STRUCTURES OF HOLOTHURIAN GLYCOSIDES WITH THE AID OF RELAXATION TIME T_1 OF THE CARBOHYDRATES

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Continuing an investigation of the application of ^{13}C NMR spectroscopy to the study of the structure of the glycosides of holothurians, we have measured the carbon-13 relaxation times of the series of glycosides (I)-(IV). Holothurian glycosides possess the feature that all the sugar residues of the carbohydrate chain are of the glucose type so that the vector of the ring C–H bonds have the same direction for each monosaccharide residue. Then, if the monosaccharide rings are regarded as absolutely rigid, it may be expected that the methine carbons of a given ring will be characterized by the same relaxation time, which will provide the possibility of determining the sequence of monosaccharide residues in the chain.

There are a number of publications on this subject [1-3]. In [1], this approach was demonstrated for K-strophanthoside – a trioside containing a cymarose residue and two glucose residues. In [2] and [3], where a linear tetraoside – desulfated holothurin A – was considered, contradictory results are presented. Thus, in [2] the values of T_1 for the car-

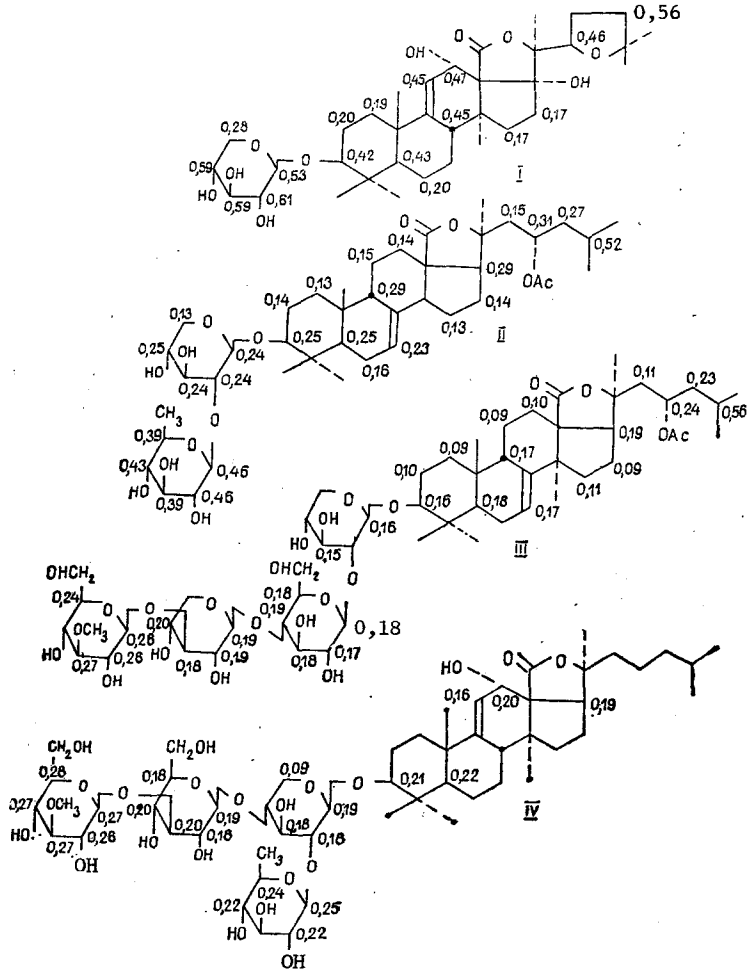

Fig. 1

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bon atoms of different monosaccharide residues overlapped to a considerable degree, which made the use of this criterion problematical. In [3], one and the same value of T_1 was obtained for the anomeric carbons of the second, third, and fourth monosaccharide residues, which was apparently due to the overlapping of their signals in the ^{13}C NMR spectra; i.e., this value was not reliable. In view of this, we have studied a monoside, a bioside, and a linear and a branched tetraoside the preparation of which has been described in [4]. The assignment of the signals in the ^{13}C NMR spectra of these compounds was made in [4]. Figure 1 gives the values of the relaxation times of the methine and methylene carbon atoms giving overlapping signals in the spectra.

The spectra were taken on a WM 250 instrument with a working frequency for carbon of 62.9 MHz in pyridine-d_5 at 55°C. The experimental intensities of the signals obtained from the pulse sequence 180°–τ–90° were approximated by the three-parameter exponent $I = A + B\exp(-\tau/T_1)$ through the minimization of the function $Fx = \frac{1}{n}\sum_{i=1}\frac{1}{\sigma_i^2}\times(I_{calc} - I_{exp})^2$ on an ES 1060 computer, where n is the number of experiments (in our case, n = 9-10), and σ is the noise level.

It must be mentioned that in view of the fact that pyridine-d_5 was used as the solvent, vibrations around the glycosidic bonds were not effected by the monosaccharide residues themselves but by their complexes with pyridine. It can be seen from the facts presented that in the case of the monoside it is possible reliably to differentiate the signals of the aglycon and the carbohydrate moiety, while in the case of the bioside the values of NT_1 of the first monosaccharide residue and of the aglycon coincide, which is due to the above-mentioned effects of complex-formation. This also, apparently, explains the fact that in the case of tetraosides it is not the aglycon moiety but the first monosaccharide that has the smallest NT_1 values. It can also be seen from these facts that in the case of a bioside the values of NT_1 permit a reliable differentiation of the signals of the individual monosaccharide residues. In the case of a linear tetraoside the values of NT_1 for the second and third monosaccharide residues coincide. For the branched glycoside (IV) the NT_1 values provide the possibility of distinguishing the signals of the outer monosaccharide residues from those of the inner residues.

Thus, in certain cases, a series of partially relaxed ^{13}C NMR spectra of holothurian glycosides permits the assignment of signals to outer or inner monosaccharide residues and a determination of the sequence of monosaccharide residues in a carbohydrate chain.

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