INFLUENCE OF UV IRRADIATION ON CERTAIN CARDIAC GLYCOSIDES AND THEIR ANALYSIS BY CHROMATOGRAPHIC, SPECTROPHOTOMETRIC, AND BIOLOGICAL METHODS

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It is known that during irradiation by sunlight, by UV light, and by the action of radioactive radiation, cardiac glycosides decompose [1-3], and their biological activity decreases. During the study of the decomposition of several glycosides in aqueous solutions, it was shown [3-5] that the irradiation of solutions of cardiac glycosides by UV light can serve as a test of aging during the study of the influence of light on their stability. However, the mechanism of the decomposition of glycosides during irradiation by light has been little studied. In [6-8], a chromatographic separation of the digitalis glycosides was carried out. By using chromatographic and spectral methods of analysis, it is possible to follow the changes in the compound under the influence of light to determine the decomposition products, and to evaluate the activity of the irradiated preparations without biological methods of analysis.

In the present work, we studied the influence of UV radiation on certain glycosides by analyzing the initial glycosides and the irradiated samples by liquid chromatography, UV spectroscopy, photocolometric, and biological methods. This complex application of different methods made it possible to compare their suitability for the analysis of cardiac glycosides.

As the source of UV radiation, we used the Minuvis 131200 apparatus from the Desaga firm, with a mercury lamp and light filter with a 254 nm wavelength. For the investigation we used 0.05% solutions of the glycosides in aqueous-alcoholic mixtures. The solutions were irradiated in open vessels at a distance of 50 mm from the source for 3 and 6 h, and corelborin for 3 and 9 h.

The biological activity of the solutions before and after irradiation was determined on grassy frogs [9]. The spectrophotometric determination of the preparations from the absorption in the 210-360 nm UV-region was carried out on a Hitachi recording spectrophotometer, as in [10]. The analysis of the glycosides according to reactions for a lactone and steroid rings and sugar residue was carried out by photocolometric methods [5, 11, 12]. The chromatography of the glycosides was carried out on the Spectra-Physics 3500B liquid chromatograph with a UV detector with variable wavelength. For the detection, the 217 nm wavelength was mainly used. The column was from stainless steel, 50 cm long and 2.1 mm in internal diameter, and filled with silanized silica gel LiChrosorb SI60 (LiChrosorb RP-2) with a particle size of about 30 μ m. The eluent was a solution of 18% ethanol and 82% of water. The column was filled with dry adsorbent.

Figure 1 shows the UV spectra of glycoside solutions before and after irradiation by UV light for different periods of time. We can see that with increase in the irradiation time, the absorption of these substances in the 220 nm region decreases. A similar change in the absorption is also observed during the irradiation of corelborin, which has a six-membered lactone ring and has two bands in its UV spectrum (Fig. 2). Such an appreciable change in the UV spectra of the glycosides makes it possible to use the spectrophotometric method for the determination of the content of glycosides in the solutions and the change in their content under the action of UV light. However, the absorption in this region of the spectrum is not characteristic, since the decomposition products of the glycosides can also absorb here. In particular cases, this interferes with the use of the spectrophotometric method. However, a combination of UV spectroscopy with separation of the mixture into components in a chromatographic column on a liquid chromatograph with a UV detector and a monochromator made it possible to analyze the glycoside solutions qualitatively and quantitatively. Figure 3 shows the chromatograms of certain crystalline glycosides before and after irradiation by UV light for 3 and 6 h. We can see that after irradiation, the height of the peaks of the main components decrease, which indicates a decrease in their concentration in the solution. On the chromatograms of the irradiated substances, peaks appear whose height increases with increase in the irradiation time. These peaks apparently correspond to the decomposition

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Fig. 1

Fig. 1. UV spectra of g-strophanthin (a), erizimoside (b), olitoriside (c), and k-strophanthin (d) before and after irradiation of their solutions by UV light. Time of irradiation: 0 h (1), 3 h (2), 6 h (3). Length of the optical path 1 mm, concentration of glycosides a, b, and d was 0.05%, and of glycoside c, 0.04%.

Fig. 2

Fig. 2. UV spectra of corelborin before and after irradiation by UV light. Time of irradiation: 0 h (1), 3 h (2), 9 h (3). Length of optical path 1 mm, concentration 0.05%.

products of the glycosides as the result of irradiation. The absorption of these products in the UV region of the spectrum is somewhat lower than that of glycosides at 217 nm, and therefore the peaks corresponding to these substances are relatively low in height.

Figure 4 shows the chromatograms of a corelborin solution before and after irradiation. Since corelborin absorbs in a broad spectral region, the detection was carried out at three wavelengths: 217, 254, and 300 nm. After irradiation for 3 h, the concentration of corelborin decreased by a factor of nearly 2, and after irradiation for 9 h, practically no corelborin remained in the solution. The decomposition products of corelborin had a very low absorption at these wavelengths, so that it was difficult to detect them by the UV detector.

The study of the biological activity of the glycoside solutions before and after irradiation on grassy frogs showed that with increase in the time of irradiation, the biological activity of the solutions decreased, which indicates a decrease in the concentration of the biologically active glycosides in the solution, and their conversion into inactive derivatives. The method of liquid chromatography used makes it possible to directly determine the decrease in the concentration of the glycosides after irradiation by UV light, and the appearance of any new compounds. A decrease in the concentration of the glycosides is observed in the UV spectra of their solutions also, but it was impossible to notice the appearance of the decomposition products.

It was interesting to compare the changes in the concentrations of the glycosides in the solution, observed by biological, chromatographic, and spectrophotometric methods, as well as to compare these data with the results of the determination of the concentration of the glycosides by methods which are at present used for the analysis of glycosides. These are mainly photocolorimetric methods for the analysis of glycosides, during the application of which reactions with picric acid and bis- (2,4-dinitrophenyl) sulfone, reactions with different reagents for the steroid ring, and for the sugar residue are used.

Such a comparison is given in Table 1. This table shows the percent content of the glycoside which remained after irradiation for 3 and 6 hours, compared with the initial solution in which it was assumed that the content of the glycoside is 100%. The biological method made it possible to determine the content of the glycosides expressed in frog units of action (FUA) per g of glycoside. The initial glycosides contained 50,000 FUA of G-strophanthin, 62,000 FUA of erizimoside, 48,000 FUA of olitoriside, 50,000 FUA of K-strophanthin, and 66,000 FUA of corelborin. The optical density was determined spectrophotometrically at 220 nm for G-

Fig. 3. Chromatograms of G-strophanthin (a), erizimoside (b), olitoriside (c), K-strophanthin (d) before and after irradiation by UV light. Time of irradiation is indicated in the figure. The eluent contains 18% of ethanol and 82% of water, the flow rate of the eluent is $0.08 \text{ cm}^3/\text{min}$, temperature of the column 50°C , wavelength at which the glycosides were determined was 217 nm.

Fig. 4. Chromatograms of corelborin before and after irradiation by UV light, obtained at 217 nm (1), 254 nm (2), and 300 nm (3). The conditions of the chromatography are the same as in Fig. 3.

strophanthin, erizimoside, olitoriside, and K-strophanthin, and at 300 nm for corelborin. The area of the peak corresponding to the given glycoside was determined chromatographically, and the optical density corresponding to the absorption maximum of the colored compounds, photocolormetrically.

Fig. 5. Chromatograms of ampullaceous samples of corelborin, stored in the dark (a), and in the light (b). The conditions of the chromatography are the same as in Fig. 3. 1) Nonidentified impurity; 2) conservant (nipagin); 3) corelborin.

TABLE 1. Results of Determination by Different Methods of the Change in the Content of Glycosides in Solution (in %) during Irradiation by UV Light for Different Periods of Time

Glycoside	3 h of irradiation								6 h of irradiation							
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
G-Strophanthin	60	72	68	85	81	103	—	102	42	44	51	72	64	120	—	127
Erizimoside	74	75	77	86	92	110	98	—	65	51	67	78	83	128	100	—
Olitoriside	54	57	56	78	72	157	107	—	38	33	39	62	64	185	107	—
K-Strophanthin	58	51	65	79	75	175	—	114	40	26	47	64	59	206	—	143
Corelborin	44	40	47	—	—	69	—	75	0*	3*	18*	—	—	48*	—	52*

*The irradiation was carried out for 9 hours.

Note. Methods: 1) biological on frogs (subcutaneous administration), 2) liquid chromatography, 3) spectrophotometric, according to the absorption in the UV region of the spectrum, 4) photocolormetric, according to the reaction with sodium picrate, 5) the same, according to the reaction with bis- (2,4-dinitrophenyl) sulfone, 6) the same, according to the reaction with sulfuric acid, 7) the same, according to the reaction with xanthidrol, 8) the same, according to the reaction with a FeCl_3 solution in glacial acetic acid and concentrated sulfuric acid. The content of the glycosides in the initial solution was 100%.

Table 1 shows that similar results are obtained when biological, spectrophotometric, and chromatographic methods are used, which indicates the possibility of determining the content of the cardiac glycosides in preparations without using the biological method. The photocolormetric methods used at present for the analysis of glycosides are not very suitable for the analysis of glycosides containing the decomposition products and can be used only for the analysis of pure glycosides. It should be noted that the above method of liquid chromatography has several advantages over the spectrophotometric method, since it makes it possible not only to isolate impurities, but also to detect the decomposition products of the glycosides, which cannot be done by the UV spectra only.

Figures 3 and 4 show the chromatograms of solutions of glycosides before and after irradiation by UV light, which served as a test for the aging of glycosides under the action of light. Figure 5 shows the chromatograms of ampullaceous corelborin, which was stored in the dark and in the light. The chromatograms show that in a sample which was stored in the dark, there are peaks of the conservant, corelborin, and an unidentified impurity, while in a sample stored in the light, the peak of the conservant only was observed. Thus, without study of the biological activity of the ampullaceous solutions, it can be stated that the contents of the ampuls with corelborin stored in the light will be biologically inactive.

It thus follows from the data obtained that UV radiation inactivates the solution of glycosides, and that during irradiation of glycosides by UV light under the given experimental conditions, changes in the lactone

ring occur, which lead to the formation of products with no biological activity. Hence, in this case we can use physicochemical methods to detect the changes taking place in the lactone ring. Results comparable with the data of the biological determination were obtained by using methods of UV-spectrophotometry and liquid chromatography on an adsorption column. Purely chemical methods, based on the reaction of the lactone ring of the glycosides with bis-(2,4-dinitrophenyl) sulfone and picric acid, as well as with xanthidrol, for the determination of the sugar component, and those based on the reaction with sulfuric acid for the determination of the steroid part of the glycosides, are time consuming and because of large discrepancies in the results of the analysis, cannot be used for the quantitative determination of glycosides.

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AEROSOL CONTAINERS FOR LOWERING THE MICROBIAL CONTENT OF AIR DURING RESPIRATORY VIRAL INFECTIONS

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The prophylaxis of human illnesses in many cases depends on the degree of the participation of the whole population in the improvement of the sanitary-hygienic working and living conditions. The fight against respiratory infections, in particular, against influenza and parainfluenza viruses present in the air, should be directed to the decomposition of the microbial aerosol. During the continuous influx of the infection into the atmosphere, a constant introduction of clean and conditioned air into the premises is most effective. However, to effect conditioning, the appropriate devices are not always available, and therefore air-conditioning is often replaced by ventilation, which is also not always possible.

One of the ways of fighting the aerogenic infections, by no means universal, can be the periodic introduction of aerosols containing antimicrobials into the premises.

Under domestic conditions, aerosol containers containing deodorants can be used for this purpose. The present work deals with the study of the virucidal properties of aerosols of these compounds. It can be assumed that the removal of the microbial sources from the air will be related to the biocidal action of biologically active compounds, a more intense coagulation of the aerosols, and subsequent settling of the particles.

As deodorants, glycols (propylene glycol, triethylene glycol, and their mixtures) are most widely used. Special odorants (aromatized fumigant substances) are present in the aerosol containers together with them.

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