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Anticancer Compounds of *Plantago asiatica* L.

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ABSTRACT : The cytotoxicity-guided fractionation of the seed of *Plantago asiatica* extracts led to the isolation of four compounds, responsible for the cytotoxicity against four human tumor cell lines, i. e., A 431 (Human Epidermoid carcinoma), KHOS-NP (Human Osteosarcoma). SNU-1 (Human stomach carcinoma), SNU-C4 (Human large intestine carcinoma). The structure were elucidated by the phsyco chemical data : β-sitosterol (C1), cholest-5-en-3β-ol (C2), rutin (C3), coumarin-7-O-β-glucopyranoside (C4). IC_{50} values of compound C2 were 14. 6, 13. 5, 10. 3, 17. 8 μg/ml, and compound C3 and C4 showed activity, having IC_{50} values ranging from 10. 3 to 20. 14 μg/ml.

Key words : *Plantago asiatica*. β-sitosterol. 3β-cholesterol. rutin. coumarin-7-O-β-D-glucopyranoside.

INTRODUCTION

Extract of the seed of *Plantago asiatica.* has been applied for the treatment of urination and urolith in Chinese Traditional Medicin (Kim. 1998). We have found recently that the extracts of the herb exhibited a significant cytotoxity against human tumor cell lines cultured *in vitro.* Thus, the phytochemical investigation has been undertaken for the isolation of active constituents of the herb by way of the cytotoxicity oriented fractionation procedure combined with the SRB (sulforhodamine B) methods (Doyle. 1992). In this work, we have isolated four compound responsible for the cytotoxicity against four human tumor cell lines, i. e, A431 (Human epidermoid carcinoma), KHOS-NP (Human osteosarcoma). SNU-1 (Human stomach carcinoma), SNU-C4 (Human large intestine). These compounds were newly isolated from this plant and characterized the cytotoxicity of active components.

MATERIALS AND METHODS

Genaral

^{1}H and ^{13}C-NMR spectra were run at 500MHz, 400MHz and 125MHz, 75MHz. The EI-MS (70

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eV) and FAB-MS were determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). The UV spectrum was recorded on Shimadzu UV-visible spectrophotometer. IR spectrum was measured on JASCO FT/IR-5300 Infrared spectrophotometer. TLC and preparative column chromatography were carried out on Merck precoated silica gel F_{254} plates. All other chemicals and solvents were analyticlal grade and used without further purification

Plant material

The seed (1.5Kg) of *Plantago asiatica* were purchased in June 1998 from Kyungdong market. The voucher specimen (No. SKKU-9806-11), identified from the division of Herbology at Korea Pharmaceutical Reaserch Center (KPRC) was preserved in the Herbarium of the Sung Kyun Kwan University, College of Pharmacy.

Extraction and Isolation

The air-dired plant material (1.5kg) was extrated with MeOH (3 *l*) for 15 days at room temperature and concentrated under pressure below 40℃, and then the syrupy product (56g), which was suspended in H_2O and partitioned successively with n-Hexane (12g), CH_2Cl_2 (2g), EtOAc (3g) and BuOH (13g). By the evaluation of cytotoxicity of each fraction, only CH_2Cl_2, EtOAc souble fraction was active and investigated extensively through serial fraction by preparatve column chromatography followed by the cytotoxicity monitoring, which finally led to the isolation of following active constituents : The CH_2Cl_2 extract was subjected to CC over SiO_2 with n-hexane : EtOAc (9 : 1) as solvents to give compound C1 (25mg), C2 (14mg). The EtOAc extract was subjected to CC over SiO_2 with EtOAc : MeOH (9 : 2) and EtOAc : MeOH : water (6 : 4 : 1) as solvents to give compound C3 (25mg), compound C4 (45mg) which were found to exhibit a cytotoxicity (Table 1).

Table 1. Inhibition of tumor cell proliferation by four compounds from *Plantago asiatica*.

Compound	IC_{50} (㎍/㎖)[a]			
	KHOS-NP	SNU-1	A431	SNU-C4
Hexane Fr	〉100	〉100	〉100	〉100
CH_2Cl_2	21.5	25.3	78.6	45.8
Fr EtOAc Fr	31.5	45.8	〉100	65.7
BuOH Fr	〉100	〉100	〉100	〉100
β-sitosterol.	〉100	78.5	〉100	〉100
cholest-5-en-3β-ol	14.6	13.5	10.3	17.8
rutin.	〉100	17.8	〉100	20.8
coumarin-7-O-β-D-glucopyranoside.	13.5	16.8	20.14	〉100

[a] IC_{50} value of compound against each cancer cell line, which was defined as a concentration (*μg/ml*) that caused 50% inhibition of cell proliferation *in vitro*.

Test for cytotoxicity in vitro

Sulforhodamin B Bioassay (SRB) was used as cytotoxicity screening method. Activities of fractions were monitored in serveral concentration level against four kinds of clutured human tumor cells, *i. e.*, KHOS-NP, SNU-1, A431, and SNU-C4 *in vitro*. (Detailed experiment procedures are in Ryu *et al.* 1988)

Physical and spectra data

Compound C1 - mp 137-139℃ ; ^{1}H-NMR, (400MHz, $CDCl_3$) δ : 0.67 (3H, s, 18, $-CH_3$), 0.76-0.86 (9H, 26, 27, 29-CH_3), 0.90 (3H, d, *J*=6.6Hz, 21-CH_3), 1.04 (3H, s, 19-CH_3), 3.52 (H, m, H-3), 5.32 (1H, d, *J*=5.6Hz, H-6) ; MS (EI 70eV) *m/z* 414 (M$^+$), 396, 381, 329.

Compound C2 - mp 149-150℃ ; IR υ^{KBr}_{max} 3400 (OH), 2938 (-CH), 1476 (C=C), ; ^{1}H-NMR, (400MHz, $CDCl_3$) δ : 0.68 (3H, s, 18-CH_3), 0.73 (6H, d, *J*=6.6Hz, 26, 27-CH_3), 0.83 (3H, d, *J*=6.8Hz, 21-CH_3), 0.98 (3H, s, 19-CH_3), 3.

50 (1H, m, H-3), 5.29 (1H, d, J=1.8Hz, H-6); ^{13}C-NMR (100MHz, $CDCl_3$) δ36.82 (C-1), 30.46 (C-2), 72.86 (C-3), 43.71 (C-4), 140.76 (C-5), 121.78 (C-6), 32.59 (C-7), 31.64 (C-8), 50.07 (C-9), 36.28 (C-10), 22.04 (C-11), 29.97 (C-12), 43.21 (C-13), 56.72 (C-14), 31.68 (C-15), 39.42 (C-16), 56.09 (C-17), 11.74 (C-18), 19.36 (C-19), 35.56 (C-20), 18.46 (C-21), 35.23 (C-22), 22.64 (C-23), 31.97 (C-24), 27.65 (C-25), 22.32 (C-26), 22.43 (C-27); MS (EI 70eV) m/z 386 (M+), 368 (M^+- H_2O), 301, 275, 273, 231.

Compound C3 - mp 210-212℃; Mg-HCl, Zn-HCl, $FeCl_3$ solution test : positive; IR υ_{max}^{KBr} 3400 (OH), 1668 (C=O), 1623, 1510, 1432 (aromatic C=C) cm^{-1} UVλ_{max} (MeOH) 212, 254 (sh), 348nm, (+NaOMe) 273, 333, 414nm, (+$AlCl_3$) 275, 332, 437nm, (+$AlCl_3$/HCl) 259, 311 (sh), 358nm (sh), (+NaOAc) 276, 316, 392nm, (+NaOAc/H_3BO_3) 265nm; 1H-NMR (400MHz, CD_3OD) δ : 1.12 (3H, d, J=6.9Hz, Rhamnose-Me), 5.12 (1H, s, Rhamnose H-1), 6.18 (1H, d, J=6.7Hz, Glucose H-1), 6.37 (1H, d, J=2.2Hz, H-6), 6.95 (1H, d, J=8.4Hz, H-5′), 7.59 (1H, d, J=2.6Hz, H-2′), 7.62 (1H, d, J=10.92Hz and 2.52Hz, H-6′); ^{13}C-NMR (100MHz, CD3OD), 157.3 (C-2), 133.5 (C-3), 178.2 (C-4), 163.0 (C-5), 98.6 (C-6), 165.3 (C-7), 95.2 (C-8), 158.7 (C-9), 104.8 (C-10), 123.4 (C-1′), 115.6 (C-2′), 144.8 (C-3′), 149.7 (C-4′), 117.2 (C-5′), 123.1 (C-6′), 104.3 (C1-Glu), 75.6 (C2-Glu), 77.9 (C3-Glu), 72.4 (C4-Glu), 77.4 (C5-Glu), 68.6 (C6-Glu), 102.5 (C1-Rha), 71.9 (C2-Rha), 71.5 (C3-Rha), 73.6 (C4-Rha), 69.8 (C5-Rha), 18.1 (C6-Rha); MS (EI 70eV) : m/z 610 (M^+).

Compound C4- mp 203-204℃; IR υ_{max}^{KBr} 3400 (OH), 1426 (aromatic C=C), 1180 (glycosidic C-O) cm^{-1}; UVλmax (MeOH) 236, 302, 334nm, ; ^{1}H-NMR (400MHz, DMSO-d_6) δ : 3.1-3.7 (Sugar H), 7.96 (1H, d, J=9.6Hz H-4), 7.64 (1H, d, J=8.8Hz, H-5), 7.01 (2H, m, H-6, 8), 6.32 (1H, d, J=9.8Hz H-3), 5.04 (1H, d, J=7.6Hz H-1′); ^{13}C-NMR (100MHz, DMSO-d_6), 162.64 (C-2, 7), 155.42 (C-8a), 144.76 (C-4), 130.82 (C-5), 116.24 (C-6), 114.71 (C-4a), 113.64 (C-3), 100.32 (C-8), 103.42 (C1-Glu), 77.64 (C3-Glu), 76.42 (C5-Glu), 73.62 (C2-Glu), 70.86 (C4-Glu), 61.21 (C6-Glu); MS (EI 70eV) : m/z 324 (M^+).

RESULTS AND DISCUSSION

Each compound 1-4 was identified by direct comparision of its physical and spectra properties with those in the literature 1 (Rubinstein *et al.* 1976), 2 (Pojak *et al.* 1977, Wegmann *et al.* 1978, Berghofer *et al.*. 1987), 3 (Agrawal *et al.* 1989), 4 (Murray *et al.* 1982, Steck *et al.* 1976) and all of them had been found previously. However examined tumor cells were significantly inhibited in their proliferation. Cholest-5-en-3β-ol demonstrated a inhibition on each tumor cell proliferation. The IC_{50} values of each compounds on the proliferation of four tumor cells are summarized in Table 1. Even though cholest-5-en-3β-ol have been reported to have both cytotoxic and antitumor activity (Aiello *et al.* 1982). C1, C3, C4 has not been previously examined for their cytotoxic effect on human tumor cells.

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摘 要

차전자의 메탄올추출물을 헥산, 클로로포름, 에틸아세테이트, 부탄올로 계통분획하여, 활성을 검정하고, 그 중 활성분획인 헥산과 에틸아세테이트 분획을 크로마토그라피하여 4종의 화합물을 분리하였으며, 각종 기기분석과 이화학적 분석을 통하

여 β-sitosterol(C1), cholest-5-en-3β-ol(C2), rutin(C3), coumarin-7-O-β-glucopyranoside(C4) 임을 확인 하였으며, 4종의 화합물 중 cholest-5-en-3β-ol, rutin(C3)이 주요 항암 성분 이었다.

LITERATURES CITED

Aiello, A., E. Fattorusso., S. Magno. and M. Menna. 1991. Steroids of the marine sponge *Cinachyra tarentina*: Isolation of cholest-4-ene-3, 6-dione and (24R)-24- ethylchloest-4-ene-3, 6-dione. *J. Nat. Prod.* 54 : 218-285.

Agrawal, P. K. 1989. Carbon-13 NMR of Flavonoids. Elsevier. Amsterdam. P 446Bergh fer, R. and J. H ö lzl. 1987. Biflavonoids in *Hypericum perforatum*; Part 1. Isolation of I 3, II 8-Biapigenin. *Planta Medica.* 53 : 216-217.

Doyle, A. 1992. Cell and tissue culture Lab procedure. Wiley. USA, P 1306.

Do, J. C., K. H. Son, and S. S. Kang. 1988. Studies on the constituents of the roots of *Rubus parvifolius*(I), isolation of the (-)-epicatechin. Kor. *J. Pharmacogn*, 19 : 170

Kim, Chang-Min. 1998. Chungyakdaesacheon. . Cheongdam. Seoul. Korea. PP 5214-5216

Murray, R. D. H. 1982. The natural coumarins, occurrence, chemistry and biochemistry. John Willey and Sons Ltd. New York. USA. P 357.

Pojak, G., J. Edmon., A. L. Anet, and N. R. Easton. 1977. Carbon 13-NMR studies on cholesterol boisynthesis from ^{13}C mevalonate. *J. Am. Chem. Soc.* 99 : 931-939.

Rubinstein, I., L. J. Goad., A. D. Clague., and L. J. Mulberim. 1976. The 220 MHz nmr spectra of phytosterol. *Phytochemistry.* 15 ; 195-201.

Ryu, S. Y. and O. P. Zee, 1992. An antitumor activity of *Psoralea corylifolia.* Arch. Pharm. Res. 15, 356-359.

Steck, W. and M. Mazureck. 1972. Identification of natural coumarins by NMR spectroscopy. 35 : 418-439. Lliyodia.

Wegmann, A. 1978. Variation in mass spectral *fragnentation* produced by active sites in mass specirometer source. *Anal Chem.* 50 : 830-838.