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Thin-Layer Chromatographic Determination of Panaxadiol and Panaxatriol by Ultraviolet Derivatization[1]

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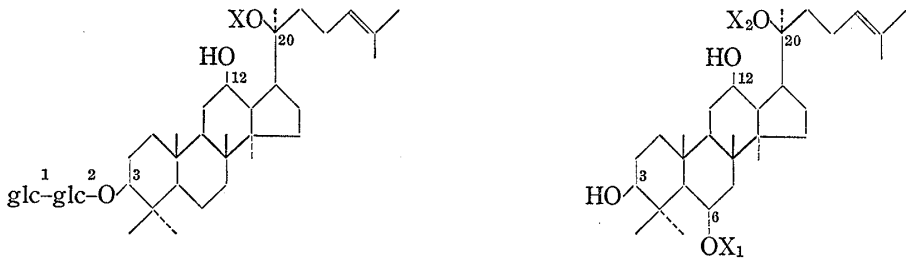
Panaxadiol (I) and panaxatriol (II) yielded on hydrolysis of ginseng saponins with 5% H_2SO_4/H_2O–EtOH (3:1) quantitatively reacted with a hundred fold excess of β-naphthoyl chloride in pyridine at room temperature, respectively. Completely stable derivatives, which were obtained as β-naphthoates, were analyzed by a thin-layer chromatography-dual chromatoscanner. This procedure was applied to the evaluation of the commercial ginseng.

Keywords——panaxadiol; panaxatriol; ultraviolet-derivatization; β-naphthoates; dual chromatoscanner; quantitative analysis of saponins; *Panax ginseng* C.A. Meyer

Ginseng saponins having dammarane type triterpene as their sapogenins are divided into two groups, ginsenoside —Rb_1, —Rb_2, —Rb_3, —Rc and —Rd which possess 20(*S*)-protopanaxadiol as the genuine aglycone and ginsenoside —Re, —Rf, —Rg_1 and —Rg_2 which possess 20(*S*)-protopanaxatriol as the genuine aglycone. Nagai *et al.*[3a,b] showed that with dil. mineral acid ginsenoside Rbcd group affords panaxadiol (I) and ginsenoside Refg group yields panaxatriol (II). The structures of these saponins have been established by Shibata *et al.*,[4a–c] and ginsenoside R_0 composed of oleanolic acid was isolated.



Rb_1: X=glc(6)-glc
Rb_2: X=glc(6)-ara(pyr)
Rb_3: X=glc(6)-xyl
Rc : X=glc(6)-ara(fur)
Rd : X=glc

Re : X_1=glc(2)-rham, X_2=glc
Rf : X_1=glc(2)-glc, X_2=H
Rg_1: X_1=glc, X_2=glc
Rg_2: X_1=glc(2)-rham, X_2=H

glc=β-D-glucopyranosyl, ara(pyr)=α-L-arabinopyranosyl,
rham=α-L-rhamnopyranosyl, ara(fur)=α-L-arabinofuranosyl.

Fig. 1. Structures of Ginsenoside

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4) *a*) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *Chem. Pharm. Bull.* (Tokyo), **22**, 421 (1974); *b*) S. Sanada, N. Kondo, J. Shoji, O. Tanaka, and S. Shibata, *ibid.*, **22**, 2407 (1974); *c*) Y. Nagai, O. Tanaka, and S. Shibata, *Tetrahedron*, **27**, 881 (1971).

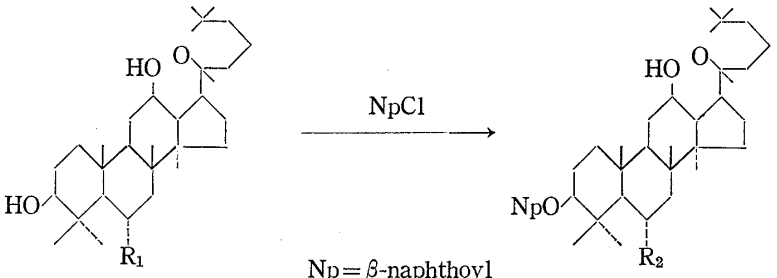
Recently, qualitative and quantitative evaluation of the commercial ginseng and related crude drugs have been investigated.[5a,b] Sakamoto *et al.*[6] reported the gas chromatographic determination of trimethylsilyl (TMS)-panaxadiol and TMS-panaxatriol, using diacetylhederagenin methyl ester as the internal standard.

In the present paper, we wish to report the quantitative analysis of panaxadiol (I) and panaxatriol (II) ultraviolet (UV)-derivatized with β-naphthoyl chloride using a thin-layer chromatography (TLC)-dual chromatoscanner.

Results and Discussion

Recently, chemical derivatization is frequently used for the analysis of carbohydrates[7] and hydroxy steroids[8] by high-performance liquid chromatography. Panaxadiol (I) and panaxatriol (II) obtained by acid hydrolysis of saponins have no UV absorption, so acylation of I and II with aromatic acid chlorides were conducted for so-called UV-labeling.

The UV labeling reagents used in this work were benzoyl chloride, 4-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride, anthraquinone-β-carbonyl chloride and β-naphthoyl chloride. Benzoyl chloride, 4-nitrobenzoyl chloride, 3,5-dinitrobenzoyl chloride and anthraquinone-



I: R_1=H
II: R_1=OH
III: R_2=H
IV: R_2=ONp

Chart 1. Labeling Reactions of Panaxadiol and Panaxatriol

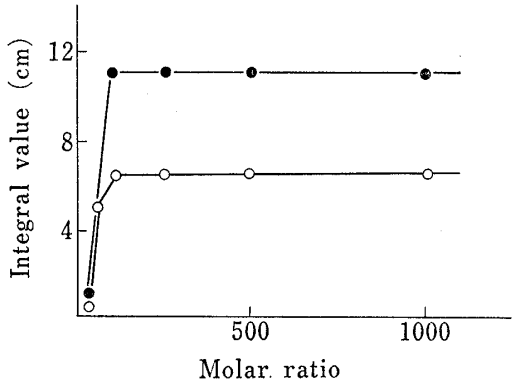


Fig. 2. Molar Ratio of β-Naphthoyl Chloride in the Labeling Reaction

○; panaxadiol, ●; panaxatriol.

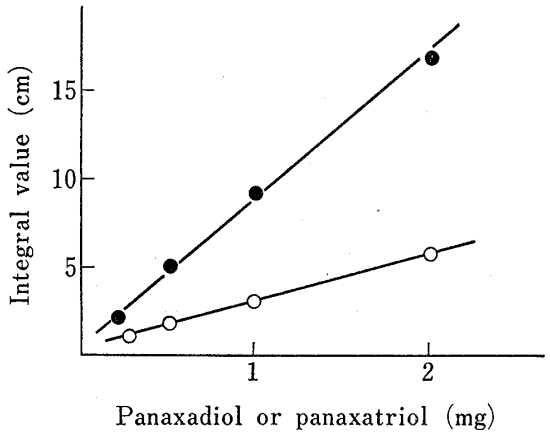


Fig. 3. Calibration Curves of β-Naphthoylated Panaxadiol and Panaxatriol

○; panaxadiol, ●; panaxatriol.

5) *a*) T. Namba, M. Yoshizaki, T. Tomimori, K. Kobashi, K. Mitsui, and J. Hase, *Yakugaku Zasshi*, **94**, 252 (1974); *b*) Y. Saito, S. Sanada, J. Shoji, and S. Shibata, Abstracts of Papers, 23th Annual Meeting of The Japanese Society of Pharmacognosy, Hiroshima, Nov., 1976, p. 25.
6) I. Sakamoto, K. Morimoto, and O. Tanaka, *Yakugaku Zasshi*, **95**, 1456 (1975).
7) J. Lehrfeld, *J. Chromatogr.*, **120**, 141 (1976).
8) F.A. Fritzpatrick and S. Siggia, *Anal. Chem.*, **45**, 2310 (1973).

β-carbonyl chloride reacted with I to afford a mixture of several products by TLC, respectively. In the case of quantitative analysis by chemical derivatization, it is necessary that the reaction of one substrate with labeling reagent affords the corresponding one derivative. Accordingly, the above reagents are not suitable for the UV derivatization of I and II. β-Naphthoyl chloride selectively reacted with I and II in pyridine to afford one product, respectively. In this reaction, β-naphthoic acid and β-naphthoic anhydride were also obtained as the by-products. The anhydride was developed near the solvent front on silica gel plate of TLC, accompanying the tailing. As the TLC spots of labeled I and II are affected by the tailing of the anhydride, it seems that the removal of the anhydride is the major problem. This problem was solved by the filtration through the silica gel column with chloroform. The influence of β-naphthoic acid can be neglected because of its low *Rf* value.

sapogenins
↓ β-naphthoyl chloride/C_5H_5N, r.t. 3 hr, 5% $NaHCO_3$ (4-DMAP)[a], 0.5 N HCl, H_2O
$CHCl_3$ extract
↓ filtered through silica gel column
eluate
↓ TLC, $CHCl_3$–C_6H_6–AcOEt (10: 10: 1)
chromatoscanner
λ_R=365 nm, λ_S=285 nm

Chart 2. Labeling Procedure

a) 4-DMAP; 4-dimethylaminopyridine.

The dependence of the acylation of I and II on reagent concentration was shown in Fig. 2. From the data in Fig. 2, it becomes clear, that with a reagent excess from a hundred fold to thousand fold in molar ratio, quantitative derivatization with β-naphthoyl chloride can be achieved. The process of acylation was monitered by TLC and was found to complete within three hours at room temperature.

Calibration curves of the labeling reaction of I and II with β-naphthoyl chloride gave a good linearity, respectively, shown in Fig. 3.

The labeling procedure is summarized in Chart 2. According to Chart 2, the recoveries were investigated, so that the good results were obtained as shown in Table I. These derivatives were stable for over 10 days at room temperature.

Table 1. Recoveries of Panaxadiol and Panaxatriol

	Added weight (mg)	Recovered weight (mg)	Recoveries (%)
Panaxadiol	1.29	1.27	98.4
	1.79	1.77	99.1
	1.57	1.55	98.5
Panaxatriol	1.50	1.55	103.5
	1.63	1.62	99.3
	1.66	1.65	99.4

The structures of these naphthoates (III, IV) were deduced by infrared (IR) and proton magnetic resonance (PMR) spectra. The IR spectra of III and IV in chloroform showed bands at 3280 cm^{-1} (intramolecular hydrogen bonding OH), 1705 cm^{-1} (ester C=O), and 1635 cm^{-1} (aromatic C=C). Both I and II have also similar absorption bands around 3280 cm^{-1}. The PMR spectrum of III in deuteriochloroform exhibited the peaks at δ: 8.6—7.5 (m, 7H), 4.8 (t, 3α-H) and 3.8—3.4 (m, 12α-H). The chemical shift of C-12 proton of I is as same as that of its derivative. The similar result was obtained in the PMR spectra of II and IV. Consequently, it seems that III and IV can be formulated as panaxadiol 3-(β-naphthoate) and panaxatriol 3,6-di(β-naphthoate), respectively.

In determining the content of saponin, it is necessary to obtain the calibration curves of III and IV obtained from the hydrolysis of saponins. Ginsenoside Rb_1 for Rbcd group and Re for Refg group were respectively hydrolyzed by Sakamotos' method,[6] and the reac-

tion products were labeled according to Chart 2. Calibration curves for the hydrolysis of saponins also gave a good linearity as shown in Fig. 4.

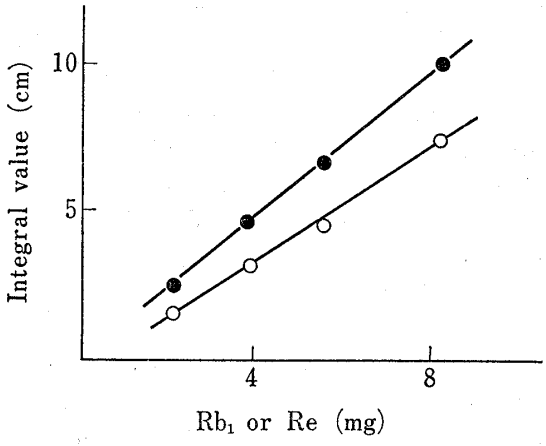


Fig. 4. Calibration Curves of Panaxadiol 3-(β-naphthoate) from Rb_1 and Panaxatriol 3,6-di(β-naphthoate) from Re

○; panaxadiol, ●; panaxatriol.

Table II. Analytical Results of Crude Drugs of Ginseng

Ginseng	Calcd. for Rb_1 (%)	Calcd. for Re (%)	Total (%)
White ginseng A	0.8	0.5	1.3
B	0.6	0.3	0.9
Root hair (Aizu)	6.5	1.3	7.8
(Korea)	8.3	1.2	9.5

This procedure was applied to the evaluation of the commercial ginseng, and the results are summarized in Table II. From the data in Table II, the saponin content of root hairs of ginseng is about 6 to 10 times as much as that of white ginseng. As is already known, the ratio of white ginseng is higher than that of root hairs of ginseng in relative content of ginsenoside Refg group against ginsenoside Rbcd group.

This procedure is suitable for the semi-micro or micro analysis of ginseng saponin. In addition, this chemical derivatization will be applicable also to the determination of non-absorbing components of other crude drugs.

Experimental

All the melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. IR absorption spectra were obtained with a Hitachi Model 215 spectrophotometer and JASCO IRA-1 spectrophotometer in $CHCl_3$. PMR spectra were measured with a JEOL JNM-PMX60 (60 MHz) spectrometer and JNM-PS100 (100 MHz) spectrometer in $CDCl_3$. The chemical shifts are given in δ (ppm) scale with tetramethylsilane as internal standard. The density of the chromatograms was measured with a Shimadzu CS-910 dual chromatoscanner.

Saponins and Sapogenins——Ginsenoside-Rb_1, -Re, I and II were prepared according to the methods given in references. Ginsenoside Rb_1; mp 202—205° (lit.[4a] 197—198°), ginsenoside Re; mp 203—205° (lit.[4b] 201—203°), panaxadiol; mp 250° (lit.[9] 251°), panaxatriol; mp 237—239° (lit.[10] 238—239°).

Reagents——C_5H_5N used in the derivatization reaction was redistilled over sodium hydroxide and commercial β-naphthoyl chloride was used in the reaction without further purification. All other reagents and solvents were analytical grade. For TLC, commercial Merck Silica gel $60F_{254}$ (0.25 mm) plates were used. Wakogel C-200 (Wako Pure Chemical Ltd.) was employed for filtration of the reaction mixture.

Labeling Procedure——A 0.5 to 5 mg of sapogenin was dissolved in 1 ml of C_5H_5N. A hundred fold molar excess of β-naphthoyl chloride, based on the amount of hydroxyl group, was added to the solution. The reaction mixture was allowed to stand for three hours at room temperature, and poured into 50 ml of 5% $NaHCO_3$ aq. soln. containing 1 mg of 4-dimethylaminopyridine and extracted with 50 ml of $CHCl_3$. The $CHCl_3$ phase was washed twice with 50 ml of 0.5 N HCl and 50 ml of H_2O. $CHCl_3$ was evaporated off and the reaction mixture was separated by the filtration through silica gel column with $CHCl_3$. The eluate containing the UV-labeled derivatives was spotted on TLC plate and the plate was developed by C_6H_6–$CHCl_3$–AcOEt (10: 10: 1). The chromatogram was measured on a dual chromatoscanner at λ_S=285 nm and λ_R=365 nm.

9) S. Shibata, M. Fujita, H. Itokawa, O. Tanaka, and T. Ishii, *Tetrahedron Lett.*, 1962, 419.
10) S. Shibata, O. Tanaka, K. Soma, Y. Iida, T. Ando, and H. Nakamura, *Tetrahedron Lett.*, 1965, 207.

UV-Labeled Derivatives——Panaxadiol 3-(β-naphthoate) (III); mp 280—282° (from petroleum ether–C_6H_6). IR, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3280 (conc. independent, 12-OH), 1705 (ester), 1635 (arom.). PMR, δ^{CDCl_3}: 8.6—7.5 (m, 7H), 4.8 (t, 3α-H), 3.8—3.4 (m, 12α-H).

Panaxatriol 3,6-di(β-naphthoate) (IV); mp>300° (from petroleum ether–C_6H_6). IR, $\nu_{max}^{CHCl_3}$ cm^{-1}: 3280 (conc. independent, 12-OH), 1705 (ester), 1635 (arom.). PMR, δ^{CDCl_3}: 8.6—7.5 (m, 14H), 6.0—5.7 (m, 6β-H), 4.9 (t, 3α-H), 3.8—3.4 (m, 12α-H).

Hydrolysis of Saponins——Ginsenoside-Rb_1 and -Re were hydrolyzed by Sakamotos' method,[6] respectively.

Determination of the Saponin Content——Ginseng (powdered) was extracted three times with hot MeOH, and the MeOH solution was evaporated to dryness. The MeOH extract was suspended in H_2O and defatted twice with $(C_2H_5)_2O$. The aqueous phase was extracted four times with *n*-BuOH (saturated with H_2O) and the *n*-BuOH layer was evaporated off and dried over at 80° in vacuum. Total saponins (*n*-BuOH fraction) were hydrolyzed and labeled according to Chart 2.