人蔘사포닌의 毛髮에 미치는 效果에 關한 研究

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SYNOPSIS

Tensile strength and elongation of the ginseng saponin-treated hair were compared with those of collagen hydrolysate-treated hair. The absorbed of ginseng saponin into hair was measured by high speed liquid chromatography and identified by using the liquid scintillation counter. Sorption of collagen hydrolysate to hair was rapid in the first 30 min, but the rate nearly remained constant after one hour. On the contrary, the sorption of ginseng was increased with increased immersion time. The results indicate that saponin has better effect on hair than collagen hydrolysate. Also, the mechanism of absorption and increase of the tensile strength were suggested from the analysis of the strain-stress curve and surface activities.

I. INTRODUCTION

Ginseng has been used since ancient times in orient as a miraculous panacea (1) for many kinds of illness and disease and also accepted as one of the most valuable cosmetic ingredients in Asia.

The effect of ginseng extract for skin care was reported by many authors (2,5). For hair care, ginseng not only prevents or retards loss of hair (6) but prevents hair from turning to gray (7). It has been reported that ginseng has the effects of protective action on damaged hair, better manageability, combability and strength(6). Lee (8) studied the effectiveness of ginseng for the hair growth.

In the light of a number of recent papers concerning the pharmacology of ginseng, however, the dammarane triterpene glycoside (ginseng saponin) has been considered as the most important component of the biologically active substance in ginseng (9,10). Shibata and his coworkers (11) designated saponin (13 glycoside spots) as ginsenoside RX (X, o, a, b_1, b_2, c, d, f, g_1, g_2, g_3, h_1, h_2) in order of increasing Rf values, elucidated their full structure, and found that their genuine aglycone was protopanaxadiol and protopanaxatriol (Fig. 1).

Kim (12) has studied toxicity of ginseng saponin and found no adverse skin reaction for guinea pigs and rabbits.

A considerable amount of work has been carried out on the pharmacological effectiveness of ginseng saponin and its mechanism, but no systematic research on ginseng saponin for cosmetics has made. This paper reports the results of a series of experiments where the effect of ginseng saponin on hair was compared with that of collagen hydrolysate, which was known as the most substantive on hair. In the present work, the tensile strength and elongation of the saponin treated hair was measured as functions of concentration, immersion time, and bleaching degree. The amount of ginseng saponin absorbed into hair was also measured by using high-speed liquid chromatography under the same condition mentioned above and identified by ^{14}C-labelled saponin analysis. The sorbed collagen hydrolysate was analyzed by means of hydroxyproline.

II. MATERIALS AND METHODS

Human hair: Korean black virgin hair with diameter from about 80 to 90 um was previously shampooed with 1% aq. solution of Triton X 100 (Rohm & Haas), rinsed with distilled water and air-dried over night.

Hydrogen peroxide solution for bleach: 34.01% H_2O_2 solution with distilled water to 6.0% NH_4OH and stabilized with 0.1% of dry salt of tetrasodium ethylenediamine tetra-acetate dihydrate.

Ginseng saponin: Crude ginseng saponin was partitioned in a BuOH-H_2O(1 : 1) mixture as usual and n-BuOH layer was evaporated in vacuum to give residue, which was dissolved as usual and MeOH-H_2O solution. Methanol soluble portion was then treated with a large amount of ether to produce precipitate, which was collected, and dissolved again in BuOH-H_2O mixture and butanol layer is dried in vacuum.

^{14}C-labelled ginseng saponin: ^{14}C-labelled ginsenoside Rg_1 (13) was procured from Natural Product Research Inst. of Seoul National University and was turned out to be pure by thin layer chromatography and isotope dilution analysis (Fig. 2). Its specific activity was 0.1uCi/mg.

Collagen hydrolysate: Crotein A (Croda, M.W. : 2000-3000) was used.

Hyamine hydroxide solution for hair solubilizer: Hyamine 1632 (Rohm & Haas) was reacted with methanolic KOH solution, filtered and adjusted to 1 M hyamine hydroxide solution in toluene.

Bleach of hair with hydrogen peroxide solution: The selected hair was immersed in hydrogen peroxide solution at 37°C for a given time. The ratio of weight of hair (g) to volume of bleach solution (ml) was 1 : 25. Bleached hair was rinsed with distilled water and dried over-night at 60% RH.

Measurements of tensile strength and elongation: RHEO METER (Yokogawa Electric Works, Ltd.) was used to measure tensile strength and elongation at 22±2°C, 60% RH. Under the condition of initial length of hair 50mm, the rate of elongation was 1mm/sec, and full scale 200g. (Fig. 3 & 4). The tensile strength was determined in grams when hairs were broken and elongation was calculated using the following equation.

$$E=\frac{t}{50}\times 100$$

where E=elongation%

t =time in sec.

Hair fibers were secured firmly with a rubber faced screw clamp to protected hair damage at the jaw face.

Measurements of the absorbed ginseng saponin using high speed liquid chromatography: High speed liquid chromatography (Hitach Model 635) was used to determine the absorption amount of ginseng saponin. 500mg. of the bleached hair (about 2 cm long) was immersed in 50ml. of ginseng saponin solution of the given concentrations at 22±2°C for a given time. The tress was rinsed 5 times; every time the hair was rinsed with 50ml. of distilled water for 2 min. and air-dried. Ginseng saponin absorbed was extracted from the saponin-treated hair with 30ml. of 80% methanol solution 3 times, and evaporated in vacuum. Residue dissolved into 2ml. of 80% MeOH solution is eluted with $CH_3CN:H_2O$ (60 : 40) solvent using Lichrosorb RP 18 column (2.6×25cm) and measured at 254nm (flow rate : 1.0ml/min)

Measurements of the absorbed ginseng saponin by ^{14}C analysis: 50mg. of the bleached hair (about 1cm long) was immersed in 2ml. of ^{14}C-labelled saponin solution of different concenrations at 37°C for a given time. The tress was rinsed 5 times; every time the hair was rinsed with 5ml. of distilled water for 2 min. and air-dried over-night. After the sample was digested in 1ml. of hyamine hydroxide solution at 60°C for 4-5 days, cooled to room tempterature and then 8ml. of scintillation cocktail (p.p.o. and p.o.p.o.p. in toluene) was added to it. The radioactivity was determined using liquid scintillation counter (Model 6860 Mark 1, Nuclear Chicago Corp.) and correction was made for quenching by an external standard source. The amount of ^{14}C-saponin absorbed into hair was determined from calibration curve.

Determination of collagen hydrolysate absorption: 50mg. of the bleached hair was treated in same way as in ^{14}C analysis except it was immersed in 2ml. of collagen hydrolysate solution. The amount of sorbed collagen hydrolysate was measured by means of hydroxyprolin analysis (14).

Determination of C.M.C. of ginseng saponin: The C.M.C. of ginseng saponin was determined by surface tension method (Du Nouy Tensiometer) and dye adsorption method using 0.005w/v aq. solution of benzophenone 4B at 540nm.

III. RESULTS

The tensile strength and elongation

There was a substantial variation in diameter of hairs even if extracted from the same person at the same area. Therefore, the hairs with diameter between 80 to 90 um were selected microscopically, as far as possible, to eliminate errors arising from variation of diameter, and each experimental datum in the figures of one hundred determinations.

1) Effect of concentration

Figs. 5 and 6 show the effect of concentration of ginseng saponin and collagen hydrolysate on the tensile strength and elongation. There is no significant variation in tensile strength up to 1% solution, but a little increase above 2% for ginseng saponin, The similar results was obtained in the elongation. In the case of collagen hydrolysate, tensile strength was increased in the elongation increased up to 3%, but there was no significant difference above the point.

2) Effect of immersion time

Figs. 7 and 8 show the effect of immersion time. Tensile strength of hair treated with ginseng saponin was increased by negligible amount up to 30 min, but increased remarkably after 30 min. However, elongation was measured sharply up to 60 min. In case of collagen hydrolysate, tensile strength was measured sharply in great amount up to 30 min., but it remains constant after 30 min. For the control hair tensile strength at 30 min. showed the minimum value, but it was regarded as experimental error.

3) Effect of bleaching time

Figs. 9 and 10 show the effect of bleaching time on the mechanical properties of hair. Tensile strength and elongation were decreased remarkably with increased bleaching time.

Absorption of ginseng saponin into hair

Two methods were used to determine the amount of ginseng saponin absorption into hair, i.e. liquid chromatography and ^{14}C analysis. Several peaks appeared in the chromatograms by eluting the standard ginseng saponin solution (0.05%～1%). Calibration curve of ginseng saponin was obtained from the initial peak in good result which was almost proportional to the concentration of standard ginseng saponin solution. ^{14}C-labelled ginseng saponin which was obtained from Natural Product Research Inst. was 1% solution. Therefore, the absorption data by ^{14}C analysis was obtained only for the concentration, 0.01%-1.0%.

1) Effect of concentration

In Fig. 11, the amount of ginseng saponin, collagen hydrolysate absorbed into hair against the concentration of them has been plotted. When the absorbed ginseng saponin was analysed by liquid chromatography, data was obtained in good result. But, when it was measured by liquid scintillation counter for the reconfirmation, the results was a little lower than by L.C. and also flexible in some degree. The difference was due to the experimental and instrumental errors. However, the absorbed ginseng saponin (5.5mg/g.) was increased remarkably with increased concentration by 3 times more than collagen hydrolysate (1.8mg/g.)

2) Effect of immersion time

In Fig. 12, the amount of ginseng saponin absorbed into bleached hair at 5% solution was determined by liquid chromatography. For collagen hydrolysate, sorption was rapid in the first 30 min, and the rate decreased between 30 min. and one hour, but it nearly remained constant after one hour. On the contrary, the sorption of ginseng saponin to hair was increased with increased immersion time and saturation could no be found with in the time examined. The absorbed amount (7.6mg/g) was 3.5 time more than collagen hydrolysate (2.2mg/g.)

3) Effect of bleaching time

The BET area of bleached hair is increased about 2.5 times more than that of normal hair (18), which renders sorption of ginseng saponin or peptides increased markedly. In Fig. 13, the sorption of bleached hair was rapidly increased in the first 1 hour bleach. It was also increased 3 hour bleach even if the rate of increase was very slow, which indicated that level of maximm damage had been reached.

C.M.C. determination of ginseng saponin

Ginseng saponin examined in this paper was composed of 13 (different) kinds of ginseng saponin, indicating that there were some interference for the measuring C.M.C. value determined by the surface tension method in 1.0w/w%, and is consonant with the value obtained from dye adsorption method (Fig. 14). Surface tension of ginseng saponin at 1.0w/w% was 38.1 dyne/cm.

IV. DISCUSSION

The substantivity to hair may be defined by the amount of a substance to be sorbed on hair and its effectiveness on hair fiber. Many published data suggested that collagen-derived polypeptide was sorbed on hair to increase tensile strength and the healing effects. In this study, we confirmed that ginseng saponin was absorbed into hair 3 times more than collagen hydrolysate, effecting an increase in strength and elongation at 60% RH.

In Fig. 12, collagen-derived polypeptide was sorbed on hair rapidly in the first 30min. and the absorption rate decreased and then remained constant. The results of these investigations are in good agreement with the theory that the adsorption of protein hydrolysate is controlled by ionic equilibirum phenomena (19). That means collagen hydrolysate was mainly sorbed on hair by adsorption mechanism, not by absorption. On the contrary, it was suggested that ginseng saponin was absorbed into hair by the following reason.

Firstly, Fig. 12 shows that the absorbed amount was increased with increased immersion time and saturation point was not found up to 120 min. This property is the characteristics of absorption.

Secondly, it is possible for hydroxyl groups of ginseng saponin to form hydrogen bond with the hydrophilic groups in keratin, viz, -COOH, $-NH_2$, $-CONH_2$, -OH, -COHN, -NHC(NH)NH_2, -NH. Hydrogen bond between two other molecules adds a powerful force favoring sorption.

Thirdly, with nonionic characteristics, ginseng saponin has surface-active properties which were confirmed by mensuring the surface tension (39.1 dyne/cm) and C.M.C. (1w/w%) (Fig. 14). This surface activies allow ginseng saponin to be diffused between the keratin molecules.

Three points above support that ginseng saponin is absorbed into hair to form hydrogen bonds, but no microautoradiographic studies on this were implemented successfully because of diluted sample. Speaking of augmenting tensile strength and elongation, ginseng saponin was more effective than collagen hydrolysate and control on hair in all cases. To establish the mechanism, we studied the strain-stress curves of hair at 60% RH and investigated the water-absorption regain isotherms of hair, ginseng saponin, and collagen hydrolysate individually.

Fig. 15 shows the moisture-absorption isotherms at relative humidities up to 99% at 22° ± 2°C. In saturated water vapor, the regains of hair, collagen hydrolysate, and ginseng saponin were 32%, about 35%, and about 20% respectively, while, at 60% RH, the regains of hair and ginseng saponin were about 14% and 8.5%. Here, it was known that ginseng saponin is less hygroscopic than hair while collagen hydrolysate is more hygroscopic than hair. We, therefore, can predict that the hair treated by ginseng saponin, compared to the control hair, doesn't make any difference in water content which exerts substantial influence on the machanical properties of hair. It is demonstrated that microfibrils are fully composed of crystalline and helices; their structure is stabilized by a variety of intramolecular and intermolecular forces, including hydrogen bonds, dipole interactions, ionic bonds, and hydrophobic bonds. These microfibrils are embedded in an amorphous matrix and, presumedly, composed of randomly coiled polypeptide, chains highly crosslinked by S-S bonds(20).

In the Hooken region, the stress is related to the fiber strain almost linearly, reflecting deformations of the bond angles in the microfibrils, and the α-helices in the microfibrils begin to unfold into β-structure in the yield region(21). It is generally agreed that the stress in the post-yield region is mainly attributed to the existence of cleavage of disulfide crosslinks(22).

From the hair fiber strain-stress curve (refer to Fig. 10) based on the same condition as the case of measuring tensile strength and elongation of this experiments, it was resulted that ginseng saponin-treated hair has higher value of yield point, tensile strength, and elongation than control. This results shall be illustrated from the mechanical properties of hair affected by absorbing ginseng saponin.

A molecule of ginseng saponin is composed of hydrophobic group and about 10 hydroxyl groups which are prone to forming hydrogen bonds with polypeptide. The moisture, absorbed and penetrated in between of polypeptide molecules, eliminates some of the intramolecular and intermolecular bonds, i.e. hydrogen bond, between peptide moleclues, consequently reducing the tensile strength (23) and permitting peptide molecules to slip more easily past each other.

Ginseng saponin, however, when penetrated in polypeptide molecules, forms hydrogen bonds with polypeptide which was in hydrogen-bonded with water. At this juncture, as hydroxyl groups of ginseng saponin from hydrogen bond with water, the adjacent polypeptide molecules are connected each other by hydrogen bond with a molecule of ginseng saponin in between. Because of many hydroxyl groups in molecule of ginseng saponin, these hydrogen bonds are destroyed in accordance with the strain of hair and reform when hydroxyl groups of ginseng saponin approach to the polar groups of polypeptides, causing interactions between hydrophobic groups of polypeptide and of ginseng saponin. That is, both formation and destruction of hydrogen bond are considered to occur simultaneously this process continues and the hair breaks.

Thus, the tensile strength with ginseng saponin between polypeptide molecules is higher than that with water between polypeptide molecules. This suggestion was in good agreement that tensile strength of ginseng saponin-treated hair is higher than that of the control at 60% RH. Therefore, it is suggested that the stress in the post-yield region is due to the cleavage of disulfide, hydrogen bond, hydrophobic interaction, and the likes.

REFERENCE

1. Meyer, C.A., Bull. Phys. Math., Acad. Petersh 1,683, 1843
2. Georgin, A., Medicine tropicale. 20, 369 (1960)
3. German patent, No, 1067179
4. Well & Lubowe, Cosmetics & Skin pp. 603-604
5. K. Bergwin, Effective plant substances in Cosmetics, A.P.C. 83,41 (1968)
6. John, C. Chang, Ginseng and Cosmetics C&T., 92,50 (1977)
7. Sarah, Harriman book of ginseng the root of heaven 23
8. Lee, Y.K. JAP.J. Endocrine, 17,82, (1941)
9. Han, B.H., Chemical component of Korean ginseng, Korean ginseng science symposium (1974)
10. Breckhmann, I.I., Panex ginseng, Mediz Leningrad (1957)
11. Shibata, S., Ando, T. and Chsawa, T., Chemical studies on oriental plant drugs XIV., Chem. Pharm. Bull. 14 (1966)
12. Kim, N.D. and Woo, L,K., Effect of ginseng saponin on skin, Kor. J. Pharm. 7, 119-121 (1966)
13. Han. B.H., and Chang, I.M., Metabolism of dammarane triterpene glycosides of Korean ginseng, Kor, J. Ginseng Sci., Vol. 2 (1977)
14. S.A. Karjala, A. Karler, J.I., Williamson. The effect of pH on the sorption of collagen-derived peptides by hair, J. Soc. Cosmet. Chem. 18, 599-608. (1967)
15. E.D. Goddard, J.A. Faucher, R.J. Scott, & M.E. Turney, Adsorption of polymer JR on keratious surfaces J. Soc. Cosmet. Chem. 26, 539-50 (1975)
16. Richard, B.G.S. Kass, and C.F. Meyer, Elasticity and tensile properties of human hair, J. Soc. Cosmet. Chem. 22, 667-678 (1967)
17. M.M. Breuer, The binding of small molecules for hair, J. Soc. Cosmet. Chem., 23, 447-470 (1972)
18. Gannar S.B., Jan N. and Lemart, J., Mechanical properties of hair from patients with different types of hair disease. J. Inv. Dermat. 54. 3. (1970)

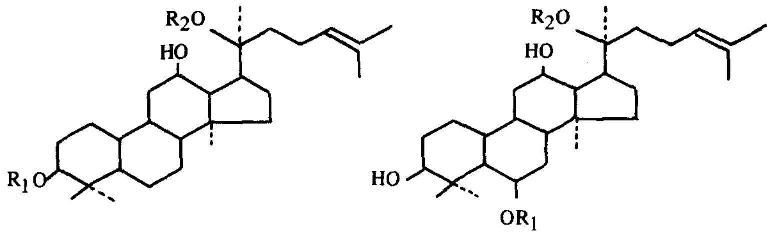
19. I. Bonadeo and G.L Variati, Affinity of hair for protein derivatives Cosm. Toil. 92, 45, (1977)
20. Lundgren, H.P., and Ward, W.H., in Boraski R., "Untrastructure of protein fiber," Academic Press, New York, 1963
21. Bemdit, E.G., Textile Research J. 30, 547 (1960)
22. Billmeyer, F.W., Jr., Tewtboook of polymer science, Interscience (1962). p.220

要 旨

人蔘사포닌으로 處理한 毛髮의 引張强度와 伸張度를 collagen 加水分解物로 처리한 毛髮과 比較하였다. 毛髮에 人蔘사포닌 吸收量은 high speed liquid chromatography로 測定하였으며 liquid scintillation counter로 確認하였다. 毛髮에 collagen 加水分解物의 吸收量은 毛髮을 collagen 加水分解物 溶液속에 沈積시켰을 때 처음 30分 동안은 많은 量의 吸着이 일어났으나 1時間 經過後부터는 더 以上의 增加는 보이지 않았다.

이와는 反對로 人蔘사포닌의 境遇는 沈積時間의 增加에 比例하여 吸收量의 增加를 나타내었다.

實驗結果는 人蔘사포닌이 collagen加水分解物보다 毛髮에 더욱 效果的임을 보여 주었고, 그리고 人蔘사포닌의 毛髮에 對한 吸收와 引張强度의 增加는 毛髮의 strain-stress曲線과 人蔘사포닌의 界面活性에 依해 說明하였다.



R_1, R_2 ; glucose

R_1 ; glucose
R_2 ; H , glucose

Fig. 1 20(S)–proto panaxadiol 20(S)–protopanaxatriol

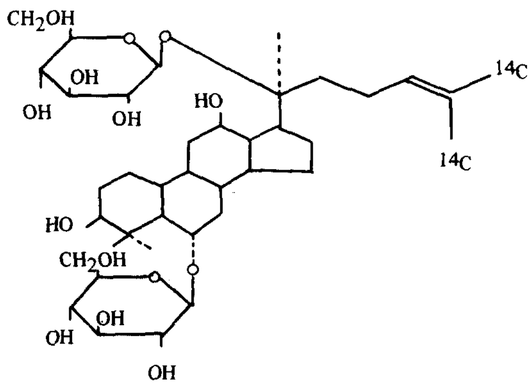


Fig. 2 ^{14}C-labelled ginsenoside Rg_1

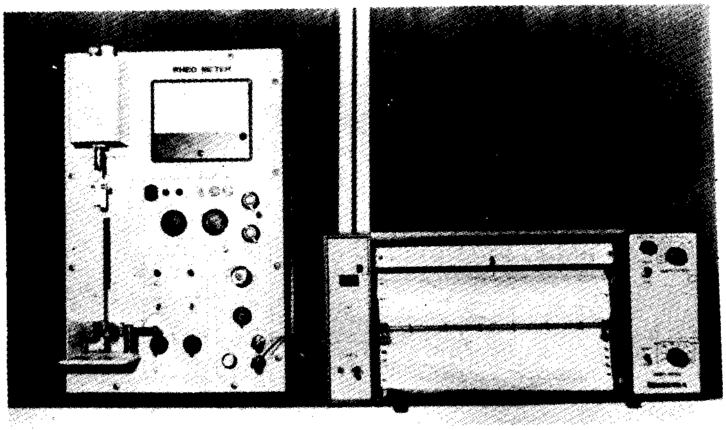


Fig. 3 RHEO METER test

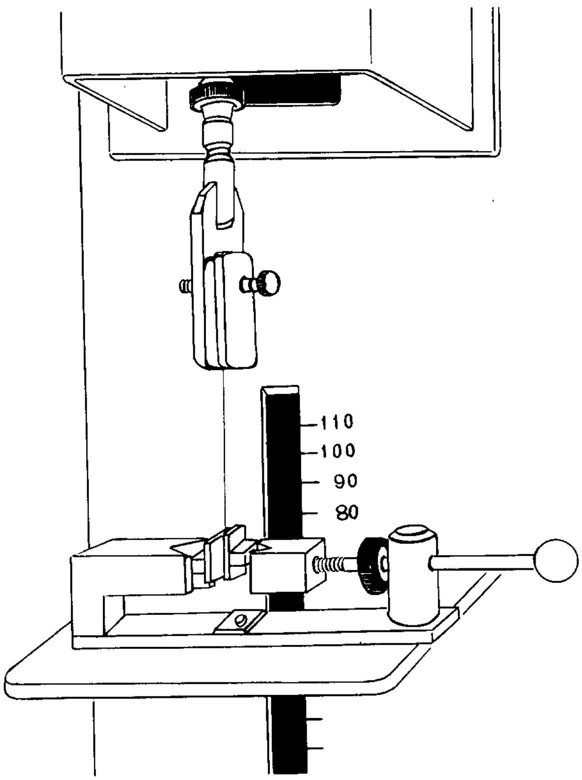


Fig. 4 Sample mount for RHEO METEP test

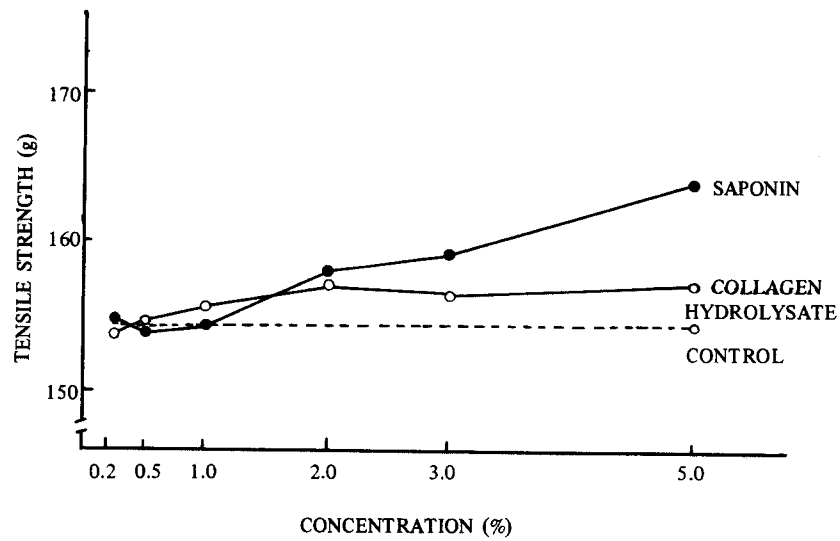


Fig. 5 The effect of concentration on tensile strength
(bleaching time; 1 hour, immersion time; 1 hour)

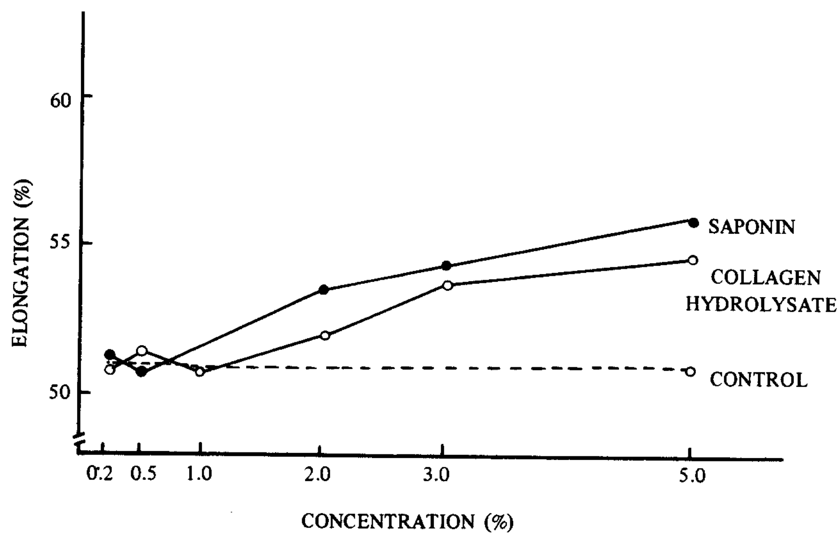


Fig. 6 The effect of concentration on elongation
(bleaching time; 1 hour, immersion time; 1 hour)

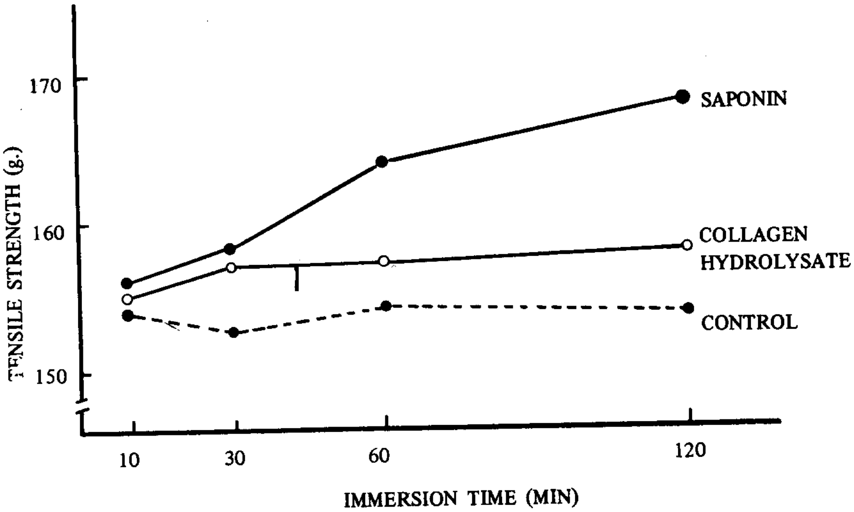


Fig. 7. The effect of immersion time on tensile strength
(bleaching time; 1 hour, concentration; 5%)

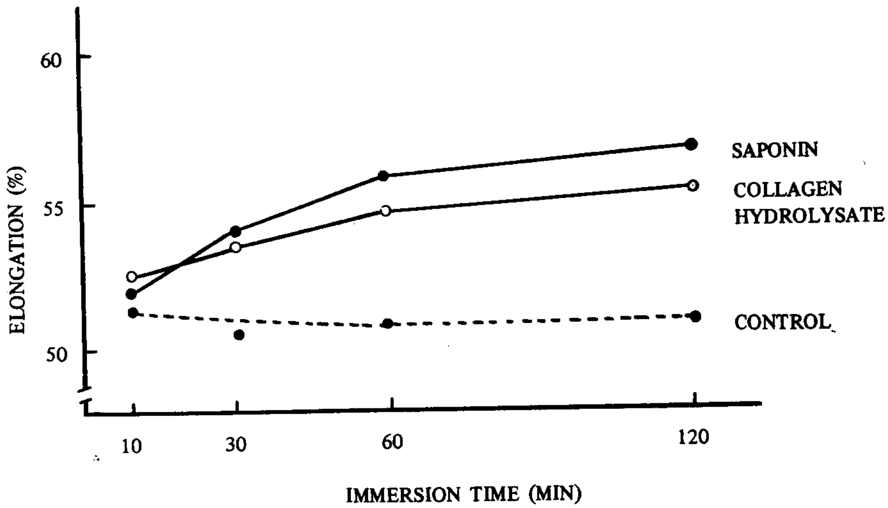


Fig. 8. The effect of immersion time on elongation
(bleaching time; 1 hour, concentration; 5%)

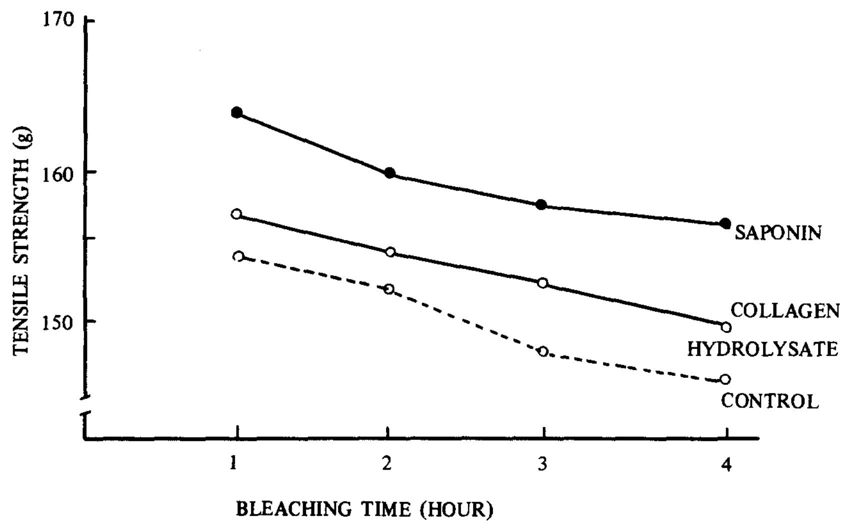


Fig. 9 The effect of bleaching time on tensile strength
(immersion time; 1 hour, concentration; 5%)

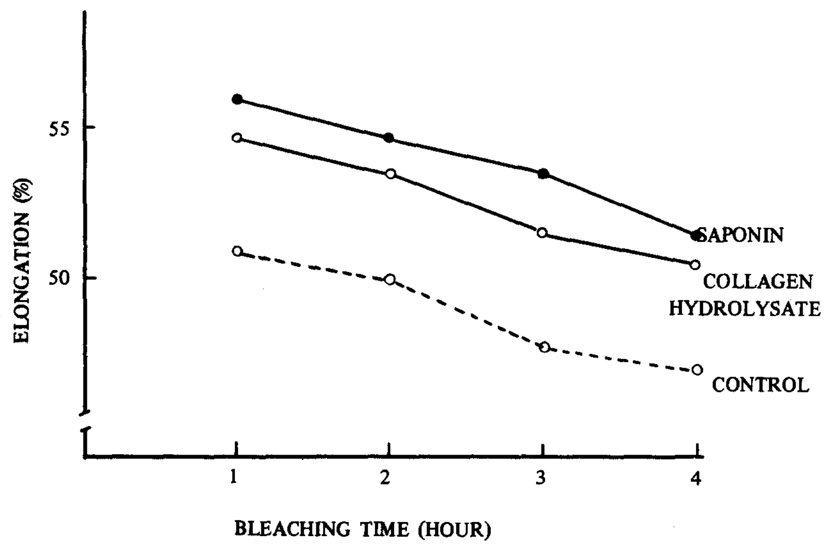


Fig. 10 The effect of bleaching time on elongation
(immersion time; 1 hour, concentration; 5%)

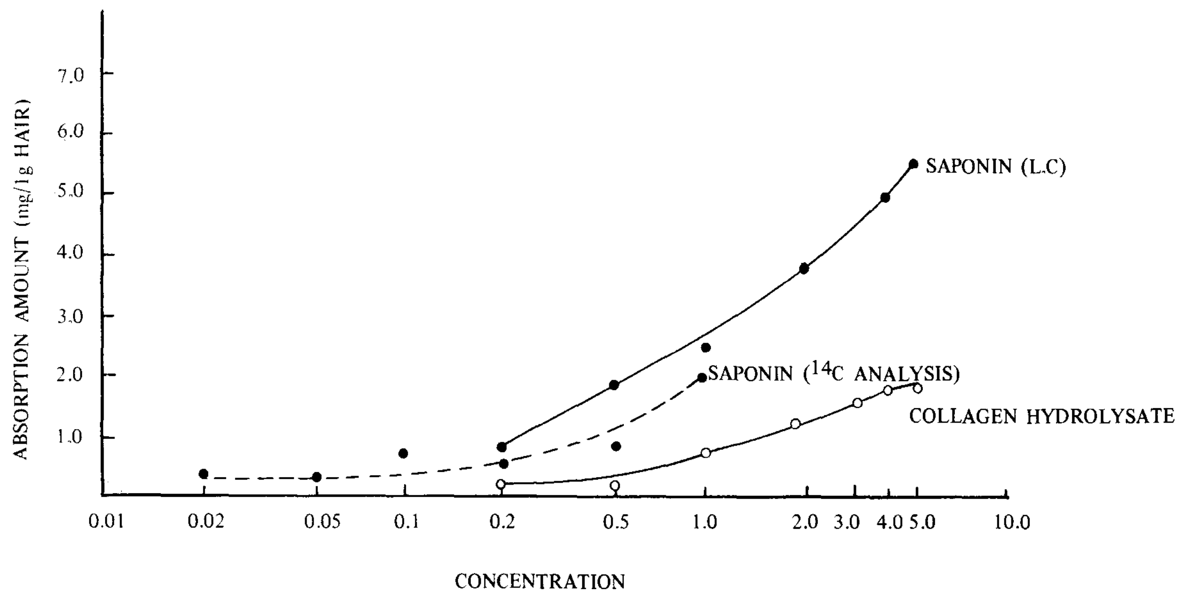


Fig. 11 The effect of concentration on absorption amount of saponin and collagen hydrolysate. (immersion; 1 hour, bleaching; 1 hour)

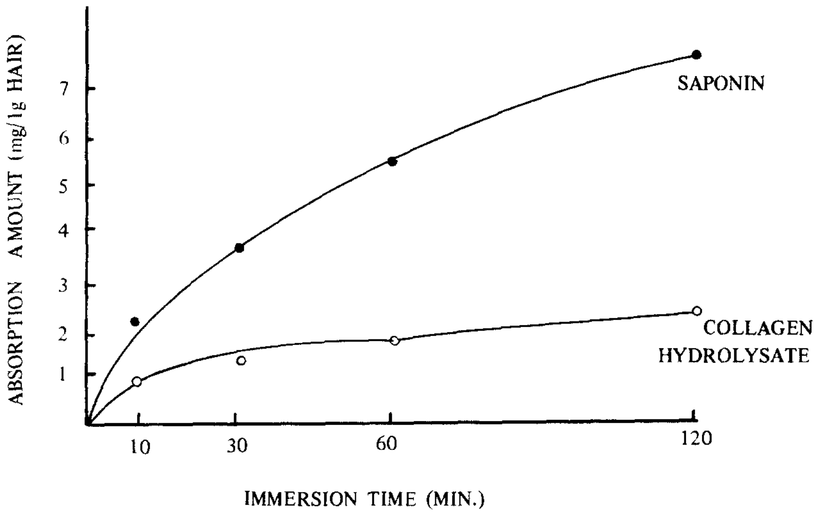


Fig. 12 The effect of immersion time on absorption amount
(bleaching time; 1 hour, concentration 5%)

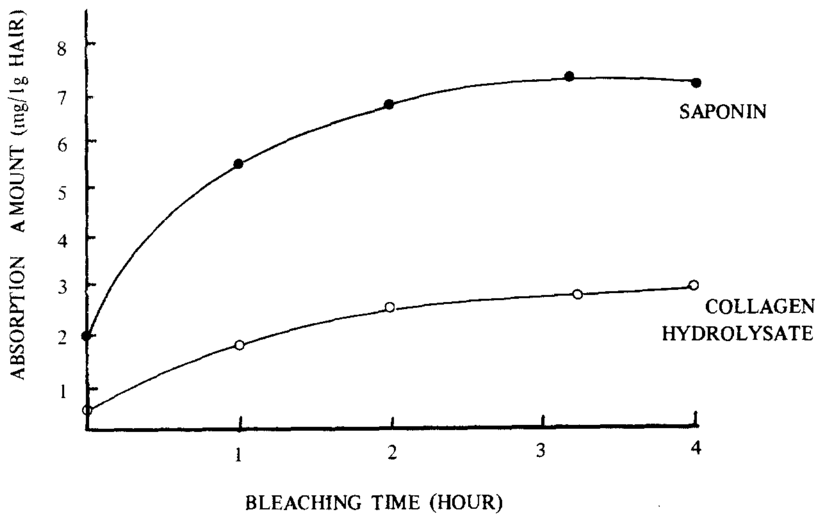


Fig. 13 The effect of bleaching time on absorption amount
(immersion time; 1 hour, concentration 5%)

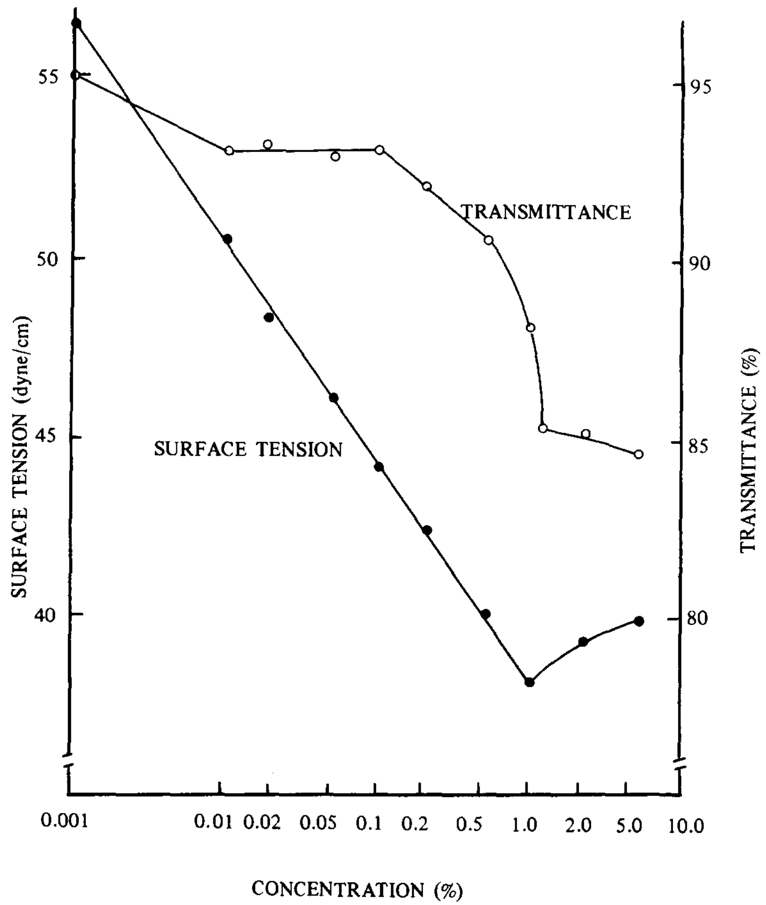


Fig. 14 C.M.C. of saponin by the surface tension method and dye adsorption method

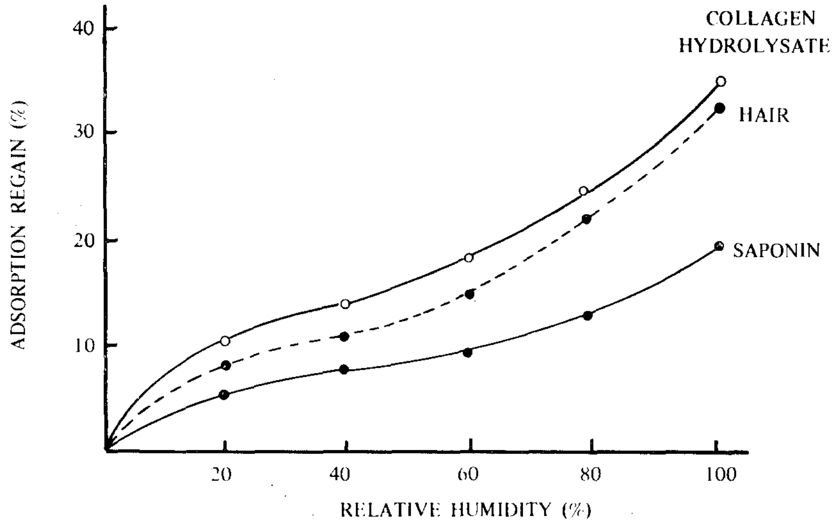


Fig. 15 The adsorption regain isotherms of hair, collagen hydrolysate and saponin

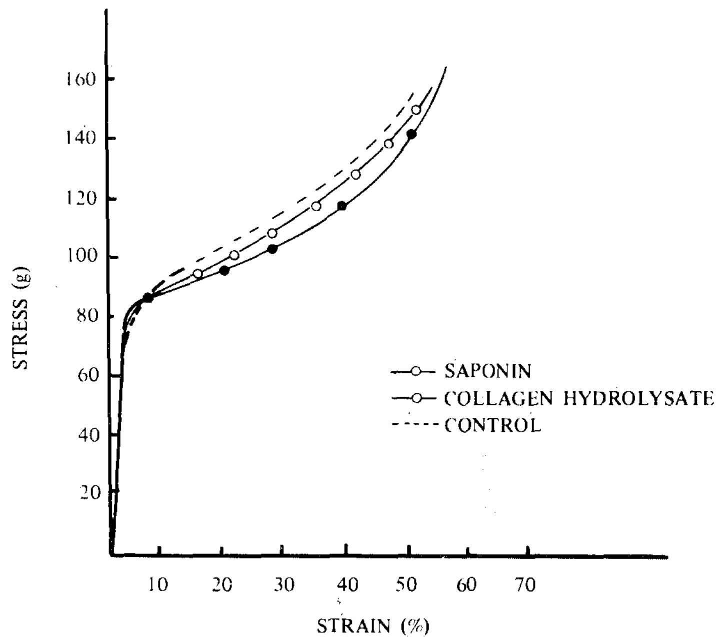


Fig. 16 The stress strain curve
(full scale; 200g, chart speed; 6 cm/min.
rate of elongation; 6cm/min.)

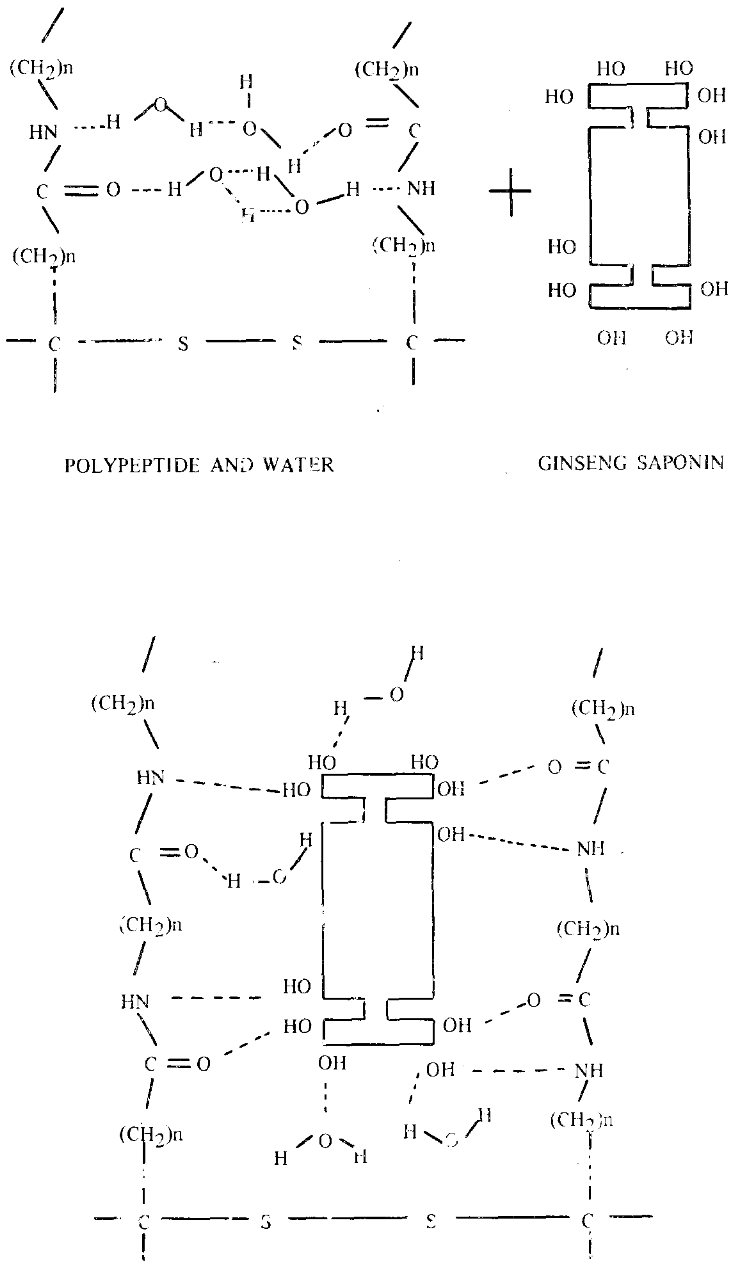


Fig. 17 Suggested schematic diagram for complexes between ginseng saponin and polypeptide