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(*S*)-Dihydrooroxylin A in human urine following oral administration of the traditional Chinese medicines: *Daisaiko-to* and *Shosaiko-to*

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Abstract: Dihydrooroxylin A was isolated and characterized from human urine collected after oral administration of traditional Chinese medicines, *Daisaiko-to* and *Shosaiko-to* (*Dachaihu-tang* and *Xiaochaihu-tang* in Chinese, respectively). Spectroscopic data (UV, CD, ^{1}H NMR and MS) and chiral HPLC analysis revealed that this compound had the *S*-configuration (80% ee). This is the first example of stereoselective hydrogenation of a substituted flavone into its corresponding flavanone in the human body. © 1997 Elsevier Science Ltd

To clarify molecular structures of chemical components which are actually absorbed into the body is important for evaluating clinical effects of traditional herbal medicines. Recently, we have determined chemical structures of nine metabolites[1] detected in human urine following oral administration of *Daisaiko-to* (TJ-8, Tsumura Co., Japan) and *Shosaiko-to* (TJ-9),[2] the most popular *Kampo*-medicines used in Japan. The structures of these metabolites were characterised as simple flavonoids and anthraquinones which were derived from the corresponding glycosides contained in the medicines through hydrolysis by intestinal bacterial flora. There are some other metabolites, the structures of which are still to be determined.

Here we report the first example of stereoselective hydrogenation of a herbal flavonoid by some of the metabolizing enzymes of intestinal bacterial flora or the human liver. We analyzed the timed urine samples collected after adminisrtration of TJ-8 and TJ-9 to healthy volunteers with a dosage of 5 g. A new metabolite **1** was detected by reversed phase HPLC as illustrated in Figure 1 (blackened peak). The highest peak of **1** was detected at 30~36 h following administration. In Figure 1, peaks of two flavones, wogonin and oroxylin A, were also observed (Peaks **2** and **3**, respectively). To determine the herbal origin of **1**, we further analyzed urine samples collected after administration of aqueous extract of the dry root of *Scutellaria baicalensis*, one of the herb ingredients of the medicines, and found the same peak as that of **1**. The amount of **1** in pure crystals (m.p. 166~168 °C) recovered from the cumulative urine samples collected at 24~42 h following administration was around 0.3 mg.[3] Using this as an authentic specimen, we analysed quantitatively the total excretion of **1** following administration of TJ-8 and the amount of **1** in TJ-8 (5 g), respectively. Since it has been reported that **1** is contained in *S. baicalensis* as an aglycone and glycosides,[4,5] we determined the total amount of **1** in TJ-8 after hydrolysis of glycosides by the use of β-D-glucuronidase and β-D-glucosidase. As a result, the excreted amount (1.87 mg) of **1** was 6 times greater than the total amount (0.33 mg) of **1** in TJ-8.

The chemical structure of the new metabolite was elucidated as follows.[6] First, EI-MS exhibited a base molecular peak at m/e 286, which was larger than those of the coexisting oroxlylin A and wogonin by 2 mass units, suggesting that a possible structure for **1** might be a dihydro-derivative of either oroxylin A or wogonin. Second, the ^{1}H NMR spectrum of **1** was superimposable on the reported spectrum of dihydrooroxylin A,[4] and there was an apparent difference in chemical shifts of the methoxyl protons between **1** and dihydrowogonin[7] (3.95 and 3.90 ppm, respectively). The UV-

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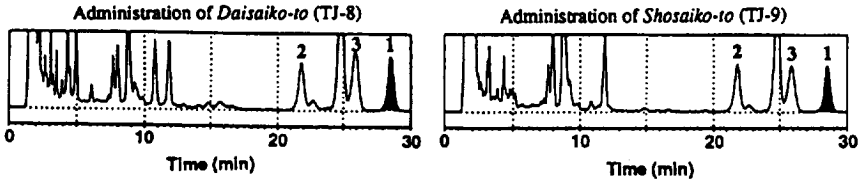

Figure 1. Chromatograms of urine samples at 30~36 h after oral administration of each herbal medicine. ODS column (see Ref.[3]) was maintained at 30 °C, H_2O/CH_3CN (67/33, v/v, containing 0.5 mM H_3PO_4) was delivered at 1.5 ml/min, and UV wavelength was set at 195~400 nm. Key: **1**, new metabolite; **2**, wogonin; **3**, oroxylin A.

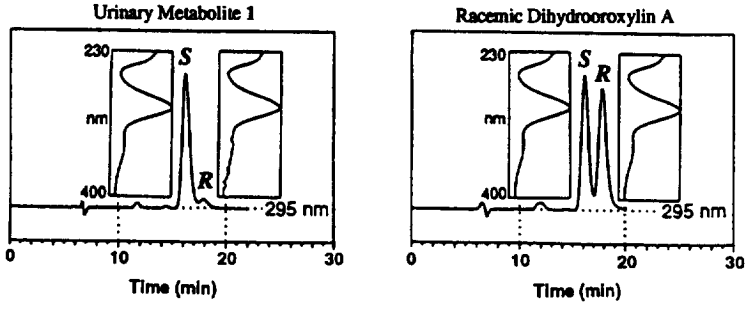

Figure 2. Enantiomeric separation of the urinary metabolite **1** and racemic dihydrooroxylin A. Chiralcel® OD column (250 mm×4.6 mm i.d.) was maintained at 25 °C, n-hexane/2-propanol (70/30, v/v, containing 0.1% AcOH) was delivered at 0.5 ml/min.

absorption spectrum of **1** was consistent with both of the candidates. Moreover, the difference in the reported melting point of the candidates was larger than 10 °C which enabled us to confirm **1** as dihydrooroxylin A (Ref.[4]: m.p. 165~167 °C) rather than dihydrowogonin (Ref.[7]: m.p. 154~155 °C).

In the next stage of our structure elucidation, we investigated the configuration of the new metabolite by means of chiral HPLC and CD. We observed that **1** consisted of almost a single enantiomer, as shown in the left chiral chromatogram of Figure 2, and exhibited a negative Cotton effect at 295 nm in the $\pi \rightarrow \pi^*$ region and a positive effect at 340 nm due to $n \rightarrow \pi^*$ transition. This suggested that the major component of the new metabolite had the *S*-configuration. To confirm the enantiomeric excess of **1**, we prepared a racemic mixture from **1** as illusrated in Scheme 1. Treatment of **1**, first in 10% KOH solution at 80 °C for 1 min and then in acidified solution by the addtion of 5% HCl, gave the racemic mixture of dihydrooroxylin A. Although this mixture showed no optical activity on its CD spectrum, each enantiomer peak (see the right chromatogram in Figure 2) exhibited the same UV spectra as expected. The amount of the stereoisomers in **1** from urine was around *S*:*R*=90:10, determined by peak-area calculation.

The enantiomeric configuration of dihydrooroxylin A contained in *S. baicalensis*[4,5] and *S. scandens*,[8] the former of which is a herb ingredient used in TJ-8 and TJ-9, was *S*. The fact that the amount of dihydrooroxylin A in the herbal medicines was much less than that excreted into the urine means that the *S*-isomer was principally formed in the human body through stereoselective transformation of oroxylin A by intestinal bacterial flora or hepatic enzymes. Since the maximum rate of formation was observed 30~36 h after administration of the medicines, some intervention of intestinal bacterial flora rather than hepatic metabolism was principally suggested. Pharmacological activities of **1** remain to be investigated.

Acknowledgements

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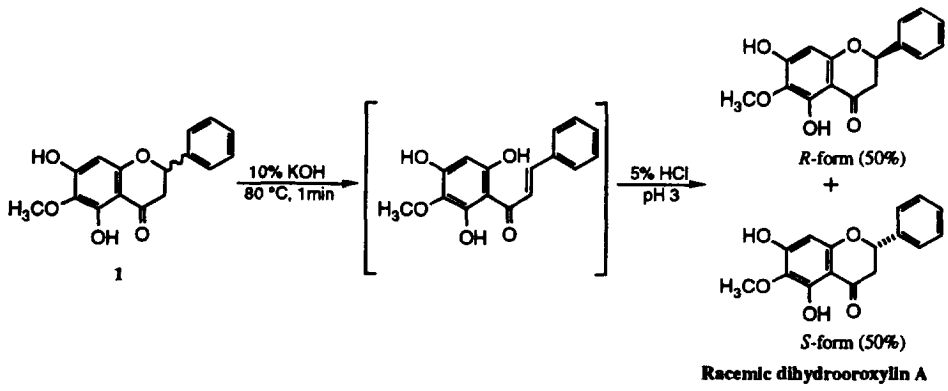

Scheme 1. Chemical transformation of **1** into racemic dihydrooroxylin A.

References

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2. *Daisaiko-to* (TJ-8) and *Shosaiko-to* (TJ-9) purchased from Tsumura Co. (Tokyo, Japan) were the aqueous extract granule preparations for ethical use. Their herb ingredients are:

Herb ingredient	TJ-8		TJ-9	
	Parts	% (w/w)	Parts	% (w/w)
Root of *Bupleurum falcatum* Linne (*Umbelliferae*)	6.0	26.1	7.0	29.2
Root of *Scutellaria baicalensis* Georgi (*Labiatae*)	3.0	13.0	3.0	12.5
Tuber of *Pinellia ternata* Breitenbach (*Araceae*)	4.0	17.4	5.0	20.8
Rhizoma of *Zingiber officinale* Rosco (*Zingiberaceae*)	1.0	4.3	1.0	4.2
Ripe fruit of *Zizyphus jujuba* Miller var. *inermis* Rehder (*Rhamnaceae*)	3.0	13.0	3.0	12.5
Rhizoma of *Rheum palmatum* L. & *R. tanguticum* M. (*Polygonaceae*)	1.0	4.3		
Immature fruit of *Citrus natsudaidai* Hayata (*Rutaceae*)	2.0	8.7		
Root of *Paeonia lactiflora* Pallas (*Paeoniaceae*)	3.0	13.0		
Root of *Panax ginseng* C.A. Meyer (*Araliaceae*)			3.0	12.5
Root of *Glycyrrhiza glabra* Linne (*Leguminosae*)			2.0	8.3

3. Isolation of **1**: An 150-ml volume of the urine sample pre-treated by β-D-glucuronidase was extracted with 1800 ml of 1,2-dichloroethane on a diatomaceous earth column (5 cm×14 cm i.d.). After the solvent was evaporated under reduced pressure, the residue was dissolved in 2 ml of ethanol. Repetitive injection of each 100-μl volume of the solution into the HPLC system which consisted of an ODS column (250 mm×4.6 mm i.d., Shiseido, Tokyo, Japan) and a mobile phase of 0.2% aq. AcOH/CH_3CN (16/9, v/v) at a flow rate of 1.5 ml/min gave 0.3 mg of **1** after the solvent of the 19-min fraction was evaporated to dryness and the residue was recrystallized with ethanol.
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6. Spectral data for **1**: UV, λ_{max} (MeOH) 290 nm, λ_{max} (MeOH+NaOAc) 294 nm, 326 nm; ^1H NMR (400 MHz, $CDCl_3$) δ 2.83 (1H, dd, J=17.1, 3.1 Hz, C3-H), 3.08 (1H, dd, J=17.2, 13.0 Hz, C3-H), 3.95 (3H, s, C6-OCH_3), 5.41 (1H, dd, J=13.0, 3.0 Hz, C2-H), 6.13 (1H, s, C8-H), 7.39–7.45 (5H, m, C2′~6′-H_5).
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