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Chiral separation, configurational identification and antihypertensive evaluation of (±)-7,8-dihydroxy-3-methyl-isochromanone-4

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ABSTRACT

(±)-7,8-Dihydroxy-3-methyl-isochromanone-4 [(±)-XJP] is a natural antihypertensive product contained in banana (*Musa sapientum* L.) peel. (−)-XJP and (+)-XJP were first obtained by chiral resolution, meanwhile circular dichroism (CD) calculations and chiral synthesis were employed to investigate the absolute configuration. The results indicated that the absolute configuration of (+)-XJP is *S*-configured and the absolute configuration of (−)-XJP is *R*-configured. Furthermore, the evaluation of antihypertensive effects in vivo proved that *R*-(−)-XJP was more potent than *S*-(+)-XJP and [(±)-XJP].

Life is heavily dependent on chirality, and chiral discrimination is frequently encountered in biological systems.[1] Although a number of chiral isomers of racemic drugs have almost identical physical and chemical properties, the enantiomers of many chiral therapeutic agents exhibit differences in activity and toxicity. Recently a great deal of attention has been focused on the separation of stereochemically pure enantiomers for the purpose of marketing drugs as single active compounds.[2]

(±)-7,8-Dihydroxy-3-methyl-isochromanone-4[(±)-XJP], isolated from the banana (*Musa sapientum* L.) peel extract,[3,4] is a structurally unique polyphenolic compound possessing a chiral center. It has been documented that (±)-XJP exerted a large variety of biological activities including antioxidative, antiinflammatory, antihypertensive and cardioprotective.[5–7] Interestingly, in our previous investigations, (±)-XJP significantly decreased blood pressure in a dose-dependent manner. In both acute and therapeutic antihypertensive tests (RHRs), the maximum antihypertensive effect of (±)-XJP at the dose of 100 mg/kg was comparable to that of captopril at the dose of 25 mg/kg.[6]

However, all the above pharmacodynamic studies were based on racemic XJP, the possible difference of the activities between the two enantiomers became interesting. Many attempts have been made to investigate the separation of stereochemically pure enantiomers for the purpose of obtaining single active moieties. Herein, we wish to report the chiral separation, absolute configurational analysis and evaluation of antihypertensive effects of XJP enantiomers.

The general methods to obtain pure enantiomers are asymmetric synthesis and resolution of racemates.[8] Several methods have been investigated to asymmetrically synthesize XJP.[3,6] However, due to keto-enol tautomerism of the carbonyl, the chiral carbon at C-3 becomes racemic easily in the process of preparation, especially under vigorous reaction conditions. In order to obtain stereochemically pure enantiomers, we tried to perform the chiral synthesis and demonstrate the absolute configuration.

As shown in Scheme 1, alkylation of **1** with methyl *S*-(−)-lactate in presence of NaH, followed by saponification of the methyl ester gave compound **3**. Intermediate **3** was treated with *n*-BuLi in THF at −85 °C, and then the temperature was allowed to rise to room temperature to give compound **4** in 58% yield.[9] After deprotection of phenolic methyl ether **4** by $AlCl_3$ in $CHCl_3$, target compound *S*-(+)-XJP was obtained. However, the content percentage of the *S*-configured intermediates and the optical rotation decreased with every reaction step, while the ultimate optical rotation was only $[\alpha]_D^{20}$ +31.58 (*c*, 0.202, MeOH). Using *R*-(+)-lactate as starting material, *R*-(−)-XJP was synthesized following the above procedure, and

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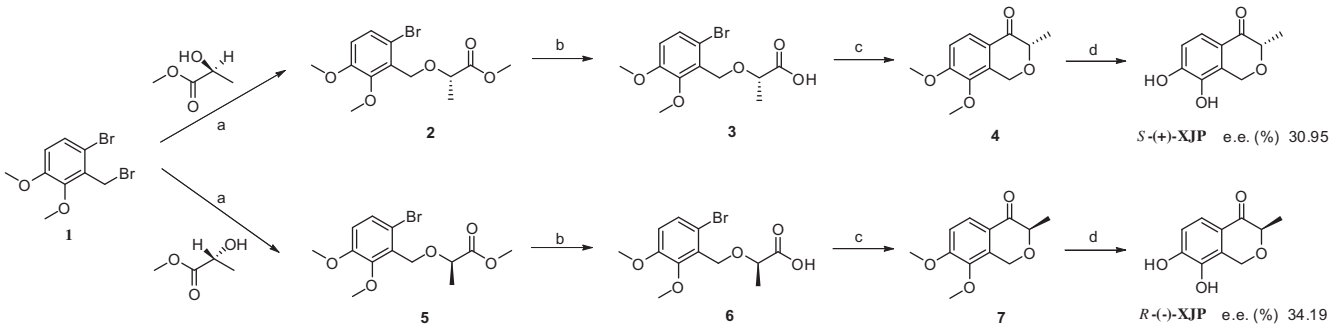

Scheme 1. Chiral synthetic routes to *S*-(+)-XJP and *R*-(−)-XJP. Reagents and conditions: (a) anhydrous DMF, NaH, 0 °C, 75–80%; (b) MeOH, 10% NaOH, rt, then 10% HCl, 90–95%; (c) *n*-BuLi, anhydrous THF, −85 °C to rt, 58–60%; (d) $AlCl_3$, NaI, $CHCl_3$, reflux, 50–55%.

the optical rotation was $[\alpha]_D^{20}$ −35.45 (*c*, 0.182, MeOH). Although part of the aimed products changed into racemic mixtures during the reaction procedures, it has been proved preliminarily that *S*-XJP is the dextro isomer, and *R*-XJP is the levo isomer. In order to get optically pure XJP enantiomers for further investigation, our group has also attempted other methods.

Resolution of racemates by chromatographic techniques is another available method for the development of isomerically pure agents in industry.[10] We performed the chiral separation of XJP enantiomers on a chiral column CHIRALPAK AD-H (5 cm I.D. × 25 cm L) by Supercritical Fluid Chromatography (SFC) and desired results were obtained. The chiral column was made of 5 μm silica gel, which was coated with starch tri[(3,5-dimethylphenyl)carbamate]. Methanol–CO_2 (15:85, *V*/*V*) was applied as the mobile phase and the detective wavelength was set at 212 nm.

As illustrated in Figure 1, (±)-XJP could be separated successfully under the above condition, the retention times of the enantiomers were 3.99 and 4.43 min, respectively. Enantiomeric excess of the two enantiomers were determined to be 98.09% and 99.84% by SFC using the same condition, respectively. Optical test proved that the first elute peak is the dextro isomer and the optical rotation is $[\alpha]_D^{20}$ +104.93 (*c*, 0.209, MeOH), and the second elute peak is the levo isomer and the optical rotation is $[\alpha]_D^{20}$ −103.68 (*c*, 0.500, MeOH).

The pure XJP enantiomers are white powder as their racemic mixtures. In order to determine the absolute configuration by X-ray diffraction analysis, although many attempts have been made, the perfect crystals were not obtained. Therefore, circular dichroism (CD) calculations, another mature method, was employed to investigate the absolute configuration.

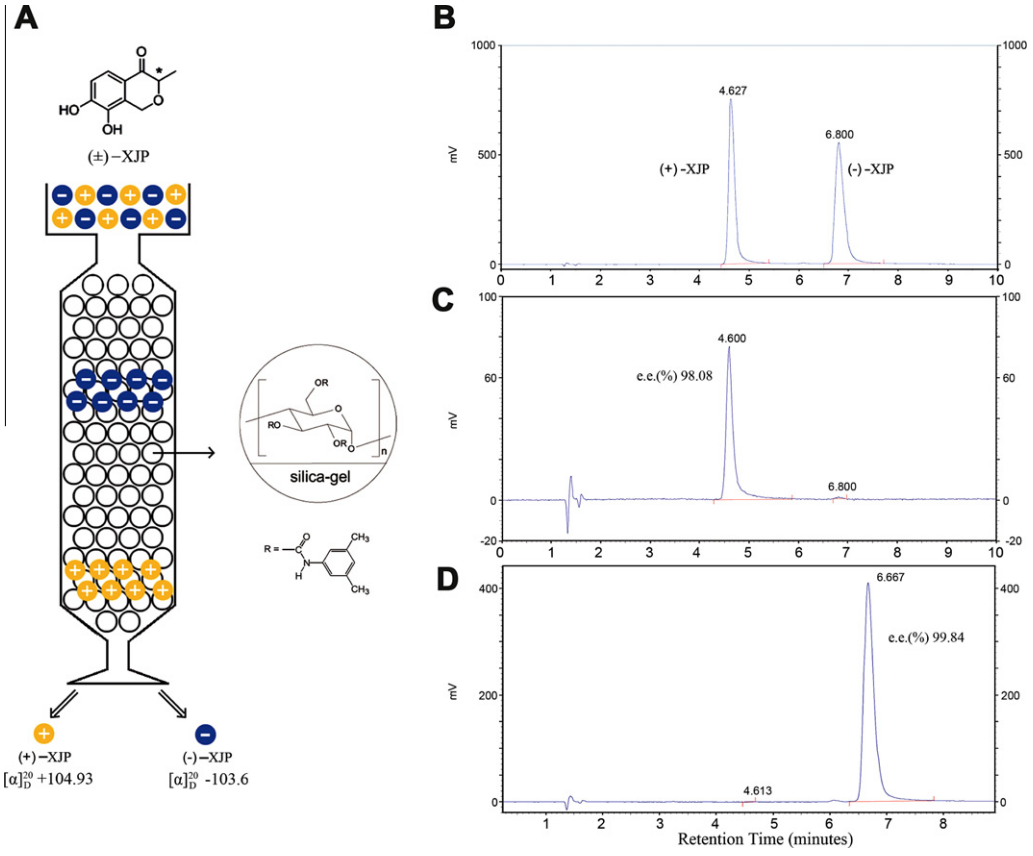

Figure 1. Chiral separation of XJP enantiomers: (**A**) The chiral column used in the separation; (**B**) Chromatograms of the separation of (±)-XJP; (**C**) Chromatograms of (+)-XJP; (**D**) Chromatograms of (−)-XJP.

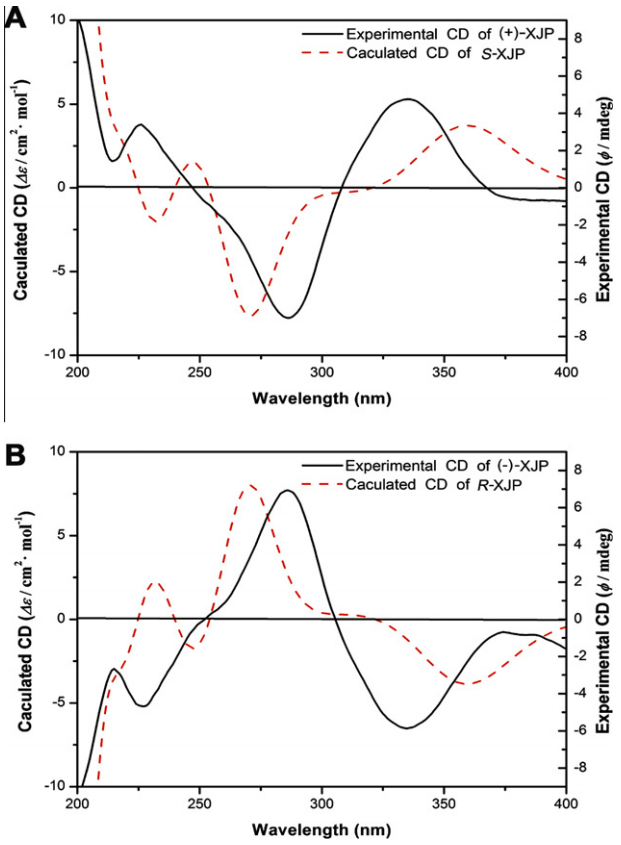

Figure 2. (**A**) Experimental CD spectra of (+)-XJP and comparison with the experimental CD spectrum of *S*-XJP; (**B**) Experimental CD spectra of (−)-XJP and comparison with the experimental CD spectrum of *R*-XJP.

Theoretical calculations of electronic circular dichroism (ECD) spectra for compounds (+)-XJP and (−)-XJP were performed with the Gaussian 09 program package.[11,12] The ECD calculations of compounds (+)-XJP and (−)-XJP were performed with time-dependent density functional theory (TDDFT) calculations at the b3pw91/6-311 + g(d, p) level of theory with the default solvent model using methanol as solvent. The calculated excitation energy (in nm) and rotatory strength, *R*, in dipole velocity (R_{vel}) and dipole length (R_{len}) forms, were simulated into an ECD curve using the:

$$\Delta\varepsilon(E) = \frac{1}{2.296 \times 10^{-39}\sqrt{\pi}\Delta}\sum_{a}\Delta E_{0a}R_{0a}exp[-(\frac{E-\Delta E_{0a}}{\Delta})^2]$$

Where Δ is half the bandwidth at 1/*e* height and expressed in energy units. The parameters ΔE_{0a} and R_{0a} are the excitation energies and rotatory strengths for the transition from 0 to *a*, respectively. The calculated CD spectrum for *S*-XJP showed an unambiguously agreed behavior in comparison to the experimental CD data of (+)-XJP, and the calculated spectrum also matched the experimental data perfectly (Fig. 2). A similar agreement applied between *R*-XJP and (−)-XJP. The calculated optical rotations of *S*-XJP and *R*-XJP were +161.99° and −161.99°, respectively, which were approximately consistent with the experimental data. The results indicated that the absolute configuration of (+)-XJP is *S*-configured and the absolute configuration of (−)-XJP is *R*-configured, which could be drawn with the same conclusions as that of chiral synthesis. So we confirmed that the XJP enantiomers are *S*-(+)-XJP and *R*-(−)-XJP, respectively.[13]

Based on the results of chiral separation and configurational identification, in vivo evaluation of antihypertensive effects in spontaneously hypertensive rats (SHRs) was performed to provide the information of possible different potency between XJP enantiomers. After oral administration of control drug captopril

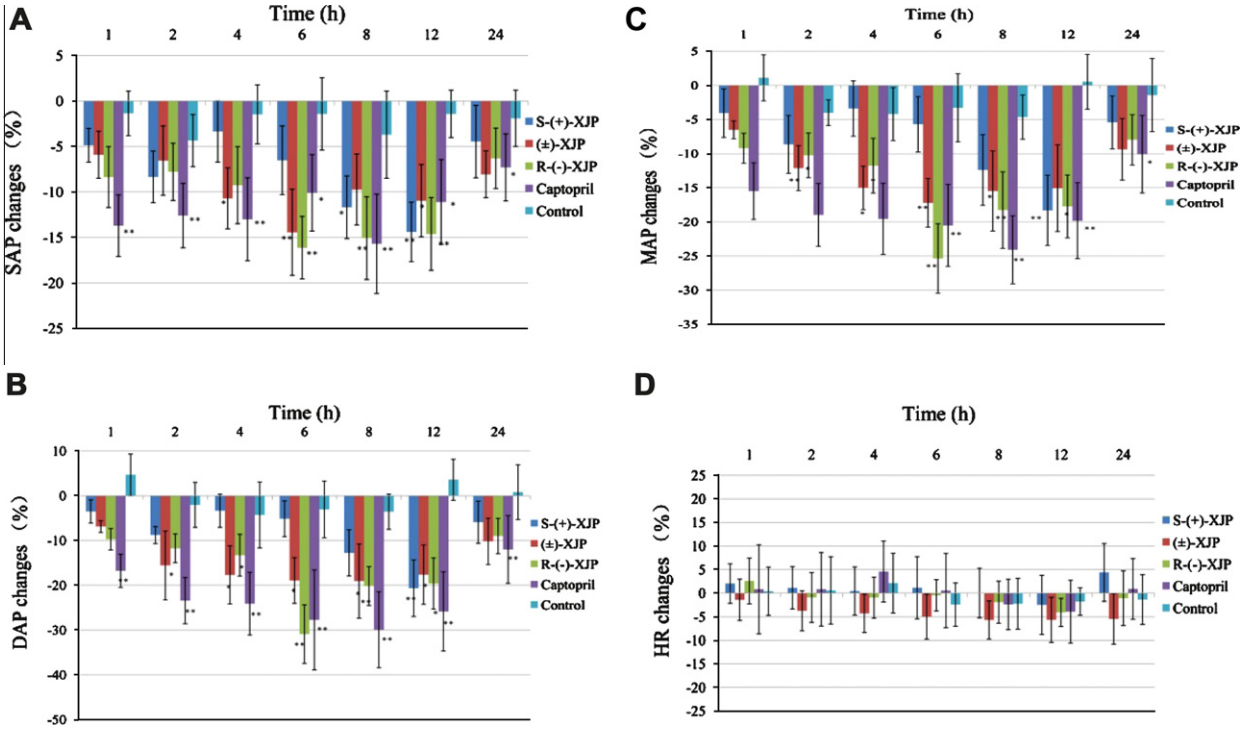

Figure 3. (**A**) The acute antihypertensive activities of (±)-XJP, (−)-XJP, (+)-XJP and captopril in SHRs (SAP, systolic arterial pressure); **B.** The acute antihypertensive activities of (±)-XJP, (−)-XJP, (+)-XJP and captopril in SHRs (DAP, diastolic arterial pressure); **C.** The acute antihypertensive activities of (±)-XJP, (−)-XJP, (+)-XJP and captopril in SHRs (MAP, mean artery pressure); **D.** The changes of heart rate (HR) of (±)-XJP, (−)-XJP, (+)-XJP and captopril in SHRs. Data are represented as mean ± SEM ($n = 8$). Significance levels $^{*}p$ <0.05 and $^{**}p$ <0.01 as compared with the respective control.

(40 mg/kg), (±)-XJP (80 mg/kg), (+)-XJP (80 mg/kg) and (−)-XJP (80 mg/kg), the blood pressure and heart rate of SHRs were detected from 0 to 24 h. The evaluation results showed that (−)-XJP exhibits more potent antihypertensive activity than (±)-XJP significantly, and the antihypertensive activity of (±)-XJP is stronger than that of (+)-XJP, which demonstrate that (−)-XJP plays a more important role in the antihypertensive effect of (±)-XJP (Fig. 3). The diastolic arterial pressure (DAP) of SHRs treated with (−)-XJP was reduced by almost 30% at 6 h, which is obviously superior to that of control drug captopril. Meanwhile, the maximum antihypertensive effect on the systolic arterial pressure (SAP) of (−)-XJP is also somewhat more effective than that of captopril. Furthermore, (±)-XJP and its enantiomers produce no observable alteration in the basal heart rate at these doses.

In summary, (−)-XJP and (+)-XJP was first obtained by chiral resolution, and CD calculations as well as chiral synthesis were employed to investigate the absolute configuration. It has been demonstrated that the absolute configuration of (+)-XJP is *S*-configured and the absolute configuration of (−)-XJP is *R*-configured. Antihypertensive effects of *S*-(+)-XJP and *R*-(−)-XJP in vivo were also evaluated. The results proved that (±)-XJP is a racemates with isomers possessing similar pharmacodynamic effects, but of different potency, and the *R*-(−)-XJP is more potent than *S*-(+)-XJP. Further investigation involving with *R*-(−)-XJP and the mechanism are currently in progress and will be reported in due course.

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13. *Analytical data for S-(+)-XJP*: $[\alpha]_D^{20}$ +104.93 (*c*, 0.209, MeOH); mp 163–164 °C (dec.); ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.29 (d, J = 6.7 Hz, 3H, –CH_3), 4.19 (q, J = 6.7 Hz, 1H, –CH–), 4.68 (d, J = 15.5 Hz, 1H, –CH_2–), 4.97 (d, J = 15.9 Hz, 1H, –CH_2–), 6.82 (d, J = 8.7 Hz, 1H, Ar-H), 7.31 (d, J = 8.7 Hz, 1H, Ar-H), 8.90 (1H, –OH), 10.40 (1H, -OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) 194.8, 150.8, 139.8, 130.3, 121.7, 119.0, 114.6, 76.8, 62.6, 15.9. HRMS (ESI) calcd for $C_{10}H_{11}O_4$ $[M+H]^+$: 195.0652, found 195.0653. Analytical data for *R*-(−)-XJP: $[\alpha]_D^{20}$ −103.68 (*c*, 0.500, MeOH); mp 163–164 °C (dec.); ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) 1.26 (d, J = 6.7 Hz, 3H, –CH_3), 4.19 (q, J = 6.7 Hz, 1H, –CH–), 4.68 (d, J = 15.5 Hz, 1H, –CH_2–), 4.92 (d, J = 15.9 Hz, 1H, –CH_2–), 6.82 (d, J = 8.7 Hz, 1H, Ar-H), 7.31 (d, J = 8.7 Hz, 1H, Ar-H), 8.92 (1H, –OH), 10.39 (1H, –OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm) 194.8, 150.8, 139.8, 130.3, 121.7, 119.0, 114.6, 76.8, 62.6, 15.9. HRMS (ESI) calcd for $C_{10}H_{11}O_4[M+H]^+$: 195.0652, found 195.0650.