Preparation and Cancer Cell Invasion Inhibitory Effects of C_{16}-Alkynic Fatty Acids

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Five C_{16}-alkynic fatty acids (2—6) were prepared and examined their inhibitory effects on cancer cell invasion. It has been found that hexadeca-6,8,10-triynoic acid (5) and hexadeca-8,10,12-triynoic acid (6) exhibit similar potent inhibitory activities with that of octadeca-8,10,12-triynoic acid (1) which was isolated from Scurrula atropurpurea (Loranthaceae).

Key words C_{16}-alkynic fatty acid; cancer cell invasion inhibitory effect; anticancer; hexadeca-6,8,10-triynoic acid; hexadeca-8,10,12-triynoic acid

The whole plant of Scurrula atropurpurea (Bl.) Dans. (Loranthaceae), a parasitic plant on the tea plant Thea sinensis L., has been traditionally used for the treatment of cancer in Java Island, Indonesia. In our previous report, we reported isolation of six C_{16}-fatty acids [(Z)-9-octadecenoic acid, (Z,Z)-octadeca-9,12-dienoic acid, (Z,Z,Z)-octadeca-9,12,15-trienoic acid, octadeca-8,10-diynoic acid, (Z)-octadec-12-ene-8,10-diynoic acid and octadeca-8,10,12-triynoic acid], besides two xanthines, two flavonol glycosides, a monoterpen glycoside, a lignan glycoside, and four flavanes. Among those C_{16}-fatty acids, octadeca-8,10,12-triynoic acid (1) showed the most potent inhibitory effect (99.4% inhibition at 10 μg/ml) on cancer cell invasion through a rat mesothelium monolayer by using MM1 cell line isolated from rat ascites hepatoma AH130 cells. Furthermore, it was found that the rise of number of unsaturation function in the fatty acids seems to strengthen the inhibitory activity.

On the other hand, we examined cancer cell invasion inhibitory effects of four saturated fatty acids, namely myristic acid (C_{14}), palmitic acid (C_{16}), stearic acid (C_{18}), and eicosanoic acid (C_{20}). Among them, palmitic acid (C_{16}) showed much stronger activity (46.8% inhibition at 10 μg/ml) than myristic acid (31.5%), stearic acid (29.5%) and eicosanoic acid (20.5%) at the same concentration.

Therefore, we here describe a simple preparation route for five C_{16}-alkynic fatty acids [hexadec-8-ynoic acid (2), hexadec-10-ynoic acid (3), hexadeca-8,10-diynoic acid (4), hexadeca-8,10,12-triynoic acid (5) and hexadeca-8,10,12-triynoic acid (6)], in order to compare their inhibitory effects with that of octadeca-8,10,12-triynoic acid (1) isolated from Scurrula atropurpurea.

Preparation of C_{16}-Alkynic Fatty Acids (2—6) Among the C_{16}-alkynic fatty acids (2—6), hexadec-8-ynoic acid (2) was synthesized by Levine et al. using alklyylation of 7-bromoheptanoic acid with 1-nonyne, and hexadec-10-ynoic acid (3) was reported by Arsequeill et al. as an intermediate for the synthesis of cyclopropane fatty acids. Also hexadeca-8,10-diynoic acid (4) was synthesized by Gunstone and Sykes using a coupling reaction of 1-bromohext-1-yne with 8-nonynoic acid. However, so far, the preparation of hexadeca-6,8,10-triynoic acid (5) and hexadeca-8,10,12-triynoic acid (6) have not yet been reported. We tried to prepare the C_{16}-alkynic fatty acids (2—6) by combination of known simple reactions as shown in Fig. 1.

A condensation of propargyl alcohol (7) and appropriate 1-bromoalkanes (8 or 11) by treatment with n-BuLi and potassium 3-aminopropylamide (KAPA)10 afforded non-8-ylnol (9) and undec-10-ynol (12) in 80% and 78% yield, respectively. Then a coupling reaction of 9 and 12 with proper 1-bromoalkanes (10 or 13) by n-BuLi treatment and subsequent CrO_{3} oxidation furnished hexadec-8-ynoic acid (2) and hexadec-10-ynoic acid (3) in moderate yields.

Hexadeca-8,10-diynoic acid (4) was prepared by use of the above-mentioned non-8-ylnol (9). A coupling reaction of 1-iodo-1-heptyne (15), which was prepared from 1-heptyne (14), with 9 by treatment with Cul and pyrrolidine and subsequent CrO_{3} oxidation furnished hexadeca-8,10-diynoic acid (4) in 57% yield from 14.

A coupling of propargyl alcohol (7) and 1-bromobutane (16) by treatment with n-BuLi and KAPA afforded hept-6-ylnol (17) in 78% yield. Then, 17 was converted into nona-6,8-diylnol (20) via 10-hydroxy-10-methylundeca-6,8-diylnol (19) by the similar procedure reported by Nakanishi et al. Finally, 20 was coupled with 1-iodo-1-heptyne (15) and oxidized by chromic acid to provide hexadeca-6,8,10-triynoic acid (5) in a moderate yield.

Undeca-8,10-diylnol (21) was prepared from 9 and 3-methyl-1-pentyn-3-ol (18) through the procedures reported by Zeni et al. 21 was coupled with 1-iodo-1-pentyn (22) in the presence of Cul and pyrrolidine followed by CrO_{3} oxidation to afford hexadeca-8,10,12-triynoic acid (6) in a moderate yield.

Cancer Cell Invasion Inhibitory Effects of C_{16}-Alkynic Fatty Acids (2—6) As a result of the assay as shown in Table 1, five synthetic C_{16}-alkynic fatty acids (2—6) exhibited stronger inhibitory effects on cancer cell invasion than palmitic acid and palmitoleic acid. Especially, two triyne derivatives (5, 6) showed potent inhibitory activities over 95% at a concentration of 10 μg/ml. Those activity were similar with that of the C_{16}-trialkynic fatty acid, octadeca-8,10,12-triynoic acid (1) which had been isolated from Scurrula atropurpurea (Loranthaceae). Furthermore, the concentration-dependent behavior of the triyne derivatives (5, 6) were examined at 5 μg/ml and 2.5 μg/ml, which indicating those C_{16}-
Table 1. Cancer Cell Invasion Inhibitory Effects of C16-Fatty Acid Derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration (µg/ml)</th>
<th>Inhibitory activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitic acid</td>
<td>10</td>
<td>46.8</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>10</td>
<td>49.7</td>
</tr>
<tr>
<td>Hexadec-8-ynoic acid (2)</td>
<td>10</td>
<td>82.4</td>
</tr>
<tr>
<td>Hexadec-10-ynoic acid (3)</td>
<td>10</td>
<td>77.2</td>
</tr>
<tr>
<td>Hexadeca-8,10-diynoic acid (4)</td>
<td>10</td>
<td>85.6</td>
</tr>
<tr>
<td>Hexadeca-6,8,10-triynoic acid (5)</td>
<td>10</td>
<td>95.7</td>
</tr>
<tr>
<td>Hexadeca-8,10,12-triynoic acid (6)</td>
<td>10</td>
<td>98.7</td>
</tr>
<tr>
<td>Octadeca-8,10,12-triynoic acid (1)</td>
<td>10</td>
<td>99.4</td>
</tr>
<tr>
<td>(−)-Epigallocatechin-3-O-gallate (EGCG)</td>
<td>10</td>
<td>82.8</td>
</tr>
</tbody>
</table>

Fig. 1. Preparation of C16-Alkynic Fatty Acids (2—6)
alkynic fatty acids showed over 50% inhibitory effects even at 2.5 μg/ml.

Although no drug possessing cancer cell invasion inhibitory activity has been produced, (—)-epigallocatechin-3-O-gallate (EGCG, 82.8% at 10 μg/ml inhibition, 59.7% inhibition at 5 μg/ml, 40.1% inhibition at 2.5 μg/ml), 1) genistein (10 μg/ml; 80.5% inhibition, 5 μg/ml; 64.0% inhibition, 2.5 μg/ml; 55.7% inhibition) 2) and ginsenoside Rg1 (25 μg/ml; 98.8% inhibition) 3) have so far been reported as natural occurring materials showing the inhibitory activity.

The present work has indicated that the C18-tryne fatty acid (1) and the C16-tryne fatty acids (5, 6) are potent cancer cell invasion inhibitory materials in spite of the simple chemical structures.

It should be noted that the C18- and C16-alkynic fatty acids (1–6) show no cytotoxicity to the cancer cells used in the present assay. 1, 2)

Experimental
The instruments used to obtain physical data and experimental conditions for chromatography were the same as in our previous paper. 3)

Myristic acid, palmitic acid, stearic acid, eicosanoic acid and palmitoleic acid were purchased from Wakó Pure Chemical Industries, Ltd.

Non-8-ynol (9) To a solution of propargyl alcohol (7, 1.96 g, 35.0 mmol) in tetrahydrofuran (THF, 32 ml) and hexamethylphosphoric triamide (HMPA, 1.0 ml) was added n-BuLi (1.6 M in hexane, 43.8 ml, 70.0 mmol) at −78 °C. After the reaction temperature allowed to reach at −30 °C, 1-bromohexane (8, 6.36 g, 38.5 mmol) was added to the mixture and stirred at room temperature for 12 h. The reaction mixture was treated with aqueous saturated NH4Cl and extracted with CHCl3. The CHCl3 extract was worked up in the usual manner to give a product (3.0 g). Purification of the product by silica gel column chromatography (SiO2 40 g, hexane:EtOAc 5:1) afforded undec-2-ynol (2.12 g, 12.6 mmol), which was treated with Et3O and Hexadec-8-ynol (390 mg, 1.64 mmol). The chromic acid reagent 4) (1.7 ml, 3.4 mmol) was added to the solution of hexadec-10-ynol (390 mg in acetone (3.5 ml) and the whole was stir at −10 °C for 30 min. The reaction mixture was poured into ice-water and extracted with CHCl3. The CHCl3 extract was worked up in the usual manner to give a product (3.0 g). Purification of the product by silica gel column chromatography (SiO2 40 g, hexane:EtOAc = 3:1) gave hexadec-10-ynic acid (3, 310 mg, 82% yield). The physicochemical properties were identical with those in the literature. 5)

Hexadeca-8,10-diynic Acid (4) To a solution of 1-phenyl-4 (146, 960 mg, 10.0 mmol) in THF (8.0 ml) was added n-BuLi (1.6 M in hexane, 6.25 ml, 10.0 mmol) at −78 °C. Then, iodine (2.79 g, 11.0 mmol) in THF (5 ml) was added to the reaction mixture at −30 °C and stir at room temperature for 30 min. The reaction mixture was poured into aqueous saturated NH4Cl and extracted with EtOAc. The EtOAc extract was treated with 2-propanol and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (430 mg). Purification of the product by silica gel column chromatography (SiO2, hexane:EtOAc = 4:1) afforded hexadeca-8,10-diynic acid (4, 317 mg, 1.28 mmol, 57% from 14). 1H- and 13C-NMR data of 4 are given here, since the spectra have not been reported in the literature. 6)

4: 1H-NMR (300 MHz, CDCl3) δ: 0.89 (3H, t, J = 7.1 Hz), 1.25—1.42 (8H), 1.47—1.55 (4H), 1.64 (2H, quintet, J = 7.3 Hz), 2.22—2.27 (4H), 2.35 (2H, t, J = 7.4 Hz). 13C-NMR (75 MHz, CDCl3) δ: 179.7, 77.7, 68.3, 65.4, 62.5, 32.9, 31.0, 28.5, 28.8, 28.1, 28.4, 22.5, 19.2, 19.1, 13.9.

Hept-6-ynol (17) n-BuLi (1.6 M in hexane, 15.6 ml, 25.0 mmol) was added to a solution of 7 (700 mg, 12.5 mmol) in THF (10 ml) and HMPA (6.5 ml) at −78 °C and then the reaction temperature allowed to −30 °C. 1-Bromobutane (16, 1.89 g, 13.8 mmol) was added to the solution and stirred at room temperature. After 12 h, the reaction mixture was poured into aqueous saturated NH4Cl and the whole was extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (2.5 g). Purification of the product by silica gel column chromatography (SiO2, hexane:EtOAc = 4:1) gave hept-2-ynol (1.18 g, 10.5 mmol), which was treated with 2-propanol and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (1.19 g). Purification of the product by silica gel column chromatography (SiO2, hexane:EtOAc = 3:1) afforded heptadeca-6-ynic acid (17, 503 mg, 1.25 mmol). To a solution of hexadeca-8,10-diynol (400 mg, 1.71 mmol) in acetonitrile (4.0 ml) was added the chromic acid reagent 4) (1.7 ml, 3.4 mmol) and the whole was stir at −10 °C for 15 min, then treated with 2-propanol and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (430 mg). Purification of the product by silica gel column chromatography (SiO2, 30 g, hexane:EtOAc = 5:2) and HPLC (Wakosil 5 SIL, hexane, EtOAc = 4:1) afforded heptadeca-6,8,10-diynic acid (4, 317 mg, 1.28 mmol, 57% from 14).
EtOAc=9 : 1) afforded hept-6-ynol (17, 1.09 g, 9.73 mmol, 78% yield from 7).

To a solution of 17 (900 mg, 8.0 mmol) in THF (6.0 ml) was added n-BuLi (1.6 m in hexane, 10.0 ml, 16.0 mmol) at −78 °C. I2 (2.24 g, 8.8 mmol) in THF (3 ml) was added to the mixture at −30 °C and stirred at room temperature for 30 min. The reaction mixture was treated with aqueous saturated NH4Cl and extracted with Et2O. The Et2O extract was worked up in the usual manner to give 7-iodohept-6-ynol (1.83 g). To a solution of 7-iodohept-6-ynol (1.83 g) and 3-methyl-1-pentyn-3-ol (18, 967 mg, 9.6 mmol) in pyridylone (8.0 ml) was added CuI (152 mg, 0.80 mmol) and the whole was stirred at room temperature for 30 min. The reaction mixture was treated with aqueous saturated NH4Cl and extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product (2.23 g), which was purified by silica gel column chromatography (SiO2 200 g, hexane : EtOAc 4: 1) to afford hexadeca-6,8,10-triynol (81 mg, 0.305 mmol) and 1-iodopent-1-yne (21, 71 mg) in pyrrolidine (1.0 ml) was added Cud (5.8 mg, 0.031 mmol) and the whole was stirred at room temperature for 30 min. The reaction mixture was treated with aqueous saturated NH4Cl and extracted with EtOAc. The EtOAc extract was washed with brine and dried over MgSO4. Removal of the solvent gave hexadeca-8,10,12-triynol (85 mg), which was purified by silica gel column chromatography (SiO2 10 g, hexane : EtOAc 2: 1) to afford hexadeca-8,10,12-triynol (63 mg, 0.27 mmol). To a solution of hexadeca-8,10,12-triynol (63 mg, 0.27 mmol) in acetone (1.0 ml) was added the chromic acid reagent8) (0.350 ml, 0.70 mmol) and HPLC (Wakosil 5 SIL, hexane : EtOAc=4: 1) afforded hexadeca-8,10,12-triynol acid (6, 49 mg, 0.201 mmol, 66% yield from 21).

6. Colorless needles. mp 70—72 °C (from ether). IR (film) cm−1 : 3300—
2500 (br), 2216, 1695. H-NMR (300 MHz, CDCl3) δ : 0.99 (3H, t, J=7.3 Hz), 1.30—1.47 (4H), 1.50—1.65 (6H), 2.26 (2H, t, J=7.0 Hz), 2.29 (2H, t, J=7.0 Hz), 2.36 (2H, t, J=7.4 Hz). 13C-NMR (75 MHz, CDCl3) δ : 179.1, 79.3, 79.0, 65.9, 65.8, 60.5, 60.3, 33.8, 28.5, 28.4, 27.8, 24.5, 21.6, 21.4, 19.3, 13.5. El-MS m/z (%): 244 (M+, 24), 128 (100). High-resolution El-MS m/z: Calcd for C14H20O2 244.1463. Found: 244.1462 [M+].

The detail of invasion assay procedure was described in our previous paper.10

References

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References