Ammonia as Extractant and Reactant for Ginsenosides

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Abstract

In different approaches ginsenosides were extracted from Korean ginseng roots by ammonia and for comparison with methanol-water and water. The extracts have been analyzed qualitatively and quantitatively to evaluate yield and selectivity of extractions of ginsenosides. Water supplied the lowest yield. The yields of extracts with liquid ammonia were higher than those with methanol-water. However, this is partly due to the conversion of malonyl ginsenoside to normal ginsenosides by ammonia. It was proved by HPLC that malonyl-ginsenosides m-Rb$_1$, m-Rb$_2$, m-Rc and m-Rd were converted to the corresponding neutral ginsenosides. Furthermore, ginsenosides from ginseng roots were extracted by alkaline methanol-water (60%) solutions. Alternatively, the extracts of the methanol-water (60%) extraction were treated with sodium hydroxide solution. Both methods also convert the malonyl-ginsenosides to neutral ginsenosides.

Introduction

Ginsenosides or triterpene-saponines are considered to be the compounds of the ginseng plant responsible for the pharmaceutical effects. The ginsenoside are on a analytical scale to separate and to identify by High Performance Liquid Chromatography (HPLC), Thin Layer Chromatography(TC) or Gas Chromatography (GC). These saponines are soluable in polar solvents and are usually extracted by alcohols or by water. However, these extraction processes are of limited selectivity and more over often the solvent has to be separated from the extract by an additional process. These are the reasons why in many analog cases the new technique of "supercritical fluid extraction" (SFE) or extraction with compressed gases mostly using carbon dioxide as a solvent has been tried. The polar ginsenoside can practically not be solved and moved by dry or even humid carbon dioxide [1]. Even opposite carbon dioxide can be used to extract pesticides from ginseng materials without touching the valuable components. However, ammonia can be

used as an extractant. NH_3 is a good solvent, particularly for nitrogen-containing compounds, and has a still convenient critical point (T_c = 132.24°C, P_c = 113.53 bar, V_c = 72.8 cm^3 mol.) Hence ammonia is also one of the compounds suggested as a mobile phase for chromatographic processes [2]. Ammonia is fairly reactive and easily it can damage natural products as e.g. hops or coffee. Fortunately ammonia is not attacking the neutral ginsenoside as proved by our analysis. We have used ammonia at sub-critical conditions mostly at around 333°C corresponding to a pressure of around 26 bar. The supercritical condition of 418 K and 130 bar leads to a decomposition of the ginsenosides.

Materials

The roots of the ginseng plant (Araliaceae, *Panax ginseng* C.A. Meyer) have been bought 1998 in Taegu, Korea. The age of this material was 5 years. The natural humidity was 3% by weight. The particle diameter of the milled roots was between 0.063 and 0.63 mm.

We used the ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rg_1 and Rf supplied by Roth (Karlsruhe/Germany) as standards. Ammonia (supplied by Messer-Griesheim) was better as 99,8% pure. The water used was bidistilled and has had an electrical conductance of 1.6 µS/cm. Methanol for analysis was supplied by J. T. Baker. KOH and NaOH for analysis was bought from Riedel-de Haën.

Extraction and Analysis

For the ammonia extraction an apparatus of the Jennings-type [3] has been used. However, the apparatus has been modified in some important features, the main modification being the construction of window [4] [5] to control the correct function of the apparatus through visual observation of the condenser and the siphon. In addition the vapor line of the Soxhlet apparatus has been removed, since this line is necessary only on an open laboratory bench but not in a sealed autoclave. By omitting this line, the space available within the autoclave can be used more efficiently.

The high pressure part of the apparatus consists of a cylindrical autoclave (i.d. 63 mm, o.d. 80 mm height of the cylinder 340 mm, volume 935 cm^3) from stainless steel (German code 1.4571) with closures at bottom and top. In the cylinder walls sapphire windows were placed in order to

allow the observation of the condenser, the siphon and the flask. The light of a small electric lamp was passed through on of the windows not used for the actual visual observations. The windows are from synthetic sapphire (h 10 mm, d 12 mm) sealed by an o-ring. The top closure contains the cooling finger and the high pressure tubes connecting a gauge and a valve. the valve is used for loading and discharging the compressed solvent.

The inner extraction apparatus is built completely from glass. It consists of an extraction thimble with a siphon for the periodical removal of the extractant and extract and a separate glass vessel serving as reservoir for the evaporating extractant and for the storage of the extract. This glass vessel must have a volume larger than the volume of the extractant delivered periodically by the siphon.

The apparatus has to operate in the two phase region below the critical temperature. The working temperature is most easily detected and controlled by the operating pressure. The temperature corresponding to the operating pressure can be read off from the well known vapor pressure curve of ammonia. The temperature is mainly influenced by the temperature of the coolant circulating in the cooling finger. In Germany the water available from the normal water tape can be used as coolant. Small changes in this temperatures will also change the extraction conditions. For using lower temperature cooling equipment or a ice-water bath have to be used. The bottom of the apparatus was kept in a water bath at at higher temperatures in order to ensure the evaporation of the extractant.

In different experiments ginseng has also been extracted in pressure resistant hydraulic tubes and the extracts can be analyzed by conventional methods. The elevated pressure is indicated by a small gauge and after extraction reduced to normal pressure by a small capillary, which can be connected to be bulk volume of a syringe in order to collect the extract in this syringe. The extract is then transferred into an analytical apparatus [6].

Extraction at normal pressure with methanol-water (60% methanol) at temperature between 333 and 348 K and with water at 373 K were carried out in a normal Soxhlet apparatus.

Our analytical methods have developed from simple thin-layer chromatography [1] and high-performance thin-layer chromatography (HPTLC) [7] to HPLC with UV-detection [8] and HPLC with MS-detection [9] just following the development of analysis as described in literature.

Results

An important result of an extraction process is the yield. The extraction with water has a yield

from only ca. 5% w/w, with 60% - methanol 13% w/w and with liquid ammonia 16% w/w. The extraction-time was 4 hours in all cases. The yields of the summarized ginsenosides Rb_1, Rb_2, Rc, Rd, Re, Rf and Rg_1 follow the same schema and are 0,5% (water), 1,6% (60% methanol) and 2,7 % (ammonia).

The HPLC-chromatograms of a 60%-methanol-extracts show the neutral ginsenosides and in addition four components. These additional components are not present in the ammonia-extracts. The explanation of these results is the existence of malonyl-ginsenosides in the methanol-extracts. Ammonia transforms the malonyl-ginsenosides to neutral ginsenosides leading to a higher concentration of the neutral ginsenosides Rb_1, Rb_2, Rc and Rd in the ammonia extract.

We have had no standards for the malonyl-ginsenosides. Hence we have used the graphical method in plotting the difference in the retention-time of the normal ginsenosides to the still unidentified malonyl-ginsenosides against the retention-time of the ginsenosides or of the unknown components. Both plots give straight lines and have the same intersect of 2,9 minutes.

The ammonolysis of the malonyl-ginsenosides may be important if the neutral ginsenosides have a higher pharmaceutical importance. The malonyl-ginsenosides are roughly in the same order of magnitude present in the ginseng plant as the neutral ginsenosides. That means that the ammonolysis increases the amount of the neutral ginsenosides remarkable. Ammonia is easily to evaporate and if necessary compounds of the malonic acid could be removed e.g. by a reversed phase material.

We let the 60%-methanol extracts react with NaOH-, KOH- or NH_3- solutions and analyzed the products with HPLC. In all cases the pH-value decreases with the reaction time and also the areas of the peaks of the malonyl-ginsenosides decrease. After 24 hours we could not detect any malonyl-ginsenosides. The neutral ginsenosides are stable in contact with strong alkaline solutions.

The different extractions demonstrate that the yields of ginsenosides depend from the time of extraction using the same extractant and depend also from the extractant at comparable times of extraction. This are possibilities of fractionating of the ginsenosides. As a rule for all extractants in the first period of the extraction the panaxatriols Re, Rg_1 and Rf are better transported as the panaxadiols Rb_1, Rc, Rb_2 and Rd. These diols are extracted much better with ammonia than with water.

Finally with the HPLC/MS-analysis we could show that in the ammonia-extracts there are still small quantities of malonyl-ginsenosides not to detect by the HPLC/UV-method. Also with the HPLC/MS-method we could identify the small quantities of the ginsenoside Ro in the extracts.

Literature

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