THE REPLACEMENT OF THE HYDROGEN ATOMS OF THE CH_2 GROUP OF THE BUTENOLIDE RING OF A CARDENOLIDE BY DEUTERIUM

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It has been shown previously [1] that in the mass spectra of the products of the deuteration of cardenolides with deuteromethanol there is a partial additional shift of the peaks by 1-2 mu. This phenomenon has been explained by the replacement of the hydrogen at C_{21} of the butenolide ring. It appeared of interest to confirm these results by other methods, in particular by NMR spectroscopy in which the CH_2 group of a butenolide is usually shown by a signal at about 5.0 ppm [2] (the NMR spectra were obtained on a JNM-4H-100/100 MHz instrument with HMDS as internal standard).

As the subjects of investigation we used strophanthidin acetate (I) and periplogenin (II). The conditions of the experiments were varied. A solution of (I) in CD_3OD was heated to 100°C, and after some time the deuteromethanol was evaporated off, the residue was dissolved in C_5D_5N, and the NMR spectrum was recorded with the addition of a few drops of CD_3OD. In another experiment, after the elimination of the bulk of the deuteromethanol, a solution of (I) in CD_3OD previously heated (150°C) for 1 h was placed under the conditions usually used for the mass spectrometry of cardenolides (150°C, $2\text{-}3 \cdot 10^{-7}$ torr, 0.5 h), after which the sample was dissolved in CD_3OD and the NMR spectrum was recorded. Periplogenin (II) was heated in CD_3OD solution in a sealed tube at 105°C for several days and was then recrystallized from the same solvent until a chromatographically homogeneous sample had been obtained, after which the NMR spectrum was obtained in CD_3OD.

In all cases, the signals of the protons of the CH_2 group of the butenolide were located in the usual region for them, forming a quartet in the spectrum of a solution of (I) in C_5D_5N at δ 4.88 and 5.15 ppm with J_{gem} = 17.0 Hz. The signal of the proton in the HCOAc group appeared in the same region. In the spectrum of a solution in CD_3OD, the signals of these protons were found in the δ 4.60-5.00-ppm range, and that of the olefinic proton ($C_{22}-H$) at δ 5.78 ppm. In the spectrum of (II), the signal from $HO-C_3H$ appeared at δ 4.17 ppm, and the quartet of the CH_2 group in the interval from 4.70 to 5.15 ppm. The signals of the unsubstituted tertiary OH groups that appear in the range under consideration were shifted by heating the solutions to 50-95°C during the recording of the spectra.

By comparing the integral intensities of the olefinic proton and the CH_2 and HCOAc protons in (I) and the CH_2 protons in (II) it was established that within the limits of accuracy of integration, the intensity of the CH_2 signal does not decrease. To check this, we also compared the intensities of the olefinic proton and the protons of the angular methyl groups. Thus, in the experiments with the NMR spectra deuterium exchange of the CH_2 group of the butenolide ring in a neutral medium was not confirmed. Consequently, the deuteration of the methylene group took place only under the conditions of mass spectrometry. For a more detailed study of this phenomenon, we performed some additional experiments and calculations. Thus, deuterated samples were treated with ordinary methanol; after treatment, sometimes accompanied by brief heating, the solvent was distilled off and the mass spectrum was obtained, which was practically identical with that of the initial sample. In order to exclude the possibility of an additional shift of the peaks through the formation of impurities on deuteration, the mass spectrum of a sample repeatedly recrystallized from deuteromethanol was recorded, and similar results were obtained.

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TABLE 1. Relative Contents of Labeled Ions and of the Ion with m/e 111 in the Mass Spectra of Some Deuterocardenolides

Compound	Positions of the OH groups	Relative content, %		
		111 (D=0)	112 (D=1)	113 (D=2)
Strophanthidol	3β, 5β, 14β, 19	44	32	24
Periplogenin	3β, 5β, 14β	46	39	15
Strophanthidin acetate	5β, 14β	42	42	16
Diffugenin	3β, 5β	57	36	7
Digitoxigenone	14β	56	33	11
Uzarigenin acetate	14β	64	26	10
DASE	–	81*	14*	5*

* Mean values for several groups of peaks.

The absence of a change in the signal of the CH_2 group of the butenolide ring in the NMR spectra permits the assumption that its deuterium exchange takes place outside the tube containing the sample – directly in the inlet system. To prove this, a special experiment was performed: a nondeuterated sample of periplogenin (II) was evaporated in the inlet system of the mass spectometer and then from a separate reservoir a current of CD_3OD was passed through the same system and, after 1 min, the spectrogram of (II) was recorded. The spectrum obtained repeated the spectra of samples previously treated with deuteromethanol [1]. Then the spectrum of the residue of the sample, rapidly transferred to another instrument, was recorded. This spectrum showed the absence of the label both in the OH group and in the CH_2 group at C_{21}.

It is necessary to answer the question of the source of the deuterium, since on deuteration in solution practically all the deuteromethanol is pumped out in the lock system of the mass spectrometer.

The table gives the values calculated by Biemann's method [3] of the proportions of isotopes in the peak of the ion with m/e 111 including the lactone ring with the $C_{17}-C_{16}$ chain [4] for a number of cardenolides differing by the number of hydroxy groups which they contain. The figures in the table were obtained with the deuteration of the samples under similar conditions (~ 100°C, 0.5 h in a sealed tube, solution in CD_3OD). On analyzing the figures given it is possible to see a tendency to a decrease in the proportion of deuterium-substituted ions on passing to substances with a smaller number of hydroxy groups. The deuterium derivative of dianhydrostrophanthidin ethylal (DASE), the molecule of which has no OH groups, contains the smallest amount of labeled molecules – 19%. Consequently, two conclusions may be drawn: in the first place, the presence of labile deuterons of OD groups of a steroid may in some way favor the increase in the proportion of molecules labeled at C_{21}; and in the second place a process favoring the inclusion of the label in the methylene group must take place even in the absence of other labile hydrogen atoms in the molecule.

In agreement with the first conclusion we may assume the existence of deuterium exchange between molecules sorbed on heated surfaces of the inlet system, which leads to the partial loss of the label from the OD groups and to its accumulation in the methylene group through the primary isotope effect [5]. This is in harmony with the results of preceding work [1], i.e., with the decrease in the values of A and B in deuterocardenolides. The phenomenon observed obviously depends on features of the structure of the cardenolides, since in the case of other steroid compounds no additional shift has been observed.

A second, but not less important, source of deuterium consists of residues of deuteromethanol strongly retained in the crystal lattice of the cardenolides. The residual CD_3OD increases the amount of OD groups in the cardenolide molecules, and also partially replaces the hydrogen at C_{21} in the excited state, even in the case of a hydroxyl-free compound. The results of a similar experiment on the deuteration of DASE in the inlet system showed that under these conditions about 6% of the hydrogens at C_{21} are replaced by deuterium.

SUMMARY

It has been shown by the NMR-spectroscopic method that when cardenolides are treated with CD_3OD no deuterium exchange of the protons of the CH_2 group of the butenolide ring takes place. The entry of the label into this position takes place in the mass spectrometer.

LITERATURE CITED

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