ヤーコン葉に含まれるラジ細胞（ヒトバーキットリンパ腫系細胞）の抗変形活性物質であるセスキテルレペンラクトン類の迅速精製法

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Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council Secretariat
Rapid Purification of Sesquiterpene Lactones, Active Chemicals for Anti-deformation of Raji Cells (Human Burkitt’s lymphoma cell line) in Yacon Leaves

Dalad SIRIWAN, Takayuki NARUSE, Jingbo CHEN, Kana HAYASHI, Akira KONDO and Hirotoshi TAMURA

Abstract

Sesquiterpene lactones such as enhydrin, sonchifolin and uvedalin from yacon (Smallanthus sonchifolius) were extracted, purified by silica gel column chromatography, and then crystallized by acetone to get the higher purity of the sesquiterpene lactones. From the crude extracts by silica gel column chromatography, the sesquiterpene lactones of enhydrin (200mg), uvedalin (90mg), and sonchifolin (120mg) from 2.9g of crude extracts have successfully been purified the proper amounts of three sesquiterpene lactones by the combination with crystallizations and ptlc chromatographies. With this kind of short steps of purification, of three sesquiterpene lactones with higher purity (95%<) were isolated as higher activity component for anti-deformation against Raji cells and Hela cells.

Keywords: Smallanthus sonchifolius; crystallization, Raji cells; Human Burkitt’s lymphoma cell, deformation; cancer prevention

Introduction

Yacon (Smallanthus sonchifolius), is a perennial herb originally cultivated in South America, and the fresh root is eaten like a fruit in this area. Yacon was introduced to Japan in 1985 via New Zealand and has been continually paid attention to due to its plentiful content of fructo-oligosaccharides in the tubers.

Recently, it has been reported that the extracts and chemical constituents of yacon leaf have antibacterial, antifungal, hypoglycemic, anti-liperoxidative and antioxidant activities due to the sesquiterpene lactones bearing a -methylene group. Its leaves were applied to tea production in many countries. However, anti-cancer activities of yacon extracts have not been reported yet. With Raji cells and Hela cells, we have already found anti-cancer and anti-tumor promoting active substances of yacon leaf extracts. For further biological tests to create a new drug for cancer prevention, we need a lot of standard chemicals. The amount of useful natural products in plant resources are usually less than 0.1% in plants. Even though the valuable chemicals are isolated in nature, we can not always supply the enough amounts of the useful chemicals from natural resources to develop new drugs and create new medicines. Higher efficient isolation technique and higher purity of chemical supply are required for the exploring the drugs. So, in this report, we would like to introduce the method of large scale of production of biological active chemicals in yacon extracts.

Materials and Methods

Chemical reagents

Chemical reagents used in this study were as follows: 12-O-tetradecanoylphorbol 13-acetate (TPA) (Sigma Chemical Co., St. Louis, U.S.A.), butyrate sodium (Wako Pure Chemical Industries LTD., Tokyo, Japan), Trypan Blue (Nacalai Tesque, Kyoto, Japan), Dulbecco’s modified eagle’s medium (ICN Biomedicals, Ohio, U.S.A.), fetal calf serum (Boehringer Mannheim K.K., Tokyo, Japan). The chromatographic material used was silica gel 60 (70-230mesh) from Nacalai Tesque (Kyoto, Japan). All chemicals were used without further purification.

Plant material

Yacon dried leaves were supplied by Mitsui Pulp Co. in 2004 and 2008. Dried leaves (1.73Kg) of yacon provided by Nihonkaisui Co. Ltd. And Mitsui Pulp Co. Japan were soaked in 5L hexane and then compressed three times with the same amount of the solvent. The total volume of the extracts was ca. 15L. Evaporation of the solvent and dryness under the
vacuum yielded 31.8g of extracts. The extracts (29g) was dissolved in 70mL of acetone and then mixed with 20.0g of Celite. The slurry dissolved in the minimum amount of hexane was applied on a silica gel column (5.5cm i.d. × 36.0cm) after exchanging the solvent from acetone to hexane. The fractionation of the eluents was started from 60% diethyl ether-40% hexane (3L), 70% diethyl ether-30% hexane (1L), 80% diethyl ether-20% hexane (1L), 90% diethyl ether-10% hexane (1L), 100% diethyl ether (1L), 100% acetone (3L), 100% methanol (1.1L). Fraction 1 (60% diethyl ether-40% hexane) to fr. 11 (70% diethyl ether-30% hexane) were separated on the basis of different color bands of the column chromatography. Each 300mL of eluents of fr. 12 and later fractions until fr. 37 was collected in individual flasks.

Analytical instruments

HPLC analysis was done with an inertsil ODS-3 column (4μ, 150mm × 4.6mm i.d.) equipped with a UV-975 Intelligent UV/VIS detector (JASCO Co.) and a Shimadzu C-R6A Chromatopac integrator. Analytical conditions were as follows: column temperature was at 40°C; mobile phase was a mixture of 40% acetonitrile and 60% H2O (v/v) and 80% acetonitrile and 20% H2O (v/v) with gradient system for 38min at 1.0mL/min of the flow rate after 2min holding the initial solvent condition; wavelength of the detector was at 230nm. Volume of injection was fixed to 1μL.

Results

Fractionation of three active components extracted from yacon leaves by a silica gel column chromatography. Acetone extract of yacon leaves that was dissolved in acetone at the concentration of 10mg/mL was injected one microliter into the 4μ ODS column (4.6mm i.d. × 150mm) at 230nm by using HPLC grade of acetonitrile and water as shown in Fig. 1. With a standard chemical and calibration curve of enhydrin, quantitative analysis of enhydrin was done and estimated enhydrin, uvedalin and sonchifolin in the acetone extract. The acetone extract (31.8g), may contain 2.5g of enhydrin, 2.4g of uvedalin, and 1.1g of sonchifolin. With a silica gel column chromatography of 29g of the extracts, thirty-eight fractions were separated and showed the individual weights of each fraction in Fig. 2. The sesquiterpene lactones identified in former paper such as enhydrin, uvedalin and sonchifolin were analyzed by ODS-HPLC. It was found that enhydrin was rich in the fractions between fr. 19 and fr. 25. Uvedalin was found mainly in the fr. 13 and fr. 14. Sonchifolin was detected only fr. 6 and fr. 7.

Purification of three sesquiterpene lactones. Crystalization of three sesquiterpenes were conducted as follows, Sonchifolin: Fraction 6 (1.0g) containing sonchifolin was isolated by the column chromatography described above.

![Fig. 1 HPLC analysis of acetone extracts of yacon leaves. ODS column 4μ, 4.6mm i.d. × 150mm. Concentration of sample was 10mg/mL in acetone.](image-url)
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Fig. 2 Fractions of column chromatography.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Sample weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
</tr>
<tr>
<td>13</td>
<td>0.5</td>
</tr>
<tr>
<td>15</td>
<td>0.5</td>
</tr>
<tr>
<td>17</td>
<td>0.5</td>
</tr>
<tr>
<td>19</td>
<td>0.5</td>
</tr>
<tr>
<td>21</td>
<td>0.5</td>
</tr>
<tr>
<td>23</td>
<td>0.5</td>
</tr>
<tr>
<td>25</td>
<td>0.5</td>
</tr>
<tr>
<td>27</td>
<td>0.5</td>
</tr>
<tr>
<td>29</td>
<td>0.5</td>
</tr>
<tr>
<td>31</td>
<td>0.5</td>
</tr>
<tr>
<td>33</td>
<td>0.5</td>
</tr>
<tr>
<td>35</td>
<td>0.5</td>
</tr>
<tr>
<td>37</td>
<td>0.5</td>
</tr>
</tbody>
</table>

60% ethyl ether-40% hexane, 70% diethyl ether-30% hexane, 80% diethyl ether-20% hexane, 90% diethyl ether-10% hexane, 100% diethyl ether, 100% acetone, 100% methanol were used as the eluants.

Fifty milligrams of fr. 6 were developed on 12 plates of PTLC (20cm × 20cm, thickness 0.5mm) with 20% hexane and 80% diethyl ether dissolving it in 0.8mL of acetone. Crystallization of sonchifolin from fr. 6 could not be succeeded. So, purification of one hundred sixty-four grams of fr. 6-7 was accomplished by the repetition of the PTLC with the same preparative conditions as described before. Finally, 75mg of sonchifolin was isolated with 100% purity confirmed by HPLC at 230nm of a UV detector.

Enhydrin: From fr. 19 (604mg) to fr. 25 (503mg), enhydrin and uvedalin were detected as the major component (33.6% to 69.1% for enhydrin and 6.9% to 13.8% for uvedalin). The crystallization of enhydrin and uvedalin was conducted by the mixture of diethyl ether and hexane (3 : 2) at the concentration of 20mg/mL or a small amount of diethyl ether. The solution was cooled down at 4°C for one hour. The crystal obtained from fr. 19 (604mg) for example was 323mg and showed the improvement of enhydrin and uvedalin contents from 39.0% and 13.1% to 74.6% and 23.9%, respectively. It meant that initial sample (fr. 19) contained 52.9% of impurity but after crystallization, 98.5% of the crystal mixture was composed of both chemicals and 1.5% of impurity was found in it. So, crystal of enhydrin (74.6%) and uvedalin (23.9%) mixture excluded the impurity from the crystal. Improvement of purities of enhydrin and uvedalin was also observed in other fractions as shown in Table 1. The final purification and separation of both chemicals (50mg out of 230mg) was done by one PTLC plate (20cm × 20cm, thickness 0.5mm) with 97% dichloromethane and 3% acetone after dissolving it in 1mL of acetone to afford 19.2mg of enhydrin (100% purity) and 7.8mg of uvedalin (97.3% purity).

Discussion

Co-crystals have the potential to be much more useful in pharmaceutical products and food ingredients than solvates
Table 1. Co-crystals of enhydrin and uvedalin

<table>
<thead>
<tr>
<th>Fr.</th>
<th>Weight (mg)</th>
<th>Yield (%)</th>
<th>HPLC Area Percentage (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Enhydrin</td>
</tr>
<tr>
<td>Fr.19</td>
<td>604</td>
<td></td>
<td>39</td>
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<tr>
<td>Fr.19 crystal</td>
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<td>53.5</td>
<td>74.6</td>
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<tr>
<td>Fr.20</td>
<td>697</td>
<td></td>
<td>69.1</td>
</tr>
<tr>
<td>Fr.20 crystal</td>
<td>346</td>
<td>49.6</td>
<td>83.3</td>
</tr>
<tr>
<td>Fr.21</td>
<td>744</td>
<td></td>
<td>63.4</td>
</tr>
<tr>
<td>Fr.21 crystal</td>
<td>157</td>
<td>21.2</td>
<td>83.3</td>
</tr>
<tr>
<td>Fr.22</td>
<td>715</td>
<td></td>
<td>44.7</td>
</tr>
<tr>
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<td>235</td>
<td>32.9</td>
<td>75</td>
</tr>
<tr>
<td>Fr.23</td>
<td>716</td>
<td></td>
<td>37.5</td>
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<tr>
<td>Fr.23 crystal</td>
<td>142</td>
<td>19.9</td>
<td>83.9</td>
</tr>
<tr>
<td>Fr.24</td>
<td>713</td>
<td></td>
<td>33.6</td>
</tr>
<tr>
<td>Fr.24 crystal</td>
<td>233</td>
<td>32.7</td>
<td>41.8</td>
</tr>
<tr>
<td>Fr.25</td>
<td>503</td>
<td></td>
<td>44.9</td>
</tr>
<tr>
<td>Fr.25 crystal</td>
<td>231</td>
<td>45.9</td>
<td>81.6</td>
</tr>
</tbody>
</table>

or hydrates\(^{[6,9]}\). Examples of co-crystals have existed in conductive organic crystals, non-linear optical crystals, dyes, photographic materials pigments, anthocyanins, and agrochemicals. Three recent papers by Fleischman et al.\(^{[11,12]}\) and Tamura et al.\(^{[13]}\) emphasize the importance of understanding "supramolecular synthons" in synthesizing co-crystals containing pharmaceutical agents and foods ingredients. Co-crystallization of sesquiterpene lactones is seldom reported in recent papers. It will be investigated that what kind of interaction can be supported the molecular association between the sesquiterpene lactones of enhydrin and uvedalin in future.

References

(6) Sirivan, D., Miyawaki, C., Miyamoto, T., Naruse, T., Tamura, H. Anti-tumor Promoting and cytotoxic activities of sesquiterpene lactones (SLs) isolated from *Smallanthus sonchifolius* (yacon) leaves. Submitted (2010).
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ヤーコン葉に含まれるラジ細胞（ヒトバーキットリンパ腫系細胞）の抗変形活性物質であるセスキテルベンラクトン類の迅速精製法

シリワンダラ・成瀬孝行・陳 静波・林 加奈・近藤 昭・田村啓敏

要約

ヤーコン（Smallanthus sonchifolius）葉に含まれるエンヒドリン、ウベダリン、ソヒホリンなどのセスキテルベンラクトンは抗発ガンプロモーター活性を有するが、これらの成分をアセトン抽出後、シリカゲルクロマトグラフィー及びアセトンによる結晶化により、簡便に精製できることが分かった。2.9gのアセトン抽出物からエンヒドリン200mg、ウベダリン90mg、ソヒホリン120mgをジエチルエーテルとヘキサンを用いた結晶化とPTLCにより95%以上の純度で単離できた。