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Isolation of Linoleic Acid and Ferulic Acid from Alkaline Hydrolysate of a Saponin Rich Fraction of *Panax ginseng*

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Abstract□Linoleic acid and ferulic acid were isolated from alkaline hydrolysate of a crude ginseng saponin fraction of *Panax ginseng* C.A. Meyer roots.

Keywords□*Panax ginseng* C.A. Meyer, saponin, linoleic acid, ferulic acid

Introduction

A butanol soluble fraction of *Panax ginseng* C.A. Meyer roots is known to mainly contain ginseng saponin, so is referred to as total ginseng saponin fraction or crude ginseng saponin fraction.[1] We reported that ginseng possessed potent anti-oxidant and antifatigue characteristics which could be due to certain phenolic substances rather than to pure ginsenosides.[2-7]

In order to isolate the phenolic components, crude ginseng saponin was divided into two sub-fractions, ginseng saponin poor (GSP) and rich (GSR) fractions, through the procedure of dissolution in methanol and then precipitation with ethyl acetate.[7] Three compounds, isomaltol α-D-glucopyranoside, ketopropyl α-D-glucopyranoside and adenosine were isolated from GSP which was the soluble fraction in a mixture of methanol and ethyl acetate. No phenolic compounds were isolated from GSR which was insoluble in the solvent mixture.

This paper describes the isolation of linoleic acid and ferulic acid from the alkaline hydrolysate of GSR.

Experiment

Instrumental analysis

Mps were determined on a Mitamura-Ricken apparatus and are uncorrected. IR absorption spectra were obtained in KBr pellets on a Perkin-Elmer Model 283B spectrophotometer. A recording spectrophotometer, Gilford Type 2600, was used for the measurement of UV/visible absorption spectra. ^{1}H-NMR spectra were taken at 25 °C using Varian FT-80A (80MHz), and chemical shifts are given as δ (ppm). EIMS spectra were obtained on a Hewlett Packard GC/MS spectrometer, Type 5985B.

Preparation of ginseng saponin rich fraction (GSR)

Fresh ginseng roots were refluxed with MeOH for 4 hrs at 60 °. The extraction was repeated four times. All the extracts were combined and MeOH was removed into water, and were extracted with benzene and then chloroform. The aqueous layer was extracted with butanol four times. All the butanol extracts were combined and freed from solvent under vacuum below 60 ° to gave drude ginseng saponin. 500g of it was dissolved in methanol (2L) and then ethyl acetate (2L) was added with stirring. The precipitate was dried under vacuum to yield ginseng saponin rich fraction (GSR)(430g).

Alkaline hydrolysis of GSR

GSR (50.4g) was dissolved in the mixture of EtOH (250 m*l*) and 1.0N NaOH (250 m*l*), and then was refluxed for 4 hrs under N_2 stream. The hydrolysate was freed from EtOH, acidified to pH 3 with 2N H_2SO_4, and extracted with hexane (300 m*l* × 2 times). Hexane layers were combined and

concentrated to yield an oily extract (2.3g, 4.5% yield). The aquous layer was extracted with ether (300 m*l* × 3 times). Ether layers were combined and concentrated to give a residue (1.1g, 2.2%).

Isolation of compounds 1 and 2

Compound 1 (linoleic acid)

Hexane extract showed a single spot (Rf 0.5) on a silica gel plate developed with solvent of hexane/ethyl acetate/acetic acid (=20:10:1) when visualized with H_2SO_4. It served as compound **1** without further purification. MS (relative intensity): *m/z* 280M$^+$, 12.5), 91(100). Methylation of **1** with ethereal diazomethane gave a monomethyl ester (oily). MS: *m/z* 294 (M$^+$, 6.6), 263(M$^+$-31, 3.3), 81(84.5), 67(100).

Compound 2 (ferulic acid)

Ether extract was chromatographed on a silica gel column, using an eluent of chloroform/methanol (5:1). UV-absorbing fractions which were positive by Pauly reagent (diazotized sulfanilic acid solution) were combined and concentrated to yield compound **2**. Crystallized from ethyl acetate (yield, 0.5g). mp: 162-164°. UVλ_{max}: in MeOH 292.5, 320 nm; in NaOH 304.340 nm. IR ν(cm^{-1}): 3410, 3010, 1690, 1600, 1510, 1270, 1200, 940, 845. Acetylation of **2** with acetic anhydride/pyridine gave an acetate (**2a**). mp: 182-184°. Methylation of **2a** with ethereal dizomethane yield a monomethyl ester (**2b**). ^{1}H-NMR ($CDCl_3$): 2.31(3H, s, CH_3CO), 3.80, 3.86 (3H × 2, each s, OCH_3 and $COOCH_3$), 6.37, 7.65 (2 × 1H, each d, J = 16Hz), 7.08(3H, br. s, aromatic H). MS: *m/z*250(M$^+$, 8.8), 208(100), 177(33.3), 145 (16.5).

Determination of ferulic acid in GSR

One m*l* of 2% GSR water solution was taken into a test tube, and one m*l* of an alkaline solution (K_2CO_3 or NaOH; 0.2N or 2.0N) was added to it. The tube was heated on a boiling water bath for 1 to 3 hours. After cooling, the hydrolysate was mixed with one m*l* of 10% Na_2CO_3 solution and 0.5 m*l* of diazotized sulfanilic acid solution. Optical density was measured at 500nm.

Results and Discussion

Ginseng saponin rich fraction (GSR) showed maximal absorption peaks at 267.5 and 279 nm in its UV spectrum as shown in Fig. 1, indicating the presence of phenolic components in GSR. The fact was confirmed by TLC which exhibited discrete bands as shown in Fig. 2. Some of UV-absorbing bands were overlapped with those being positive in Pauly reaction, and with those of ginseng saponins. Isolation of the UV-absorbing or Pauly positive substances has not succeeded yet, since they tend to degrade to less polar substances during separation. The lowest band which was positive in Pauly reaction (Fig. 2), was proved to be spinacine, which was previously isolated from water soluble fraction of ginseng.[8]

Thus, GSR was hydrolyzed with NaOH, and then acidified for solvent fractionation with hexane and ethyl ether. Hexane extract showed a single spot on TLC, serving as compound **1** without further purification.

Compound **1** showed the same Rf value as fatty acids such as palmitic acid and linoleic acid, when thin layer chromatographed with a developing solvent of hexane/ethyl acetate/acetic acid (=20:10:1). Molecular ion peaks of compound **1** and its methyl ester were found *m/z* 280 and 294 in their MS spectra, respectively. Mass fragmentation pattern of the methyl ester was superimposed with that of linoleic acid methyl ester.[9] Therefore, compound **1** was identified as linoleic acid. Its yield was about 4.5% in GSR.

It is well known that ginseng contains various kinds of fatty acids, which are easily transferred in ether when extraction of ginseng with the solvent,[10] and does phospholipids.[11] Hexane extract from the alkaline hydrolysate of GSR only contained linoleic acid. It is unlikely that the unsaturated fatty acid derived from phospholipids, because each type of phospholipid can exist in many different chemical species differing in their fatty acid substituents, and usually there is one saturated and one unsaturated fatty acid.

Ether extract obtained from the alkaline hydro-

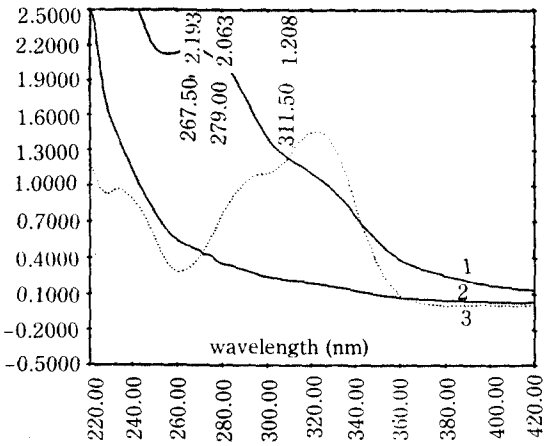

Fig. 1. UV spectra of ginseng saponin rich fraction (GSR) and ferulic acid in ethanol. 1, GSR 2.5 mg/ml; 2, GSR 0.25 mg/ml; 3, ferulic acid 70μM.

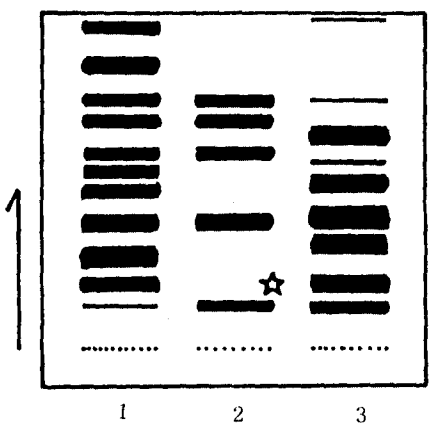

Fig. 2. Thin layer chromatogram of GSR. TLC was carried out on a silica gel plate using a developing solvent of chloroform/methanol/water (=15:10:2.5). Detection: 1, UV lamp; 2, Pauly reagent; 3, d-H_2SO_4. *The spot of spinacine.

lysate of GSR also showed a single spot on TLC when visualized with Pauly reagent, but did several minor spots toghther with the main spot when visualized under a UV lamp. Thus, the extract was subjected to column chromatography to yield the main component, compound **2**.

Compound **2**, mp 162-164°, showed maximum peaks at 292.5 and 320 nm in UV spectrum, and absorption bands at 3410 (OH), 3010, 1600, 1510 (aromatic H), and 1690 cm^{-1} (free acid) in IR spectrum. Acetylation and then methylation of compound **2** gave a methyl ester acetate (**2b**). ^{1}H-NMR of **2b** showed the signals of an acetyl at δ2.31 (3H,s), a methoxy at δ3.80 (3H,s), a methyl ester at δ3.86 (3H, s), two olefine protons at δ6.37 and 7.65 (2 × 1H, each d, J = 16 Hz), and three aromatic protons at δ7.08 (3H, br.s). These properties of compound **2** are identical with those of ferulic acid. Its content in GSR was measured about one %, when assessed under the optimal hydrolyzing condition that GSR was treated with 0.1N NaOH for one hour at 100° (Table 1).

Table 1. Analysis of ferulic acid in ginseng saponin rich fraction (GSR)

Final concentration of alkali	Amount of ferulic acid (%)* Hydrolyzing time (hr) 1	2	4
0.1N K_2CO_3	0.87	0.80	0.82
1.0N K_2CO_3	0.94	0.83	0.87
0.1N NaOH	1.00	0.91	0.92
1.0N NaOH	0.58	0.64	0.61

* Each tube containing 20mg of GSR was heated on a boiling water bath, and then ferulic acid was determined by Pauly reaction. Each test was performed in duplicate.

The presence of ferulic acid was reported in the ether soluble fraction of ginseng extract by Choi,[12] who could detect only a trace amount of it, utilizing a GC/MS technique. In our work, it was isolated from the alkaline hydrolysate of GSR with a relatively high yield.

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