TECHNICAL COMMUNICATIONS

Spectrophotometric Determination of Saponin in Yucca Extract Used as Food Additive

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A spectrophotometric method was developed for the determination of saponin in Yucca extract or its preparation for food additive use. A saponin fraction of Yucca extract was prepared by column chromatography with porous polymer, and hydrolyzed with a 2 mol/L mixture of hydrochloric acid–ethanol (1 + 1) to generate sapogenin. Sapogenin amounts were determined by measuring absorbance at 430 nm, based on the color reactions with anisaldehyde, sulfuric acid, and ethyl acetate. Recoveries from Yucca extracts were 91.5–95.1%, and the detection limit was 10 μg/kg. Commercial Yucca extracts for food additive use were composed of 5.6–6.4% (w/w) saponin, making it a minor component.

The Yucca species grows widely in North and Central America. It has been used as a foodstuff (1) and folk medicine (2) for many years. The presence of saponins in *Yucca schidigera* was reported (3), some of which have been isolated and identified (4). Yucca is listed in the code of federal regulations (cfr, United States) § 172.510 "Natural flavoring substances and natural substances used in conjunction with flavors" (5).

Yucca extract (extract of whole plant of *Yucca arborescens* Tres. or *Yucca schidigera* Roezl ex Orlgies) is listed in the "Lists of Existing Food Additives" in Japan (6). Because steroidal saponins in Yucca were reported to exhibit antiyeast or antifungal activities (4), Yucca extract has been added to food as a "shelf life extender" in the Japanese market. However, a procedure to ensure its quality for food additive use "food additive specification" has not been established yet.

In this study, we used a spectrophotometric method (7) to determine total amounts of saponin in Yucca extracts.

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Experimental

Materials

(**a**) *Samples*.—Yucca extracts (n = 4) and Yucca extract preparations (n = 2) were donated by Maruzen Pharmaceuticals Co. Ltd. (Hiroshima, Japan).

(**b**) *Standards*.—Standard saponin YE-2 (4, 8), the major saponin in Yucca extract, which showed a single spot on TLC, 25(R,S)-5β-spirostan-3β-ol 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside was donated by Maruzen Pharmaceuticals Co. Ltd. The aglycones of the saponin are sarsasapogenin and smialgenin, respectively. Crude saponin (95% methanol fraction of Yucca extract from Diaion HP-20, which contains ca 50% saponin) was also donated by Maruzen Pharmaceuticals Co. Ltd. Sarsasapogenin, the structure of which is shown in Figure 1 (minimum 98%), was from Sigma-Aldrich Japan (Tokyo, Japan).

(**c**) *Reagents*.—Diaion HP-20 (a styrene-divinylbezene copolymer, HP-20) was obtained from Mitsubishi Chemicals Co. Ltd. (Tokyo, Japan). *p*-Anisaldehyde and dextrin (for chemical use) were from Wako Pure Chemical, Industries Ltd. (Osaka, Japan).

(**d**) *Instrument*.—Shimadzu UV-2200 spectrophotometer (Shimadzu Co.; Kyoto, Japan).

Sample Preparation

Twenty mL of HP-20 resin was soaked in methanol overnight and packed in a glass column of 15 mm diameter with methanol. The resin was washed with 100 mL methanol and 200 mL water successively. Yucca extract, or its preparation (ca 200 mg), was charged to the column with a small amount of water, and washed with 100 mL water and 100 mL 40 % methanol successively.

Saponin was eluted with 100 mL 95% methanol solution. The solvent in the saponin fraction was removed under reduced pressure. The residue was dissolved with methanol and made up to 20 mL for the hydrolysis step.

Hydrolysis

Five to 20 mL of the methanol solution of saponin was placed in a short neck Kjeldahl flask. Methanol was removed under reduced pressure. Ten mL of 2 mol/L HCl and 10 mL ethanol were added to the residue and hydrolyzed for 3 h at

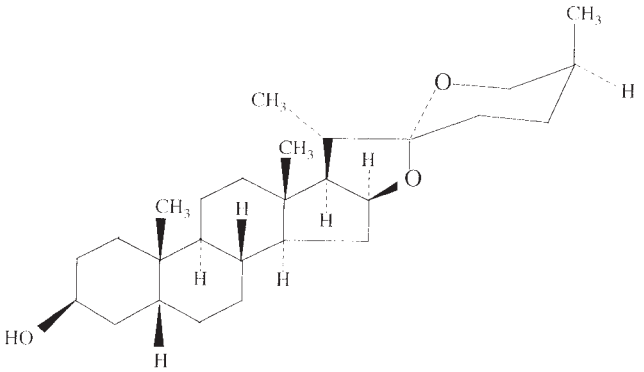

Figure 1. Chemical structure of sarsasapogenin.

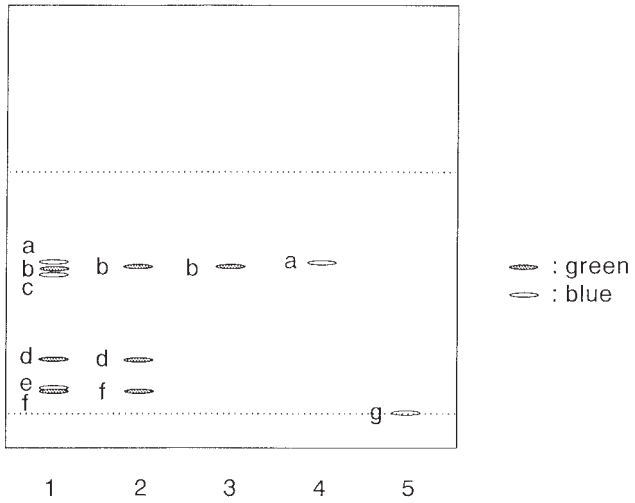

Figure 2a. TLC of hydrolyzed Yucca extract. (1) Yucca extract hydrolyzed with 2 mol/L HCl–ethanol (1 + 1) without purification by HP-20; (2) Yucca extract purified with HP20 and hydrolyzed with 2 mol/L HCl–ethanol (1 + 1); (3) standard sarsasapogenin; (4) sitosterol and stigmasterol; (5) saccharides; and c–f were found to correspond to other sapogenins and hydrolysis by-products (Uematsu et al., to be published).

90°C, cooled, and extracted twice with 80 mL diethyl ether. The saponin fraction in the ether layer was washed with 20 mL water and dehydrated with 20 g sodium sulfate anhydride. The ether was removed under reduced pressure and the residue which contained sapogenin was dissolved with ethyl acetate and made up to 10 mL for spectrophotometry.

Spectrophotometric Determination

The following color developing reagent solutions were prepared: (A) 0.5 mL *p*-anisaldehyde and 99.5 mL ethyl acetate, and (B) 50 mL concentrated sulfuric acid and 50 mL ethyl acetate. The ethyl acetate solution containing sapogenin was diluted with ethyl acetate to contain 2.5 to 10 μg/mL sapogenins. Two mL diluted sapogenin solution was placed in a 10 mL test tube. One mL each of reagent solutions (A) and (B) were added and the test tube sealed with a glass stopper. After stirring, the test tube was placed in a water bath maintained at 60°C for 10 min to develop color, then allowed to cool for 10 min in a water bath maintained at room temperature. Because the boiling point of ethyl acetate is 76°C, the water bath temperature should be controlled accurately. The absorbance of the color-developed solution was measured. Ethyl acetate was used as a control for the measurement of absorbance. As a reagent blank, 2 mL ethyl acetate was placed in a test tube and assayed in a similar way as the sapogenin solution.

Solutions containing 2–40 μg sarsasapogenin in 2 mL ethyl acetate were used to obtain a calibration curve.

Results

Sample Preparation

Because Yucca extract or its preparations for food additive use contain dextrin, sugar, or organic acids in their matrixes, spectrophotometric interference from these substances or from the ones naturally occurring in Yucca, such as sterols, was a concern. In fact, sugars, sterols, and several unidentified substances developed color on thin-layer chromatography (TLC) with a reagent containing *p*-anisaldehyde and sulfuric acid (Figure 2a).

Purification by column chromatography was, therefore, performed on an open column packed with HP-20. Yucca extracts were dissolved in water and charged to the column. The elution pattern with methanol solutions was obtained by increasing the methanol ratio from 0 to 100% (Figure 2b). Saponins started to elute with 60% methanol, and 98% of saponins were eluted with 95% methanol. Hence, we purified saponins from Yucca extract or its preparations by a stepwise elution method; the sample was charged to the column with a small amount of water, washed with 100 mL water and 100 mL 40% methanol, successively, then saponin was eluted with a 95% methanol solution. We then examined whether the 40 to 95% methanol fractions contained other ingredients. Dextrin and sugar were eluted with water, and organic acids were eluted with water and 40% methanol (Figure 2c). Sterols did not elute from the column. The completeness of the purification was confirmed further by TLC, in which spots other than

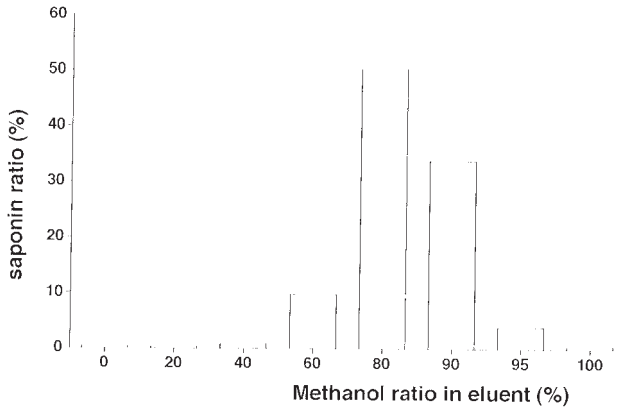

Figure 2b. Elution profiles for saponin from HP-20.

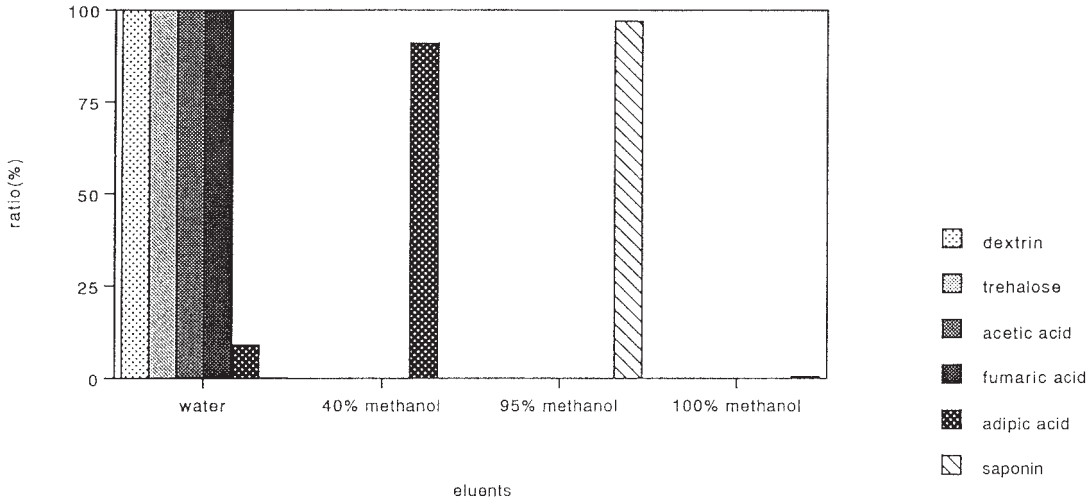

Figure 2c. Elution profiles for saponin and matrix substances in Yucca extract and food additive preparations from HP-20.

sapogenins were hardly observed in the purified Yucca extract (Figure 2a). TLC conditions: plate, silica gel 60 (HPTLC, 10 × 10 cm, layer thickness 0.2 mm, Merck KGaA, Darmstadt, Germany); developing solvent, hexane–ethyl acetate (2 + 1) mixture; color developing reagent, 0.5 mL *p*-anisaldehyde was dissolved in 9 mL ethanol, to which 0.5 mL sulfuric acid and 0.1 mL acetic acid was added.

Hydrolysis Reagent Concentrations and Yield

Conditions for the hydrolysis of saponins in Yucca extract purified with HP-20 were investigated. Saponins were hydrolyzed with various concentrations of HCl (3, 9, 10). We decided to hydrolyze with a 2 mol/L HCl–ethanol (1 + 1) mixture for 3 h at 90°C because of the relatively good yield obtained under these conditions.

We then quantitated the extent of hydrolysis. Because the saponins in Yucca extract were known to be a mixture of various types, a few of which have been identified (4), we used one of the major saponins in the extract, YE-2 (4), to investigate the hydrolysis yield. An amount of standard saponin YE-2 was accurately weighed, hydrolyzed, and its absorbance was measured according to the procedure described in the *Experimental* section. The corresponding amount of standard sarsasapogenin, which was calculated based on the molecular mass of YE-2 (872) and sarsasapogenin (416), was also spectrophtometrically determined. The yield, calculated as a ratio of the absorbance of hydrolyzed YE-2 to that of corresponding sarsasapogenin, was 98–108%.

Spectrophotometric Determination of Sapogenins

To determine the most suitable color developing time, the relationship between color developing time and absorbance was investigated using a 5 μg/mL sarsasapogenin solution. The results are shown in Figure 3. The reaction was completed in a very short time. The increase in absorbance with increase in color developing time was mainly due to the increase in the absorbance of the reagent blank.

Thus, we decided to maintain the color developing time used for the reagent blank, to be as accurate as possible. Ten minutes was chosen for simplicity of manipulation: 10 min was needed for the reaction mixture to cool to room temperature in water. The absorbance of the reagent blank was subtracted from that of the sapogenins.

The calibration curve was linear between 2 to 40 μg sarsasapogenin. The detection limit in Yucca extract was 10 μg/g. Because Baccou et al. reported that the molar absorbance coefficients of several steroidal sapogenins were similar (7), we decided to determine the total amount of sapogenin by using a calibration curve of sarsasapogenin, which is available commercially.

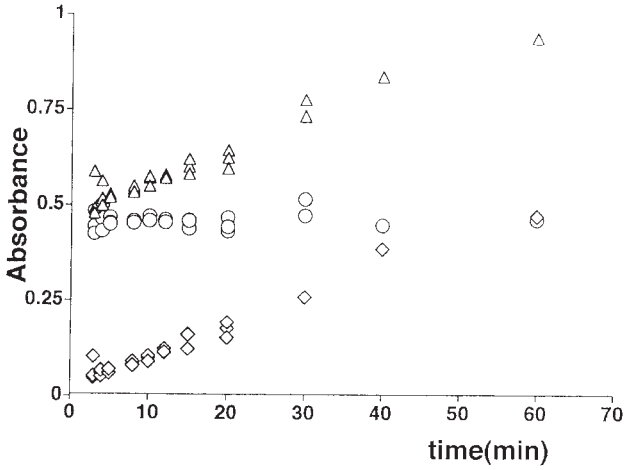

Figure 3. Time course of color development with sarsasapogenin. Δ = sapogenin; ◊ = blank; and ○ = sapogenin – blank. Color was developed with 5 μg/mL sarsasapogenin according to the method described in the *Experimental* section except for color developing time.

Table 1. Saponin amounts in commercial yucca extracts and their preparations for food additive use calculated from sapogenin amounts

Sample	Sapogenin amount, %	Saponin amount, %
Extract 1	2.7	5.6
Extract 2	2.8	5.9
Extract 3	2.9	6.1
Extract 4	3.0	6.4
Preparation 1	0.7	1.6
Preparation 2	0.5	1.1

Recoveries

The crude saponin (containing about 50% saponin) was used for the recovery test. Because dextrin is often mixed with Yucca extract for food additive use, the crude saponin was added to dextrin. Crude saponin (80 mg) was dissolved in 50 mL methanol and 10 mL of the solution was added to 200 mg dextrin and left overnight for the recovery test. The methanol was removed under reduced pressure, and saponin recovery was determined according to the procedure described in the *Experimental* section. Recoveries of saponin from the dextrin mixture were 91.5–95.1%.

Commercial Yucca Extract Analysis

The amount of saponin in 4 Yucca extracts and 2 Yucca extract preparations for food additive use was determined. The results are shown in Table 1. The hydrolyzed Yucca extract contained 2.7–3.0% sapogenin. The saponin value was calculated to be 5.6–6.4%, based on the molecular mass of saponin YE-2 and that of sarsasapogenin. In the food additive preparations, sapogenin values were 0.5 and 0.7 % and saponin values were 1.1 and 1.6%.

Discussion

Natural food additives, that is, those not chemically synthesized, had been used in Japan without any restrictions until 1995 when the Japanese Food Hygiene Law was amended. Prior to an amendment for labeling in 1991, natural food additives were not required to be listed on packages. As a result, numerous natural food additives were used in Japan. Most were plant extracts, some with a long history of use as food and, thus, considered safe. However, the rest had not been consumed as food, and neither their chemical properties nor toxicities have been well characterized.

Because food additive specifications ensure the quality of the food, they should be established for all food additives. But specifications for most existing natural food additives, such as Yucca extract, have not been established. Specifications should include identification, purity, and assay tests. Although a method which determines the amount of effective compound may be important, it is sometimes difficult to develop because the effectiveness of natural products is not due to a single chemical compound.

To determine the amount of saponin in Yucca extract, we chose a spectrophotometric method that can measure the total amount of effective compounds. Spectrophotometric methods are practical and simple, making them preferable for specification testing. Thorough purification was performed to avoid overestimation of the saponin amounts due to possible interference from other substances, which likely occurs in spectrophotometry. The standard substance is available commercially, which is also important for an official method such as specification.

This is the first report on the determination of saponin in Yucca extract for food additive use. The extracts contained only 5–6% saponin, making it a minor component. Even though approximately 30% dextrin is known to be added to Yucca extract for food additive use, the present study revealed that more than 60% of the Yucca extract consists of unknown substances. Because these unknown substances are ingested together with the saponin from foods, it is important to identify them, or purify the extract further so that the intake of possibly harmful substances is reduced.

Acknowledgment

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