Arch. Pharm. Res. 4(1), 25~32(1981)

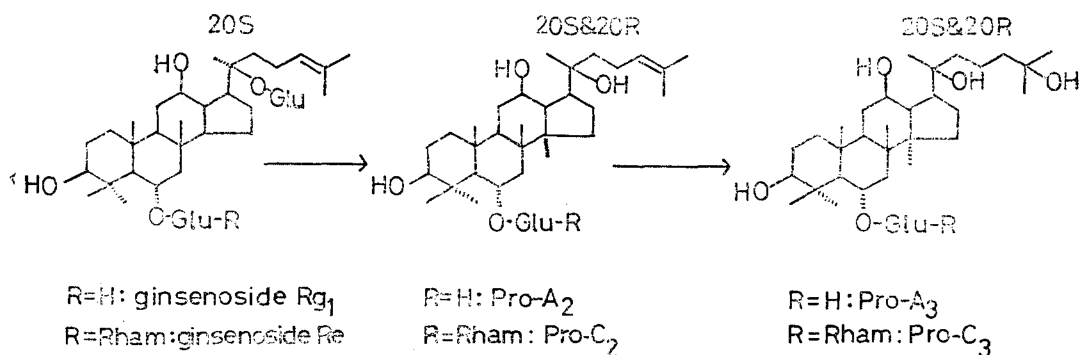
The Transformation of Ginsenosides by Acid Catalysis in Gastric pH

Byung Hoon Han, Myung Hwan Park, *Yong Nam Han, Lin Keun Woo
**Ushio Sankawa, Shoji Yahara and ***Osamu Tanaka

Nautral Products Research Institute, Seoul National University, Seoul 110, Korea, Department of Chemistry, College ot Natural Science, Kangweon National University, Chuncheon 200, Korea, **The Faculty of Pharmacy, The University of Tokyo, Japan and ***Institute of Pharmaceutical Sciences, School of Medicine, Hiroshima University, Hiroshima, Japan*

(Received 3 April 1981)

Abstract□ 1. The ginsenosides of Korean ginseng decomposed profoundly to produce artifact products of prosapogenin A_1, A_2 and A_3 from ginsenoside Rg_1, prosapogenin C_1, C_2 and C_3 from ginsenoside Re, and prosapogenin E_1, E_2 and E_3 from ginsenoside Rb_1 by the acid treatment under physiological condition such as 37°C incubation in 0.1 N HCl. 2. The chemical structures of the artifact substances were determined by the analysis of CMR and mass spectra of TMS-derivatives as following;



Keywords□Ginsenosides, Panax gineseng, ^{13}C-NMR spectroscopy, prosapogenins acid-catalyzed transformation

The ginsenosides of Korean ginseng have been shown to elicit a wide variety of the physiological responces, such as the stimulation on protein synthesis[1-3], the growth of nerve cells[4], the production of red blood cells[1-3,5], and the activities of anti-stress[6], anti-fatigue[7], anti-inflammation[6,8] and central nervous stimulation or depression[9-11], etc.

These activities were mostly observed by the experiments in which ginsenosides were directly applied to the response systems, or in which ginsenosides were parenterally administered to animals. These experimental designs seem to obviate a due consideration on the possible chemical modification of ginsenosides by the interaction with gastric acid in the gastro-intestinal tract.

Present paper deals with the estimation

on what extent of acid catalyzed modification of ginsenosides may take place at the physiological condition of gastric pH, and also with the structure elucidation of the transformed products of ginsenosides.

EXPERIMENTAL

All melting points were taken on a heat block apparatus and given uncorrected values. CMR data were obtained in pyridine-d_5 or DMSO-d_6 solution using TMS as internal standard on JEOL-PET-100 NMR spectrometer (25.15 MHz) and recorded by ppm. unit.

Mass spectra were taken at 75eV on JEOL 01-SG-2 spectrometer as the TMS-derivatives. Densitogram data were obtained by Dual Wave Length TLC-Scanner.

Purification of Ginsenoside Rg_1, Re and Rb_1

Ginsenoside Rg_1, Re and Rb_1 were isolated from Korean ginseng by the combined methods of solvent fractionation and silica gel column chromatography as followings. All chromatographic effluents were monitored by TLC using the authentic ginsenoside standards as references. The butanol soluble fraction of ginseng methanol extracts was chromatographed over silica gel by using the solvent of chloroform/methanol/water (75 : 25 : 2.5). Ginsenoside Rg_1 was first eluted and then ginsenoside Re as a single spot. Ginsenoside Rg_1 was isolated as deca-acetate (mp 250°C) from methanol after acetylation by usual way, and then it was deacetylated by ordinary saponification. Ginsenoside Re was purified by repeated crystallization from water (mp 190–2°C). Ginsenoside Rb_1 was isolated by silica gel column chromatography using the solvent of chloroform/methanol/water (70 : 30 : 4) as eluent. The isolated ginsenosided Rb_1 was crystallized from water (mp 205–9°C).

Time course acid treatment on ginsenosides at gastric pH

Ginsenosides Rg_1, Re and Rb_1 were dissolved in water and then mixed with N-HCl to make the final concentration of 0.5–2.5 mg ginsenosides/ml and 0.1N HCl, respectively. The resulting solutions were incubated at 37°C for the definite time length given in Table I. An aliquot of the incubation mixtures was neutralized by adding 5% Na_2CO_3 solution, and then chromatographed over a silica gel plate by using the solvent of chlorofolm/methanol/water (75: 25: 2.5). The plate was sprayed with d-H_2SO_4 solution and heated in 105°C oven to maximum color development. The color densitogram data were obtained by Shimadzu dual wave length TLC-scanner(Emax 522nm, reference 730nm).

Isolation of Acid-catalized Decomposition Products(prosapogenins)

Ginsenosides Rg_1 (0.9 g) and Re (3 g) were dissolved in water, respectively, and added with 1N-HCl to make the final concentration of 2.5 mg ginsenosides/ml and 0.1N-HCl. The reaction mixtures were incubated at 37°C for 2 hours and then neutralized by the addition of 10% Na_2CO_3 solution. The reaction products were extracted with butanol and concentrated to dryness. The residues were chromatographed

over silica gel column using the eluent of chlorofolm/methanol/water(75: 25: 2.5), and prosapogenins were obtained in a chromatographically pure state by ordinary treatment.

RESULTS AND DISCUSSION

Time Course Rate of Degradation of Ginsenosides.

Ginsenoside Rg_1, Re and Rb_1 were chosen as the representative components of ginsenosides present in Korean ginseng and treated with 0.1N HCL at 37°C for various time intervals. On this treatment, every ginsenosides produced three additional TLC-spots as shown in the thin layer chromatograms of Fig. 1.

They were tentatively designated as prosapogenin A_1, A_2, and A_3 (from Rg_1), C_1, C_2, and C_3 (from Re), E_1, E_2, and E_3 (from Rb_1) in the order of increasing polarity. The time course rates of the prosapogenin production were monitored by TLC-densitometry using a dual wave length TLC-scanner and the results were illustrated in Table I.

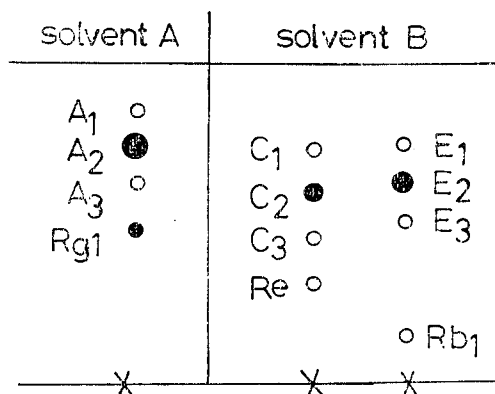


Fig. 1: Thin layer chromatograms of the acid treatment products of dammarene glycosides; ginsenoside Rg_1, Re and Rb_1.
TLC solvent A: $CHCl_3/MeOH/H_2O=70:20:2$
B: $CHCl_3/MeOH/H_2O=70:25:2.5$

Table I: Densitogram of time course acid-hydrolysates of ginsenoside Rg1, Re and Rb1.

spots \ Incub. Time (Min)	0	15	30	45	60	90	120
Rg1	100%	65.5	37.9	28.5	18.7	14.6	10.6
Pro-A_3	0	0.7	1.51	3.57	9.3	15.8	28.1
Pro-A_2	O	24.6	45.4	50.0	53.1	48.8	51.3
Pro-A_1	0	9.9	15.1	17.9	18.7	20.7	10.0
Re	100%	85.9	75.8	65.6	47.2	38.5	44.5
Pro-C_3	0	0	1.7	4.5	7.5	12.4	14.8
Pro-C_2	o	12.5	19.0	23.9	36.7	40.0	33.6
Pro-C_1	0	1.6	3.5	6.0	8.6	9.1	7.1
Rb1	100%	65.0	48.3	36.4	23.5	17.1	13.1
Pro-E_3	0	0	3.5	7.2	7.8	11.4	19.0
Pro-E_2	0	24.1	35.1	40.0	51.0	54.4	52.4
Pro-E_1	0	10.9	13.1	16.4	17.9	17.1	15.5

As shown in Table I, the decomposition of ginsenoside Rg_1 took place more than 34% at 15min., 62% at 30 min, and 89.4% at 120 min. Similar tendencies were observed in the data of ginsenoside Re and Rb_1. These results suggest that a significant decomposition of ginsenosides should take place in the gastro-intestinal tract before any appreciable absorption of genuine ginsenosides proceeds.

Isolation of Prosapogenins

We attempted to isolate the prosapogenins produced by the acid catalysis under physiological condition and to determine their chemical structures.

Ginsenoside Rg_1 (0.9g) and Re (3g) were treated with 0.1N HCl at 37°C for 120 min. and applied to silica gel column to obtain prosapogenin A_1 (Pro–A_1) 100mg, Pro–A_2, 300mg, and Pro–A_3 190mg (from Rg_1) and Pro–C_1 455mg, Pro–C_2 851mg and Pro–C_3 540mg (from Re) in a chromatographically pure state. Upon concentration of the eluate, Pro–A_1 and Pro–C_1 were further decomposed to yield very complicated mixture of spots on TLC, therefore they could not be subjected to the structure determination.

The Structure of Prosapogenin A_2

The CMR spectra of Pro–A_2 were assigned by referring to the already assigned CMR spectra of ginsenoside Rh_1[2] and tabulated in Table II. As shown in Table II, all the CMR peaks of Pro–A_2 are superimposable with those of ginsenoside Rh_1, except that the CMR spectra of Pro–A_2 show additional peaks in the neighbour of the peaks of C_{13-23}. S. Yahara *et. al*[12] isolated ginsenoside Rh_1 from the root of Panax ginseng, fully assigned the peaks in CMR spectra of the substance, and demonstrated its chemical structure as 20S–protopanaxatriol–6–O–glucoside. On the other hand, J. Asakawa et al[13] studied C_{20} *chirality* inducd chemical shift changes of ginseng sapogenins. Referring to these data, Pro–A_2 was shown to be the mixture of ginsenoside Rh_1 and its C_{20}–R–epimer.

The Structure of Prosapogenin A_3

The structure of Pro-A_3 could be envisaged by the comparison of CMR spectra of Pro-A_3 with those of Pro–A_2. Every peaks in the spectra of Pro-A_3 is superimposable with those of Pro–A_2 except the peaks of C_{24-27}. The olefinic carbon peaks at 126.2 ppm and 130.6 ppm observed in the CMR spectra of Pro-A_2 were replaced in the spectra of Pro-A_3 by the tertiary alcoholic carbon peak at 69.8 ppm. This suggests the presence of additional tertiary alcohol group in carbon-25 of Pro-A_3 produced by the hydration at the olefinic bond of Pro-A_2. Identification of the C-25 hydroxyl group in Pro-A_3 is also possible by comparison of

Table II: ^{13}C–NMR Chemical shifts.

Comp. / Carbon No.	Pro–A2 (DMSO–d_6)	Pro–A2 (C_5D_5N)	Pro–A3 (C_5D_5N)	Pro–A3 (DMSO–d_6)	Pro–C2 (DMSO–d_6)	Pro–C3 (DMSO–d_6)	Ginsenoside Rh1 (C_5D_5N)	Ginsenoside Re (DMSO–d_6)
1	38.9	39.5	39.5	38.8	38.8	38.8	39.4	38.5
2	26.9[a)]	27.6	27.7	26.8[a)]	28.8	26.6[a)]	27.9	26.7[a)]
3	76.6[d)]	79.9[c)]	79.9[d)]	78.5[d)]	77.7p)	77.6[d)]	78.6	77.7[c)]
4	39.1	40.1	40.2	39.0	38.8[b)]	38.8	40.3	38.5
5	60.4	61.3	61.3	61.3	60.3	60.0	61.4	59.7
6	77.5[d)]	78.5[c)]	78.5[d)]	77.3[d)]	73.2	73.2	78.0	73.2
7	44.2	45.0	45.5	44.3	44.7	44.8	45.2	44.5
8	40.3	41.0	41.0	40.2	40.3	40.3	41.1	40.2
9	49.3	50.1	50.1	49.2	48.9	48.9	50.2	48.5
10	38.9	39.5	39.5	38.8	38.6[b)]	38.3	39.6	38.5
11	31.2[c)]	31.5[a)]	31.8[c)]	31.1[b)]	31.1	31.1	3.20	30.1[b)]

Aglycone Sugar

12	69.9	70.9	70.9	69.8	69.8	69.8	71.0	69.0
12	47.4	48.0	48.1	47.3	47.3	47.2		
	(48.0)	(48.6)	(48.7)	(47.9)	(47.9)	(47.8)	48.2	48.1
14	51.5	51.5	51.5	58.9	51.0	51.0	51.6	58.7
15	30.9[c)]	31.2[a)]	31.2[c)]	30.7[b)]	31.1	31.1	31.1	31.0[b)]
16	25.9[a)]	26.8	27.0	26.7[a)]	26.0	26.0[a)]	27.2	25.7[a)]
17	53.6	54.5	54.5	53.5	53.5	53.5	54.7	50.7
	(49.7)	(50.4)	(50.5	(40.6)	(49.5)	(49.5)		
18	17.6[b)]	17.6[b)]	17.6[a)]	17.0[c)]	17.4[a)]	16.9[b)]	17.6[a)]	16.7
19	17.0[b)]	17.3[b)]	17.3[a)]	17.0[c)]	16.9[a)]	16.5[b)]	17.6[a)]	16.7
20	72.1	73.0	73.3	72.3	72.0	72.3	73.0	82.1
21	26.6	26.8	26.8	26.0	26.4	26.6	26.8	23.6
	(21.6)	(22.5)	(22.5)	(22.1)	(21.6)	(22.1)		
22	35.0	35.6	36.3	35.6	34.9	35.6	35.8	35.2
	(42.2)	(42.2)	(42.9)	(43.5)	(43.0)	(42.2)	(42.9)	
23	22.0	22.5	19.0	18.1	21.9	18.1	23.0	22.1
	(21.6)	( )	(18.6)	(17.6)	( )	(17.3)		
24	125.6	126.2.	45.3	44.1	125.5	44.3	126.3	125.2
	(125.4)	(125.9)	(45.5)	(44'6)	129.9	63.9	130.6	130.0
	(125.4)	(125.9)	(45.5'	(44.6)	(125.2)	( )		
25	130.0	130.6	69.8	68.9	129.9	68.9	130.6	130.0
26	25.5	17.0[b)]	29.7[b)]	29.3	25.4	29.3	25.3	25.3
27	17.0[b)]	25.8	30.0[b)]	29.3	16.5[a)]	29.3	17.4[a)]	16.7
28	30.9	31.5	31.5	30.7	31.1	31.1	31.7	30.1
29	16.7[b)]	16.7[b)]	16.7[a)]	16.7[c)]	16.5[a)]	16.5[b)]	16.4[a)]	16.7
30	15.5[b)]	16.2[b)]	10.2[a)]	15.4[c)]	16.5[a)]	16.5[b)]	16.8[a)]	16.7
Glu-1	104.4	105.7	105.7	104.3	100.0[c)]	99.9	105.9	99.8
2	74.3	75.1	75.1	74.1	77.0[d)]	77.0[d)]	75.4	77.7[c)]
3	76.6[d)]	77.8[c)]	77.8[d)]	76.5[d)]	77.3[d)]	77.4[d)]	79.5[b)]	77.7[c)]
4	70.6	71.6	71.6	70.5	71.0	71.1	71.3	70.9
	77.9[d)]	79.2[c)]	79.3[d)]	77.8[d)]	77.5[d)]	77.6[d)]	80.0[b)]	77.2[c)]
6	61.6	62.8	62.9	61.4	61.7	61.7	63.1	61.6
Rhom-1					99.8[c)]	99.9		99.8
2					70.5	70.6		70.3
3					70.5	70.6		70.3
4					72.2	72.3		72.1
5					86.0	78.0		67.9
6					17.7	17.7		17.7
C-20-qlucose								
C-1								96.7
2								73.2
3								76.9[c)]
4								70.3
5								76.4[c)]
6								61.3

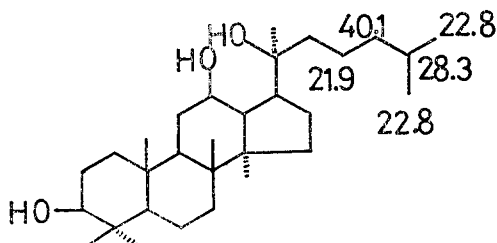


Fig. 2 Chemical shifts of C_{23}-C_{27} of betulafoliane-triol.[13)]

the chemical shifts of the neighbouring carbons in Pro-A_3 with thoe of betulafoliane-triol, whose CMR spectra were fully assigned by J. Asagawa *et al.*[13)]

All CMR assignments of Pro-A_3 are also supported by the mass spectra of the TMS-derivative of the compound. As shown in Fig. 3, mass spectra of TMS-Pro-A_3 give

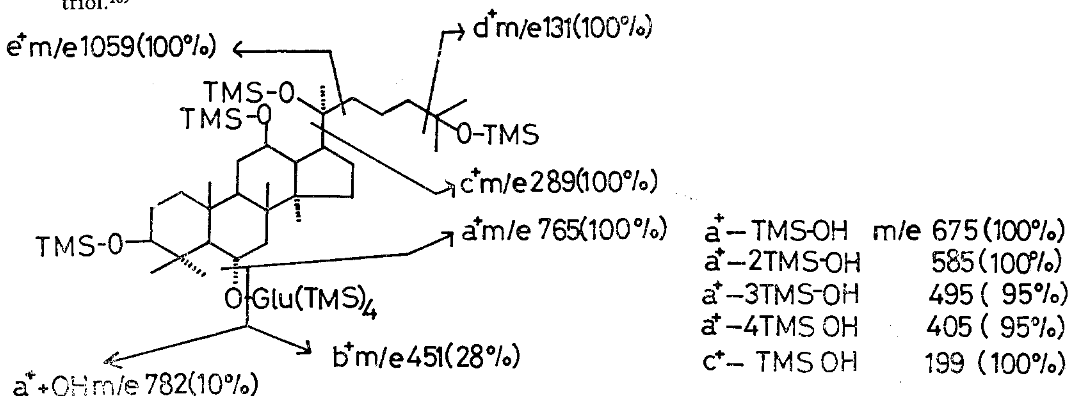


Fig. 3 Mass spectrum of TMS-Pro-A_3.

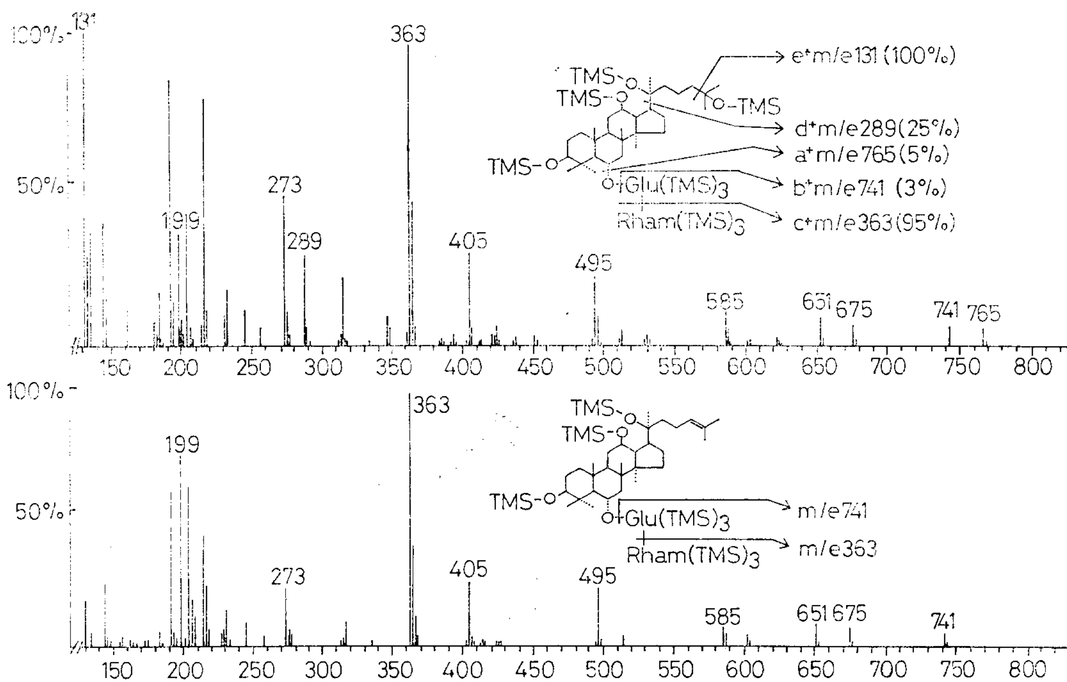


Fig. 4: Mass Spectra of TMS-Pro-C_2 and TMS-Pro-C_3.

the fragment ions of d^+ m/e 131 (TMS-isopropanol) and C^+ m/e 289 which suggest two TMS-groups in the side chain of TMS-Pro-A_3. Considering also the additional CMR peaks in the neighbour of C_{13-23}, Pro-A_3 was identified as the mixture of C-25-hydroxy derivative and its C-20R epimer produced by the hydration of Pro-A_2 on C_{24-25} olefinic bond.

The Structure of Prosapogenin C_2 and C_3

The structure of Pro-C_2 was determined by comparison of CMR specta of Pro-A_2 and Pro-C_2. The chemical shifts of side chain carbons are same in both Pro-A_2 and Pro-C_2. However, those of sugar carbon peaks of Pro-C_2 are almost same with those of ginsenoside Re. The mass spectra of TMS-Pro-C_2 give the fragment ions of TMS-glucosyl rhamnose b^+ m/e 741 and TMS-rhamnose C^+ m/e 363 (Fig. 4)

Considering the above results, the structure of Pro-C_2 corresponds to ginsenoside Rg_2 and its C-20 R epimer which were produced by the decomposition of C–20 glycoside bond of ginsenoside Re.

The structure of Pro-C_3 was formulated as the C-25-hydroxy derivative of Pro-C_2 by the comparison of the CMR spectra and the mass spectra of Pro-C_3 with those of Pro-C_3. Similar discussions are possible in case of the relation between Pro-A_2 and Pro-A_3. The mass spectra of TMS-Pro-C_3 give fragment ions e^+ m/e 131 and d^+ m/e 289 as base peak, suggesting the presence of two TMS-groups in the side chain of Pro-C_3, and fragment ions b^+ m/e 741 and C^+ m/e 363 as prominent peaks, suggesting also the presence of the TMS-rhamno-glucosyl group in Pro-C_3. The fragement ions of e^+ and d^+ seem to have a diagnostic value for the structure identification of Pro-C_3 and Pro-A_3 since Pro-C_2 doesn't give those paeaks.

LITERATURE CITED

1) Yamamoto, M. *Metabolism and Disease* (Japan), **10,** 119(1973).
2) Han, B. H., Kim, C. H and Han, Y. N. *Korean Biochem. J.*, **6,** 63(1973)
3) Oura, H. and Hiai, S. *Metabolism and Disease* (Japan), **10** 102(1973).
4) Saito, H. and Lee, Y. M. *Proc. 2nd Internl. Ginseng Symp.*, Korea Ginseng Res. Inst., Seoul, Korea P–109(1978).
5) Yamamoto, M., Kumagi, A. and Yamamura, Y. *Proc. Internl. Ginseng Symp.,* The Central Res. Inst., Office of Monopoly, R. O. K. P–129(1974).
6) Petkov, W. and Staneva, K. *Proc. 2nd Internl. Pharmacol. Meeting.* 39–45, Prague, 20–23th August, 1963.
7) Bykov, V. T. and Naidenova, I. N. *Chung Yao Tong Pao,* **3,** 101(1957).
8) Han, B. H., Han, Y. N. and Woo, L. K. *J. Pharm. Soc. Korea,* **16,** 129 (1972).
9) Takagi, K. *Proc. Internl. Ginseng Symp.,* The Central Res. Inst., Office of monopoly, R. O. K. P–119(1974).
10) Hong, S. A. and Cho, H. Y. *Korean Ginseng Science Symposium;* Korea Soc. Pharmacog., Seoul, Korea, 113(1974).
11) Brekhman, I. I., Dardymov I. I. *Ann. Rev. Pharmacol.,* **9,** 419(1969).
12) Yahara, S., Kaji, R. and Tanaka, O. *Chem. Pharm. Bull.,* **27,** 88(1979).
13) Asakawa, J., Kasai, R., Yamasaki, K. and Tanaka O. *Tetrahedron,* **33,** 1935(1977).