QUANTITATIVE DETERMINATION OF THE TOTAL LACTONES AND TAUREMISIN OF ARTEMISIA TAURICA

N. A. Kechatova, P. M. Loshkarev, and K. S. Rybalko

Khimiya Prirodnykh Soedinenii, Vol. 3, No. 2, pp. 83-86, 1967

With the introduction into medical practice of tauremisin (a new medicinal substance), the necessity has arisen for developing a method for its quantitative determination in plant raw material.

Tauremisin is a sesquiterpene lactone of the endesman type; its empirical formula is $C_{15}H_{20}O_4$, mp 176°-177° C (from alcohol), $[\alpha]_D^{20}$ +42.7° (c 6.8; chloroform) [1, 2].

The raw material for the production of tauremisin is the epigeal part of A. taurica consisting of small downy leaves, small (3.5 mm long and about 2 mm wide) flower heads, and thin green sprigs. The latter contain no tauremisin, but it is difficult to separate them and therefore they are included in the composition of the raw material according to MRTU-42 No. 3377-65.

In addition to tauremisin, two other sesquiterpene lactones have been isolated in small amounts from A. taurica, mibulactone [3] and lactone x, which we are now studying.

Table 1

Influence of the Time of Extraction on the Content of Total Lactones

Extraction period, min.	Content of total lactones in the absolutely dry raw material, %	
	expt. 1	expt. 2
30	1.493	1.486
60	1.516	1.498
90	1.366	1.350
120	1.351	1.343

We have developed a gravimetric method for the quantitative determination of tauremisin in the plant raw material which consists of extracting the total alkaloids from the raw material with hot water with subsequent isolation of the tauremisin. Experiments showed that a single treatment of the raw material with water in a ratio of 1:20 at 80° C for 1 hr ensured a sufficiently complete extraction of the lactones (Table 1); on more prolonged extraction, they underwent decomposition.

Water extracts not only the lactone but also other materials including many substances of an acidic nature. To separate the latter we have made use of the lactone characteristic of not being saponified by cold alkalies; the acid substances of the extracts readily form water-soluble salts and, therefore, are not extracted with chloroform from aqueous solutions, while the lactones are readily extracted. The removal of the chloroform by distillation gave a yellowish brown crystalline residue with a high lactone content. A quantitative determination of the total lactones was carried out acidimetrically after the lactones had been saponified with alkalies.

Table 2

Determination of Tauremisin in the Raw Material

Sample number	Repetition	Tauremisin content, % of the absolutely dry raw material	Mean content	Deviation from the mean,	Mp of the crystalline substance, °C
1	1	0.433	0.429	±2.33	170—172
	2	0.437			170—172
	3	0.419			170—172
2	1	0.308	0.322	±4.64	170—172
	2	0.323			170—172
	3	0.337			170—172
3	1	0.417	0.420	±0.95	171—172
	2	0.424			170—172
4	1	0.239	0.232	±3.45	—
	2	0.228			
	3	0.234			
	4	0.235			
	5	0.224			
	6	0.234			
	7	0.230			

The experiments on the determination of tauremisin in the raw material were carried out with a sample of the herb A. taurica collected in the budding stage in various regions of the Caucasus and the Crimea (Tables 2 and 3).

It can be seen from Table 2 that the agreement of parallel determinations is completely satisfactory. To investigate the relative error of the method, the tauremisin in the raw material was determined after additional amounts had been added. The results of Table 3 show that the deviations from the mean do not exceed 5%.

The proposed method has been included in the technical documentation of the production of tauremisin and has been used to develop and carry out a stepwise check on the production of this material.

Table 3

Results of Determinations of Tauremisin in the Raw Material with Additives

Weight of the raw material	Tauremisin found before addition	Pure tauremisin added	Total tauremisin	Tauremisin found after addition	Deviation	
g					g	%
100	0.4103	0.1563	0.5666	0.5581	0.006	−1.4
100	0.4103	0.1875	0.5978	0.5878	0.010	−1.7
100	0.4103	0.2087	0.6190	0.6295	0.010	+1.6

Experimental

Determination of the total lactones. A weighed sample of the comminuted raw material (25.0 g) was covered with 500 ml of water heated to 80° C and infused at this temperature for 1 hr. 300 ml of the aqueous extract was filtered through absorbent cotton, cooled to room temperature, and brought to pH 9 with a 5% solution of sodium carbonate (using universal indicator solution). The aqueous extract was then shaken five times with chloroform in a 1:10 ratio for 10 min each time. The chloroform extracts were combined and washed twice with distilled water, the final wash water being neutral. The chloroform was distilled off and the residue was dissolved in 15 ml of alcohol and treated with 3–4 drops of phenolphthalein solution, after which 0.1 N caustic soda solution was added dropwise until a red coloration appeared (neutralization of the phenols and the acidic substances of the alcohol), after which another 15 ml of 0.1 N caustic soda solution was added, and the mixture was heated on a boiling water bath for 3 min to saponify the lactones. The excess of caustic soda was back-titrated with 0.1 N hydrochloric acid until the color of the indicator disappeared. 1 ml of 0.1 N caustic soda solution corresponds to 0.02643 g of total lactones (calculated as tauremisin). A blank experiment was carried out concurrently.

To determine the tauremisin in the raw material quantitatively, the total lactones were extracted by the method described above. From the total lactones obtained, the tauremisin was separated by means of ether, in which it is practically insoluble, while the accompanying lactones and other substances passed into the ether. To separate the tauremisin more completely, it was necessary to eliminate the chloroform completely, which was done by drying the total lactones in a drying oven at 80° C for 6–7 hr.

Determination of tauremisin in the raw material. One hundred grams of the comminuted raw material was covered with 2 *l* of distilled water heated to 80° C and infused at this temperature for 1 hr. 1600 ml of the aqueous extract was filtered through absorbent cotton, cooled to room temperature, and brought to pH 9 (using universal indicator solution) with a 5% solution of sodium carbonate (approximately 75–80 ml was used). After this, the aqueous solution was extracted five times with chloroform (160, 100, 100, 80, 60 ml) for 10 min each time. The chloroform extracts were combined and washed with two 100-ml portions of distilled water, the last washing being neutral (test with litmus), and filtered. The filtrate was washed with a small amount of chloroform, and the chloroform was distilled off from the water bath in vacuum to give a volume of 15–20 ml. It was then transferred quantitatively to a weighed 30–50 ml flask and distilled until the chloroform had been completely eliminated.

The resin obtained was dried at 80° C (not above) for 7 hr. The residue was treated with 1.5 ml of ether and the resin was dissolved in it with gentle shaking. After about 30 min, crystals of tauremisin deposited; they were transferred to a weighed No. 4 glass filter and the flask was rinsed with small portions of ether, (2, 5, 5, and 5 ml) which were used to wash the residue on the filter. The flask and the filter were dried at 100°–105° C for 1 hr, weighed, and the melting point of the material determined; it must not be lower than 169° C.

The percentage of tauremisin in the raw material (X %) was calculated from the formula

$$X = \frac{a \cdot 100}{b \cdot 0.8},$$

where a is the amount of crystalline substance on the filter and in the flask, g, b is the weight of raw material, g, and 0.8 is the aliquot factor.

Summary

A quantitative gravimetric method for determining the tauremisin in the herb Artemisia taurica has been proposed. The method has been used to evaluate the quality of the raw material and for monitoring the individual stages of the production of this substance.

REFERENCES

1. K. S. Rybalko, A. I. Ban'kovskii, and M. E. Perel'son, Med. prom. SSSR, no. 10, 21, 1960.
2. K. S. Rybalko and L. Dolejs, Collect. Czechosl. Chem. Communs, 2909, 1961.
3. K. S. Rybalko, KhPS [Chemistry of Natural Compounds], 142, 1965.

23 August 1966

All-Union Scientific-Research Institute for Medicinal Plants