GLYCOSIDES OF RED-GINSENG

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ABSTRACT

From water extract of red ginseng, two new-type glucosides, A: $C_{12}H_{16}O_8$ and B: $C_9H_{16}O_7$, were isolated by fractionation with highly porous polymer ollowed by chromatography on silica gel in yields of 0.04 and 0.16%, respectivley.

The structures of A and B were elucidated by means of NMR and MS. Neither glucosides were detected in the extract of white ginseng and the mechanism of formation of A and B during the process of steaming will be discussed.

The isolation and identification of saponins of corms (head of the root) of red ginseng are also reported.

INTRODUCTION

For most of our chemical studies of Ginseng roots, peeled and dried roots, so-called White-Ginseng have been used.[1] However, in Asian countries, steamed Ginseng roots without peeling, so-called Red-Ginseng have been more common as a medicine than White-Ginseng. With regard to the difference in the secondary metabolites between White-Ginseng and Red-Ginseng, we isolated monoacetylated dammarane saponins named ginsenosides Rs1 and Rs2[2] from Red-Ginseng but not from White-Ginseng, while Kitagawa et al. obtained malonylated saponins from White-Ginseng but not from Red Ginseng. Isolation of some other less polar minor saponins as well as the characteristic polyacetylenic alcohols from Red-Ginseng was also reported by Kitagawa et al. However, study on the chemical transformation of primary metabolites during the process of the preparation of Red-Ginseng has not appeared in the literature. The present paper reports isolation and structure determination of highly water-soluble glucosides, which must be artifacats formed from primary metabolites.

SEPARATION PROCEDURE

Recent progress in application of highly porous polymer such as MCI gel Daia-ion CHP20P allows to separate saponins from more water-soluble compounds such as amino acids and mono-and oligo-saccharides. A water extract of powdered Red-Ginseng was subjected to chromatography on highly porous polymer and the resulting non-saponin fraction was chromatographed on silica gel to give two glucosides 1 and 2 in yields of 0.16 and 0.03%, respectively. As shown in Chart 1, 1 and 2 showed the similar Rf values as that of ginsenoside Rg1 on thin layer chromatogram. Both 1 and 2 could not be isolat-

ed from White-Ginseng and also from the dried roots without peeling.

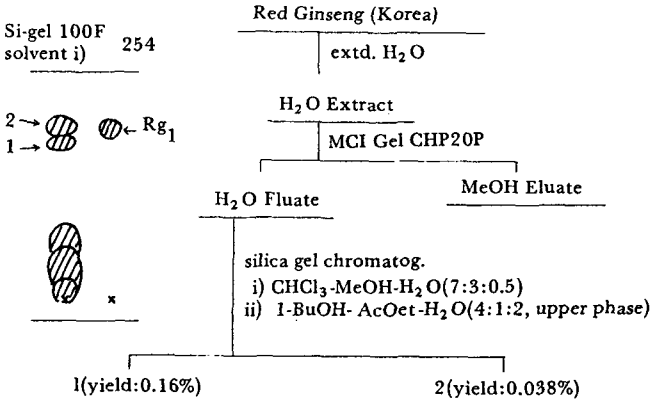
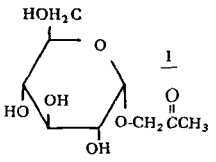


Chart 1



$[\alpha]_D^{16}$ +137.1°(c=0.24, H_2O)

acid hydrolysis(2N H_2SO_4):

D-glucose

IR(KBr): 1710cm^{-1} (C=O)

^{13}C NMR(pyridine-d_5):

δ: 100.6(g-1)
73.7(g-2)
75.3(g-3)
72.0(g-4)
75.0(g-5)
62.8(g-6)
206.4(C=O)
73.0(CH_2)
26.5(CH_3)

^{1}H NMR(pyridine-d_5):

δ:2.11 (s, 3H)
4.2-4.7(m, 8H)
5.37(d, 1H, J=3.6Hz)

CI-MS: m/z 237$(M+H)^+$

EI-MS: m/z 163
73
57

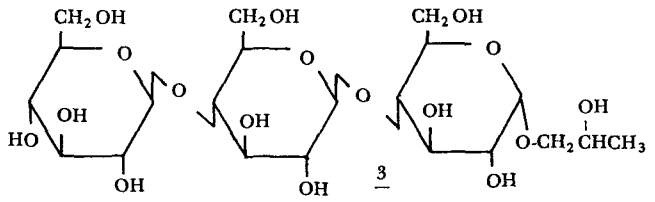


Chart 2

STRUCTURES AND PHARMACEUTICAL SIGNIFICANCE

The CI-MS of glucoside 1 showed a $(M+H)^+$ ion at m/z 237. Acid hydrolysis of 1 afforded D-glucose. The coupling constant of an anomeric proton signal and the ^{13}C NMR spectrum indicated the presence of an α-D-glucopyranoside moiety.

The IR spectrum of 1 exhibited a band attributable to a ketone and its ^{13}C NMR spectrum showed signals due to one ketone, one CH_3 and one -CH_2-O-(Chart 2). Further its proton signal indicated the presence of one CH_3 attached to a carbonyl group. These observations led to the formulation of 1 as 2-oxopropyl α-D-glucopyranoside (=acetol α-D-glucoside). The EI-MS fragment ions of 1 supported this formulation.

It should be noted that this structure is closely related to that proposed for rhynchosporoside (=1-hydroxy-2-propyl α-cellotrioside, 3), produced by a fungi, Rhynchosporium secalis.[3]

As already mentioned, this glucoside 1 seems to be formed from sugars during the process of the steaming, though the refined mechanism of its formation has been still obscure. It has been reported that the several pyrolysis products of carbohydrates such as methyl glyoxal, β-propiolactone, glycidol and propylene glycol show the significant mutagenicity in Ames test.[4] The weak mutagenicity was also reportated for acetol, the aglycone of glucoside 1. The Ames test in our present study revealed that the mutagenicity of glucoside 1 on three strains of Salmonela typhimurium with and without S-9 is neglegible (Table I).

Another compound, glucoside 2 is quite unstable in a pure state, being decomposed even on standing at room temperature to give glucose and maltol(4). Enzymic hyrolysis of 2 afforded glucose and 4. The structure of 2 was proposed by the MS, NMR and IR spectra which were taken immediately just after the purification. Its FD-MS exhibited M+H ion at m/z 307. The ion at m/z 127 in CI-MS is associated with 4 which was formed during the MS measurement. This was confirmed by CI, EI-MS linked scan method. The base peak at m/z 126 in the EI-MS was proved to be also due to 4 by means of high resolution MS experiment. All of the carbon and proton resonances of glucoside 2 appeared as a pair of signals with the similar intensity (Table II). This indicated that this glucoside must be a mixture of a couple of stereo-isomers, though it seemed to be homogeneous by thin layer chromatography. The ^{13}C NMR spectrum as well as the anomeric proton signal indicated the presence of a α-D-glucopyranoside residue. This

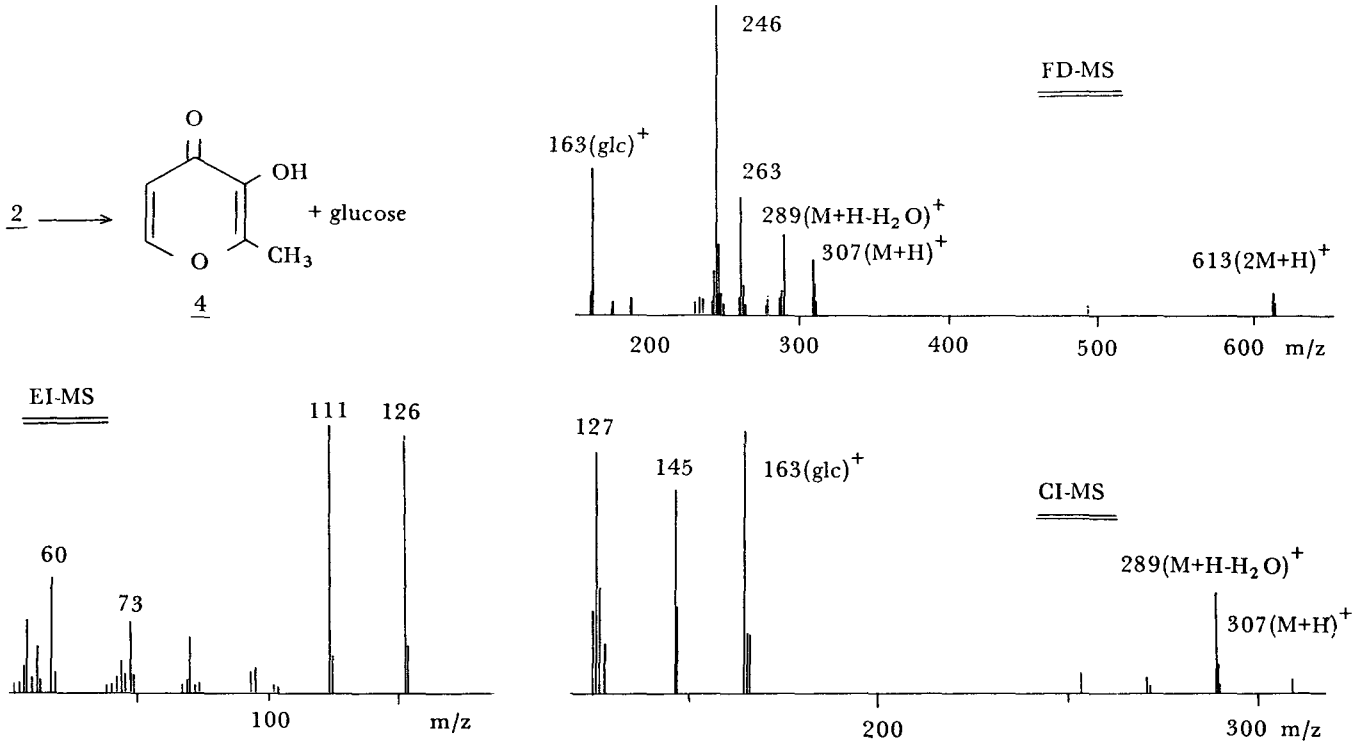


Chart 3

Table 1. Reverse Mutation Test by Pre-Incubation Method on Salmonella typhimurium

	Conc./plate (μg)	Mutagenicity (Revertants/plate) TA98 S9(−)	TA98 S9(+)	TA100 S9(−)	TA100 S9(+)	TA1537 S9(−)	TA1537 S9(+)
Control	0	19	47	108	95	6	30
	125	22	55	121	119	6	35
1	250	23	48	107	109	7	32
	500	24	46	136	151	11	26
	125	27	42	123	88	4	29
GA	250	25	44	248	123	7	20
	500	38	46	1312	201	10	28
MNNG	2	9	36	2998	97	773	25
BP	5	22	150	101	284	9	109

GA: DL-Glyceraldehyde MNNG: N-Methyl-N'-nitrosoguanidine
BP: 3, 4-Benzopyrene

was further supported by the result of GC-MS methylation analysis.

As already mentioned, 4 was obtained from glucoside 2. However, the carbon signals were inconsistent with the formulation as maltol α-D-glucoside. The ^{13}C NMR spectrum (Table II) revealed the presence of one C=0, one CH_2-O, one CH_3, and one olefinic carbon having one proton on it. In addition, a signal at δ 145.5 (and 144.9) could be assigned as an olefinic carbon having an enolic hydroxyl group of diosphenol type by comparison with that report-

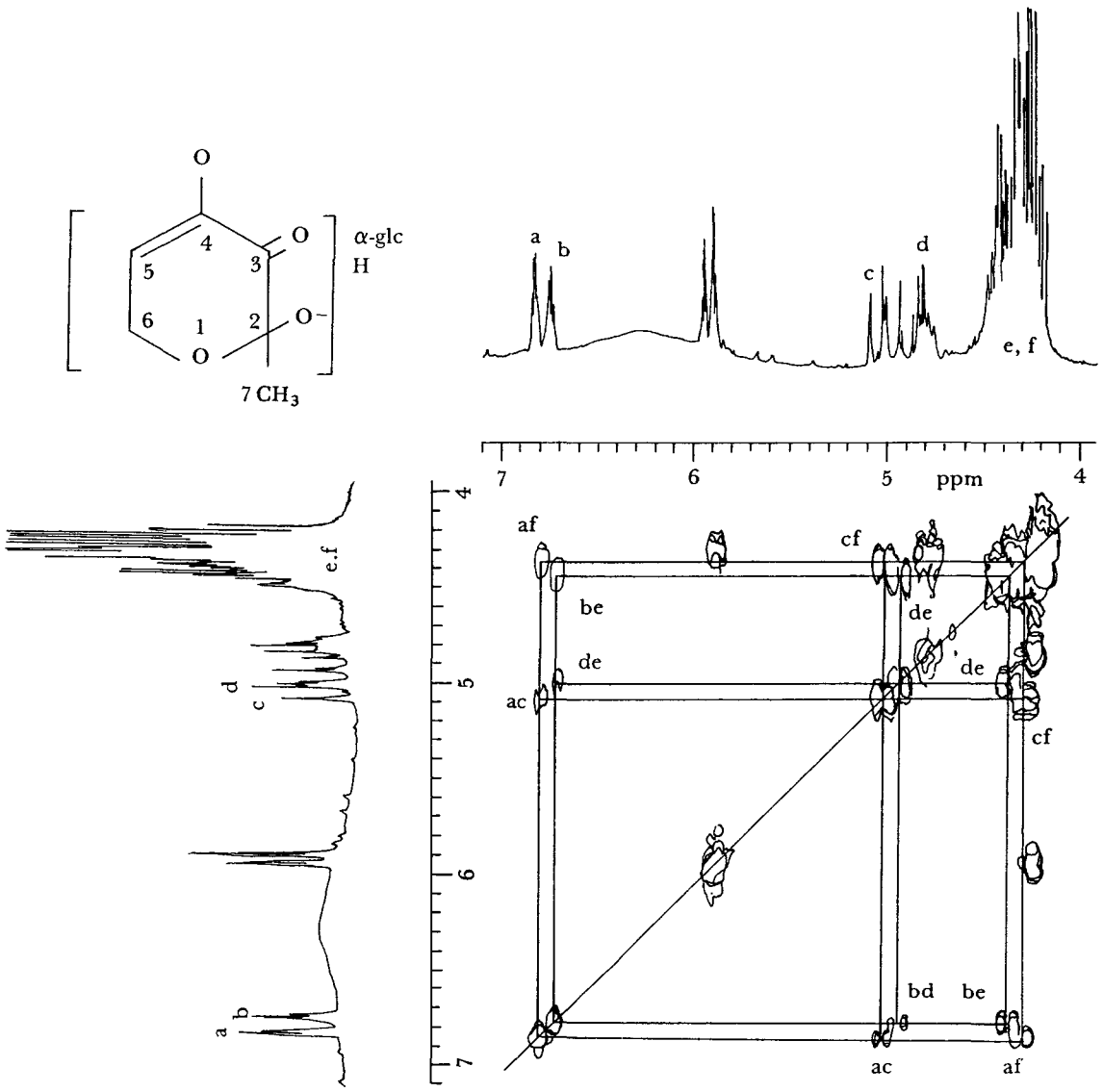


Chart 4. Proton homonuclear correlation 2D NMR spectrum of 2

ed for compounds 5 in Table II. Further, a carbon signal at δ 97 indicated the presence of a tetrasubstituted carbon having two oxygen functions. All of the signals in the 1H NMR spectrum of glucoside 2 were identified by means of proton homonuclear correlation two dimentional NMR technique(Chart 4), indicating the presence of the partial structure 6 (Table II). These spectral observation coupled the ready formation of 4 led to the formulation of glucoside 2 as an mixture of epimers at C-2 of the structure 2 (or 2') Table II.

The location of the glucosyl linkage on enolic OH was elucidated as follows. Acetylation of glucoside 2 afforded a tetraacetate(7) and a penta-acetate(8), the former of which gave the latter on further acetylation. It has been reported that acetylation of diosphenol-type compound(5) to 5a results in the deshielding of the enolic and carbonyl carbons and shielding of the olefinic carbon. The ^{13}C NMR spectra demonstrated that on going from 2 to 7, C-4 was slightly shielded and C-5 was evidently deshielded, while other singals due to the aglycone moiety remained

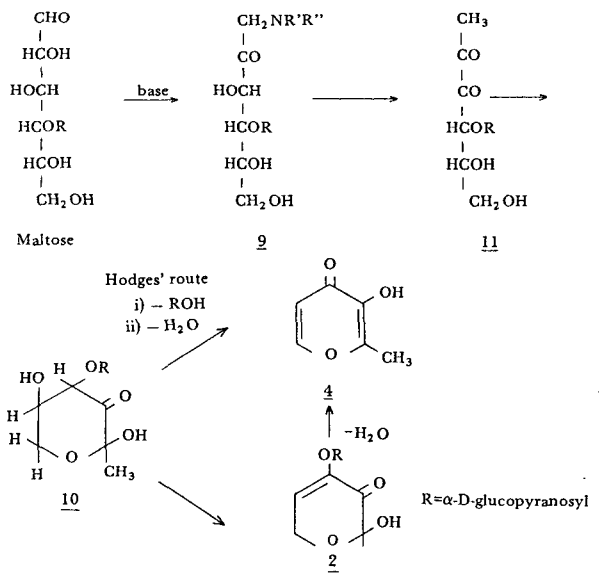


Chart 5

almost unchanged. On going from the tetra-acetate(7) to the pentaacetate(8), shielding was observed slightly for C-5 and -7 and remarkably for C-3, while C-2 and -6 were evidently deshielded and the signal due to C-4 remained almost unaffected (Table II). These acetylation shifts indicated the presence of the glucosyl linkage at its 4-hydroxyl group-(structure 2 in Table II), being inconsistent with the formulation as 2'.

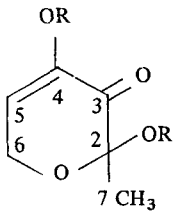
Hahn et al.[5] previously reported the isolation of 4 from Red-Ginseng which has not been identified in the fresh roots, though they did not mentioned the mechanism of the formation of 4 in Red-Ginseng. Since 2 is quite unstable and afforded 4 even on standing at room temperature, this glucoside must be a plausible intermediate of 4, an artifact formed during the process of the steaming.

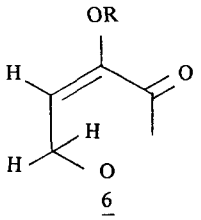
The preparation of Red-Ginseng from fresh Ginseng roots seems to be a kind of browning process whereby mixtures of sugars and amino acids affored on heating to dark-colored products, the so-called Maillard reaction. In connection with this reaction, the mechanism of the formation of 4 from maltose by heating with piperidine phosphate has been tentatively proposed as shown in Chart 3. The initially formed Amadori compound(9) may be converted into compound 10 through compound 11 and 4 may be formed from compound 10 by cleavage of the glucosyl linkage followed by isomerization and dehydratation. Based on this proposal, the mechanism of the formation of glucoside 2 on Red-Ginseng preparation process is proposed as shown in Chart 5. Maltose formed from starch reacts with an amino acid to give the similar Amadori com-

Table 2. ^{13}C-Chemical Shifts in Pyridine-d_5[a]

	2		7[b]		8[b]	
C-2	97.3	97.1	97.2		101.4	
C-3	189.0	188.9	189.0		185.6	
C-4	145.0	144.9	144.1	144.0	144.8	144.4
C-5	123.0	122.4	128.2	127.3	127.0	125.6
C-6	59.5	59.4	59.5		62.2[c]	62.1[c]
C-7	23.8	23.6	23.5	23.4	22.6	21.6
G-1	100.0	99.2	95.9	95.7	96.0	95.2
G-2	75.0[c]	74.9[c]	70.9[c]		70.9[d]	70.8[d]
G-3	75.4[c]	75.2[c]	70.4[c]		70.5[d]	70.3[d]
G-4	71.8	71.6	69.1[c]	69.0[c]	69.1[d]	
G-5	73.5[c]	73.4[c]	69.0[c]	68.9[c]	68.9[d]	
G-6	62.4	62.3	62.3	62.2	62.7[c]	62.3[c]

a : The characterization of carbon signals were made on the bases of the INEPT experiments.
b : The signals due to Ac group were abbreviated.
c, d: may be interchanged



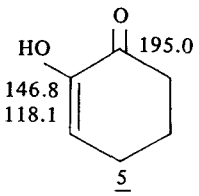


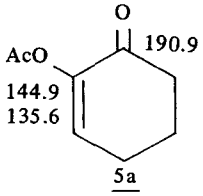
2: R=α-glc R'=H

2': R=H R'=α-glc

7: R=α-glc(OAC)4 R'=H

8: R=α-glc(OAc)4 R'=Ac





pound 9 which afforded the compound 10. Dehydration of compound 10 affords glucoside 2, which yields 4 by elimination of glucose and subsequent rearrangement.

The isolation of glucosides 1 and 2 which are characteristic of Red-Ginseng, is significant to distinguish Red-Ginseng extract from the extracts of other preparation of Ginsng roots. This paper is published in Chem. Pharm. Bull. (Japan), 32, No. 11 (1984).

ACKNOWLEDGEMENT

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홍삼의 배당체

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홍삼의 물추출물을 먼저 다공성의 polymer로 분획을 나누고 다음에 Silica gel컬럼 크로마토 그래피로 분리하여 2개의 새로운 형태의 배당체인 A($C_{12}H_{16}O_8$)와 B($C_9H_{16}O_7$)를 분리하였으며, 이때 이때 수율은 각각 0.04%와 0.16%였다.

A와 B의 구조는 NMR과 MS에 의해서 밝혀졌다. 백삼의 추출물에서는 이들 배당체는 검출되지 않았으며, 한편 증삼과정중에 A와 B의 형성기 전이 논의될 것이다.

또한 홍삼의 뇌두로부터 사포닌의 분리 및 확인이 역시 보고될 것이다.

Shibata: Do you think that the compount you isolated from red ginseng was formed during the process of making Red Ginseng from fresh *Panax ginseng?*

Is there any possibility that such compound was formed by boiling the White Ginseng for a long time?

Tanaka: Yes, that is a very interesting problem. I'll conduct that kind of experiment in the near future. We have not yet examined the formation of this type of compound by decoction of ginseng.

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