생 약 학 회 지
Kor. J. Pharmacog.
6(1) : 15~21(1975)

Studies on the Components in the Ethereal Extract of *Panax ginseng* (I)

Studies on the Free Fatty Acids

Chae-Ho Cook and Seung-Ho An

Department of Organic Pharmaceutical Manufacturing Chemistry, College of Pharmacy
Seoul National University, Seoul, Korea

人蔘의 Ether抽出物의 成分에 관한 硏究(I)

遊離脂肪酸에 관한 硏究

鞠 埰 豪·安 承 鎬

서울大學校 藥學大學 有機藥品製造化學敎室

人蔘의 ether 抽出物로부터 遊離 脂肪酸을 分離하고 이것을 diazomethane으로 처리하여 methyl ester를 만든 다음 초산수은 및 column chromatography를 利用하여 飽和 脂肪酸과 不飽和 脂肪酸으로 分離하였다. 分離된 脂肪酸을 G.L.C.에 의하여 分析하여 다음과 같은 結論을 얻었다.

1) 韓國產 人蔘 6年生根中에는 遊離 脂肪酸이 0.28% 含有되어 있다.

2) 人蔘中에서 24種類의 遊離 脂肪酸이 發見되었으며 그 中 22種類는 G.L.C.에 依하여 確認되었으나, 나머지 2種類는 G.L.C. data만 가지고는 確認할 수 없었다. 未確認의 두 脂肪酸은 그 量이 多量이었으며 天然에 흔히 存在하지 않는 unusual fatty acids라고 思料된다.

3) Lee 等은 人蔘中에 n18:3이 存在한다고 報告한 바 있으나 本實驗에서는 n18:3의 存在를 確認할 수 없었으며 그 代身 peak XVI이 n18:2와 n18:3의 사이에서 나타남을 보여준다. peak XVI은 未確認 脂肪酸이다.

There are many reports on the components and pharmacological actions of *Panax ginseng*, especially on the saponins. Takagi *et al.* [1~3] noticed that *Panax ginseng* had two complex biological actions such as sedative and stimulant actions on the central nervous system in the view of classical pharmacology and Oura *et al.*[4~7] proposed that the each component of the saponins in *Panax ginseng* had its characteristic pharmacological action on the level of modern pharmacology. But there are few reports which studied on the free fatty acids of *Panax ginseng* by gas chromatography. Manki and his coworker[8] had studied on the free fatty acids of the stem of *Panax ginseng*. They showed that n6:0, n8:0, n10:0, n12:0, n14:0 and n16:0 of free fatty acids existed in the stem. Lee and Lee[9] reported in their publication that there were n18:1, n18:2, n18:3 and several other fatty acids in *Panax ginseng*.

Choi and Hong[10] indicated that the fatty acid fraction of *Panax ginseng* decreased the cholesterol level in blood in the splague dowely 4 weeks after and recovered 8 weeks after. Many a scholar has studied on the effects of unsaturated fatty acids in the fields of medicine, pharmacy and nutrition. Peifer and Rand *et al.* [11~13] showed that specific types of fatty acid unsaturation induced hypocholesterolemic effects in the rat and rabbit.

In the analytical methods of fatty acids by gas

chromatography, various ways have been introduced. CREVAR[14] used 15% EGSS-X column in gas chromatographic analysis of fatty acids and ABURANO and his coworkers[15] used 5% DEGS column and SAKURAI[16] adopted polyethylene glycol succinate column. MIWA and his coworkers[17] chose Apiezon L and LAC-2-R 446 column in analyzing fatty acids.

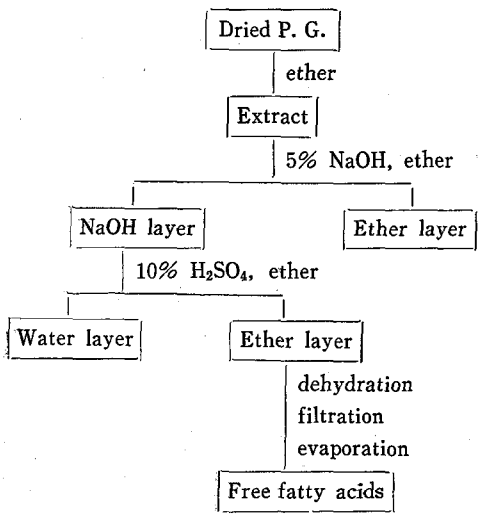
It was recently reported [14,18,19,25] that odd numbered fatty acids also exist in higher plants and animals. And in this experiment, several odd numbered free fatty acids were also identified.

According to the above mentioned reason, the study of the free fatty acids of *Panax ginseng* was undertaken, prior to the examination of the pharmacological action of the free fatty acids contained in *Panax ginseng*, the free fatty acids were isolated from the root of six-year old Korean *Panax ginseng* and their identification and quantitation were undertaken by gas chromatography.

Experimental

Extraction of Free Fatty Acids

The free fatty acids were prepared from the ethereal fraction of *Panax ginseng*. This fraction was obtained by the air-dried coarsely powdered roots of six-year old Korean ginseng with ether. The ethereal solution was extracted three times with 5% sodium hydroxide.



Scheme 1. Isolation of free fatty acids from *Panax ginseng*.

The basic aqueous solution was treated with 10% sulfuric acid, and this acidic solution was extracted again with ether. The ether layer was dehydrated with anhydrous sodium sulfate and evaporated to dryness under nitrogen gas. The total free fatty acids obtained were 0.28%. The performed procedure was shown in Scheme 1.

Separation of Methyl Esters of Saturated and Unsaturated Free Fatty Acids

The prepared free fatty acids were methylated with diazomethane(CH_2N_2). The prepared methyl esters were gas-chromatographed at 130° and 170° using polar column(Figs. 1 and 3). Quantitation as percent of each free fatty acid was achieved by the triangulation procedure[20] on the gas-chromatogram.

A sample of 20mg of dry methyl esters of the fatty acids and a portion of 100mg of mercuric acetate were placed in a culture tube(16×150mm) equipped with a teflon-lined screw cap and then 8ml of a solution containing 5% diluted water and 0.3% glacial acetic acid in methanol was added. The resulting tightly sealed tube was heated in a water bath at 60° for approximately 5 min. to ensure solution of the mercuric acetate; the tube then stored in the dark at room temperature for 24 hrs. The solvent and excess acetic acid were removed, and the residue was dried by evaporation under nitrogen gas at room temperature. The dry residue was shaken three times with 10ml benzene at 50~60°, and the extracts were filtered through glass wool onto a column of silica gel.

The silica gel column was prepared from a slurry in benzene which was poured into pasteur pipet(100mm in height). The column was eluted with the benzene to a total volume of 150ml. This eluate contained the methyl esters of saturated fatty acids, with the methyl esters of unsaturated fatty acid mercuric acetate adducts remaining at the top of the column as indicated by a yellow band. The benzene eluate was evaporated to dryness under nitrogen gas. The dried methyl esters of the saturated fatty acids were 6 mg.

The mercuric acetate adducts were eluted with 50ml of 5% glacial acetic acid in absolute ethanol. To recover the methyl esters of the unsaturated fatty acids, this eluate was treated with 10ml of 6N hydr-

chloric acid and 50ml of water, after 5 min. this mixture was diluted with 50ml water and extracted with 20 ml of benzene. The resulting benzene fractions were dried with anhydrous sodium sulfate and evaporated to dryness under nitrogen gas. The dried methyl esters of the unsaturated fatty acids were 6mg. The gas-chromatograms of those were shown in Figs. 2 and 4.

Gas chromatography

All gas chromatographical analyses were carried out by an instrument equipped with a hydrogen flame ionization detector (Shimadzu model GC-4B). The column was used with Shimalite (60～80 mesh) coated 15% DEGS film 2m glass helix shaped tube, 4mm diameter. Column temperatures were 130° (150°) and 170° (185°). Nitrogen was used as carrier gas at flow rate of 40ml/min. All quantitative works were performed by the triangulation procedure[20].

Calculation of Separation Factor

The separation factor was calculated for each component relative to the proceding saturated component, as described by LANDOWNE and LIPSKY[21], according to the respective retention time for each component. The separation factor increases as column temperature increases for the methyl esters of the unsaturated straight chain fatty acids, but decreases as column temperature increases for the saturated straight and branched chain components[21]. The semilog plot of the log of the retention time versus the carbon number of a homologous series was linear[22] (Figs. 5, 6, 7 and 8) and this fact was constructed for the identification of various components as previously described by MIWA[17], RUSEVA-ATANASONA[23] and JAMES[24]. A modified separation factor was also used as a means of identifying the di- and triunsaturated fatty acid esters. The following equations explains the separation factors in dtail.

$$\text{Separation factor} = \frac{\text{Retention time of component}}{\text{R.T. of preceding saturated comp.}}$$

$$\text{S. F. (modified)} = \frac{\text{R. T. of unsaturated acid ester}}{\text{R. T. of parent-saturated acid ester}}$$

Separation factor:

increase(+) : unsaturated straight chain fatty acids

decrease(−) : saturated straight chain fatty acids or saturated branched chain fatty acids

Scheme 2. Calculation of separation factor

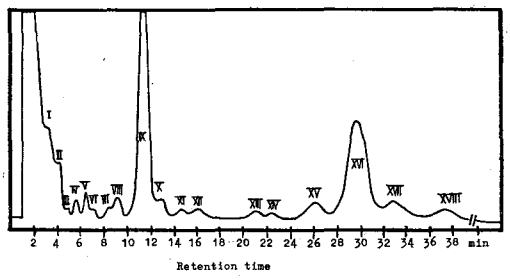


Fig. 1. Gas chromatogram of methyl esters of free fatty acids derived from *Panax ginseng*
Column : 15% DEGS, 60～80 mesh Shimalite, 2m×4mm glass, Rate of carrier gas : 40 ml/min nitrogen, Detector : F. I. D., Sensitivity: 6.4×10^{-9} a.f.s. Temperature: 130°

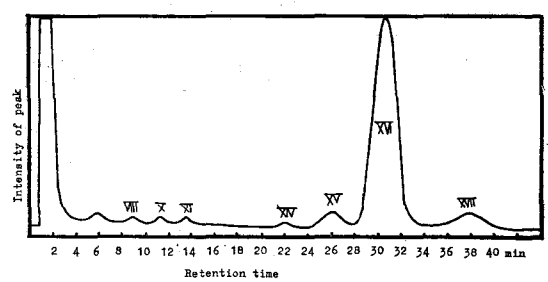


Fig. 2. Gas chromatogram of methyl esters of unsaturated free fatty acids derived from *Panax ginseng*
Column : 15% DEGS, 60～80 mesh Shimalite, 2m×4mm glass, Rate of carrier gas : 40ml/min. nitrogen, Detector : F.I.D., Sensitivity : 6.4×10^{-9} a.f.s. Temperature : 130°

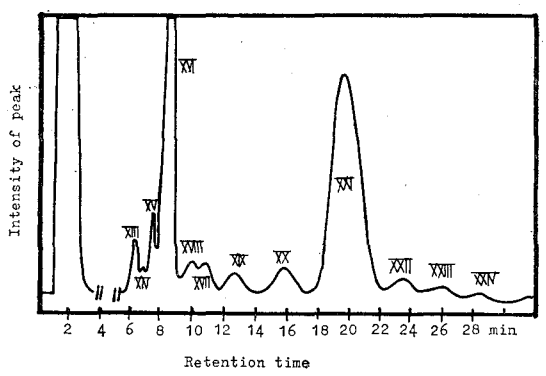


Fig. 3. Gas chromatogram of methyl esters of free fatty acids derived from *Panax ginseng*
Column : 15% DEGS, 60-80 mesh Shimalite, 2m×4mm glass, Rate of carrier gas : 40ml/min nitrogen, Detector : F.I.D., Sensitivity : 6.4×10^{-9} a.f.s., Temperature : 170°

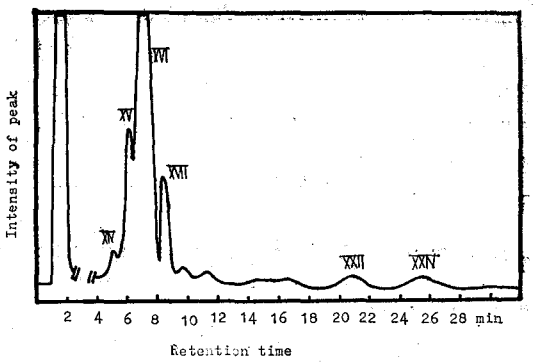


Fig. 4. Gas chromatogram of methyl esters of unsaturated free fatty acids derived from *Panax ginseng*. Column : 15% DEGS, 60~80 mesh Shimalite, 2m×4mm glass, Rate of carrier gas : 40ml/min nitrogen, Detector : F. I. D., Sensitivity : 6.4×10^{-9} a.f.s., Temperature : 170°

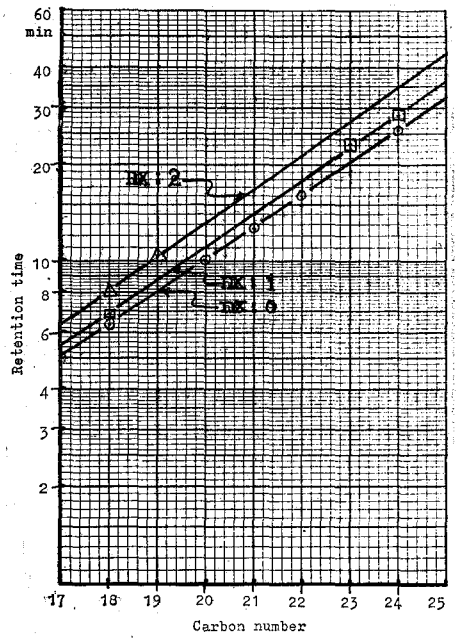


Fig. 6. Retention times of methyl esters of unsaturated free fatty acids derived from *Panax ginseng* at 130°
◇—◇ nX : 1, △—△ nX : 2

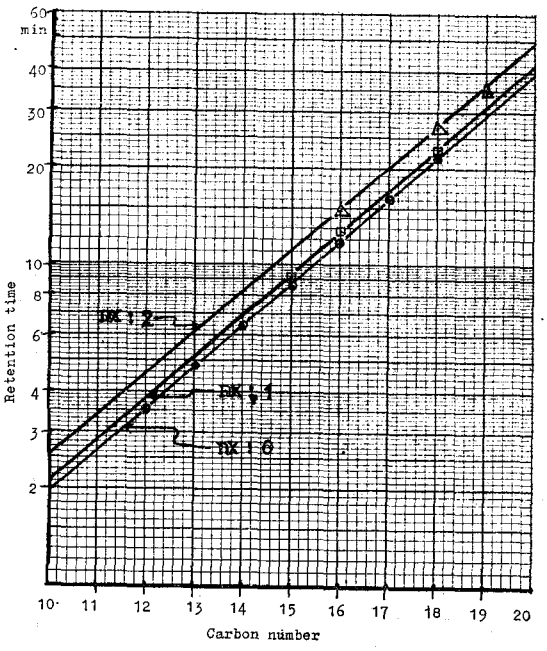


Fig. 5. Retention times of methyl esters of free fatty acids derived from *Panax ginseng* at 130°
⊙—⊙ nX : 0, ◇—◇ nX : 1, △—△ nX : 2

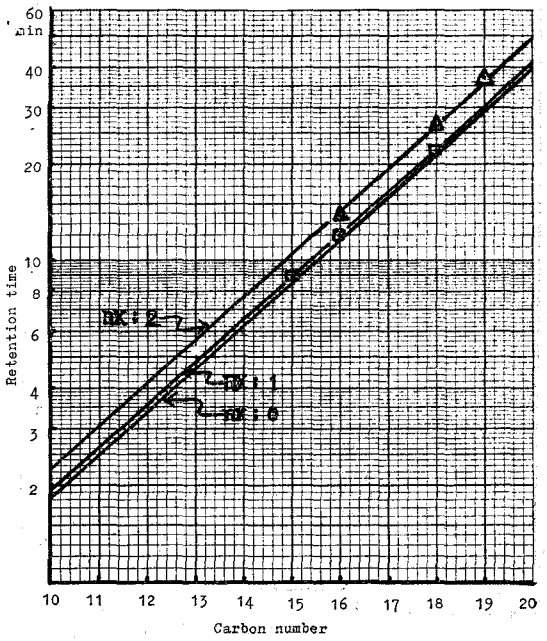


Fig. 7. Retention times of methyl esters of free fatty acids derived from *Panax ginseng* at 170°
⊙—⊙ nX : 0, ◇—◇ nX : 1, △—△ nX : 2

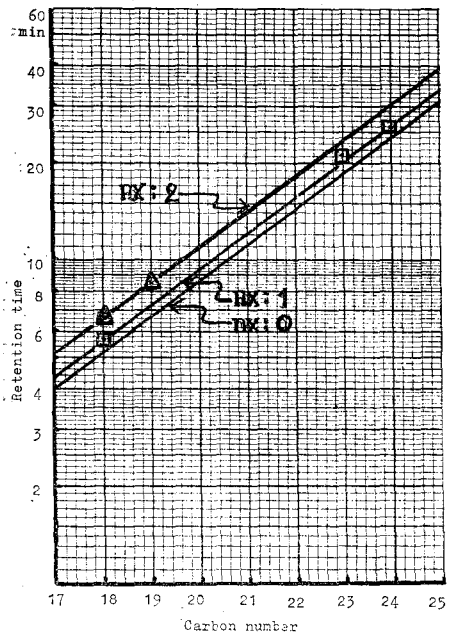


Fig. 8. Retention times of methyl esters of unsaturated free fatty acids derived from *Panax ginseng* at 170°. ◇—◇ nX : 1, △—△ nX : 2

Results and Discussion

The retention times and separation factors of methyl esters of the fatty acids of *Panax ginseng* at 130° (150°) and 170° (185°), using polar column in gas-chromatographic analysis, are listed in Tables Ⅰ and Ⅱ. The semilog graphs of the retention time versus carbon number for the methyl esters are shown in Figs. 5, 6, 7, and 8. The retention times, separation factors and graphic analyses for the methyl esters of the saturated and unsaturated fatty acids of *Panax ginseng*, which were separated by the mercuric acetate method, were in agreement with similar experiments carried out with the standard free fatty acids. The identification of the respective methyl ester of the fatty acid and the average percent composition for each component are listed in Table Ⅲ.

Landowne and Lipsky[21] showed that the separation factor for the methyl ester of a saturated fatty acid is inversely proportional to the temperature, whereas the reverse is true for the unsaturated acid ester.

Table Ⅰ. Retention times and separation factors at 130° and 150°, using polar column, of components of *Panax ginseng*.

Peak number	Retention time (min) 130°	150°	Separation factor 130°	150°	±	Identified component
Ⅰ	3.6	2.8	1.38	1.33	−	n12:0
Ⅱ	4.1	3.1	1.17	1.10	−	i13:0
Ⅲ	4.9	3.4	1.40	1.31	−	n13:0
Ⅳ	5.6	3.9	1.21	1.18	−	i14:0
Ⅴ	6.4	4.4	1.36	1.33	−	n14:0
Ⅵ	7.1	4.8	1.10	1.09	−	i15:0
Ⅶ	8.5	5.3	1.33	1.26	−	n15:0
Ⅷ	9.1	5.7	1.06	1.08	+	n15:1
Ⅸ	11.6	6.6	1.35	1.25	−	n16:0
Ⅹ	12.8	7.3	1.10	1.11	+	n16:1
Ⅺ	14.6	9.6	1.26	1.45*	+	n16:2
XII	16.0	8.5	1.38	1.29	−	n17:0
XIII	21.5	10.9	1.35	1.28	−	n18:0
XIV	22.5	12.0	1.04	1.11	+	n18:1
XV	26.5	14.6	1.23	1.35	+	n18:2
XVI	29.3	15.1	1.02	1.11	+	?
XVII	32.3	19.0	1.12	1.40*	+	n19:2
XVIII	37.4	16.3	1.30	1.20	−	n20:0

* modified separation factor

This observation was confirmed in this analysis (Tables Ⅰ and Ⅱ).

There is indication that peak XV and XVI are overlapped at 185° (Table Ⅱ). This was supported by the chromatogram of the unsaturated and saturated esters after separation by the use of mercuric acetate. The semilog plots of the homologous series also supported this observation. But peak XV and XVI are separated clearly at 130° (Fig. 1).

There was suggestion that peak XI, n16:2 was preceded by peak XII, n17:0 at 130° but this order was reversed at 150° (Table Ⅰ). And peak XVII, n19:2 was preceded by peak XVIII, n20:0 at 130° but this order was also reversed at 150° (Table Ⅰ).

Table Ⅱ. Retention times and separation factors at 170° and 185°, using polar column, of components of *Panax ginseng*

Peak number	Retention time (min) 170°	185°	Separation factor 170°	185°	±	Identified component
XIII	6.2	4.2	1.29	1.27	−	n18:0
XIV	6.8	4.5	1.06	1.09	+	n18:1
XV	8.0	5.4	1.25	1.31	+	n18:2
XVI	8.3	5.4	1.06	1.08	+	?
XVII	10.4	6.3	1.02	1.03	+	n19:2
XVIII	9.9	6.1	1.27	1.22	−	n20:0
XIX	12.4	7.4	1.22	1.21	−	n21:0
XX	15.8	9.0	1.23	1.20	−	n22:0
XXI	19.8	11.3	1.21	1.23	+	?
XXII	23.4	13.3	1.12	1.19	+	n23:1
XXIII	26.6	14.0	1.27	1.25	−	n24:0
XXIV	28.4	15.3	1.08	1.09	+	n24:1

Summary and Conclusion

The free fatty acids were prepared from the ethereal fraction of *Panax ginseng*. The prepared acids were methylated with diazomethane. The methyl esters of saturated and unsaturated fatty acids were separated by the means of mercuric acetate method and column chromatography. The separated methyl esters were gaschromatographed and analyzed. The obtained conclusions were as follows.

1. The root of six-year old Korean *Panax ginseng* contains 0.28% of free fatty acids.

2. It was found that 24 kinds of free fatty acids existed in *Panax ginseng*. Among them, 22 kinds of free fatty acids were identified by the gas chromatogram and the graphical method but the rest, 2 kinds of them were not identified by the only gas chromatographical data. The amount of each free fatty acid which was not identified was predominant andthey were supposed to be unusual free fatty acids which would not commonly exist in nature. These results were shown in Table Ⅲ.

3. Lee and Lee[9] reported that n18:3 existed in *Panax ginseng*. However, in this experiment, n18:3 did not exist in *Panax ginseng*, and instead, peak XVI appeared between n18:2 and n18:3 as shown in Fig. 9.

Acknowledgments

The author (S.H. An) would like to thank Dr. Youn-Sang Cho, Associate Professor of Pharmaceutical Chemistry, College of Pharmacy, Seoul National University, for his advice in carring out this experiment. I am also indebted to Mr. Sang-Sup Jew for his help in performing the gas chromatography. I wish to thank Mr. Jin-Hyup Chung for his assistant in this experiment.

〈Received 4 January 1975〉

Table Ⅲ. Identification of respective methyl esters of free fatty acids and percent composition

Peak number	FFA Identification	Percent composition
Ⅰ	n12:0	2.80
Ⅱ	i13:0	1.64
Ⅲ	n13:0	0.08
Ⅳ	i14:0	1.31
Ⅴ	n14:0	1.97
Ⅵ	i15:0	0.28
Ⅶ	n15:0	0.41
Ⅷ	n15:1	1.95
Ⅸ	n16:0	19.94
Ⅹ	n16:1	1.90
Ⅺ	n16:2	0.81
XII	n17:0	1.09
XIII	n18:0	1.00
XIV	n18:1	0.15
XV	n18:2	3.36
XVI	?	16.25
XVII	n19:2	1.27
XVIII	n20:0	1.58
XIX	n21:0	1.61
XX	n22:0	1.90
XXI	?	20.94
XXII	n23:1	1.00
XXIII	n24:0	0.38
XXIV	n24:1	—

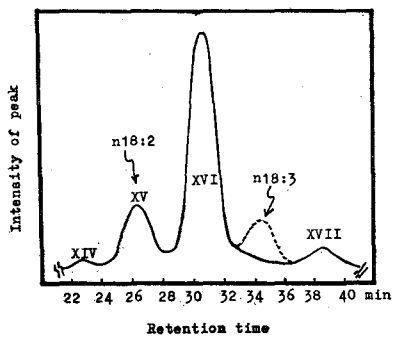


Fig. 9. Gas chromatogram of methyl esters of unsaturated free fatty acids derived from *Panax ginseng* mixed with linolenic acid methyl ester (n18:3) as standard at 130°.

References

1) Takagi, K.: *Inter, Ginseng Symp, Seoul,* (1974).
2) Han, B.H., Han, Y.N. and Woo, L.K.: *Korean J. Pharmacog.* **3**, 151 (1972).
3) Han, B.H. and Woo L.K.: *J. Phar. Soc. Korea,* **16**, 129 (1972).
4) Oura, H. and Nakashima, S.: *Chem. Pharm. Bull.* **20**, 980 (1972).
5) Oura, H. *et al.*: *Chem.* and *Pharm. Bull.* **20**, 219 (1972).
6) Hiai, S. *et al.*: *Chem.* and *Pharm. Bull.* **21**, 2705 (1973).
7) Oura, H. and Hiai, S.: *Inter. Ginseng Symp. Seoul,* (1974).
8) Komatsu, M. and Tomimori, T.: *Shoyakugaku Zasshi,* **20**, 21(1966).
9) Lee, C.Y. and Lee, T.Y.: *Symp. Phytochem.,* 171 (1961).
10) Choi, T.K. and Hong, S.A.: *Korean J. Pharmacol.* **4**, 17(1968).
11) Peifer, J.J. *et al.*: *J. Nutrition,* 88, 351 (1966).
12) Rand, P.G. and Quackenush, F.W.: *J. Nutrition,* **87**, 489 (1965).
13) Bieberdorf, F.A. and Wilson, J.D.: *J. Clinical Investigation,* **44**, 834(1965).
14) Crevar, G.E. *et al.*: *J. Pharm. Sci.* **61**, 1336 (1972).
15) Aburand, S. *et al.*: *Yakugaku Zasshi,* **92**, 1298 (1972).
16) Sakurai, Y. and Iwaguchi, T.,: *Chem. Pharm. Bull.* **15**, 771(1967).
17) Miwa, T.K. *et al.*: *Anal. Chem.*, **32**, 1739 (1960).
18) Bottino, N.R.: *J. Lipid Res.* **12**, 24 (1971).
19) Yasue, M. *et al.*: *Yakugaku Zasshi* **90**, 341(1970).
20) Funasaka, W. and Ikekawa, M.: *Modern gaschromatography.* **2**, 647 (1970).
21) Landowne, R.A. and Lipsky, S.R.: *Biochim. Biophys. Acta* **47**, 589(1961).
22) Funasaka, W. and Ikekawa, N.: *Modern gaschromatography,* **1**, 311 (1970).
23) Ruseva-Atanasona, N. and Janak, J.: *J. Chromatog.* **21**, 207(1966).