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Gomisin J with Protective Effect Against *t*-BHP-Induced Oxidative Damage in HT22 Cells from *Schizandra chinensis*

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Abstract – Four lignan compounds including gomisin J (**1**), schizandrin (**2**), gomisin A (**3**), and angeloyl gomisin H (**4**) have been isolated from the MeOH extract of *Schizandra chinensis* fruits. The evaluation for protective effect of compounds **1-4** against *tert*-butyl hydroperoxide (*t*-BHP)-induced cytotoxicity in hippocampal HT22 cell line was conducted. Compound **1** showed significant protective effect with an EC_{50} value of 43.3 ± 2.3 μM, whereas compounds **2-4** were inactive. Trolox, one of the well-known antioxidant, used as a positive control, and also showed protective effect with an EC_{50} value of 213.8 ± 8.4 μM. These results suggest that compound **1** may possess the neuroprotective activity against oxidant-induced cellular injuries.
Keywords – gomisin J, *Schizandra chinensis*, *t*-BHP-induced cytotoxicity, HT22 cells

Introduction

tert-Butyl hydroperoxide (*t*-BHP), an analog of lipid peroxide, is widely employed to induce oxidative stress in mammalian cells (Gebhardt, 1997; Hogberg *et al.*, 1975). *t*-BHP is known to be metabolized to reactive oxygen species (ROS) by microsomal cytochrome P450 system (Davies, 1989), which subsequently initiates lipid peroxidation, depletes cellular reduced glutathione (GSH) content and induces cell damage (Davies, 1989; Thornalley *et al.*, 1983). These phenomena are similar to the oxidative stress occurring in cells and/or tissue. Oxidative stress is considered to play an important role in a variety of neurodegenerative disorders of central nervous system (CNS), such as Alzheimer's disease, Parkinson's disease, Huntington's disease and ischemia (Behl *et al.*, 1994; Coly and Puttfarcken, 1993; Makkesbery, 1997; Simonian and Coly, 1996). HT22 cell line is a subclone of the HT4 hippocampal cell line, and has been used as one of the useful models for studying the mechanism of oxidative toxicity (Davis and Maher, 1994). Thus, the aim of this work was to find substances for the protection of the cytotoxic effects of *t*-BHP in hippocampal HT22 cells from medicinal plants, and would be valuable for potential therapeutic use.

Schizandra chinensis Baillon (Schizandraceae) distributes in the most Eastern part of Russia, north-eastern China, Korea and Japan (Hancke *et al.*, 1999). The fruits of this plant are used as anti-hepatotoxic, cardiovascular, and anti-bacterial agents in oriental medicine (Zhu, 1998). Recently, biological studies of this plant revealed that some of lignans showed the antioxidative activity (Choi *et al.*, 2006), protective effect against glutamate-induced neurotoxicity (Kim *et al.*, 2004), and inhibitory activity on NFAT transcription (Lee *et al.*, 2003). As a part of our continuing research to find substances with protective effect on HT22 cells from medicinal plants (Kang *et al.*, 2005), this paper describes the isolation of four lignans from the fruits of *S. chinensis*, and their protective effects against *t*-BHP-induced cytotoxicity in HT22 cells.

Experimental

Chemicals and instruments – NMR spectra were recorded in $CDCl_3$ using a JEOL Eclipse-500 MHz spectrometer (500 MHz for ^1H, 125 MHz for ^{13}C), and chemical shifts are quoted *versus* tetramethylsilane, ESI-MS spectra were measured on a Quattro LC-MS (Micromass). Column chromatography was performed on Silica gel 60 (70-230 mesh, Merck) and YMC-GEL ODS-A (S-75 μm, YMC). In TLC silica gel 60 F_{254} plate (Merck) were used. Spots were detected under UV light or after spraying with 10% H_2SO_4 reagent, followed by heating. Trolox, *t*-BHP, and 3-(4,5-dimethylthiazol-2-yl)-

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2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (St Louis, MO, USA). Cell culture materials were purchased from Gibco BRL (Gaithersburg, MD, USA).

Plant material and isolation – The dried fruits of *S. chinensis* were purchased from the University Oriental Drugstore, Iksan, Korea in June 2005. The voucher specimen (No. WP05-295) was deposited at the Herbarium of the College of Pharmacy, Wonkwang University (Korea). The dried fruits of *S. chinensis* (600 g) were extracted twice with MeOH (2 L) under the ultrasonic condition for 3 h. The MeOH extract (113 g) was suspended in H_2O and partitioned successively with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2-soluble extract (5.0 g) was subjected to column chromatography (CC) on silica gel, which was using *n*-hexane-EtOAc (2 : 1) to give five fractions (Fr. 1, 95.8 mg; Fr. 2, 182.3 mg; Fr. 3, 635.4 mg; Fr. 4, 806.6 mg; Fr. 5, 841.7 mg). The Fraction 1 was subjected to CC on YMC gel (75%, MeOH in H_2O) gave compound **1** (11.0 mg). Fraction 3 was fractionated by YMC gel CC with MeOH-H_2O (3 : 1) to afford four sub-fractions (Fr. 31-34). Fraction 32 (193 mg) was chromatographed to YMC gel CC with MeOH-H_2O (3 : 1) to give compound **2** (182.6 mg). Fraction 34 (72 mg) was subjected to CC on silica gel, eluted with *n*-hexane-EtOAc (3 : 1 → 1 : 1) to give compounds **3** (48.7 mg) and **4** (26.0 mg).

Gomisin J (1) – Colorless powder, (–)-ESI-MS *m/z* 387 $[M - H]^-$, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 6.62 (2H, s, H-4, 11), 3.92 (3H, s, OC$\underline{H}_3$-2), 3.91 (3H, s, OC$\underline{H}_3$-13), 3.51 (6H, s, OC$\underline{H}_3$-1, 14), 2.54 (1H, dd, J = 7.3, 13.7 Hz, H-6b), 2.45 (1H, dd, J = 1.8, 13.7 Hz, H-6a), 2.23 (1H, dd, J = 9.2, 12.8 Hz, H-9b), 2.01 (1H, d, J = 12.8 Hz, H-9a), 1.88 (1H, m, H-7), 1.79 (1H, m, H-8), 0.96 (3H, d, J = 7.3 Hz, C$\underline{H}_3$-17), 0.72 (3H, d, J = 7.3 Hz, C$\underline{H}_3$-18); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 150.4 (C-1), 137.7 (C-2), 147.6 (C-3), 113.2 (C-4), 134.9 (C-5), 38.9 (C-6), 33.8 (C-7), 41.0 (C-8), 35.3 (C-9), 140.3 (C-10), 110.1 (C-11), 148.8 (C-12), 137.4 (C-13), 150.3 (C-14), 121.5 (C-15), 122.5 (C-16), 21.8 (C-17), 12.6 (C-18), 60.1 (O$\underline{C}H_3$-1, 14), 61.1 (O$\underline{C}H_3$-2, 13).

Schizandrin (2) – Colorless powder, (–)-ESI-MS *m/z* 431 $[M - H]^-$, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 6.60 (1H, s, H-4), 6.53 (1H, s, H-11), 3.90 (3H, s, OC$\underline{H}_3$-14), 3.88 (6H, s, OC$\underline{H}_3$-12, 13), 3.87 (3H, s, OC$\underline{H}_3$-3), 3.58 (3H, s, OC$\underline{H}_3$-2), 3.57 (3H, s, OC$\underline{H}_3$-1), 2.65 (1H, d, J = 13.5 Hz, H-6b), 2.63 (1H, dd, J = 1.4, 14.2 Hz, H-9b), 2.37 (1H, dd, J = 6.0, 14.2 Hz, H-9a), 2.36 (1H, d, J = 13.5 Hz, H-6a), 1.87 (1H, m, H-8), 1.25 (1H, s, C$\underline{H}_3$-18), 0.81 (3H, d, J = 7.3 Hz, C$\underline{H}_3$-17); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 151.9 (C-1), 140.8 (C-2), 152.5 (C-3), 110.5 (C-4), 131.9 (C-5), 40.9 (C-6), 71.9 (C-7), 41.9 (C-8), 34.3 (C-9), 133.9 (C-10), 110.0 (C-11), 152.1 (C-12), 140.2 (C-13), 151.6 (C-14), 122.8 (C-15), 124.3 (C-16), 15.9 (C-17), 29.9 (C-18), 60.7 (O$\underline{C}H_3$-1), 61.0 (O$\underline{C}H_3$-2), 56.0 (O$\underline{C}H_3$-3), 56.0 (O$\underline{C}H_3$-12), 61.0 (O$\underline{C}H_3$-13), 60.7 (O$\underline{C}H_3$-14).

Gomisin A (3) – Colorless powder, (–)-ESI-MS *m/z* 415 $[M - H]^-$, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 6.62 (1H, s, H-4), 6.47 (1H, s, H-11), 5.96 (2H, s, -OC$\underline{H}_2$O-), 3.90 (6H, s, OC$\underline{H}_3$-3, 12), 3.83 (3H, s, OC$\underline{H}_3$-2), 3.51 (3H, s, OC$\underline{H}_3$-1), 2.67 (1H, d, J = 13.5 Hz, H-6b), 2.57 (1H, dd, J = 1.4, 14.2 Hz, H-9b), 2.34 (1H, d, J = 13.5 Hz, H-6a), 2.33 (1H, dd, J = 8.1, 14.2 Hz, H-9a), 1.86 (1H, m, H-8), 1.25 (3H, s, C$\underline{H}_3$-18), 0.81 (3H, d, J = 7.3 Hz, C$\underline{H}_3$-17); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 152.2 (C-1), 140.8 (C-2), 152.4 (C-3), 110.4 (C-4), 132.1 (C-5), 40.6 (C-6), 71.7 (C-7), 42.1 (C-8), 33.8 (C-9), 132.6 (C-10), 106.0 (C-11), 148.0 (C-12), 135.0 (C-13), 141.3 (C-14), 121.9 (C-15), 124.2 (C-16), 15.9 (C-17), 30.2 (C-18), 60.7 (O$\underline{C}H_3$-1), 61.1 (O$\underline{C}H_3$-2), 56.0 (O$\underline{C}H_3$-3), 59.7 (O$\underline{C}H_3$-14), 100.9 (-O$\underline{C}H_2$O-).

Angeloyl gomisin H (4) – Colorless prisms, (–)-ESI-MS *m/z* 499 $[M - H]^-$, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 6.68 (1H, s, H-4), 6.55 (1H, s, H-11), 5.88 (1H, m, H-3'), 3.90 (3H, s, OC$\underline{H}_3$-13), 3.87 (3H, s, OC$\underline{H}_3$-12), 3.83 (6H, s, OC$\underline{H}_3$-2, 3), 3.54 (3H, s, OC$\underline{H}_3$-1), 2.73 (1H, d, J = 13.7 Hz, H-6b), 2.69 (1H, dd, J = 1.4, 14.2 Hz, H-9b), 2.41 (1H, dd, J = 7.8, 14.2 Hz, H-9a), 2.33 (1H, d, J = 13.7 Hz, H-6a), 1.88 (1H, m, H-8), 1.75 (6H, m, H-4', H-5'), 1.24 (1H, s, H-18), 0.84 (3H, d, J = 7.3 Hz, C$\underline{H}_3$-18); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 151.9 (C-1), 140.4 (C-2), 152.6 (C-3), 110.2 (C-4), 133.1 (C-5), 40.7 (C-6), 72.1 (C-7), 42.0 (C-8), 34.3(C-9), 133.9 (C-10), 112.8 (C-11), 151.8 (C-12), 139.7 (C-13), 142.3 (C-14), 122.9 (C-15), 123.3 (C-16), 16.0 (C-17), 30.0 (C-18), 60.9 (O$\underline{C}H_3$-1), 61.0 (O$\underline{C}H_3$-2), 56.1 (O$\underline{C}H_3$-3), 56.1 (O$\underline{C}H_3$-12), 60.7 (O$\underline{C}H_3$-13), 165.8 (C-1'), 127.7 (C-2'), 137.4 (C-3'), 15.4 (C-4'), 20.4 (C-5').

Cell culture – HT22 cells were received from Dr. Inhee-Mook (Ajou University, Suwon, Korea) and were maintained at 1×10^6 cells/ml culture in DMEM supplemented with 10% heat inactivated FBS, penicillin G (100 IU/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM) and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and 95% air. The experiment was performed with three groups: the control, *t*-BHP-treated and test extracts pre-treated groups. Cells of the control group received neither *t*-BHP nor the extracts treatment. Those of the *t*-BHP-treated group were incubated with *t*-BHP at a concentration of 20 μM for 24 hours, and those of the test extracts pre-treated group received sample

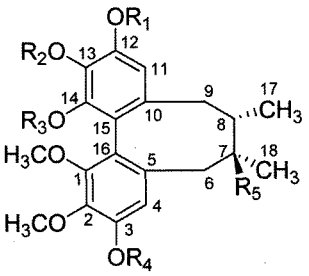
treatment for 3 hours prior to exposure to *t*-BHP. The samples were dissolved initially in DMSO (stock solution) and then diluted with the medium solution. The final DMSO concentration in each experimental and control well was kept constant at 0.1%, and this final concentration showed no relevant effects of DMSO on cellular growth and survival in our assay.

MTT assay – MTT cytotoxicity assay was performed according to the method previously described (Mosmann, 1983). MTT solution was added at a concentration 50 μg/ml into each well. After 4 hours of incubation at 37 °C, the medium was discarded and the formazan blue, which formed in the cells, was dissolved in 50 μl DMSO. Optical density at 570 nm was determined with a microplate reader. The optical density of formazan formed in control (untreated) cells was taken as 100% of viability. Trolox was used as a positive control. EC_{50} values for protective effects (defined as percentage viability versus the respective control) were calculated by linear regression using mean values, and are expressed as means ± S.D. of three independent experiments. The results were compared using one-way ANOVA and Tukey's multiple comparison test. Statistically significant differences between groups were defined as having *P* values of < 0.01. Calculations were performed using GraphPad Prism program (GraphPad Software, Inc., San Diego, CA, USA).

Results and Discussion

In the present study, we investigated the MeOH extract of the fruits of *S. chinensis* with the aim of identifying natural compounds for the protection of the cytotoxic effects of *t*-BHP in hippocampal HT22 cells. Phytochemical fractionation of CH_2Cl_2-soluble fraction led to the isolation of four compounds (**1-4**). The structures of isolated compounds were identified as gomisin J (**1**) (Seo *et al.*, 2004), schizandrin (**2**) (Kim *et al.*, 2002; Ikeya *et al.*, 1978), gomisin A (**3**) (Ikeya *et al.*, 1979), and angeloyl gomisin H (**4**) (Ikeya *et al.*, 1978) by comparing data with those previously reported (Fig. 1).

Compounds **1-4** were tested for their cytoprotective activity in the *in vitro* assay system. Of these, compound **1** showed significant protective effect in a concentration-dependent manner against *t*-BHP-induced cytotoxicity in mouse hippocampal HT22 cells with EC_{50} value of 43.3 ± 2.3 μM (Fig. 2). When the EC_{50} value of compound **1** compared to that of Trolox (EC_{50} = 213.8 ± 8.4 μM), which is one of the well-known antioxidants, and used as a positive control, it is revealed that the cytoprotective effect of compound **1** was better than that of Trolox in our *in vitro* system (Fig. 2).



1 : $R_1 = R_2 = R_3 = R_4 = CH_3$, $R_5 = OH$
2 : $R_1 + R_2 = CH_2$, $R_3 = R_4 = CH_3$, $R_5 = OH$
3 : $R_1 = R_2 = R_4 = CH_3$, $R_3 = COC(CH_3)CHCH_3$, $R_5 = OH$
4 : $R_1 = R_4 = H$, $R_2 = R_3 = CH_3$, $R_5 = H$

Fig. 1. Chemical structures of compounds **1-4**.

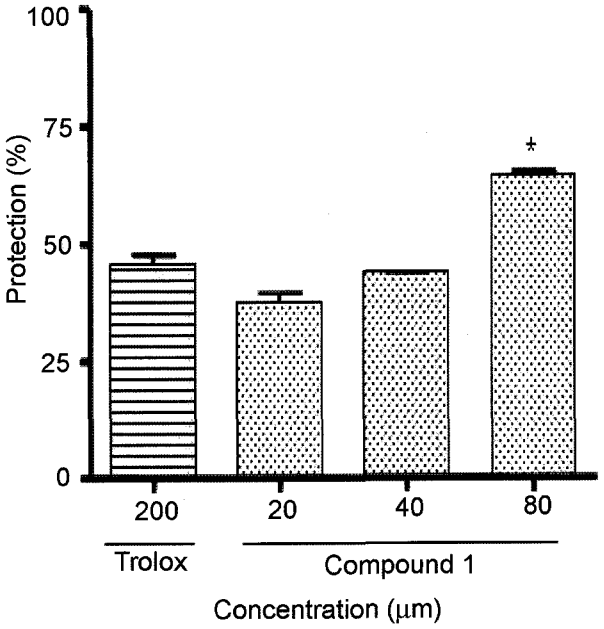


Fig. 2. Cytoprotective effect of compound **1** against *t*-BHP-induced cytotoxicity in HT22cells. Cells were treated with or without various samples for 3 hours before being challenged with 20 μM *t*-BHP for 24 hours. Each column represents the mean ± S.D. from three independent experiments. *P < 0.01 indicates significant differences from the group treated with *t*-BHP only. Trolox was used as a positive control.

Reactive oxygen species (ROS) generated endogenously or exogenously are involved in a variety of pathological events, and oxidative stress is also considered to play an important role in a variety of neurodegenerative disorders of central nervous system (CNS), such as Alzheimer's disease, Parkinson's disease, Huntington's disease and ischemia (Behl *et al.*, 1994; Coly and Puttfarcken, 1993; Makkesbery, 1997; Simonian and Coly, 1996). Thus, antioxidants may protect the aging brain against oxidative

stress damage associated with CNS disorders (Grundman *et al.*, 2002). The mouse hippocampal HT22 cell line has been used as one of the useful models for studying the mechanism of oxidative brain toxicity (Davis and Maher, 1994). It is well-known that *t*-BHP can be metabolized to free radical intermediates in physiological condition, which can subsequently initiate lipid peroxidation, resulting in cell injury (Rush *et al.*, 1985). Based on above evidences, this study was conducted to examine whether four lignans (compounds **1-4**) isolated from *S. chinensis* fruits have neuroprotective activity in the HT22 hippocampal neuron cell line.

In conclusion, we have demonstrated that a lignan gomisin J (**1**) has the concentration-dependent and significant protective effect against *t*-BHP-induced cytotoxicity in HT22 cells, and it would be need further evaluation as potential neuroprotective agents.

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References

Behl, C., Davis, J.B., Lesley, R., and Schubert, D., Hydrogen peroxide mediates amyloid β protein toxicity. *Cell* **77**, 817-827 (1994).

Choi, Y.W., Takamatsu, S., Khan, S.I., Srinivas, P.V., Ferreira, D., Zhao, J., and Khan, I.A., Schisandrene, a dibenzocyclooctadiene lignan from *Schisandra chinensis*: structure-antioxidant activity relationships of dibenzocyclooctadiene lignans. *J. Nat. Prod.* **69**, 356-359 (2006).

Coly, J.T. and Puttfarcken, P., Oxidative stress, glutamate and neurodegenerative disorders. *Science* **262**, 689-695 (1993).

Davies, M.J., Detection of peroxyl and alkoxyl radicals produced by reaction of hydroperoxides with rat liver microsomal fractions. *Biochem. J.* **257**, 603-606 (1989).

Davis, J.B. and Maher, P., Protein kinase C activation inhibits glutamate-induced cytotoxicity in a neuronal cell line. *Brain Res.* **652**, 169-173 (1994).

Gebhardt, R., Antioxidative and protective properties of extracts form leaves of the artchoke (*Cynara scolymus* L.) against hydroperoxide-induced oxidative stress in cultured rat hepatocytes. *Toxicol. Appl. Pharmacol.* **144**, 279-286 (1997).

Grundman, M., Grundman, M., and Delaney, P., Antioxidant strategies for Alzheimer's disease. *Proc. Nutr. Soc.* **61**, 191-202 (2002).

Hancke, J.L., Burgos, R.A., and Ahumada, F., *Schisandra chinensis* (Turcz.) Baill. *Fitoterapia* **70**, 451-471 (1999).

Hogberg, J., Orrenius, S., and O'Brien, P., Further studies on lipid peroxide formation in isolated hepatocytes. *Eur. J. Biochem.* **59**, 449-455 (1975).

Ikeya, Y., Taguchi, H., Yosioka, I., and Kobayashi, H., The constituents of *Schizandra chinensis* Baill. The structures of two new lignans, gomisin N and tigloylgomisin P. *Chem. Pharm. Bull.* **26**, 3257-3260 (1978).

Ikeya, Y., Taguchi, H., Yosioka, I., and Kobayashi, H., The constituents of *Schizandra chinensis* Baill. I. Isolation and structure determination of five new lignans, gomisin A, B, C, F and G, and the absolute structure of schizandrin. *Chem. Pharm. Bull.* **27**, 1383-1394 (1979).

Kang, T.H., Baek, H.Y., and Kim, Y.C., Protective effect of Jakyak-Gancho-Tang extract and its constituents against *t*-BHP-induced oxidative damage in HT22 cells. *Am. J. Chin. Med.* **33**, 181-189 (2005).

Kim, K.S., Kang, S.S., and Ryu, S.N., Quantitative analysis of lignans from fruits of *Schizandra chinensis*. *Kor. J. Pharmacog.* **33**, 272-276 (2002).

Kim, S.R., Lee, M.K., Koo, K.A., Kim, S.H., Sung, S.H., Lee, N.G., Markelonis, G.J., Oh, T.H., Yang, J.H., and Kim, Y.C., Dibenzocyclooctadiene lignans from *Schisandra chinensis* protect primary cultures of rat cortical cells from glutamate-induced toxicity. *J. Neurosci. Res.* **76**, 397-405 (2004).

Lee, I.S., Lee, H.K., Dat, N.T., Lee, M.S., Kim, J.W., Na, D.S., and Kim, Y.H., Lignans with inhibitory activity against NFAT transcription from *Schisandra chinensis*. *Planta Med.* **69**, 63-64 (2003).

Makkesbery, W.R., Oxidative stress hypothesis in Alzhiemer's disease. *Free Radical Bio. Med.* **23**, 134-147 (1997).

Mosmann, T., Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**, 55-63 (1983).

Rush, G.F., Gorski, J.R., Ripple, M.G., Sowinski, J., Bugelski, P., and Hewitt, W.R., Organic hydroperoxide-induced lipid peroxidation and cell death in isolated hepatocytes. *Toxicol. Appl. Pharmacol.* **78**, 473-483 (1985).

Seo, S.M., Lee, H.J., Park, Y., Lee, M.K., Park, J.I., and Paik, K.H., 2004. Lignans from the fruits of *Schizandra chinensis* and their inhibitory effects on dopamine content in PC12 cells. *Nat. Prod. Sci.* **10**, 104-108 (2004).

Simonian, N.A. and Coly, J.T., Oxidative stress in neurodegenerative diseases. *Ann. Rev. Pharmacol.* **36**, 83-106 (1996).

Thornalley, P., Trotta, R.J., and Stern, A., Free radical involvement in the oxidative phenomena induced by *tert*-butyl hydroperoxide in erythrocytes. *Biochim. Biophys. Acta.* **759**, 16-22 (1983).

Zhu, Y.-P., *Chinese Materia Medica*, Harwood Academic Publishers, Amsterdam, 1998.

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