

QUANTITATIVE GAS CHROMATOGRAPHIC EVALUATIONS OF PANAXADIOL AND PANAXATRIOL IN GINSENG ROOTS AND COMMERCIAL GINSENG PREPARATIONS

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For the pharmacological qualities of ginseng roots and their preparations 8–10 glycosides with saponin character are made responsible. Therefore, the quality of the commercial preparations should depend on the percentile presence of these glycosides in the root and in the extracts made of it. Thus, ginseng growers and producers of ginseng preparations have to be interested in a procedure by which the content of glycosides in ginseng can be determined in all preparations as exactly as possible.

The chemical structure of 9 ginseng glycosides is known. Therefore, the best statement could be provided by a procedure by means of which all glycosides are quantitatively covered. Because of the great similarity of these glycosides, because of the different quantity of the individual glycosides, because of their mutual influence resulting from the high interfacial activity and, finally, because of their insolubility in slightly polar organic solvents this aim can only be reached with considerable preparative difficulties. They always will reduce the quantitative accuracy.

In 1976, *Bombardelli* and his colleagues¹⁾ described a method for determining the ginsenosides by means of gas-liquid-chromatography coupled with mass-spectrometry. The mixture of ginsenosides was transferred into the trimethylsilyl ethers. According to specifications of the authors, they

prove six of the glycosides gas-chromatographically from the ginseng extracts and obtain a gas-chromatogram which, for them, is reproducible.

By the mass-spectrometrical determination the glycosides, gas-chromatographically separated, give, as is known by glycosides, only very slight or no molecular peaks. Therefore, the authors had to orient themselves on selected fragments of the mass spectrum when making their assignment.

*Bombardelli*¹⁾ considers his GC/MS procedure a useful way for judging ginseng extracts. With this method, he, up to now, has not been able to make quantitative statements concerning the percentile portion of certain ginsenosides.

Quantitative evaluations of aglycones

In other analysing instructions, the indirect way is recommended and the glycosides are determined by means of their aglycones²⁾. These aglycones will be received by acid hydrolysis of the glycosides. As aglycones, they contain:

Ginsenoside Ro: oleanolic acid (1), after hydrolysis we received it unchanged, ginsenosides Rb₁, Rb₂, Rb₃, Rc, Rd, contain as aglycones: dammar-24-en-3, 12, 20-triol (2) (= 20(S) protopanaxadiol), ginsenosides Re, Rf, Rg₁ and Rg₂: dammar-24-en-3, 6, 12, 20-tetraol (3) (= 20(S) protopanaxatriol). These two aglycones undergo

by hydrolysis a chemical alteration. Under the influence of protons, their unsaturated side chain cyclizes and results in two new compounds with four rings. The artefacts are 20 (R)-panaxadiol and 20(R)-panaxatriol. As investigations by other study groups have shown, these artefacts are the only transformation products of ginsenoside aglycones protopanaxadiol and protopanaxatriol respectively.

It may be that a color reaction with vanillin sulphuric acid (90 %) for panaxadiol, recommended by *Hiai*³⁾, is suitable for the quantitative determination of the pure, separated aglycone. However, it is not characteristic enough since color products with an equal maximum are also formed with panaxatriol and with oleanolic acid. For judging the commercial preparations, however, only statements concerning the percentile portion of glycosides of protopanaxadiol and protopanaxatriol are significant. Additionally, the recommended separation procedure for the fraction giving the color reaction has a lot of stages and, therefore, is not suitable for a quantitative determination.

However, we expected a quantitative NMR spectroscopy to be of greater success. For the content determination we intended to use the acetyl protons of the peracetylated aglycones. This, however, cannot be done. The H of the OH-group at the C-12 of any of the two aglycones is exchanged by D₂O only after an acting for five hours. In these circumstances it seems evident that an acetylation of the C-12 OH-group cannot be obtained because of sterical hindrance. The acetyl signals of the C-3-OAc- and C-6-OAc-groups of the panaxatriol diacetate coincide to a single signal. Therefore, a differentiation between panaxadiol and panaxatriol is not possible by NMR spectroscopy.

Encouraged by the existing equipment of my laboratory, we tried to make a gas chromatographic division of the ginsenosides recognized as being important for application by means of their two aglycones panaxadiol and panaxatriol and to determine their respective portions by integrating the corresponding peak areas. In order to provide a better admission of the glycosides to the hy-

drolysis and to be able to cover the aglycones quantitatively by this, we started from a root powder fully degreased by means of dichloro-methane. In case of ginseng preparations we extracted the ginsenosides with n-butanol saturated with water. Degreased root powder or n-butanol extracts are hydrolyzed with 3N-sulphuric acid/dioxane (6 + 4) for three hours in order to set free the aglycones oleanolic acid, panaxadiol and panaxatriol. All three aglycones are extracted from the hydrolysate by performance with dichloro-methane. Previous tests had proved that, in the gas chromatogram, oleanolic acid and panaxatriol show almost the same retention times.

Therefore, the real value of panaxatriol can only be discovered if oleanolic acid was previously removed from the dichloro-methane extract. According to our experiences, this can be obtained by means of a highly basic anion exchange-resin (Lewatit 600). Agitating the dichloro methane extract with a little quantity of exchange-resin for five minutes will be enough to remove oleanolic acid almost quantitatively (remainder 3-5 %). The quantities of panaxadiol and panaxatriol remain unchanged in this procedure. The ion exchange-resin also removes co-extracted acidic components of the ginseng preparations. They must be separated, because they change the base line of the chromatogram to such an extent that an integration of panaxadiol and panaxatriol becomes impossible. After treatment with ion exchange-resin from dichloro-methane extract of roots and commercial preparations chromatograms were obtained which quantitatively could be evaluated very well.

As an internal standard we chose cholesterol. It is added to the extracts before hydrolysis. After evaporation of the dichloro-methane extract and after separation of oleanolic acid, panaxadiol and panaxatriol are gas-chromatographed as trimethylsilyl ether together with cholesterol. A computer connected to the gas-chromatograph refers to the peak areas of a standard solution which contains defined quantities of cholesterol, panaxadiol and panaxatriol.

One of the analysed (15) commercial ginseng

preparations showed a gas chromatogram, in which the signal of the cholesterol coincided with an unknown substance in the extract. Instead of cholesterol, in this case, morin is suitable as a standard substance. When using morin, the treatment with the exchange-resin has to be done already before adding the standard. Otherwise, morin would be removed again by the exchange-resin because of its slightly acidic reaction.

The values established for panaxadiol and panaxatriol were transferred to the ginsenosides Rb₁ and Re which, in the case of all kinds of ginseng, represent the main ginsenoside in each of the two glycoside groups.

We obtained the hydrolysis rates and the reproducibility of the procedure from the pure ginsenosides Rb₁ and Re. Then, 18.1% panaxadiol

is obtained from the ginsenoside Rb₁, and 22.3% panaxatriol from the ginsenoside Re.

Two different charges of each of the commercial preparations listed in the Table 1 were tested. The indicated values of panaxadiol and panaxatriol represent the mean of three determinations each. These values were transferred to their corresponding amounts of Rb₁ and Re respectively. In this way we were able to draw conclusions from the initial quantities of ginseng root declared or not clearly declared by the producers.

As can be seen from Table 1, the 15 ginseng preparations contain very different quantities of the representative ginsenosides Rb₁ and Re for each 100 g of the declared root powder. This gives reason to the conclusion that different quantities of ginseng roots of different qualities were used

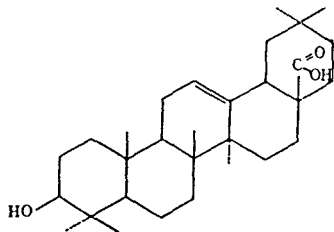
Table 1.

Ginseng-preparations	mg panaxadiol in 100 g preparation	mg Rb ₁ in 100 g preparation	mg Rb ₁ in 100 g root (declart.)	mg panaxatriol in 100 g preparation	mg Re in 100 g preparation	mg Re in 100 g root (declart.)
1	5.2	28.7	672.4	4.8	20.7	485.0
	4.3	23.5	550.6	5.1	21.9	513.1
2	334.6	1,847.0	1,847.0	314.5	1,355.5	1,355.5
	273.6	1,510.3	1,510.3	251.3	1,083.1	1,083.1
3	10.3	56.7	1,312.2	8.5	36.7	849.3
	9.7	53.4	1,235.8	10.6	45.6	1,055.3
4	not to be evaluated					
5	4.4	24.0	1,062.4	3.5	15.2	672.9
	4.3	23.5	1,040.3	4.8	20.8	920.8
6	content below the limit of accuracy					
7	0.5	2.8	no declarat. of the producer	0.7	2.9	no declarat. of the producer
8	not to be evaluated					
9	4.3	23.7	no declarat. of the producer	3.0	12.7	no declarat. of the producer
	6.6	36.5		5.5	23.9	
10	5.3	29.2	534.4	4.6	20.0	366.1
	3.4	18.4	336.8	3.4	14.6	267.2
11	content below the limit of accuracy					
12	11.0	60.7	1,011.7	9.6	41.2	686.7
	26.9	148.5	2,475.0	16.7	72.0	1,200.0
13	not to be evaluated					
14	content below the limit of accuracy					
15	91.0	502.0	426.1	84.0	362.0	307.3
	83.1	458.7	389.4	89.9	387.5	328.9

for the production of the individual commercial ginseng preparations.

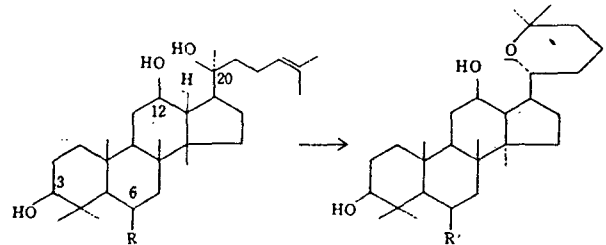
In the interest of maintaining the reputation of vegetable drugs the producers of ginseng preparations are earnestly entreated to choose a phrasing for the declaration that indicates clearly the drug content.

Compared to the procedure of *Sakamoto*²⁾, the reported procedure has some advantages. *Sakamoto* removes the oleanolic acid with diluted sodium hydroxide solution. According to our experience this result shows considerable losses of panaxadiol and panaxatriol. To use dichloromethane instead of ether has the advantage that the silylization of the aglycones is not disturbed by existing water traces. Moreover, a strong separation



1 Oleanolic acid

of phases is obtained with dichloro-methane. As a standard substance, *Sakamoto* only uses the hardly obtainable diacetylhederaenin-methylester which limits the possibilities of copying his procedure by third persons.



2 R' = H = Dammar-24-en-3,12,20-triol → Panaxadiol

3 R' = OH = Dammar-24-en-3,6,12,20-tetraol → Panaxatriol

References

1. Bombardelli, E.; Bonati, A., Gabetta, B., Martinelle, E. M., Mustich, G.: *Fitoterapia* **3**, 99(1976).
2. Sakamoto, J., Morimoto, K., Tanaka, O.: *Yakugaku Zasshi* **95**, 1456(1975).
3. Hiai, S., Oura, A., Hamanaka, H., Odaka, S.: *Planta Medica* **28**, 131(1975).