Stereospecific Association of C-20 Epimers of 3β-Hydroxy-16-oxo-24-nor-17-azachol-5-eno-23-nitryle

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The cryoscopic measurements show that title compounds are strongly associated in CHCl₃ solutions. The association of the 20R epimer is distinctly less pronounced than that of the 20S epimer. Self-association of the 20S epimer leads to the formation of very large complexes. The 20R epimer forms associates via water molecules. The dissimilarity may be explained in terms of different accessibility of the lactam carbonyl groups in the two epimers for the association. It is proposed that the association process is controlled by the configuration at the carbon atom C(20) and conformation around the C(20)-C(22) bond. Populations of side chain conformations of both epimers were determined by means of proton nuclear magnetic resonance. It was found for the 20R epimer that the t and the −g rotamers are almost equally populated, and the rotamer +g is excluded. For the 20S epimer the +g rotamer predominates over the t one, and the −g rotamer is excluded. The NMR data are fully consistent with the results of the molecular modelling studies.

Introduction

It is known that the formation of the intermolecular hydrogen bond is frequently the driving force of the association process [1]. We have recently shown [2] that the title compounds (see Fig. 1) are hydrogen bonded in the solid state. For the 20S epimer the hydrogen bond is formed between the hydrogen atom of the 3β-OH group of one molecule and the amide carbonyl oxygen atom of the D ring of another with the oxygen-oxygen distance of 2.82 Å. The hydrogen bonded molecules of the 20R epimer are separated with the crystallisation water molecules. One water molecule forms three hydrogen bonds, serving as a donor in two of them and as a receptor in one of them. The oxygen-oxygen distances are: 2.79 Å (a bond between hydrogen atom of water and oxygen atom of the 3β-OH group of the steroid molecule), 2.81 Å (a bond between hydrogen atom of water and oxygen atom of the carbonyl group of the steroid), and 2.73 Å (between hydrogen atom of the 3β-OH group of the steroid and oxygen atom of water). The aim of this study is to find if the substantially different hydrogen bonding patterns of both epimers in crystals are preserved in their solutions.

Experimental

Synthesis. The synthesis of both epimers was described earlier [2]. The purity of both epimers was...
checked with NMR (for the presence of the opposite epimer), and with the mass spectra (for the presence of other impurities). The results of elemental analyses were used to calculate the amount of water present in the samples. The amount of water present in the sample was always much smaller than the water solubility in chloroform [3].

NMR measurements. The spectra were measured in CDCl₃ (99.8%, Sigma) or in CF₃CH₂OH (GC 99%, Aldrich-Chemie)/CD₃OD (99.8%, Sigma) 3:1 v/v mixture as a solvent. All measurements were recorded on a Bruker AM 500 spectrometer at ambient temperature. TMS (Uvasol) was used as internal ¹H NMR reference. ¹H NMR spectra were acquired using 90 degrees flip angle pulses and 64k data points to achieve a digital resolution of 0.16 Hz per point.

Molecular modelling calculations. A standard molecular mechanics program HyperChem™ release 4 for Windows (Hypercube Inc.) was used for calculations. The program utilises MM+ force field and the Polak-Ribiere algorithm (a conjugate gradient method).

CD measurements. CD spectra were measured at room temperature in C₂H₅OH and CF₃CH₂OH (Uvasol, Merck) and CH₃CN (Chromasolv, Riedel-deHaen) solutions at concentration 1×10⁻³ M with Aviv 62 DS spectrophotometer using 0.1 and 1.0 cm pathlength (spectral band width 1.5 nm, sensitivity 5×10⁻⁶ or 10×10⁻⁶ [DA unit/nm]). Curve smoothing was done with the home written software from the Institute of Organic Chemistry applying the Golay-Savitzky algorithm [4]. Δε is expressed in [L*M⁻¹*cm⁻¹] units.

Cryoscopic measurements. The freezing point method was used. The apparatus and procedure was described elsewhere [5]. All measurements were done in freshly prepared CHCl₃ solutions. Chloroform was washed with concentrated H₂SO₄, water, dried with anhydrous MgSO₄ and distilled twice prior to measurements [6]. The amount of solution sample used was 0.3 g. The method consists of linear programming of apparatus temperature as a function of time with simultaneous measurement of the temperature inside the solution sample and the temperature of surrounding thermostat [5]. The temperature scanning velocity was kept as low as possible to approach the thermodynamic equilibrium conditions. The value of 0.2 [K/min] was used. As a result the melting curves as functions of temperature were obtained. The numerical analysis [7] of the cryometric curves facilitates determination of the values of ΔT. The mean molecular weight of the steroid was calculated using the following equation:

\[ M_n = K_o \times 1000W_2 / (\Delta T \times W_1) \]

where: \( K_o \) – cryoscopic constant of the solvent, the value of 4.9 [K*g/1000 mol] [6] was used for CHCl₃; \( W_1 \) – amount of solvent; \( W_2 \) – amount of steroid; \( \Delta T \) – difference of solvent and solution melting temperatures.

Results and Discussion

NMR studies

The ¹H NMR spectra of the 20R and the 20S epimers reveal changes in chemical shifts of signals assigned to the H(15α) and the H(15β) protons with increasing polarity of the solvent (see Fig. 2).
Table I. Chemical shifts (ppm) and averaged coupling constants (Hz) of the signals corresponding to the selected ring D and side chain protons of the 20R and the 20S epimers.

<table>
<thead>
<tr>
<th>Epimer</th>
<th>Solvent</th>
<th>H(22 pro-R) δ</th>
<th>J_{20,22}</th>
<th>H(22 pro-S) δ</th>
<th>J_{20,22}</th>
<th>H(15α) δ</th>
<th>J_{14,15}</th>
<th>H(15β) δ</th>
<th>J_{14,15}</th>
</tr>
</thead>
<tbody>
<tr>
<td>20R</td>
<td>b)</td>
<td>3.05</td>
<td>7.4</td>
<td>3.05</td>
<td>7.4</td>
<td>2.17</td>
<td>5.9</td>
<td>2.12</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>c)</td>
<td>3.19</td>
<td>8.4</td>
<td>2.93</td>
<td>6.9</td>
<td>2.19</td>
<td>6.2</td>
<td>2.22</td>
<td>13.5</td>
</tr>
<tr>
<td>20S</td>
<td>b)</td>
<td>3.37</td>
<td>10.1</td>
<td>2.57</td>
<td>5.1</td>
<td>2.19</td>
<td>6.7</td>
<td>2.09</td>
<td>13.5</td>
</tr>
<tr>
<td></td>
<td>c)</td>
<td>3.31</td>
<td>10.2</td>
<td>2.66</td>
<td>5.1</td>
<td>2.20</td>
<td>6.7</td>
<td>2.19</td>
<td>13.5</td>
</tr>
</tbody>
</table>

a) The NMR spectra were analyzed with a simulation program, see ref. 9; b) in CDCl3 at ambient temperature, data of ref. [2]; c) in CF3CH2OH/CD3OD (3:1 (v/v)) at ambient temperature.

The solvent induces no meaningful changes in the ring D conformation. The changes of chemical shifts indicate that the carbonyl group of each epimer undergoes solvation by the polar solvent molecules from the β-face of the steroid molecule.

It was shown that the conformation around the N(17)–C(20) bond of the 20R and the 20S epimers in CHCl3 solution is the same as that found for the epimers in the solid state [2]. Therefore it is likely that the conformation with a small value of the torsion angle C(13)–N(17)–C(20)–H(20) is also preserved in the solvent of higher polarity.

The region of the 1H NMR spectra which differs more significantly with varying solvent polarity is assigned to the side chain protons [2]. The assignment of the signals to the H(22 pro-R) and the H(22 pro-S) protons [8] was made on the basis of the 1H 1D difference NOE spectrum of the 20S epimer in CDCl3 solution. The irradiation of the H(22) signal at higher field gave 3.4% relative enhancement at the H(20) signal. The irradiation of the H(21) methyl signal gave 1.6% and 1.0% relative enhancement at the H(22) higher and lower field signals, respectively. These allowed us to assign the higher field signal to the H(22 pro-S) proton and the lower field signal to the H(22 pro-R) one (see Fig. 2 and Table I). As can be seen in the Fig. 2 the chemical shifts of the H(22 pro-R) and the H(22 pro-S) protons strongly depend on the solvent used. The differences in the chemical shifts of the signals amount to as much as 0.80 ppm and 0.65 ppm (see Table I) for the 20S epimer, and 0.00 ppm and 0.26 ppm for the 20R epimer in less and more polar solvent, respectively. The apparent vicinal coupling constants essentially remain unchanged for the 20S epimer and change slightly in the spectra of the 20R epimer (see Table I). The assignment of the prochiral protons is consistent with the observed changes in their chemical shifts with varying solvent polarity.

Averaged homonuclear vicinal coupling constants (Table I) were obtained from the 1H 1D NMR spectra with the use of the fitting procedure [9] and were utilised to determine the rotamers distribution. The analysis of conformation of the C(20)–C(22) bonds has been performed with the use of the discrete model [10]. The three possible rotamers of the side chains of both epimers are shown in Figures 3 and 4. The dependence of vicinal coupling constants for proton H(20) with both the H(22 pro-R) and the H(22 pro-S) protons on the dihedral angle was generated using Altona program [11,12]. The analysis of the three possible rotamers of the C(20)–C(22) bond of the 20R epimer allowed us to exclude the +g conformer and find the remaining −g and t conformers to be nearly equally populated (see Fig. 3 and Table II). In the solid state the −g conformer was found for that epimer [2]. For the 20S epimer the analysis showed that the −g conformer (see Fig. 4), is excluded, and the conformer +g prevails with the ratio 4:1 over the t one, with the latter found in crystals [2].

Molecular modelling studies

In order to determine the favoured conformation of the side chain of the epimeric nitriles in vacuo the molecular mechanics calculations were performed. The X-ray crystal structures of both epimers were used as starting structures with torsion angles N(17)–C(20)–C(22)–C(23) of −52.3°.
for the 20R epimer and of 172.6° for the 20S epimer. Two other rotamers with the torsion angles bigger and smaller by 120° were used as well for each molecule. A systematic search was conducted for the conformations that correspond to the minimum value of the total steric energy. Three conformers were found for each epimer with dihedral angles of the side chain corresponding to the t, −g, and +g rotamers (see Table III). It can be easily seen that for the +g rotamer of the 20R epimer and for the −g rotamer of the 20S epimer distinctly higher energies were calculated and both rotamers may virtually be excluded. These two rotamers were not observed in the NMR spectra. For the t and the −g rotamers of the 20R epimer, and for the t and the +g rotamers of the 20S epimer similar values of energies were obtained, which corresponds to equilibria measured for both epimers in their NMR spectra. The inspection of O(lactam)-C(nitrile) distances for each rotamer reveals that the nitrile group in the −g rotamer of the 20R epimer is in closer proximity to the lactam oxygen atom than in the t rotamer. The same is true for the +g and the t rotamers of the 20S epimer. It can be therefore assumed that the conformation around the C(20)-C(22) bond is the factor determining accessibility of the lactam oxygen atom for association. The 20S epimer molecules have relatively longer intramolecular distances between carbonyl and nitrile groups and may undergo free association, whereas for the 20R epimer the hydrogen bond is formed with the help of the solvating water. The dissimilarities in the association propensities of the two epimers originate from the different arrangements of the resi-

Table II. Relative populations of C(20)-C(22) rotamers calculated for the 20R and the 20S epimers using data of Table I.

<table>
<thead>
<tr>
<th>Epimer</th>
<th>Solvent</th>
<th>Rotamer</th>
<th>t</th>
<th>−g</th>
<th>+g</th>
</tr>
</thead>
<tbody>
<tr>
<td>20R</td>
<td>less polar</td>
<td>0.50</td>
<td>0.47</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>more polar</td>
<td>0.60</td>
<td>0.40</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>20S</td>
<td>less polar</td>
<td>0.18</td>
<td>0.01</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>more polar</td>
<td>0.18</td>
<td>0.00</td>
<td>0.82</td>
<td></td>
</tr>
</tbody>
</table>

a Calculated using following equations; refs. 11 and 12 (see Fig. 3):
1. \( J_{H(20)H(22\text{ pro-S})} = 11.8 P_t + 2.9 P_{-g} + 3.4 P_{+g} \)
2. \( J_{H(20)H(22\text{ pro-S})} = 3.6 P_t + 11.8 P_{-g} + 2.6 P_{+g} \)
3. \( P_t + P_{-g} + P_{+g} = 1 \)

(b) Calculated using following equations; refs. 11 and 12 (see Fig. 4):
1. \( J_{H(20)H(22\text{ pro-S})} = 11.8 P_t + 3.4 P_{-g} + 2.9 P_{+g} \)
2. \( J_{H(20)H(22\text{ pro-S})} = 3.6 P_t + 2.6 P_{-g} + 11.8 P_{+g} \)
3. \( P_t + P_{-g} + P_{+g} = 1 \)

(c) in CDCl₃, at ambient temperature;
(d) in CF₃CH₂OH/CD₃OD (3:1 (v/v)) at ambient temperature.
dues on the C(20) carbon atoms of both compounds. The configuration at this carbon atom influences the conformations of the side chain, controlling the access to the oxygen atom of the lactam carbonyl group.

**CD studies**

CD spectra of both epimers measured in several solvents are presented in Table IV and Fig. 5. In all cases, the band II is characterised by a positive, and the band I, below 200 nm, by a negative Cotton effect. The apparent maximum of the band II (of \(n\pi^*\) origin) is red shifted with decreasing solvent polarity, that is from 2,2,2-trifluoroethanol (TFE) to acetonitrile. This feature of \(n\pi^*\) transition is a consequence of the stabilisation of the ground state of the \(n\)-orbital by the solute-solvent association. The band I shows no systematic variation with solvent polarity. The red shift is observed when going from TFE to ethanol. However, for both epimers in acetonitrile, this band is blue shifted by ca. 3 nm relative to TFE. Since the lactams in question are insoluble in hydrocarbons and other non-polar solvents, we were not able to study this problem more systematically. For that reason the band I assignment to the \(\pi\pi^*\) transition, cannot be proved [13]. The positive sign of \(n\pi^*\) transition is in agreement with the ring helicity rule for lactams [14]. In addition the Konno rule [15] suggests, that in \(\gamma\)-lactams the sign and the magnitude of the CD signal may be determined by two effects: substituent effect at the \(C_\alpha\) position and the ring chirality. Since the lactams studied have no \(C_\alpha\) substituent, the chirality of the D ring determines the CD spectra (second chiral sphere according to the Snatzke’s doctrine of spheres [16]). The pyrrolidine ring of the 20\(R\) and the 20\(S\) \(\gamma\)-lactams is not planar, as was shown by the X-ray analysis [2]. Thus, the planar amide group is incorporated into a chiral ring. The conformation of the ring is an intermediate between

![Fig. 5. The CD spectra of the 20\(R\) epimer (left) and the 20\(S\) epimer (right) in various solvents at room temperature: acetonitrile (---), ethanol (-----), 2,2,2-trifluoroethanol (-----).]
14α-envelope and a 13α,14β-half-chair for the 20R epimer and nearly pure 14α-envelope in the case of the 20S epimer. Lower magnitude of the nπ* Cotton effect for the 20R epimer relative to the 20S one may be caused by conformational equilibrium of the two conformers in solution (14α-envelope with strong positive and 13α,14β-half-chair with weak negative contribution). However, the influence of the chiral center at the C-20 carbon in the side chain (chiral third sphere) on the magnitude of this transition cannot be excluded.

**Cryoscopic studies**

Cryoscopic method [7,17] was applied in the study of the association process of the 20R and the 20S epimers in CHCl₃ solutions. Samples of the 20R epimer were more soluble and they always contained some crystallisation water, which could be removed only partially and some amount always remain in the sample, despite of the drying of the samples at 110 °C under the vacuum. Therefore it was not possible to compare self-association behaviour of both epimers. In this analysis we included a sample containing 1.5 [weight%] of water (e.g. 1:3 molar water/steroid ratio, see Table V and Fig. 6; the molar water/steroid ratio in the 20R epimer crystals used for crystallographic measurements [2] amounted to 0.5:1, e.g. 2.5 [weight%] of water). For comparison the sample with the same water/steroid ratio for the 20S epimer was analysed. It can be seen from the Fig. 6 that the association propensities of the 20R epimer are clearly weaker than those of the 20S one. There is no definite model of water-steroid association. The observed concentrational dependence of the average molecular weight may be accounted for the presence of water molecules in several ways. One can consider the model in which water molecules do not co-associate with the steroid molecules and only self-associate [18]. On the other hand several models of mixed association of steroid-water, two-

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**Table V. Concentrational dependence of the number-average molecular weight of the epimers 20R and 20S in CHCl₃ solutions.**

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>20R (1.5%)[^c]</td>
<td>0.0897</td>
<td>364</td>
<td>20R (6.6%)[^c]</td>
<td>0.0970</td>
<td>610</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2472</td>
<td>690</td>
<td>0.1587</td>
<td>866</td>
<td></td>
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<tr>
<td>0.3999</td>
<td>1253</td>
<td>0.2258</td>
<td>887</td>
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<tr>
<td>0.5654</td>
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<td>1248</td>
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<tr>
<td>0.6736</td>
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<tr>
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<td>0.7837</td>
<td>1548</td>
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</tr>
<tr>
<td>20S</td>
<td>0.0699</td>
<td>356</td>
<td>20S (1.5%)[^c]</td>
<td>0.0992</td>
<td>463</td>
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</tr>
<tr>
<td>0.1421</td>
<td>1787</td>
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<td>1158</td>
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<tr>
<td>0.2338</td>
<td>1135</td>
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<td>1519</td>
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</tr>
<tr>
<td>0.3458</td>
<td>1977</td>
<td>0.4160</td>
<td>1949</td>
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<tr>
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<td>0.4947</td>
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<tr>
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<td>0.6921</td>
<td>11015</td>
<td>0.7480</td>
<td>3388</td>
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</tr>
</tbody>
</table>

[^a] c – the concentration of the steroid expressed in [weight %];[^b] Mₙ – the number-average molecular weight of the steroid expressed in [g/mol];[^c] numbers of percents refer to the percent ratio of mass of water present in the sample to the mass of steroid.
solute system, may be considered [19]. Although the full analysis of our data is not possible, they clearly show different association propensities of both epimers.

The increasing amount of water in the sample of the 20R epimer causes a small decrease in measured average molecular weight (see data for 6.6 [weight%] of water sample in Table V).

The water free samples were measured for the 20S epimer. The self-association of the epimer in solution was characterised by means of the association number γ, as described by Prigogine [20]. The values of the number-average molecular weights are given in Table V. The mean association numbers are shown in Fig. 7. The data clearly indicate that the 20S epimer undergoes self-association which is strongly concentration dependent. This association behaviour is characteristic of very large complexes formation which may lead to phase separation [20]. On the basis of the crystallographic data [2] one can propose for the 20S epimer the head-to-tail model of association with the 3β-OH group presumably acting as a donor, and the carbonyl oxygen atom being an acceptor.

Conclusions

With the use of the cryoscopic data it was shown that the title compounds undergo association in CHCl₃ solution. However, the association patterns of both epimers are markedly different. Self-association of the 20S epimer leads to very large complexes. The head-to-tail arrangement with hydrogen bond formation is assumed for the 20S epimer following data obtained for crystals [2]. The association propensities of the 20R epimer are much less pronounced then those for the 20S epimer.

NMR and CD data obtained for both epimers as well as molecular modelling carried for them suggest that the dissimilarity observed is caused by the different conformation of the steroid side chain. It is proposed that the configuration at the C(20) carbon atom and the conformational equilibrium about the C(20)–C(22) bond, both control the access to the lactam oxygen atom and in this way control association propensities of both epimers.

Acknowledgement

We wish to thank Mrs. Maria Galka for her help in the cryoscopic measurements.

[9] Program SYMULANT provided by Professor A. Gryff-Keller, Department of Chemistry, Technical University, Warsaw, Poland.