Antimutagenic Activity of 5 α -Cholest-7-en-3 β -ol, a New Component from the Starfish *Asterina pectinifera*

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From the butanol fraction of the starfish *Asterina pectinifera* MÜLER et TROSCHER (Asteriidae), we have isolated a new component, 5 α -cholest-7-en-3 β -ol. Its antigenotoxic and antimutagenic activities were examined by the SOS chromotest with *Escherichia coli* PQ37 and by Ames test with *Salmonella typhimurium* TA1538, respectively. 5 α -Cholest-7-en-3 β -ol showed potent antigenotoxic activity against the mutagens, both MNNG and NQO. For 100% of antigenotoxicity, the concentration of the compound applied against MNNG and NQO were 10 μ g and 5 μ g per reaction tube, respectively. Its antimutagenic activity with *S. typhimurium* TA1538 against the mutagen MNNG was very effective. When its concentrations were varied from 1 up to 10 μ g dose per plate, the inhibition ratio of revertant CFU of TA1538 per plate was increased accordingly, from 25.2 to 99.2%. These results suggest that 5 α -cholest-7-en-3 β -ol possesses antigenotoxic and antimutagenic activity and might be useful as a chemopreventive agent.

Key words *Asterina pectinifera*; antigenotoxicity; antimutagenicity; 5 α -cholest-7-en-3 β -ol

Many natural antimutagenic compounds have been determined, such as ascorbic acid,¹⁾ α -tocopherol,²⁾ β -carotene,³⁾ taxol,⁴⁾ and aburaturbolactam C.⁵⁾ Either inactivating mutagens or interfering in the process of mutagenesis leads to their antimutagenicity by reducing the frequency or rate of spontaneous or induced mutation. Antimutagenic substances may prevent cancer because they can either destroy mutagens in or out of body cells or block mutagens which damage DNA and cause mutations in cells.

In order to search for antimutagenic agents from natural sources, we have previously investigated the antimutagenic activities of the total extract and several fractions of the starfish *Asterina pectinifera* by using the SOS chromotest and Ames test.⁶⁾ Among the tested fractions, the butanol fraction showed a low induction factor, which means this fraction increased the antigenotoxicity against the base substitution mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG).

The starfish *Asterina pectinifera* has been used to provide a crude drug for the treatment of rheumatism and as a tonic⁷⁾ in Chinese medicine, and has been reported to exhibit antimicrobial⁸⁾ and antitumor activities.^{9–11)} From the starfish many kinds of steroidal glycosides (asterosaponins)^{9,10,12,13)} in both sulfated and nonsulfated forms, polyhydroxylated steroids (aglycones),¹⁴⁾ and lectins¹¹⁾ have been isolated.

In this study, we isolated a new component from the butanol fraction of *Asterina pectinifera*, examined its antimutagenic activity, and explored its possible antigenotoxic and antimutagenic properties against the mutagens, MNNG and NQO(4-nitroquinoline *N*-oxide) using the test strains *E. coli* PQ37 and *S. typhimurium* TA1538.

MATERIALS AND METHODS

Materials The starfish was collected at the Kampo area located in the southeastern sea of the Korean peninsula. *E. coli* PQ37 (*sfhA::Mud(Ap lac)cts lac*ΔU169 *mal*⁺, *uvrA*, *galE galY*, *PhoC*, *rfa*) and *S. typhimurium* TA1538

(*hisD3052 rfa-uvrB*) were used as the test strains for the antigenotoxicity of SOS chromotest and for the antimutagenicity of Ames test, respectively. The mutagens MNNG and NQO were purchased from Sigma Co.

Assay MNNG and NQO were dissolved in dimethylsulfoxide at a concentration of 100 μ g/ml. Except for the reaction volume tested (600 μ l), the antigenotoxicity test was performed by the method of Quillardet and Hofnung.¹⁵⁾ The induction factor means SOS induction by a mutagen and is defined as the value of Rs divided by Ro, where Rs is the enzyme activity ratio of β -galactosidase activity and alkaline phosphatase with sample and/or a mutagen, and Ro is the spontaneous Rs without sample or mutagen. The antimutagenicity test was performed essentially as described by Maron and Ames.¹⁶⁾ The inhibition ratio of revertant colony forming unit (CFU) per plate was calculated by the equation as follow: % inhibition ratio = [1 – (CFU_{sm} – CFU_p) / (CFU_m – CFU_p)] × 100, where CFU_{sm} is CFU with mutagen and sample, CFU_m is CFU with mutagen, and CFU_p is a spontaneous CFU with no mutagen and sample.

Extraction and Isolation The animal (300 g) was cut into small pieces and extracted with hot methanol (500 ml) for 5 h. The total extract (48.2 g) was fractionated successively with hexane, chloroform and butanol. The butanol fraction (5.3 g) was chromatographed on a column of LiChroprep RP-18 (40–63 μ l) with a discontinuous gradient of 80 to 50% methanol to yield five fractions. The last fraction, which had the most antimutagenic activity, was further purified over the above column and eluted with methanol to give an amorphous solid which was recrystallized with acetone.

5 α -Cholest-7-en-3 β -ol Liebermann–Burchard test (positive). Colorless needles (acetone). mp 125–128 °C (Lit., mp 121 °C).¹⁷⁾ IR (nujol) cm^{–1}: 3268 (OH). ¹H-NMR (200 MHz, CDCl₃) δ : 0.65 (3H, s, C18-CH₃), 0.80 (3H, s, C19-CH₃), 0.86 (6H, d, *J* = 6.6 Hz, C26,27-CH₃), 0.92 (6H, d, *J* = 6.6 Hz, C21-CH₃), 3.60 (1H, m, C3-H), 5.17 (1H, br s, C7-H). ¹³C-NMR (50 MHz) δ : 139.58 (C-8), 117.40 (C-7), 71.08 (C-3), 56.20 (C-17), 55.08 (C-14), 49.50 (C-9), 43.44 (C-13), 40.30

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Fig. 1. The Chemical Structure of 5 α -Cholest-7-en-3 β -ol Isolated from the Starfish *Asterina pectinifera*

Fig. 2. Induction Factor of SOS Chromotest with Butanol Fraction and 5 α -Cholest-7-en-3 β -ol

The concentration of sample applied was 3 μ g/tube.

(C-5), 39.56 (C-4, C-24), 38.04 (C-12), 37.20 (C-2), 36.26 (C-20, C-22), 34.26 (C-10), 31.53 (C-1), 29.72 (C-6), 28.01 (C-16, C-25), 23.98 (C-23), 23.03 (C-15), 22.89 (C-27), 22.62 (C-26), 21.62 (C-11), 18.91 (C-21), 13.12 (C-19), 11.93 (C-18). EI-MS m/z : 386 (M^+), 371, 273, 255, 231, 228, 213.

RESULTS AND DISCUSSION

The active fraction showing antimutagenic activity was found to contain a steroidal compound (Liebermann-Burchard test) which has been identified as 5 α -cholest-7-en-3 β -ol (Fig. 1) by comparison of its instrumental data with those reported previously.¹⁸⁾ This compound has been introduced either by synthesis¹⁸⁾ or by isolation from *Ambrosia elatior*.¹⁷⁾ However, this kind of steroid has been isolated for the first time from the starfish, although 5 α -cholest-7-en-3 β , 6 β -diol is known to exist in other kind of starfish *Echinaster sepositus*.¹¹⁾ The antigenotoxic and antimutagenic activities of these steroids from starfishes have not yet been reported.

When the final concentration of 3 μ g per reaction tube of the butanol fraction and 5 α -cholest-7-en-3 β -ol was applied to *E. coli* PQ37, the compound showed much more effective antigenotoxic activity against the mutagens used, both against MNNG and NQO (Fig. 2). When the concentration of 5 α -cholest-7-en-3 β -ol was increased from 0 up to 10 μ g per reaction tube, its antigenotoxic effect also increased. The final concentrations of 5 and 10 μ g per reaction tube showed 100% antigenotoxicity against MNNG and NQO, respectively (Fig. 3).

Fig. 3. Effect of Concentration of 5 α -Cholest-7-en-3 β -ol on Induction Factor of SOS chromotest

Fig. 4. Effect of Concentration of 5 α -Cholest-7-en-3 β -ol on Inhibition Ratio of Revertant Forming *S. typhimurium* TA1538

For antimutagenic activity, the inhibitory effect of the butanol fraction and 5 α -cholest-7-en-3 β -ol was examined in a plate incorporation assay by using *S. typhimurium* test strain TA1538 without S9 activation. No mutagenic activity of the butanol fraction or the compound was detected (data not shown). These results indicate that the samples tested are not mutagenic in the Ames test. The number of histidine revertants induced by the mutagen without any extract was given as 100%. The percentage of revertants remaining in the samples was then calculated accordingly. When a 3 μ g dose of two samples per plate was applied against the mutagen MNNG, 5 α -cholest-7-en-3 β -ol showed much more effective antimutagenicity than the butanol fraction (*i.e.*, inhibition ratio of revertant CFU per plate with this compound and the butanol fraction was 63.2 and 46.3%, respectively). When the concentration of the compound was increased from 0 up to 10 μ g per plate, antimutagenic activity was accordingly increased. The final concentration of 10 μ g dose per plate showed 99.2% antimutagenic activity (Fig. 4).

When the compound was added into LB broth to give a final concentration up to 10 μ g/ml, the cell growth of *E. coli* PQ37 and *S. typhimurium* TA1538 after 24 h was not inhibited. This result suggests that the antigenotoxic and antimuta-

genic effects of the compound are not due to a killing effect of the compound in the presence of a mutagen. The present results indicate that the new component, 5 α -cholest-7-en-3 β -ol, from the starfish *Asterina pectinifera* possesses antimutagenic activity and might be useful as a chemopreventive agent against cancer.

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