SEPARATION OF CONFORMATIONAL ISOMERS IN THE 5,10-SECOCARDENOLIDE SERIES. I.

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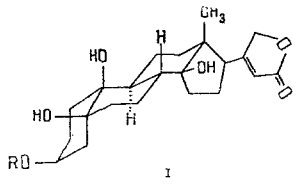
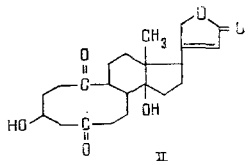
A rare case of separable conformational isomers in the 5,10-diketo-5,10-secocardenolide series has been found. Thus, the periodate oxidation of 10β-hydroxy-19-norperiplogenin has given 3β,14β-dihydroxy-5,10-diketocard-19-nor-5,10-seco-20(22)-enolides which in fact consist of a mixture of conformers formed as the result of the inversion of the AB ring. A method for separating the four conformers, their properties, their IR, mass, and PMR spectra, the kinetics of their acetylation reactions, and their heats of transformation are described. Conformational structures as the most probable are put forward on the basis of the experimental results obtained.

Convenient starting compounds for obtaining 5,10-secocardenolides are 10β-hydroxy-19-norcardenolides (I) containing a cis-α-glycol grouping involving tertiary OH groups at C-5 and C-10. Compound (I) is obtained, in its turn, by the autooxidative decarbonylation of 19-oxo compounds [1, 2].



I



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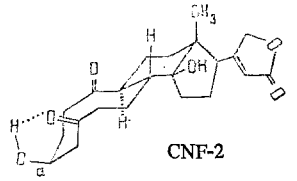
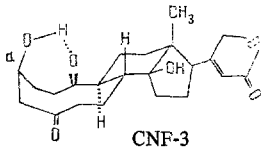
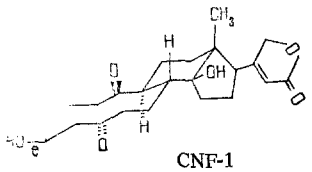


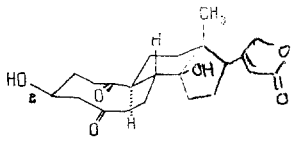
CNF-2



CNF-3



CNF-1



CNF-4"

Having prepared 10β-hydroxy-19-norperiplogenin (I, R=H) by this method [1] we have used it in the periodate oxidation reaction with the aim of obtaining 3β,14β-dihydroxy-5,10-diketo-19-nor-5,10-secocard-20(22)-enolide (II). It was unexpectedly found that the reaction product was a mixture of substances consisting of not less than four compounds revealed by reagents for the butenolide ring of cardenolides — the Raymond, Kedde, and Legal reagents. When the mixture was stored, heated, or adsorbed on alumina, it was observed that the ratio of the individual components changed.

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Assuming that we were dealing with conformational isomers (CNFs), we made an attempt to separate them and describe their properties. Satisfactory results in separation were achieved by the use of column chromatography with silica gel activated at 105°C. Separation was performed at 18-20°C with continuous working of the column and a high rate of elution. In view of the thermolability of the conformers, the eluates were evaporated without heating. In this way we isolated in the chromatographically individual state four conformers and designated them as CNF-1, -2, -3, and -4". Their properties are given in part as follows:

Conformer	Mol. wt. and mol. formula	mp, deg C	$[\alpha]_D$ deg	Time of 50% acetylation, min
CNF-1	390; $C_{22}H_{30}O_6$	233–268	−18 8±2 (in Py)	15
CNF-2	390; $C_{22}H_{30}O_6$	109–112		150
CNF-3	390; $C_{22}H_{30}O_6$	125–130/215–220		255
CNF-4"	390; $C_{22}H_{30}O_6$	227–230	−10 8±2 (in MeOH)	3

The elementary analyses of all the conformers were identical and corresponded to the composition $C_{22}H_{30}O_6$. Their molecular weights were also identical, 390. Their mass spectra contained strong peaks of the molecular ions with m/z 390 and the fragments, *m/z*: 372 (M—H_2O), 354 (M—2H_2O), 344 (M—H_2O—CO), 336, 326 (M—2H_2O—CO), 219, 201. At the same time, the conformers isolated were readily distinguished by their chromatographic properties, melting points, rates of acetylation, and PMR spectra. The absorption bands of the C=O and C=C groupings in the IR region were (cm^{-1}):

Conformer	*C=O* of the butenolide ring	*C=C* of the butenolide ring	*C=O* at *C-5* and *C-10*
CNF- 1	1610, 1720	1623	1685, 1690, 1708
CNF- 2	1780, 1740	1624	1688, 1700, 1730
CNF- 3	1780, 1740	1625	1690, 1705, 1720
CNF- 4"	1780 1740	1625	1690 1698, 1710

The IR spectra of conformers 2, 3, and 4" had bands characteristic for cardenolides: 1780 and 1740 cm^{-1} (C=O of a butenolide ring) and 1625 cm^{-1} (C=C of a butenolide ring). However, for CNF-1 a definite anomaly was observed in the position of the bands of the butenolide ring. The carbonyl groups at C-5 and C-10 gave three absorption bands in the 1685-1730 cm^{-1} region, and, while for CNF-1 and -4" these bands were adequately resolved, for CNF-2 and -3 they fused with the C=O absorption of the butenolide ring forminga shoulder on a single broad unsymmetrical absorption band.

The PMR spectra taken at a frequency of 100 MHz were individual for each of the conformers but they also had common signals:

5.90-5.95 ppm — one-proton singlet, due to 22-CH;

4.90-4.95 ppm — two-proton singlet, due to 21-CH_2;

0.77-0.80 ppm — three-proton singlet, due to 18-CH_3.

In the 1.7-3.4 ppm region there was a complex, inadequately resolved, spectrum of signals due mainly to the methylene protons at C-1, C-4, C-6, and C-9.*

An investigation of the kinetics of the acetylation of the conformers by acetic anhydride (Fig. 1) enabled useful information for conformational analysis to be obtained. The times of "half-reaction" for CNFs-1 and -4" amounted to 15 and 3 min, respectively, which shows [3] the equatorial positions of the OH groups at C-3 in these conformers. The times for 50% acetylation of CNFs-2 and -3 were 150 and 255 min, respectively, which is characteristic for the axial positions of the OH groups in them [3]. The difficulty in the acetylation of CNF-3 is also connected with the fact that the axial OH group in it is obviously included in an intramolecular hydrogen bond with one of the carbonyl groups. The presence of a hydrogen bond was confirmed by the IR spectrum, which had a broad unsymmetrical absorption band in the region of OH groups.

*This region of the PMR spectrum, which is the most informative for conformational analysis, will be discussed together with an x-ray structural analysis in a subsequent communication.

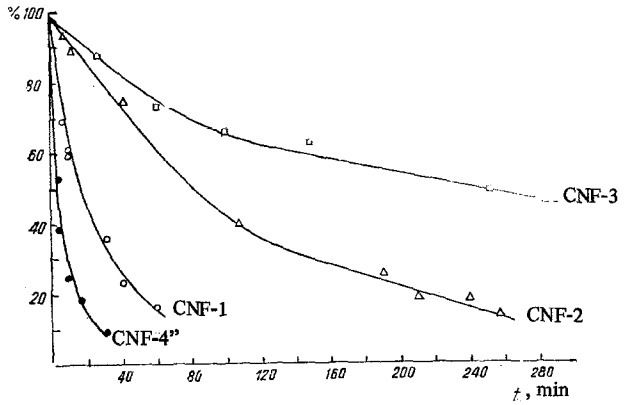

Fig. 1. Kinetic curves of the change in the amounts of the conformers in the acetylation reaction.

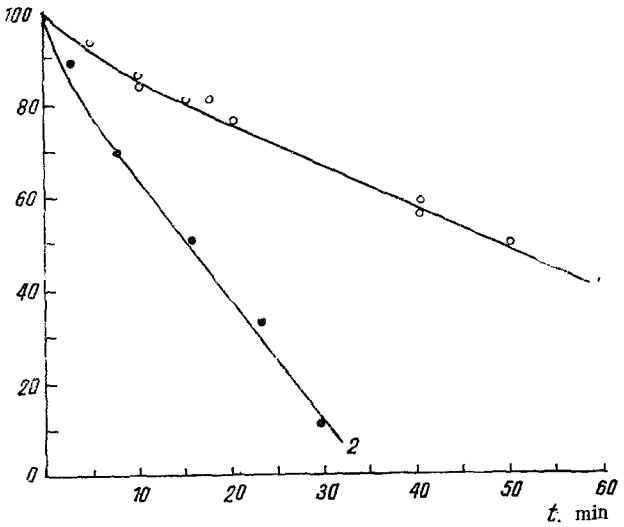

Fig. 2. Transformation of conformer 3 at 40°C (1) and 56°C (2).

The unusually high reactivity of the acetylatable OH group in CNF-4" showed not only its equatorial position but also the existence of an effect of the coparticipation of the carbonyl groups in the reaction.

CNFs-1 and -4", which were the most stable, could be kept in the crystalline state at room temperature without visible changes, CNF-2 and -3 were unstable, being transformed predominantly into CNF-1 and could be kept only at a temperature below 0°C in the crystalline state.

Figure 2 shows kinetic curves of the transformation of conformer 3 at 40 and 56°C. The stability of solutions in ethanol was studied. As was to be expected, the kinetic curves obeyed a first-order equation with n = 0.99 and 1.13 for curves 1 and 2, respectively. The rate constants calculated from the formula [6]

$$k = \frac{2.303}{t} \cdot \frac{C_0}{C},$$

were $k_1 = 0.012$ min^{-1} and $k_2 = 0.043$ min^{-1} for temperatures of 40 and 56°C, respectively.

The activation energy of the transformation of CNF-3 into more stable conformers, mainly CNF-1, was calculated from the formula [6]:

$$E = \frac{R \cdot \ln \frac{k_2}{k_1}}{1/T_1 - 1/T_2},$$

E = 16.0 kcal/mole = 67.2 kJ/mole.

On the basis of the results obtained, it may be assumed that the conformers had the structure shown in the scheme. All the conformers given were constructed in Briegleb–Stuart models, this being particularly easy for CNFs-1 and -4". CNF-2, having an axial OH group at C-3 and a cisoid structure of ring AB, is apparently the initial product of periodate oxidation.

In CNF-3, the axial OH group is apparently linked by an intramolecular hydrogen bond with the carbonyl group at C-10. In this case, it proves to be most highly screened spatially, which explains the special difficulty of its acetylation. The formation of CNF-3 from CNF-2 which is actually observed experimentally can be represented as the result of the transformation of part of ring AB – that part of which belonged to ring A – into the boat form.

CNF-1, the most stable conformer, can be apparently be represented by a formula in which ring AB is present in the form of an elongated crown, free to the maximum degree from angular stresses, in which the hydroxyl at C-3 is equatorial. The formation of the crown shape is not difficult to represent from conformations 2 and 3. It is performed both on molecular models and experimentally by the transformation of CNFs-2 and -3 into CNF-1. The C-atoms 2, 4, and 6 are present in one plane, and 1,3,7 in another. The carbonyl groups assume orientations opposite to one another. The keto group at C-5 is oriented in the α-position.

CNF-4" is a stable conformer and, as mentioned, has an equatorial hydroxyl with an increased reactivity. The choice of the conformation shown above for it enables these experimentally observed features to be explained. The C_{10} carbonyl group is present in the bent part of ring AB oriented in the direction of the C_4 atom and close to it. The influence on C-4 of two carbonyl groups simultaneously leads to the formation of a fractional positive charge on it. In its turn, this causes an inductive shift of the electron density from C-3 to C-4 and the "protonation" and activation of the C_3-OH group.

Speaking in general of the reasons for the relative stability of the conformers in the 5,10-secocardenolide series, one must bear in mind primarily the fact that part of the "macrocycle" is rigidly fixed; this is the part that is attached to ring C and has the C-8 and C-9 carbon atoms in common with it. The hydrogen atoms at these tertiary C atoms must have the axial orientation, i.e., the orientation that existed in the initial steroidal structure of cardenolide 1. Other factors are: the presence of intramolecular hydrogen bonds in CNF-2 and -3, the interaction of $C_{10}{=}O^{\delta-}$ and $C^{\delta+}_4$ in conformation 4", and the most rational construction of ring AB in CNF-1, apparently leading to a free energy minimum.

EXPERIMENTAL

General Remarks. The elementary analysis of the conformers was performed with the aid of a Hewlett–Packard C-H-N analyzer. The results of the analyses corresponded with those calculated for the structures given. Mass spectra were taken on Varian CH-8 and MKh-1303 instruments; PMR spectra, on a Tesla BS-497 instrument (100 MHz) in C_5D_5N (0 – TMS); and IR spectra, on a JR-27G instrument, the substances being tableted with KBr.

Preparation of 3β,14β-Dihydroxy-5,10-diketo-19-nor-5,10-secocard-20(22)-enolide (II). A solution of 2 g of 10β-hydroxy-19-norperiplogenin (I, R = H) in 50 ml of methanol was mixed with a suspension of 4 g of sodium metaperiodate in 100 ml of water acidified with sulfuric acid to pH 4.5. The reaction mixture was shaken on a shaking apparatus for 44 h, the process being monitored with the aid of TLC (on Silufol) in the chloroform–methanol–water (85:15:0.7) system. After the end of the reaction, the oxidation products were extracted with chloroform (150 ml × 2), and then the upper phase was saturated with sodium chloride and was additionally extracted with chloroform–ethanol (3:1; 150 ml × 5). The combined ethanol–chloroform extracts were dried over anhydrous sodium sulfate and evaporated without heating in evaporating dishes in a current of air in a fume cupboard. The residue, 1.69 g, consisting of compound (II) or, more accurately, of a mixture of conformers, was then subjected to chromatographic separation.

Separation of the Conformers. The products of periodate oxidation were chromatographed on a column containing 425 g of silica gel that had been activated by heating at 105°C for 1 h. Chloroform and chloroform–methanol (9:1–90:10) were used as eluants. Chromatography was continuous from the beginning to the complete elution of all the conformers; 40-ml fractions were collected automatically, the rate of elution being 0.6 liter per hour. The fractions were analyzed by paper chromatography in the methyl ethyl ketone–m-xylene (1:1)/formamide system; the eluates were evaporated without heating. The conformers were crystallized from aqueous methanol. The unstable conformers CNFs-2 and -3 were stored in the freezing chamber of a domestic refrigerator at a temperature of –(5-15)°C.

Kinetic Measurements. A. The kinetics of the transformation of CNF-3 were determined on ethanolic solutions. The solutions were filled into glass tubes and placed in a boiling solvent — methylene chloride, bp 40°C, or acetone, bp 56.2°C. After predetermined intervals of time, the tubes were extracted successively and each was rapidly cooled to −(7-10)°C. The quantitative analysis of the samples was carried out by the use of paper chromatography by a known method [3, 4].

B. The acetylation of the conformers was performed with acetic anhydride in pyridine at 20°C. The quantitative analysis was similar to that described in [3, 5].

SUMMARY

A rare case of separable conformational isomers (conformers) has been found in the 5,10-diketo-19-nor-5,10-secocardenolide series. The method of separating the four conformers, their properties, the IR, mass, and PMR spectra, the kinetics of the acetylation reactions, and the kinetics of thermal transformations are described. Conformational structures as the most probable have been put forward on the basis of the experimental results obtained.

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QUATERNARY 7-OXO-4,5,6,6a-TETRAHYDROHOMOPROAPORPHINE ALKALOIDS OF *Cochicum kesselringii*

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On the basis of additional information obtained by PMR and mass-spectral methods and chemical transformations, the structures of regelinone and isoregelinone, isolated from *Cochicum kesselringii* Rgl., have been established as quarternary 7-oxotetradehydrohomoproaporphine alkaloids.

We have previously [1, 2] reported on the isolation from *Colchicum kesselringii* Rgl. of regelinone (I) and isoregelinone (II), for which probable structures were proposed as epimeric 7-oxohomoproaporphines. Additional information that we have now obtained has permitted their complete structures to be established.

In the PMR spectrum of regelinone (Fig. 1) the signals stand out of three aromatic protons (7.76 ppm, s; 8.47 and 8.36 ppm, dd, J = 6.8 Hz); of two methyl groups (3.85 and 4.80 ppm); and of a tertiary proton (4.34 ppm, s). In addition, in the PMR spectrum taken in trifluoroacetic acid a two-proton singlet appears at 2.90 ppm which is due to the protons of a methylene group located next to a carbonyl group (at C_8).

A signal at 7.76 ppm relates to the H_3 proton of the benzene ring A, and signals at 8.47 and 8.63 ppm forming an AB system correspond to the H_4 and H_5 protons. On the basis of the fact that in the spectrum of an isoquinoline the signal of the H_α proton with respect

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