A PRACTICAL METHOD FOR DETERMINING THE MOLECULAR WEIGHTS OF CARDENOLIDES BY UV SPECTROMETRY

I. F. Makarevich and S. G. Kislichenko

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The presence in cardenolides of a butenolide ring leads to pronounced absorption in the UV region with a maximum at 216-220 nm. The constancy of the molar extinction of the majority of cardenolides permits the absorption in this region of the UV spectrum to be used for determining their molecular weights (mol. wts.) (see, for example, [1]).

At equal weight-volume concentrations, the optical density D_{max} depends only on the molecular weight of the substance [2]; it is inversely proportional to the molecular weight. For those cardenolides which have no groupings affecting the extinction of the butenolide ring, what has been said can be represented in the form of the relation $M \times D_{max} = f$. The magnitude of f (it may be called the molecular absorption at a given concentration C) is constant for a given concentration (weight-volume) of cardenolides. Thus, for 0.003% solutions it is 444. However, f changes according to the concentration. This relationship is shown graphically in Fig. 1.

Workers who are going to make use of the method described for determining molecular weights will find it desirable to plot this graph on a large scale, which will enable the values of f to be read from it with a greater accuracy than is possible from Fig. 1. We give the figures for the plotting of the graph: for a weight volume concentration of 0.001%, f is 152; at 0.002% it is 298; at 0.003% 444, and at 0.004% 598. The range of concentrations is selected in such a way that the measurement can be performed in the optimum

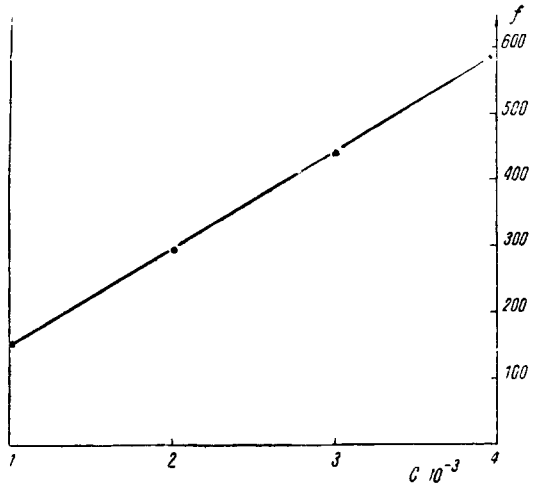

Fig. 1. Dependence of f on c ($f = D_{max} \times M$; c of the cardenolides in wt.-vol. % · 10^{-3}).

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TABLE 1

Cardenolide	M		Error, %
	found	calc.	
Erychroside	664,7	666,8	−0,3
Erycordin	691,9	698,8	−1,0
Desglucoerycordin	532,6	536,7	−0,8
Erythriside	841,4	828,9	+1,5
Alliotoxin	540,0	536,7	+0,6
Alliside	550,5	552,7	−0,4
Glucoalliside	708,4	714,8	−0,9
Cheiranthoside	538,2	534,6	+0,7
Glucoerysimosol	859,9	860,9	−0,1
Erychrosol	671,7	668,8	+0,4
Alliotoxigenin	395,7	390,5	+1,3
3-Epistrophanthidin	405,2	404,5	+0,2
3-Epistrophanthidol	408,5	406,5	+0,5
Evonogenin	402,0	406,5	−1,1
Glucoevonogenin	563,4	568,7	−0,9
Neoglucoerysimoside	867,1	858,9	+1,0

region of the scale of the instrument and, consequently, with the smallest error. Furthermore, it ensures the possibility of determining the molecular weights of cardenolides within wide limits – from aglycones to polyglycosides.

All the measurements were performed on SF-4 and SF-4A instruments using absolute ethanol and quartz cells (1 cm). In the plotting of the graph (see Fig. 1) the optical density (D_{max}) was measured in the 216-220 nm region for a large number of known cardiac aglycones and mono-, di-, and triglycosides, i.e., for cardenolides with different molecular weights.

In investigating new glycosides, we determined D_{max} in the 216-220 nm region under the same conditions and calculated the molecular weight from the formula

$$M = \frac{f}{D_{max}}.$$

The value of f used is that obtained from the graph for the actual concentration of the cardenolide under investigation that was used.

The error of the method did not exceed 1.5%. Table 1 gives the results of measurements for some of the new cardenolides isolated.

As mentioned above, the method is applicable to those cardenolides that contain no groupings affecting the extinction of the butenolide ring. Such groupings are a C=C bond in the steroid part of the molecule and an alcoholic grouping at C-16 (whether free or esterified). An angular carboxy group (at C-10) and an epoxide ring also change the intensity of absorption of the butenolide ring, although to a smaller extent. In determining the molecular weights of such cardenolides (and they are found comparatively rarely), it is desirable to plot other graphs similar to that given in the present paper using for this purpose known compounds containing the functional groups mentioned.

LITERATURE CITED

1. N. K. Abubakirov and S. D. Nikonovich, Khim.-Farmats. Zh., 3, No. 1, 40 (1967).
2. A. Gillem and E. Stern, An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry, Arnold, London (1954).