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POLAROGRAPHIC DETERMINATION OF TAUREMISIN IN ARTEMISIA TAURICA

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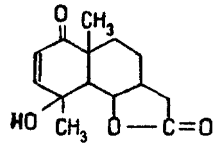
The main pharmacologically active substance in Artemisia taurica Willd. (Tauric wormwood), which has been called tauremisin [1, 2], is a sesquiterpene lactone with the composition $C_{15}H_{20}O_4$ and the structure 4-hydroxy-1-oxo-eudesm-2-en-6,13-olide.



Since tauremisin possesses cardiotonic properties [3], the necessity has arisen for developing a method for its quantitative determination in plant raw material.

A gravimetric method proved unsuitable. Consequently, we have sought a simpler and more reliable method for routine determinations. We have found that tauremisin is capable of undergoing reduction at a dropping mercury cathode (under various conditions) with the formation of a clear polarographic wave (Fig. 1). However, it was necessary to ascertain the possibility of determining tauremisin directly in an aqueous extract of wormwood. It has been found experimentally that when a mixture of the aqueous extract, acetone, and 2 N hydrochloric acid in a ratio of 2 : 1 : 0.5 is polarographed, a clear maximum of tauremisin with a half-wave potential $E_1/_2$ = −1.0 V with respect to SCE is formed on the derivative chromatogram (Fig. 2). This mixture was used in subsequent experiments on the quantitative determination of tauremisin.

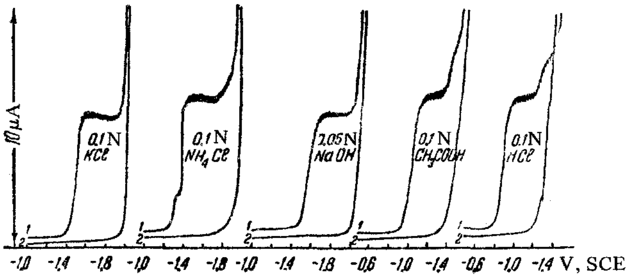


Fig. 1. Polarograms: 1) 1.13 × 10^{-3} M solution of tauremisin in water with supports of various electrolytes; 2) polarograms of the corresponding supports.

Tauremisin was determined polarographically from a calibration graph. The accuracy of the method was checked by an analysis of extracts with the addition of the pure material of them. The results of the analysis of the raw material with additions of the pure material are as follows:

Tauremisin, mg			
Content in the raw material	Added	Found	Relative error, %
5	2.1	7.3	2.8
5	4.2	8.8	4.3
5	6.3	11.6	2.6

The reproducibility of the method was determined specially and amounted to ± 4.13% rel.

In addition to tauremisin, Tauric wormwood contains two other sesquiterpene lactones: mibulactone, with the composition $C_{15}H_{22}O_4$, mp 229-231° C, $[\alpha]^{20}_D$ +166° (c 1.2; ethanol), and a lactone of undetermined structure [4]. These substances, and also the essential oil of Tauric wormwood, do not form polarographic waves under the conditions of the reduction of tauremisin (Fig. 3), which confirms the possibility of determining the latter directly in an aqueous extract. For a definitive study of the influence on the polarogram of tauremisin of substances corresponding to it, a method for the chromatographic separation of extract on a thin nonfixed layer of alumina in the benzene–methanol (9 : 1) system was developed. Under these conditions R_f for mibulactone is 0.35, for tauremisin 0.51, and for the third lactone 0.90.

To study the completeness of the elution of tauremisin from alumina, various solvents were used: acetone, methanol, ethanol, mixtures of these solvents with 1 N hydrochloric acid, and solutions of hydrochloric acid of various concentrations. Complete elution (~100%) was achieved when the alumina was heated with 1 N hydrochloric acid in the boiling water bath.

In this case, the calibration curve was plotted from the points obtained in the chromatography of a solution of standard tauremisin. The standard tauremisin had mp 176–177° C, $[\alpha]^{20}_{D}$ +42.7° (c 6.8; chloroform), and was chromatographically homogeneous. The accuracy of the determination of tauremisin using chromatography was checked by analyzing the raw material with the addition of the standard samples.

The reproducibility of the method with preliminary chromatography was ±4.48% rel.

The results of a check on the accuracy of the method using thin-layer chromatography were as follows:

Tauremisin, mg Content in the raw material	Added	Found	Relative error, %
5.7	2.1	8.1	3.8
5.7	4.2	9.6	3.1
5.7	6.3	12.2	1.6

Samples of crude Tauric wormwood of various forms (typical, spreading, and broad-pannicled) collected in the stem-growth, budding, flowering, and fruiting stages in the region of the village of Tolstov Yurty, Checheno-Ingush Autonomous Soviet Socialist Republic were analyzed by the two variants of the polarographic method described. The results of the analysis were compared with those obtained by the gravimetric method. In the isolation of tauremisin by the gravimetric method, losses of substance are possible because of its incomplete extraction from the mother solution, and therefore a considerably larger amount of it is found by the polarographic method. On comparing the results of the analysis between the two variants of the polarographic method, we found no appreciable discrepancies.

Wormwood of the provenance described above contains substances similar in structure to tauremisin which do not interfere with the polarographic determination. However, because of the possibility of the polymorphism of the plant, deviations in its chemical composition are possible which may affect the reliability of the analytical determination of tauremisin. Consequently, in the analysis of samples of wormwood with a known chemical composition it can be recommended to carry out the polarographic determination directly on the aqueous extract but in other cases to use thin-layer chromatography.

Experimental

The work was carried out on a "Radiometer" (Denmark) PO-4 polarograph.

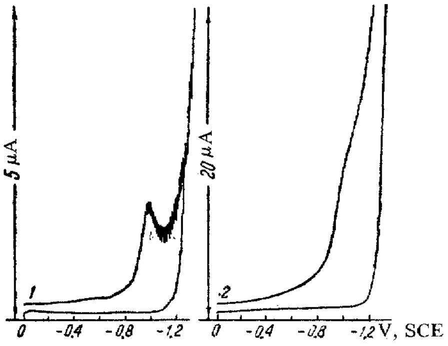


Fig. 2. Derivative (1) and ordinary (2) polarograms of a mixture of an aqueous extract of a sample containing 0.62% of tauremisin with acetone and 2 N hydrochloric acid (2 : 1 : 0.5).

Procedure for the polarographic determination of tauremisin in an aqueous extract. About 1 g (accurately weighed) of an average sample of the raw material was placed in a flask with a capacity of 30–40 ml, 10 ml of water was added with a pipet, and the mixtue was heated in the boiling water bath under reflux for 15 min. After the flask had been cooled to room temperature, with a pipet 2 ml of the extract, 1 ml of acetone, and 0.5 ml of 2 N hydrochloric acid were transferred to the electrolyzer, the mixture was purged with a current of nitrogen for 5 min, and the derivative polarogram was recorded in the range of voltages from -0.5 to -1.1 V SCE. The temperature of the solution was kept constant at the temperature for which the calibration graph had been plotted. The height of the polarographic wave was measured and the percentage of tauremisin in the raw materials studied was determined from the calibration graph. The time for an analysis was 30–40 min.

Construction of the calibration graph. Accurately weighed samples of 20 and 30 mg of standard tauremisin were dissolved in water in 25-ml measuring flasks (standard solution). Two series of solutions of the following dilutions were prepared:

1) 2 ml of the standard solution, 0 ml of water, 1 ml of acetone, and 0.5 ml of 2 N hydrochloric acid;

2) 1.5 ml of the same, 0.5 ml of water, 1 ml of acetone, and 0.5 ml of 2 N hydrochloric acid;

3) 1.0 ml of the same and 1.0 ml of water, 1 ml of acetone, and 0.5 ml of 2 N hydrochloric acid;

4) 0.5 ml of the same and 1.5 ml of water, 1 ml of acetone, and 0.5 ml of 2 N hydrochloric acid;

The prepared solutions contained, for the 20 mg sample, 2, 4, 6, and 8 mg of tauremisin in 10 ml of water or, respectively, 0.2, 0.4, 0.6, and 0.8% of tauremisin, and, for the 30 mg sample, 3, 6, 9, and 12 mg in 10 ml of water or respectively, 0.3, 0.6, 0.9, and 1.2% of tauremisin in a gram of the raw material analyzed. Each solution obtained was polarographed under the conditions of the method of determination and a calibration graph was constructed by plotting along the axis of abscissas the content of tauremisin (mg) in 10 ml of water or the corresponding percentage of tauremisin in 1 g of raw material and the height of the wave (mm) along the axis of ordinates.

Procedure for the polarographic determination of tauremisin in eluates from chromatograms. About 1 g (accurately weighed) of the comminuted raw material (0.5-mm sieve) was extracted with 96% ethanol in an apparatus of the Soxhlet type for 3 hr. The extract was evaporated to a volume of 3-5 ml, cooled and filtered into a 10-ml pycnometer. It was chromatographed on a nonfixed layer of neutral alumina (Brockmann activity grade IV) on 8 × 15 cm plates. The extract (0.05 ml) was deposited by means of a micropipet on the plate in a continuous band and adjacent to it was placed a standard ethanolic solution of tauremisin. Chromatography was carried out in the benzene–methanol (9 : 1) system. After chromatography, the chromatogram to be analyzed was covered with a glass plate and only the zone of the standard solution of tauremisin was developed in the moist state with a 0.5% solution of potassium permanganate in 0.5% sulfuric acid. Then the limits of the tauremisin zone were marked on the chromatogram at the level of the band that had appeared.

For greater certainty, the chromatogram to be analyzed was developed above and below the assumed tauremisin zone, and where a band appeared beyond the limits marked out the chromatogram was rejected. The sorbent with the tauremisin was transferred quantitatively into a 10-ml flask, 3 ml of 1 N hydrochloric acid was added and elution was carried out in the boiling water bath for 15 min with constant vigorous shaking. After standing for 5-10 min and cooling, the solution was poured into the electrolyzer and polarographed as described above. The time for an analysis was 5-6 hr.

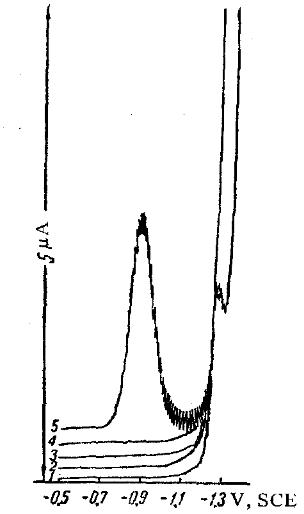


Fig. 3. Derivative polarograms: 1) mixture of water, acetone, and 2 N hydrochloric acid (2 : 1: 0.5) (support); 2) 20 mg of the essential oil of Tauric wormwood dissolved in 25 ml of the supporting solution; 3) 1.87 · $\cdot 10^{-3}$ mole of mibulactone; 4) 2 mg of the third lactone in 4 ml of the supporting medium; 5) 1.89 · 10^{-3} mole of tauremisin.

Construction of a calibration graph for the determination of tauremisin in the eluates from chromatograms. A 50-mg sample (accurately weighed) of standard tauremisin was dissolved in ethanol in a 25-ml measuring flask (ethanolic solution). The following amounts of the (ethanolic) solution were deposited with a pipet on alumina plates: 0.005, 0.010, 0.015, 0.020, 0.025, 0.030, and 0.035 ml, or 10, 20, 30, 40, 50, 60, and 70 γ of tauremisin, respectively, which corresponds to 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4% of tauremisin per gram of the raw material analyzed. The subsequent procedure was as described above.

Conclusions

A polarographic method has been developed for determining tauremisin in Artemisia taurica Willd. directly in aqueous extracts or in eluates after chromatography on a thin layer of alumina.

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