

# Polarographic determination of tropolones

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The total amount of tropolones in the presence of thymol can be determined by D.C. polarography. The content of stipitatic acid in the presence of stipitatic acid can be determined voltammetrically. The amount of stipitatic acid can be determined from the difference in the total amount of tropolones and that of stipitatic acid. The possibility to use A.C. polarography has also been investigated.

Tropolones, *i.e.* stipitatic acid (*I*, 3,6-dihydroxy-5-oxo-1,3,6-cycloheptatriene-1-carboxylic acid) and stipitonic acid (*II*, 3,6-dihydroxy-5-oxo-1,3,6-cycloheptatriene-1,2-dicarboxy anhydride) can be spectrophotometrically determined at 277 nm. By combining spectrophotometry and spectrofluorimetry, the content of *I* and *II* in the mixture was determined [1]. Another method takes advantage of the solubility of *II* in acidified chloroform by the use of which this substance can be selectively extracted. From the difference in the total amount of tropolones before the extraction and the amount of *I* after the extraction, the amount of *II* can be calculated [2]. Electrochemical properties of tropolones have been investigated by Zuman *et al.* [3] and Šantavý *et al.* [4] who confined their studies to the elucidation of the electrode reaction of tropolones. Since the tropolones *I* and *II* occur mostly together we have investigated the possibility to use polarography for the determination of these substances in the mixture.

## Experimental

### *Instruments and materials*

The instruments used were D.C. polarograph LP-7 (Laboratorní přístroje, Prague), D.C. polarograph OH-102 and A.C. adapter OH-993 (Radelkis, Budapest). Compounds *I* and *II* were made by purification of crude products obtained by cultivation of *Penicillium stipitatum* THOM and were characterized by melting points and u.v. spectra [5, 6]. Other chemicals were commercial products (reagent grade) used as supplied.

### *D.C. polarographic studies*

The measurements were performed in an acetate buffer in the presence of thymol (0.001%) as a surfactant at 22.0°C. Depending upon the pH, compounds *I* and *II* give 1–4 waves. At pH 4.72 both compounds are reduced at  $E_{1/2} = -1.05$  V and give a single wave. A linear dependence of the wave height upon the concentration has been observed.

*Voltammetric measurements*

Methanol (2.0—A ml) was added to a stock solution of *I* or *II* (A ml, 1 mg/ml) in methanol and filled up to 10 ml with 0.3 N sodium hydroxide. The derivative voltammetric curve was recorded at a scan rate of 200 mV/min, sensitivity 0.5  $\mu$ A and 22°C. All measurements were done using a platinum electrode. The reproducibility of the voltammetric curve-recording (0.47%) was determined statistically taking into account the wave heights from 10 runs under identical conditions. The total error of the determination of *I* and *II* in the concentration range 10—200  $\mu$ g/ml was about 2.80%. Voltammetric curves of a mixture of *I* and *II* (prepared by mixing A ml of a stock solution of *I* with 2.0—A ml of the solution of *II* in methanol and filling the obtained solution up to 10 ml with 0.3 N sodium hydroxide) were recorded under the same conditions. The wave height of *I* at  $E_{1/2} = +0.49$  V corresponds to the wave height read from the calibration curve with the same reproducibility and with the same total error when the excess of *I* in the mixture with *II* is in the range of 5.8 : 1—1.9 : 1 (w/w), the concentration being 10—200  $\mu$ g/ml (Tables 1 and 2).

Table 1

Voltammetric dependence of the wave height *h* upon the concentration of *I* and *II*

<i>n</i>	<i>c</i> [ $\mu$ g/ml]	<i>I</i>		<i>II</i>	
		<i>h</i> [mm]	$E_{1/2}$ [V]	<i>h</i> [mm]	$E_{1/2}$ [V]
1	200	232	+0.50	150	+0.41
2	175	221	+0.50	137	+0.42
3	150	197	+0.49	122	+0.42
4	125	170	+0.49	105	+0.41
5	100	150	+0.49	92	+0.39
6	75	118	+0.49	76	+0.39
7	50	84	+0.49	61	+0.39
8	25	54	+0.49	37	+0.39
9	10	23	+0.48	19	+0.39

Table 2

Wave height *h* and the total error  $\Delta I$  in a mixture with *II*

<i>n</i>	$c_I$ [ $\mu$ g/ml]	$c_{II}$ [ $\mu$ g/ml]	$c_I : c_{II}$	<i>h</i> [mm]	$\Delta$ [%]
1	75.0	12.9	5.8 : 1	130	2.78
2	100.0	25.8	3.9 : 1	154	2.77
3	87.5	25.8	3.4 : 1	134	2.84
4	75.0	38.8	1.9 : 1	120	2.80
5	50.0	51.7	0.97 : 1	92	5.46

*A.C. polarographic measurements*

The stock solution of *I* and *II* was made by dissolving *I* (11 mg) in 0.025 N sodium hydroxide (1 ml) and making the solution up to 10 ml with 0.4 N acetic acid. Solutions of the wanted concentration were made by dilution of the stock solution with 0.4 N

acetic acid. A.C. polarographic waves were recorded at the frequency of 60 Hz and superposed A.C. potential of 10 mV at 22.0°C. Compound *I* gives a double wave with the peak potentials  $E_{s1}$  and  $E_{s2}$ ; compound *II* gives a single wave. A linear concentration dependence of the wave height with  $E_{s2} = -0.86$  at *I* and  $E_s = -0.50$  at *II* has been observed. The reproducibility of the recording was in both cases 0.82% with an approximate total error 3.50%. A.C. polarographic waves of the mixture of *I* and *II* were measured under identical conditions. The wave height in *I* at  $E_{s2} = -0.86$  corresponds to the wave height read from the calibration curve with the same error when the excess of *II* in the mixture with *I* is in the range 9.43 : 1–1 : 1 (w/w) in the concentration region 10–110 µg/ml.

### Discussion

Stipitatic (*I*) and stipitatonic (*II*) acids can be reduced polarographically under acidic conditions or voltammetrically oxidized under alkaline conditions. D.C. polarography can be used for the determination of both these substances as a sum of the total amount of tropolones, the measurement being done in the presence of thymol as the surfactant. The pH most suitable for the determination is around 4.7 under which conditions a single wave of  $E_{1,2} = -1.05$  is formed in the case of both substances. It is important to keep the constant temperature during determination since the reduction of tropolones is a kinetic-controlled process [3]. In A.C. polarography at pH 2.73–6.11 and the frequency of 60 Hz, *I* gives a double wave only one of which (at  $E_{s2} = -0.86$  V) is concentration dependent, whereas the other one is induced by adsorption phenomena. The presence of *II* manifests itself by a single wave having at pH 2.73  $E_s = -0.50$  V, which is superimposed with the adsorption wave of *I* when this substance is also present in the analyzed mixture. In a mixture of *I* and *II* containing a high excess of *II*, substance *I* can be determined by A.C. polarography with the total error of 3.47%. More advantageous is the use of voltammetric oxidation on a platinum electrode. To achieve better separation of waves, derivative circuit was applied. In this manner compound *I* and *II* could be independently determined in a concentration range of 10–200 µg/ml with a recording reproducibility about 0.47% and total error 2.8%. Substance *I* can be determined in the mixture with *II* with the same accuracy when the former is present in an excess of 5.8 : 1–1.9 : 1 (w/w). For practical purposes it is more advantageous to use voltammetry, since naturally occurring materials usually contain mixtures of *I* and *II* where *I* predominates by a factor of 2–3.

The fact that *I* and *II* can be reduced only under acidic conditions, or oxidized only under alkaline conditions suggests that the mechanism of the electrode reaction of *I* and *II* is the same as proposed for the tropolone itself [3].

### References

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