Studies on Aldose Reductase Inhibitors from Natural Products. V.[1] Active Components of Hachimi-jio-gan (Kampo Medicine)

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Aldose reductase (AR) inhibitory activity-directed fractionation of Hachimi-jio-gan has led to the isolation of 5-(hydroxymethyl)-2-furfuraldehyde (1) and ellagic acid (2). 2 was reported to be a strong AR inhibitor in this series of study on AR inhibitors, but 1 is the first isolation from a natural source and as an AR inhibitor. The AR inhibitory activity of the eight crude drugs which constitute Hachimi-jio-gan, and a comparison of their components by TLC, were also examined. Corni Fructus was found to be one of the important drugs having an AR inhibitory effect, and only in this drug were compounds 1 and 2 contained together.

Keywords Hachimi-jio-gan; Kampo medicine; aldose reductase inhibitor; ellagic acid; 5-(hydroxymethyl)-2-furfuraldehyde; Corni Fructus

Hachimi-jio-gan (Kampo medicine 八味地黃丸), which is composed of eight crude drugs, is a very important Chinese medicinal prescription for senile cataracts and diabetic complications as well as for diabetes.[2] This medicine has suppressive effects on osmotic hydration and galactitol accumulation in the lens,[3] as well as a potent inhibitory effect on aldose reductase (AR).[4] However, its active constituents and mechanisms of action remain unclear. AR plays a significant role in the reduction of aldose to alditol under abnormal conditions such as diabetes, leading to a loss of functional integrity of the lens and subsequent cataract formation. Thus, AR inhibitors may possibly be effective for treating diabetic complications such as cataracts, neuropathy, retinopathy and nephropathy.[1] This paper reports the isolation and identification of the active components of Hachimi-jio-gan inhibiting rat lens AR. Eight constituent crude drugs of Hachimi-jio-gan were compared for their AR inhibitory activity.

AR Inhibitors of Hachimi-jio-gan An extract of Hachimi-jio-gan (A) prepared from eight crude drugs, Cinnamomi Cortex, Aconiti Tuber, Rhemaniae Radix, Hoelen, Corni Fructus, Dioscorea Radix, Alismatis Rhizoma and Moutan Cortex, was suspended in water and extracted successively with Et_2O, AcOEt and n-BuOH to obtain an Et_2O extract (fr. B), AcOEt extract (fr. C), n-BuOH extract (fr. D) and residue (fr. E) (Fig. 1). Among these extracts, frs. B and C expressed a strong inhibitory effect on AR, approximately forty times of more that of the original A (Fig. 1). Frs. B and C were obtained in low yields, therefore, another method for extraction must be developed. Dried extract (A) was powdered and extracted with AcOEt using Soxhlet's extractor to afford extract C′. This extract was compared with frs. B and C for AR

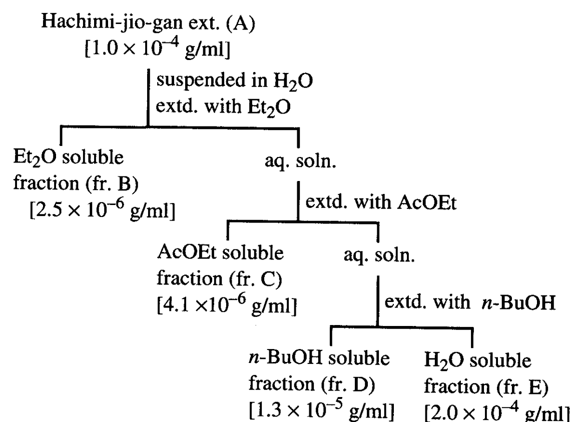

Fig. 1. Fractionation of Hachimi-jio-gan Extract
[ ] indicates AR inhibitory activity (IC_{50}).

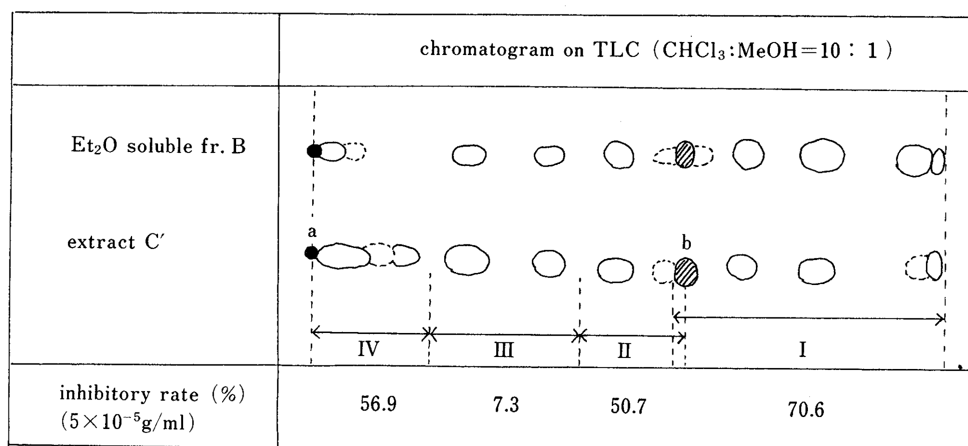

Fig. 2. TLC Pattern of Fr. B and Ext. C′ from Hachimi-jio-gan and AR Inhibitory Rate of Frs. I to IV from Fr. C′

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each Et_2O soluble fraction	chromatogram on TLC ($CHCl_3$:MeOH=10 : 1)	IC_{50}(g/ml)
Hachimi-jio-gan (fr.B)		2.5×10^{-6}
Dioscoreae Radix (Sanyaku)		1.9×10^{-5}
Rehmanniae Radix (Dioo)		8.5×10^{-6}
Alismatis Rhizoma (Takusya)		7.0×10^{-5}
Hoelen (Bukuryoo)		1.7×10^{-5}
Moutan Cortex (Botanpi)		8.1×10^{-6}
Cinnamomi Cortex (Keihi)		1.0×10^{-5}
Corni Fructus (Sansyuyu)		5.1×10^{-6}
5-hydroxymethyl-2-furfuraldehyde (1)		2.3×10^{-5}
ellagic acid (2)		2.0×10^{-7}

Fig. 3. TLC Pattern of Crude Drugs Contained in Hachimi-jio-gan and Their Inhibitory Activities on AR

inhibitory activity and TLC patterns. Extract C′ contained essentially the same components as frs. B and C from TLC results. Two conspicuous spots were found on TLC by spraying dil.H_2SO_4 followed by heating: one (a) was dark green at the *Rf* of 0.6 and the other (b) was dark brown at the origin. Extract C′ was applied onto a column of silica gel and four fractions (frs. I, II, III and IV) were obtained (Fig. 2). The inhibitory rates in fr. I to IV were 70.6, 50.7, 7.3 and 56.9% at 5×10^{-5} g/ml. Fr. I and IV were column chromatographed again using the same container and developing solvent to afford compounds **1** from fr. I and **2** from fr. IV. Compound **1** corresponded to spot b and compound **2** to spot a.

Compound **1**, a yellow oil, exhibited a peak at *m/z* 126 with a mass spectrum (MS). Its molecular formula was $C_6H_6O_3$. The infrared (IR) spectrum of **1** showed absorption bands due to hydroxyl and conjugated aldehyde functions. The proton and carbon nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectral data suggested the structure of **1** to be a two substituted furan derivative. Compound **1** was thus concluded to be 5-(hydroxymethyl)-2-furfuraldehyde from the above results and physical and spectral data.[4] It was isolated here for the first time from a natural source.

Compound **2**, yellow needles recrystallized from pyridine, exhibited a positive color reaction to $FeCl_3$ and was identified as ellagic acid by comparison with an authentic sample.

Results and Discussion

Ellagic acid had a strong inhibitory effect on AR activity (IC_{50}: 2.0×10^{-7} g/ml). It was previously isolated from other plants as a highly potent AR inhibitor.[1,6,7] The effect of 5-(hydroxymethyl)-2-furfuraldehyde (IC_{50}: 2.3×10^{-5} g/ml) was about four times that of the original extract (A) (IC_{50}: 1.0×10^{-4} g/ml). To determine the type of inhibition, the kinetics of inhibition of AR by **1** were plotted according to Lineweaver–Burk, and **1** was found to be a non-competitive inhibitor at the concentration of 2.5×10^{-5} and 2.5×10^{-6} M as was seen in the case of **2**.[6] The inhibitory effect of **1** on AR was also examined in the presence of excess bovine serum albumin. **1** did not lessen the effect of this compound, suggesting that **1** inhibits the activity of lens AR, even in the presence of other proteins.

Eight crude drugs constituting Hachimi-jio-gan were also extracted with Et_2O under the above conditions. Aconiti Tuber had no effect on AR activity. The seven other Et_2O extracts were compared for TLC patterns and only Corni Fructus showed two spots a and b similar to those observed in the TLC of fr. B. Compared to the other extracts, Corni Fructus markedly inhibited AR activity (IC_{50}: 5.1×10^{-6} g/ml). (Fig. 3). Other active constituents are presently being sought. 5-(Hydroxymethyl)-2-furfuraldehyde appears to possibly be involved in the AR inhibiting effect of this medicinal prescription, as does ellagic acid, although the activity is somewhat weaker than that of the Et_2O soluble fraction from Corni Fructus.

Experimental

IR and UV spectra were obtained with a Hitachi 260-10 and Hitachi 220 S spectrometer, respectively. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with a JEOL FX 90Q (90 MHz) spectrometer and a JEOL FX 90Q (22.5 MHz) spectrometer in $CDCl_3$, respectively. Chemical shifts are given in δ (ppm) relative to internal tetramethylsilane. MS were obtained on a JEOL-JMS-D 200 instrument. TLC was conducted using a Kieselgel 60 F_{254} plate (Merck). Spots were detected under a UV lamp and by heating after spraying with 10% H_2SO_4.

Materials The extracts of Hachimi-jio-gan (A) and eight constituent crude drugs were purchased from Nihon Funmatsu Co., Ltd., Osaka, Japan.

Bioassay The inhibitory activity of the samples on crude rat lens AR was assayed by the method previously reported.[8] The reaction was initiated by the addition of 20 μl of crude AR solution (1.6—1.9 units/ml) in 0.1 M sodium phosphate buffer containing 0.104 mM NADPH and 10 mM DL-glycelaldehyde in a total volume of 1.5 ml at 25 °C. Samples were dissolved in dimethylsulfoxide, which was found to have no effect on the enzyme activity at a concentration below 1%.

Extraction and Fractionation 300 g of extract (A) were suspended in 300 ml of H_2O and extracted with Et_2O (700 ml × 3), AcOEt (700 ml × 3) and BuOH (700 ml × 3) successively to yield extracts B (30 mg), C (200 mg) and D (40 g). 100 g of A were alternatively powdered and extracted with AcOEt using Soxhlet's extractor to give 100 mg of extract C′. Frs. I—IV for bioassay were obtained by preparative TLC developed with $CHCl_3$–MeOH (10 : 1). Compounds **1** and **2** were isolated from fr. I and IV by silica gel column chromatography by eluting $CHCl_3$ and $CHCl_3$–MeOH (10 : 1).

5-(Hydroxymethyl)-2-furfuraldehyde (1) Yellow oil. MS *m/z*: 126 (M^+). UV λ_{max}^{EtOH} nm: 278. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2850, 1670. ^{1}H-NMR ($CDCl_3$): 9.45 (–CHO), 7.16, 6.48 (each 1H, d, J=3.5 Hz, H-3,4), 4.60 (2H, s, –CH_2OH). ^{13}C-NMR ($CDCl_3$): 177.9 (d,–CHO), 161.2 (s), 152.2 (s), 123.3 (d), 110.0 (d), 57.3 (t).

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References and Notes

1) Part IV: S. Terashima, M. Shimizu, S. Horie, N. Morita, *Chem. Pharm. Bull.*, **39**, 3346 (1991).
2) K. Fujimura, *Jpn. J. Oriental Medicine*, **24**, 1 (1973).
3) A. Kamei, S. Iwata, *J. of the Eye*, **1**, 106 (1984).
4) A. Aida, H. Shindo, M. Tawata, T. Onaya, *Clin. Endocrinol.*, **33**, 1163 (1985); *idem, Planta Medica*, **53**, 131 (1987).
5) W. N. Haworth, W. G. M. Jones, *J. Chem. Soc.*, **1944**, Part II, 667.
6) M. Shimizu, S. Horie, S. Terashima, H. Ueno, T. Hayashi, M. Arisawa, S. Suzuki, M. Yoshizaki, N. Morita, *Chem. Pharm. Bull.*, **37**, 2531 (1989).
7) S. Terashima, M. Shimizu, H. Nakayama, M. Ishikura, Y. Ueda, K. Imai, A. Suzui, N. Morita, *Chem. Pharm. Bull.*, **38**, 2733 (1990).
8) M. Shimizu, T. Itoh, S. Terashima, T. Hayashi, M. Arisawa, N. Morita, S. Kurokawa, K. Itoh, Y. Hashimoto, *Phytochemistry*, **23**, 1885 (1984).

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