Studies on Aldose Reductase Inhibitors from Natural Products. IV.¹⁾ Constituents and Aldose Reductase Inhibitory Effect of *Chrysanthemum morifolium*, *Bixa orellana* and *Ipomoea batatas*

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The hot water extracts of *Chrysanthemum morifolium*, *Bixa orellana* and *Ipomoea batatas*, were found to have potent inhibitory activity towards lens aldose reductase (AR). Ellagic acid (4) was isolated from *C. morifolium* and *I. batatas*, isoscutellarein (7) from *B. orellana* and 3,5-dicaffeoylquinic acid (10) from *I. batatas*, respectively, as potent inhibitors.

Keywords *Chrysanthemum morifolium*; *Bixa orellana*; *Ipomoea batatas*; aldose reductase inhibitor; ellagic acid; isoscutellarein; 3,5-dicaffeoylquinic acid

Aldose reductase (AR) is the enzyme which, along with the coenzyme nicotinamide adenine dinucleotide phosphate (NADPH), catalyzes the reduction of glucose to sorbitol. AR is likely to be responsible for the increased intracellular accumulation of sorbitol found in many tissues, such as lens, nerve, retina and kidney of diabetic animals. It has currently been expected that AR inhibitors will play an important role in the management of diabetic complications such as cataracts,²⁾ neuropathy,^{3,4)} retinopathy⁵⁾ and nephropathy.⁶⁾ Carboxylic acids,^{7,8)} flavonoids,⁹⁻¹¹⁾ hydantoin analogues^{12,13)} and so forth have hitherto been examined as AR inhibitory drugs.

We have made screening tests on AR inhibitory effect with useful plants, for several years, among which *Chrysanthemum morifolium* (Compositae), *Bixa orellana* (Bixaceae) and *Ipomoea batatas* (Convolvulaceae) were found to show high inhibitory effects. In this paper, we report the isolation and identification of the active compounds inhibiting rat lens AR from these plants.

AR Inhibitors of *Chrysanthemum morifolium* The flower of *C. morifolium*, "kikuka," has traditionally been used for the treatment of eye disease in Japan and China and some flavonoids¹⁴⁾ were isolated without relation to the AR inhibitory effect. The hot water extract of this flower, which showed high inhibitory activity towards crude rat lens AR ($IC_{50} = 3.3 \times 10^{-6}$ g/ml), was partitioned between Et₂O and water to afford the Et₂O extract and water layer. The water layer was further extracted with AcOEt and *n*-BuOH successively to afford the AcOEt extract and *n*-BuOH extract. The AcOEt extract, which was most active (Table I), was applied to columns of silica gel and Sephadex LH-20 to give four compounds 1—4. Compounds 1—3 and 4 were identified as acacetin 7-*O*-glucoside, diosmetine 7-*O*-glucoside, luteoline 7-*O*-glucoside and ellagic acid, respectively, by comparison with published spectral data¹⁴⁾ and their authentic samples.

TABLE I. Inhibitory Activity against Rat Lens AR by Extracts

	IC_{50} (μ g)		
	<i>C. morifolium</i>	<i>B. orellana</i>	<i>I. batatas</i>
Crude ext.	3.3	3.0	1.0
Et ₂ O ext.	0.60	3.0	0.25
AcOEt ext.	0.40	0.15	0.20
<i>n</i> -BuOH ext.	5.5	2.0	2.0
H ₂ O ext.	> 10	> 10	> 10

AR Inhibitors of *Bixa orellana* The leaf of *B. orellana* has traditionally been used for the treatment of an antipyretic in South America. The IC_{50} of the hot water extract was 3.3×10^{-6} g/ml. The extract was partitioned between preceding solvents. The AcOEt extract, which was most active (Table I), was applied to columns of silica gel to give three compounds 5—7. Compounds 5 and 6 were identified as gallic acid and pyrogallol, respectively, by direct comparison with their authentic samples. Compound 7, yellow needles, exhibited a positive reaction test for flavonoids. Infrared (IR) and ultraviolet (UV) spectra of 7 showed the characteristic absorption patterns of flavone type. At the proton nuclear magnetic resonance (¹H-NMR) spectrum of 7 and 7 acetate suggested the presence of four hydroxyl groups. The positions of four hydroxyl groups on 7 were thus considered to be 5, 7, 8 and 4' by the addition of shift reagents in the UV spectrum¹⁵⁾ and comparison with published spectral data.¹⁶⁾ Consequently, 7 was concluded to be isoscutellarein.

AR Inhibitors of *Ipomoea batatas* *I. batatas*, a root called "satsumaimo," has been used for food. The hot water extract of this leaf showed high inhibitory activity towards crude rat lens AR ($IC_{50} = 1.0 \times 10^{-6}$ g/mg). The extract was partitioned between preceding solvents. The AcOEt extract, which was most active (Table I), was applied to columns of silica gel and preparative thin layer chromatography to give four compounds 4, 8—10. Compounds 8 and 9 were identified as scopoletin and caffeic acid by direct comparison with their authentic samples. Compound 10, pale yellow amorphous powder, gave a positive color reaction to FeCl₃. The UV spectrum of 10 was very similar to 9. In the IR spectrum of 10, the absorption due to α,β -unsaturated carboxylic acid group found in 9 disappeared, but the presence of another carboxylic acid group was suggested. The ¹H-NMR spectrum and ¹³C-nuclear magnetic resonance (¹³C-NMR) spectrum of 10, suggested the presence of two caffeoyl groups and one quinic acid. The position of two caffeoyl groups on quinic acid were thus considered to be 3 and 5 by comparing with published spectral data.¹⁷⁾ Consequently, 10 was determined to be 3,5-dicaffeoylquinic acid, which is the first isolation from this plant.

Results and Discussion

The isolated compounds 1—10 were tested for AR inhibitory activity (Table II). Among them, 4 exhibited the highest activity ($IC_{50} = 2.0 \times 10^{-7}$ M), which was previously reported as the high potent AR inhibitor from

TABLE II. Inhibitory Activity against Rat Lens AR by Compounds

Compd. No.	IC ₅₀ (μM)	Compd. No.	IC ₅₀ (μM)
1	>100	7	3.2
2	25	8	10
3	1.0	9	>100
4	0.20	10	0.46
5	>100	Quercitrin ^{a)}	1.2
6	>100		

a) Quercitrin was assayed previously, and was tested again as a reference in this study.

Phyllanthus niruri.¹⁸⁾ Compound **10**, which has never been tested for inhibitory activity towards AR, also showed high effect (IC₅₀ = 4.6 × 10⁻⁷ M). To determine the type of inhibition, the kinetics of inhibition of AR by **10** were plotted according to Lineweaver-Burk, and **10** was found to be a non-competitive inhibitor at the concentration of 0.1–1.0 × 10⁻⁶ M. This type was also seen in the case of **4** and other inhibitors. The inhibitory effect of **10** on lens AR was also checked in the presence of a large amount of bovine serum albumin (BSA). Compound **10** showed almost the same degree of inhibition in the presence and absence of BSA. Comparison of the activity of **1**–**3** seems to suggest that the hydroxy group in the B-ring of flavonoid is potentially concerned with AR inhibitory activity. This is in agreement with the structure-activity relationship of flavonoid in AR inhibitory activity previously reported.⁶⁾

We concluded that compound **4** is a main AR inhibitor of *C. morifolium* and *I. batatas*. In addition, compounds **3**, **7** and especially **10** are also considered to play important roles as AR inhibitors.

Experimental

Melting points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Spectral data were obtained as follows: IR spectra with a Hitachi 260-0611 spectrophotometer; UV spectra with a Shimadzu UV-260 spectrophotometer; mass spectrum (MS) with a JEOL JMS-D 200 spectrometer (70 eV); ¹H-NMR spectra with a JEOL GX-270 spectrometer (270 MHz) and ¹³C-NMR spectra with a JEOL XL-200 spectrometer (50.3 MHz) in dimethyl sulfoxide (DMSO)-*d*₆. Chemical shifts are given in δ (ppm) values referring to internal tetramethylsilane.

Plant Material *Chrysanthemum morifolium* extract was purchased from Nihon Funmatsu Co., Ltd. *Bixa orellana* has been cultivated in this medicinal plant garden. *Ipomoea batatas* was collected in Toyama, Japan.

Bioassay Crude AR was obtained from the supernatant fraction of the homogenate of rat lens according to the method of Kador and Sharpless,¹⁹⁾ and showed a specific activity of 22 unit/mg. One unit was defined as the amount catalyzing the oxidation of 1 μmol of reduced NADPH per minute. Inhibitory activity of the extracts and compounds on AR was assayed by the method previously reported.²⁰⁾ Samples were dissolved in DMSO, which was found to have no effect on the enzyme activity at a concentration below 1%.

Extraction and Isolation Hot water extract of *C. morifolium* (500 g) was partitioned between Et₂O and distilled water to afford an Et₂O extract (4 g). The aqueous layer was successively extracted with AcOEt and *n*-BuOH to give AcOEt extract (16 g), *n*-BuOH extract (38 g) and water extract (435 g), respectively. The AcOEt extract was chromatographed on a silica gel column eluting first with CHCl₃ and then with an increasing amount of MeOH to yield compounds **3** (52 mg) and **4** (24 mg). 20% MeOH elute was purified with Sephadex LH-20 column eluting with MeOH to give **1** (27 mg) and **2** (8 mg).

Dried leaf of *B. orellana* (800 g) was extracted with hot distilled water (3 h × 2) to yield 190 g of the crude extract. The crude extract was partitioned with aforesaid method, to give Et₂O extract (4 g), AcOEt extract (4 g) *n*-BuOH extract (14 g) and water extract (127 g), respectively. The AcOEt extract was chromatographed on a silica gel column eluting first

with CHCl₃ and then with an increasing amount of MeOH to yield compounds **5** (500 mg), **6** (20 mg) and **7** (25 mg).

Dried leaf of *I. batatas* (2 kg) was extracted with hot distilled water (2 h × 3) to yield 320 g of the crude extract. The crude extract (300 g) was partitioned with aforesaid method, to give Et₂O extract (4 g) AcOEt extract (33 g), *n*-BuOH extract (52 g) and water extract (200 g), respectively. The AcOEt extract was chromatographed on a silica gel column eluting first with CHCl₃ and then with an increasing amount of MeOH to yield compounds **4** (150 mg), **8** (80 mg), **9** (20 mg) and **10** (15 mg).

Acacetin 7-*O*-glucoside (**1**), diosmetin 7-*O*-glucoside (**2**), luteolin 7-*O*-glucoside (**3**), ellagic acid (**4**), gallic acid (**5**), pyrogallol (**6**), scopoletin (**8**) and caffeic acid (**9**) were identified by comparing (UV, ¹H-NMR, ¹³C-NMR) with published spectral data and by direct comparison (UV, IR and MS) with authentic samples.

Isoscutellarein (7) Yellow needles, mp 298–300 °C (CHCl₃). Mg + HCl: orange. MS *m/z*: 286 (M⁺), 168. UV λ_{max}^{MeOH} nm: 222, 278, 304. λ_{max}^{MeOH + NaOMe} nm: 222, 290, 355. λ_{max}^{MeOH + NaOAc} nm: 220, 290, 304. λ_{max}^{MeOH + NaOAc + H₃BO₃} nm: 220, 280, 304. λ_{max}^{MeOH + AlCl₃} nm: 228, 297, 324. λ_{max}^{MeOH + AlCl₃ + HCl} nm: 228, 283, 313, 342. IR ν_{max}^{KBr} cm⁻¹: 3350, 1650, 1615, 1580. ¹H-NMR (DMSO-*d*₆) δ ppm: 6.26, 6.71, 6.29, 8.00.

3,5-Dicaffeoylquinic Acid (10) Pale yellow amorphous powder, mp 169–172 °C (dec.), yellow-green to FeCl₃ reagent. UV λ_{max}^{MeOH} nm: 232, 242, 296, 325. ¹H-NMR (DMSO-*d*₆) δ ppm: 7.63, 7.59, 7.08, 6.98, 6.97, 6.793, 6.790, 6.37, 6.29, 5.50, 3.99, 2.20. ¹³C-NMR (DMSO-*d*₆) δ ppm: 175.4, 166.0, 165.5, 148.3, 148.2, 145.5, 145.1, 144.5, 144.4, 125.7, 125.6, 151.3, 151.1, 115.9, 115.8, 115.0, 114.8, 114.6, 114.1, 72.5, 70.9, 70.8, 67.8, 36.7, 34.8.

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References

- 1) S. Terashima, M. Shimizu, H. Nakayama, M. Ishikura, Y. Ueda, K. Imai, A. Suzui and N. Morita, *Chem. Pharm. Bull.*, **38**, 2733 (1990).
- 2) J. H. Kinoshita, P. E. Kador and M. Gatiles, *J. Am. Med. Assoc.*, **246**, 257 (1981).
- 3) K. H. Gabbay and J. J. Snider, *Diabetes*, **21**, 295 (1972).
- 4) R. J. Tounge, D. T. Ewing and B. F. Clarke, *Diabetes*, **32**, 938 (1983).
- 5) W. G. Robinson Jr., P. F. Kador and J. H. Kinoshita, *Science*, **221**, 1177 (1983).
- 6) A. Beyer-Mears, E. Cruz, T. Edelist and E. Varagiannis, *Pharmacology*, **32**, 52 (1986).
- 7) P. F. Kador, J. H. Kinoshita and N. E. Sharpless, *J. Med. Chem.*, **28**, 841 (1985).
- 8) J. DeRuiter, B. E. Swearingen, V. Wanderkar and C. A. Mayfield, *J. Med. Chem.*, **32**, 1033 (1989).
- 9) S. D. Varne and J. H. Kinoshita, *Biochem. Pharmacol.*, **25**, 2505 (1976).
- 10) J. Okuda, I. Miwa, K. Inagaki, T. Horie and M. Nakayama, *Biochem. Pharmacol.*, **31**, 3807 (1982).
- 11) M. Shimizu, T. Ito, S. Terashima, T. Hayashi, M. Arisawa and N. Morita, *Phytochemistry*, **23**, 1885 (1984).
- 12) I. Miwa, M. Hirano, K. Inagaki, C. Belbeoc'h and J. Okuda, *Biochem. Pharmacol.*, **36**, 2789 (1987).
- 13) R. Sarges, R. C. Schnur, J. L. Belletire and M. J. Peterson, *J. Med. Chem.*, **31**, 230 (1988).
- 14) S. Asem, R. N. Stewart and K. H. Norris, *Phytochemistry*, **14**, 1443 (1975).
- 15) T. J. Mabry, K. R. Markham and M. B. Thomas, "The Systematic Identification of Flavonoids," Springer-Verlag, Berlin, Heidelberg, New York, 1970, pp. 41–61.
- 16) M. Jay and J. F. Gonnet, *Phytochemistry*, **12**, 953 (1973).
- 17) C. J. Kelley, R. C. Harruff and M. Carmark, *J. Org. Chem.*, **41**, 449 (1976).
- 18) M. Shimizu, S. Horie, S. Terashima, H. Ueno, T. Hayashi, M. Arisawa, S. Suzuki, M. Yoshizaki and N. Morita, *Chem. Pharm. Bull.*, **37**, 2531 (1989).
- 19) P. E. Kador and N. E. Sharpless, *Biophys. Chem.*, **8**, 81 (1978).
- 20) M. Shimizu, S. Horie, M. Arisawa, T. Hayashi, S. Suzuki, M. Yoshizaki, M. Kawasaki, S. Terashima, H. Tsuji, S. Wada, H. Ueno, N. Morita, L. H. Berganza, E. Ferro and I. Basualdo, *Chem. Pharm. Bull.*, **35**, 1234 (1987).