

SPECTROPHOTOMETRIC METHOD OF ANALYZING
CARDENOLIDES OF THE STROPHANTHIDIN
GROUP WITH 2,2',4,4'-TETRANITROBIPHENYL

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One of the factors responsible for the development of colorimetric methods of analyzing cardenolides is the use of highly sensitive chromophoric reagents.

Recently, the reaction with 2,2',4,4'-tetranitrobiphenyl (TNBP) has been proposed for detecting Digitalis cardenolides [1, 2]. In the present paper we show the possibility of using this reagent for the analysis of the cardenolides of the strophanthidin series.

2,2',4,4'-Tetranitrobiphenyl was synthesized by the Ullmann reaction [1]. The influence of the concentrations of the alkali and of the reagent on the adsorption maximum (D_{\max}) of the complex in the determination of cardiac glycosides has been investigated (Table 1).

It can be seen from Table 1 that the optimum concentration of the reagent is a 0.15 ethanolic solution of TNBP and the optimum concentration of alkali is a 0.15 N aqueous solution of caustic potash. With a further increase in the concentration of either the reagent or the alkali, the stability of the complex falls sharply.

Under the influence of light, D_{\max} of the TNBP-glycoside-alkali complex decreases. Thus, if this complex is kept in the light for 15 min, D_{\max} falls by 25.8%. Consequently, in determining the amount of cardiac glycosides after the addition of the alkali the solution must be placed immediately in the chamber of the spectrophotometer or be stored in the dark.

TABLE 1. Influence of the Concentrations of TNBP and of Alkali on D_{\max} of the Absorption of the TNBP-Glycoside*-Alkali Complex

Magnitudes measured	Concentration of TNBP, %					
	0,01	0,075	0,1	0,15	0,2	0,3
D_{\max}	0,137	0,365	0,367	0,368	0,368	0,370
ϵ	11358	26317	26446	26518	26518	26817
Δt , min	∞	17	17	9	7	3
t_E , min	80	36	25	19	13	15

Magnitudes measured	Concentration of alkali, N					
	0,01	0,05	0,1	0,15	0,2	0,3
D_{\max}	—	—	0,366	0,367	0,367	0,370
ϵ	—	—	26389	26446	26446	26677
Δt , min	—	—	14	12	5	2,5
t_E , min	∞	∞	46	17	15	15

* Concentration of olitoriside 0.0248 mg/ml.

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TABLE 2. Comparative Results of the Reactions of Cardiac Glycosides of the Strophanthidin Series with TNBP and DNDPS

Glycosides	M	TNBP				DNDPS			
		ϵ	$E_{1\%}^{1\text{cm}}$	t_E	Δt	ϵ	$E_{1\%}^{1\text{cm}}$	t_E	Δt
				min				min	
Strophanthidin	404,51	26460	254,2	18-24	8-10	24650	610	3,5	1,5
Cymarín	570,83	26360	455	18-20	8-10	24800	427	4	1,5
Erysimin	570,69	26320	461	18-22	8-12	24800	474,7	4	1
k-Strophanthin- β	728,84	26360	361,6	18-23	12-13	24800	340	4,5	1,5
Erysimoside	714,82	26330	368,6	20-22	10-15	24400	341	5	2
Oltoriside	714,82	26420	369,6	22-25	9-12	24800	347	5	2

Note. The figures for the reaction with dinitro diphenyl sulfone (DNDPS) were taken from the literature [3].

The results of experiments on the determination of the cardenolides of the strophanthidin series by reaction with TNBP (averages of 10-12 determinations) are given in Table 2.

It can be seen from Table 2 that the molar extinction coefficient (ϵ) of the cardenolides of a given aglycone with TNBP, as with DNDPS, does not depend on the size and presence of the sugar moiety.

With the use of TNBP, ϵ for the cardenolides increases by 10% as compared with DNDPS. TNBP is also characterized by a longer time of constancy of D_{\max} (Δt 8-12 min) as compared with 1-2 min for DNDPS, which facilitates the measurement and permits the quantitative determination of the cardiac glycosides to be performed on a spectrometer, which increases the sensitivity of the method as compared with the photocolometric method.

EXPERIMENTAL

The work was performed on an SF-4 spectrophotometer (λ_{\max} 610 nm) using TNBP with mp 166-167°C (the results of microanalysis corresponded to the calculated figures).

A 0.15% ethanolic solution of TNBP and a 0.15 N aqueous solution of KOH were prepared. The solution of the cardenolides (chromatographically homogeneous) were prepared in spectroscopic ethanol with concentrations of 0.02-0.05 mg/ml (observance of the Bouguer-Lambert-Beer law). The cardenolide constants were determined by a known method [1, 3, 4].

CONCLUSIONS

The possibility of analyzing the cardenolides of the strophanthidin series with 2,2',4,4'-tetrinitro-biphenyl has been shown.

LITERATURE CITED

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