Isolation and Characterization of an Antiviral Flavonoid from Waldsteinia fragarioides

Mohamed Aboa-Karam, and W. Thomas Shier

J. Nat. Prod., **1992**, 55 (10), 1525-1527• DOI:
10.1021/np50088a022 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 10, pp. 1525-1527, October 1992

ISOLATION AND CHARACTERIZATION OF AN ANTIVIRAL FLAVONOID FROM *WALDSTEINIA FRAGARIOIDES*

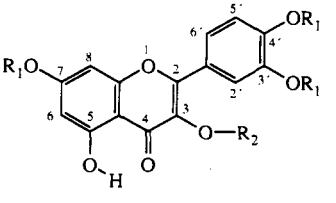
MOHAMED ABOU-KARAM[1] and W. THOMAS SHIER*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT.—The antiviral agent in a fraction from *Waldsteinia fragarioides* (Rosaceae) was purified using bioassay-guided fractionation of activity against herpes simplex type 1 virus. Structural elucidation by instrumental methods identified the active component to be the known flavonoid glycoside, isoquercitrin (3,3′,4′,5,7-pentahydroxyflavone-3β-*O*-glucoside), which had not previously been shown to possess antiviral activity.

Plants represent a large, but largely untapped, potential source of antiviral agents (1). Although there have been relatively few studies seeking antiviral agents from plants, those studies have revealed an unexpectedly frequent occurrence of antiviral activity in plants (2–6). Typically 20–30% of plants from tropical or temperate origin have been observed to possess antiviral activity. As part of a program to investigate the frequency of antiviral activity in temperate plants, we have determined the antiviral activity against herpes simplex type 1 (HSV-1, a DNA virus) and vesicular stomatitis virus (VSV, an RNA virus) in EtOH and aqueous extracts from approximately 100 plants that grow in Minnesota (2). From among these plants *Waldsteinia fragarioides* (Michx.) Tratt. (Rosaceae) was selected for detailed study because extracts from it showed much higher levels of antiviral activity than extracts from any other of the plants in the study (2), and because the aqueous fraction of the EtOH extract was observed by the National Cancer Institute In Vitro Anti-AIDS Drug Discovery Program to contain potent activity against the human immunodeficiency virus (HIV-1) (EC_{50} = 15.7 μg/ml in CEM-6 cells, in which cytotoxicity was observed at IC_{50} = 296 μg/ml). Extensive bioassay-guided (anti-HSV-1 activity) fractionation studies have been carried out on the aqueous fractions, which contain the bulk of the antiviral activity in the plant. These studies have failed to yield pure, highly active components, presumably because antiviral activity resides in numerous, difficult to isolate tannins, each with moderate activity. However, the EtOAc fraction of the EtOH extract, which contained no activity against HIV-1 in the National Cancer Institute screening system, yielded a readily purified component with antiviral activity against HSV-1.

Structural elucidation studies, primarily using uv, ^{1}H- and ^{13}C-nmr and ms spectra, and enzymatic digestion, indicated the antiviral component **1** is 3,3′,4′,5,7-pentahydroxyflavone-3β-*O*-glucoside (isoquercitrin). Comparison with commercially available isoquercitrin (Indofine Chemical Co., Somerville, NJ) revealed identical spectral (uv, ^{1}H- and ^{13}C-nmr) and chromatographic (hplc and tlc) properties. Isoquercitrin caused complete suppression of plaque forma-



1 R_1=H, R_2=β-D-glucoside
2 R_1=R_2=H
3 R_1=OAc, R_2=β-D-glucoside tetra-*O*-acetate

[1]Present address: Department of Pharmacognosy, College of Pharmacy, University of Alexandria, Alexandria, Egypt.

tion by HSV-1 virus at a concentration of 40 μg/ml and was sufficiently cytotoxic to kill half of Vero African green monkey kidney cell monolayers at a concentration of 250 μg/ml.

Isoquercitrin has previously been isolated from numerous plant sources, beginning with *Gossypium herbaceum* L. in 1909 (7). It has been shown to possess several biological activities, including inhibition of angiotensin-converting enzyme (8), inhibition of prostaglandin synthesis (9), and acting as a biosynthetic precursor for rutin (10). This is the first report of antiviral activity for isoquercitrin, although numerous other flavonoids have been reported to possess antiviral activity (11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were obtained on a Beckman DU-70. Nmr spectra were determined on a 300 MHz Nicolet-NI 300-WB. Low resolution fabms were determined on a Kratos-MS-25; other ms were determined on a VG 7070 EHF mass spectrometer. Mp's were measured in capillaries on a Mel-Temp apparatus.

PLANT MATERIAL.—Whole plants of domesticated *W. fragarioides* were collected from the University of Minnesota Landscape Arboretum, and wild *W. fragarioides* were collected near Cable, Wisconsin. The plants were identified by Dr. Anita Cholewa, Department of Botany, University of Minnesota. Voucher specimens are deposited in Department of Medicinal Chemistry, University of Minnesota.

ANTIVIRAL ASSAYS.—Antiviral assays were carried out by the simplified plaque reduction method described previously (2) with 30 plaque-forming units of herpes simplex type 1 virus in confluent cultures of Vero African green monkey kidney cells in 96-well trays. Cytotoxicity was measured (12) in the same cultures as loss of uninfected Vero cells from the monolayers surrounding plaques.

EXTRACTION.—Plant material (1360 g) was homogenized and extracted with 95% EtOH (2 × 6 liters). The combined EtOH extracts were evaporated under reduced pressure. The residue was slurried in 1 liter of H_2O and shaken successively in a separatory funnel with petroleum ether (6 × 0.5 liters), Et_2O (4 × 0.5 liters), $CHCl_3$ (4 × 0.5 liters), EtOAc (8 × 0.5 liters), and *n*-BuOH (4 × 0.5 liters). The combined fractions with each solvent and the residual aqueous solution were evaporated to dryness under reduced pressure and stored at −65° until used. The EtOAc and aqueous fractions were found to contain antiviral activity against HSV-1, but only the aqueous fraction contained activity against HIV-1.

ISOLATION AND IDENTIFICATION.—EtOAc extract (8 g) was dissolved in the minimum of MeOH and adsorbed to 5 g of polyamide-6. The solvent was evaporated leaving a free-flowing powder, which was added to the top of a polyamide-6 column (100 g). The column was eluted slowly with H_2O and increasing concentrations of aqueous MeOH, Me_2CO, NaOH (pH 11), DMF, and urea (to 5%). Antiviral activity was found in fractions eluted with 75% MeOH. Further purification by preparative tlc on Si gel using the solvent system EtOAc-MeOH-HOAc (15:2:1) yielded a component (R_f 0.5) with antiviral activity. Crystallization from H_2O afforded **1** as 5 mg of a yellow microcrystalline powder, mp 223–225°.

The uv spectrum of the antiviral agent in MeOH contained 2 peaks at 257 nm and 356 nm characteristic of a flavonoid (13). Use of the uv shift reagents $NaOCH_3$, $AlCl_3$, $AlCl_3$/HCl, NaOAc, and NaOAc/H_3BO_3 indicated the presence of 4′-OH, absence of alkali-sensitive groups, and probable presence of 5-OH, 7-OH and *o*-dihydroxy groups in the B ring (13,14). ^{1}H nmr (CD_3OD) contained resonances at δ ppm 7.70 (1H, s, H-2′), 7.57 (1H, d, J = 8 Hz, H-6′), 6.87 (1H, d, J = 8 Hz, H-5′), 6.39 (1H, d, J = 2 Hz, H-8), 6.20 (1H, d, J = 2 Hz, H-6), and 5.24 (1H, d, J = 7 Hz, anomeric proton of glycoside), and a multiplet centered at 3.5 (6H, m, glycoside protons). The chemical shifts and spin-spin decoupling results were consistent with the hydroxylation pattern 3,3′,4′,5,7-pentahydroxyflavone [**2**]. Low resolution fabms contained a peak at *m/z* 465 $[M+H]^+$ and the base peak at *m/z* 303 $[aglycone+H]^+$. Hrfabms was consistent with a molecular ion with the formula $C_{21}H_{21}O_{12}$ (glycoside) and the base peak with the formula $C_{15}H_{11}O_7$ (aglycone).

Acetylation of **1** with Ac_2O and pyridine gave **3**. The fabms molecular ion at *m/z* 757 and the nmr were consistent with the acetylated derivative **3**. This indicates the presence of a free 5-OH group which was not acetylated under the conditions used because of strong intramolecular hydrogen bonding with the 4-keto group. Eims contained fragments consistent with the hydroxylation and acetylation pattern in **3**.

The site of glycosylation was implied from the uv; specifically the absence of alkali-sensitive groups requires glycosylation of either the 3- or 4′-OH, but the presence of a free 4′-OH was confirmed with three of the uv shift reagents. Hydrolysis of **1** with HCl/MeOH and acetylation of the carbohydrate fraction gave pentaacetyl-β-glucose

with the same retention time on gc as an authentic sample (Aldrich). The presence of a β-*O*-glucoside was confirmed by hydrolysis of **1** with β-glucosidase (Sigma).

ACKNOWLEDGMENTS

This research was partially supported by fellowships to MAK from AMIDEAST and the Egyptian government. We gratefully acknowledge the assistance of Dr. Gordon M. Cragg, Developmental Therapeutics Program, National Cancer Institute, Besthesda, MD for determining anti-HIV-1 activity of *W. fragarioides* extracts.

LITERATURE CITED

1. J.B. Hudson, "Antiviral Compounds from Plants," CRC Press, Boca Raton, Florida, 1990.
2. M. Abou-Karam and W.T. Shier, *J. Nat. Prod.*, **53**, 340 (1990).
3. D.A. van den Berghe, V.A. Vlietinck, and L. van Hoff, *Bull. Inst. Pasteur, Paris*, **84**, 101 (1986).
4. D.A. van den Berghe, M. Ieven, F. Mertens, and V.A. Vlietinck, *Lloydia*, **41**, 463 (1978).
5. N.R. Farnsworth, L.K. Henry, G.H. Svoboda, R.N. Blomster, M.J. Yates, and K.E. Euler, *Lloydia*, **29**, 101 (1966).
6. N.R. Farnsworth, L.K. Henry, G.H. Svoboda, R.N. Blomster, H.H.S. Fong, M.W. Quimby, and M.J. Yates, *Lloydia*, **31**, 237 (1968).
7. A.G. Perkin, *J. Chem. Soc.*, **95**, 2181 (1909).
8. K. Kameda, T. Takaku, H. Okuda, Y. Kamura, T. Okuda, T. Hatano, I. Agata, and S. Arichi, *J. Nat. Prod.*, **50**, 680 (1987).
9. P.H. Chanh, N. Ifansyah, R. Chahine, A. Mounayar-Chalfoun, J. Gleye, and C. Moulis, *Prostaglandins Leukotrienes Med.*, **22**, 295 (1986).
10. G.A. Barber and E.J. Behrman, *Arch. Biochem. Biophys.*, **288**, 239 (1991).
11. T.N. Kaul, E. Middleton, and P.L. Ogra, *J. Med. Virol.*, **15**, 71 (1985).
12. W.T. Shier, *Am. J. Pharm. Educ.*, **47**, 216 (1983).
13. K.R. Markham, "Techniques of Flavonoid Identification," Academic Press, New York, 1982.
14. J.B. Harborne, T.J. Mabry, and H. Mabry, "The Flavonoids," Academic Press, New York, 1975, Part 1.

Received 15 April 1992