix least-squares on *F*[2] values using the programs found in the NOMCSDP package.[10] Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. The absolute configuration of **2** was set based on the known configuration of the optically pure (−)-menthol (one of the starting materials for the synthesis).

Acknowledgements

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