Communications to the Editor

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STUDY OF RED GINSENG: NEW GLUCOSIDES AND A NOTE ON THE OCCURRENCE OF MALTOL

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Two new glucosides **1** and **2** were isolated from a non-saponin fraction of a water extract of Red Ginseng (steamed ginseng root). The structurs of the glucosides was elucidated by MS and by ^{1}H and ^{13}C NMR spectrometry. Neither glucoside was detected in White Ginseng, being artifacts formed during the processing of Red Ginseng. Since **2**, an unstable epimeric mixture, is readily decomposed to give maltol, this glucoside must be an intermediate in the formation of maltol from maltose during the steaming process.

KEYWORDS —— Red Ginseng; Panax ginseng; Araliaceae; acetol α-D-glucoside; maltol formation intermediate

In Asian countries, Red Ginseng (steamed and dried ginseng roots without peeling) is more common as a medicine than White Ginseng (roots dried after peeling). The differences in the secondary metabolites of White Ginseng and Red Ginseng, have recently been reported with comparisons of dammarane-saponins[1,2] and polyacetylenic alcohols.[2] However, no studies of the chemical transformation of the primary metabolites during the steaming process have appeared in the literature. We now report the isolation and structure determination of the highly water-soluble glucosides which are characteristic of Red Ginseng.

A water extract of powdered Red Ginseng was subjected to chromatography on a highly porous polymer and the resulting non-saponin fraction was chromatographed on silica gel to give two new glucosides, **1**, white powder $[\alpha]_D^{16}$ +137.1° (H_2O) and **2**, colorless syrup $[\alpha]_D^{27}$ +89.4° (MeOH) in yields of 0.16 and 0.038%, respectively. Neither **1** nor **2** could be isolated from White Ginseng or from the dried ginseng roots without peeling and steaming.

The CI-MS of **1** showed a $(M+H)^+$ ion at m/z 237. Acid hydrolysis of **1** gave D-glucose. The coupling constant of an anomeric proton signal (in C_5D_5N, δ 5.37, J=3.6Hz) and the ^{13}C NMR spectrum (Table I) indicated the presence of an α-D-glucopyranosyl moiety in **1**. The IR spectrum of **1** exhibited a band attributable to a ketone at 1710cm^{-1}(KBr) and its ^{13}C NMR spectrum (Table I) showed signals due to one ketone, one CH_3 and one CH_2-O-. Further, its proton signal (in C_5D_5N) at δ 2.11(3H s) 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 (ions at m/z 163(glucosyl ion), 73($O-CH_2COCH_3^+$) and 57($CH_2COCH_3^+$)) supported this formulation. It should be noted that this structure is closely related to that proposed for rhynchosporoside (**3**, Chart 1)), a phytotoxin produced by the causal agent of scald deseases in barley, *Rhynchosporium secalis*.[3)]

This glucoside **1** seems to be formed from sugars during the process of the steaming, though the detailed mechanism of its formation is obscure. It has been reported that the several sugar-pyrolysis products such as methyl glyoxal, β-propiolactone, glycidol and propylene glycol are significantly mutagenic without S-9 in *Salmonella typhimurium* TA100. Weak mutagenicity in the same strain was also observed for acetol.[4)] The Ames test revealed that **1** showed negligibly weak mutagenicity in *Salmonela typhimurium* TA100 with and without S-9 and no mutagenicity in the strains TA98 and TA1537.

Another glucoside **2** which showed UVmax at 256nm (ε 4,700, in MeOH), is quite unstable after isolation, being decomposed even on standing at room temperature to give glucose and maltol (**4**). Enzymic hydrolysis of **2** afforded glucose and **4**. Its FD-MS exhibited ions at m/z 613$(2M+H)^+$, 307$(M+H)^+$, 289$(M+H-H_2O)^+$ and 163$(glc)^+$ and its CI-MS showed ions at m/z 307$(M+H)^+$, 289$(M+H-H_2O)^+$, 163$(glc)^+$ and 127. The ion at m/z 127 was assigned as **4**+H, which was confirmed by the CI, EI-MS linked scan method. The base peak at m/z 126 in the EI-MS of **2** was also assigned as the ion due to **4**, $C_6H_6O_3$ by means of a high resolution MS experiment. As shown in Table II, all of the carbon and proton resonances of **2** appeared as a pair of signals with similar intensity. This indicated that **2** must be a mixture of a couple of stereo-isomers, though the separation into each isomer has not yet been achieved. A set of carbon signals from δ 62 to 100 as well as a pair of anomeric proton signals near δ 5.9 indicated the presence of an α-D-glucopyranosyl residue.

Although **4** was obtained from **2**, the carbon signals of **2** (Table II) were inconsistent with the formulation maltol α-D-glucoside. The ^{13}C NMR spectrum of **2** revealed the presence of one C=O, one $-CH_2-O-$, one CH_3, and one -CH=. 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 reported for 2-hydroxy-2-cyclohexenone (**5**, δ 146.8 in $CDCl_3$) and brucein B (**6**, δ 144.1 in DMSO-d_6).[5)] Further, a carbon signal of **2** at δ 97.3 (and 97.1) indicated the presence of a tetrasubstituted carbon having a two oxygen function ($-O-\overset{|}{\underset{|}{C}}-O-$). In the ^1H NMR spectrum of **2** in C_5D_5N, a signal due to a methyl group on a quarternary carbon was observed at a relatively low field (at δ 1.86 (and 1.87)). A pair of proton signals at δ 6.82(1H dd, J=2.1, 4.6Hz) and 5.04(1H dd, J=2.1, 18.0Hz) accompanied by a similar pair at δ 6.74(1H dd, J=2.1, 4.6Hz) and 4.96(1H dd, J=2.1, 17.7Hz) indicated the presence of the system **7** shown in Chart 2 in which a signal due to one of the methylene group could not be identified because it overlapped the signals due to the glucosyl moiety. These spectral observations, coupled with the ready formation of **4**, led to the formulation of this glucoside as a C-2-epimeric mixture of the structure **2** or **2'** in Chart 2.

The location of the glucosyl linkage in **2** was elucidated as follows. Acetylation of **2** afforded a tetraacetate (**8**) and a pantaacetate (**9**), the former of which gave the latter (**9**) on further acetylation. The ^{13}C NMR spectra demonstrated that on going from **2** to **8**, C-4 was slightly shielded and C-5 was evidently

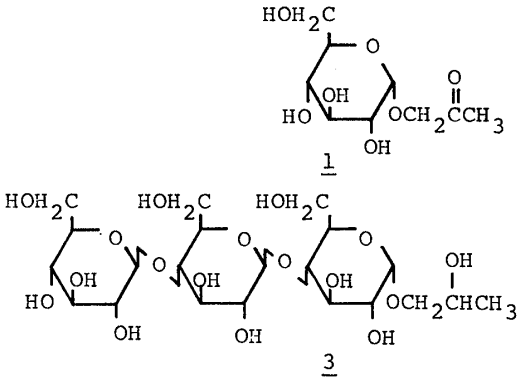


Chart 1

Table I. ^{13}C-Chemical Shifts[a]

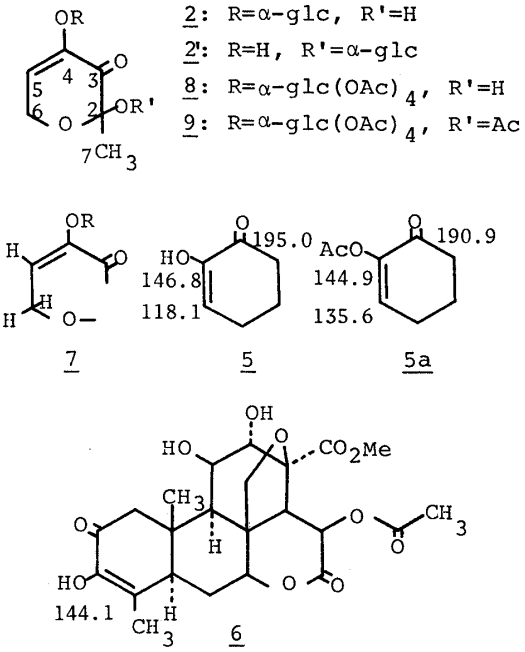
	1[b]	Me α-glc[c]
C-1	73.0	
C-2	206.4	
C-3	26.5	
G-1	100.6	101.3
G-2	73.7	73.8
G-3	75.3	75.3
G-4	72.0	72.1
G-5	75.0	74.0
G-6	62.8	62.8

a) Measured in C_5D_5N.
b) These signals were assigned by use of 2D-NMR technique.
c) Methyl α-D-glucopyranoside.

Table II. ^{13}C-Chemical Shifts in C_5D_5N[a]

	2		8[b]		9[b]	
C-2	97.3	97.1	97.2		101.4	
C-3	189.0	188.9	189.0		185.6	
C-4	145.5	144.9	144.1	144.0	144.8	144.4
C-5	123.0	122.4	128.2	127.3	127.0[c]	125.6[c]
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[c]	99.2[c]	95.9[c]	95.7	96.0[d]	95.2[d]
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[c]	71.6[c]	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 was made on the bases of the INEPT experiments.
b) The signals due to the Ac group were abbreviated.
c,d) May be interchanged.



2: R=α-glc, R'=H
2': R=H, R'=α-glc
8: R=α-glc(OAc)$_4$, R'=H
9: R=α-glc(OAc)$_4$, R'=Ac

Chart 2

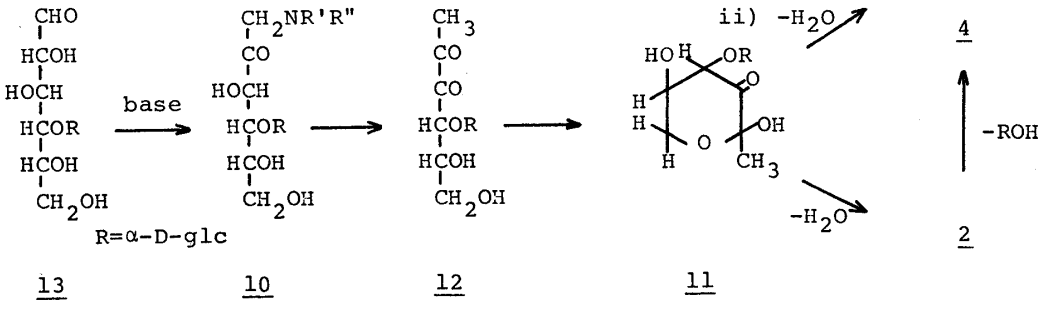


Chart 3

deshielded, while other signals due to the aglycone moiety remained almost unchanged. On going from **8** to **9**, shielding was observed slightly for C-5 and -7 and markedly for C-3, while C-2 and -6 were evidently deshielded and the signal due to C-4 remained almost unaffectd. These acetylation shifts indicated the presence of the glucosyl linkage of **2** at its 4-hydroxyl group (structure **2** in Chart 2), being inconsistent with the formulation as **2'**. It should be noted that acetylation of **5** to its acetate (**5a**) results in the deshielding of the enolic and carbonyl carbons and shielding of the olefinic carbon.[5]

Han[6] previously isolated **4** as an anti-oxidant from Red Ginseng. Since **2** readily afforded **4** even on standing at room temperature, this glucoside must be a plausible intermediate of **4**, an artifact formed during the steaming process. The preparation of Red Ginseng from fresh ginseng roots seems to be a kind of browning process whereby mixtures of sugars and amino acids are transformed into dark-colored products (the Maillard reaction). In connection with the Maillard reaction, the mechanism of the formation of **4** from maltose by heating with piperidine phosphate has been proposed as follows.[7] The initially formed Amadori compound (**10**) may be converted into **11** through 4-O-α-D-glucosyl-1-deoxy-2,3-diulose(**12**) and then **4** may be formed from **11** by cleavage of the glucosyl linkage followed by isomerization and dehydration. Based on this proposal, the mechanism of the formation of **4** in the Red Ginseng preparation process is proposed as shown in Chart 3; maltose(**13**) reacts with an amino acid to give the similar Amadori compound such as **10** which gives **11**. Dehydration of **11** affords **2**, which yields **4** by the elimination of glucose accompanied by rearrangement.

The isolation of glucosides **1** and **2** which are characteristic of Red Ginseng is significant in distinguishing Red Ginseng extract from the extracts of other preparation of ginseng roots.

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