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Beckmann rearrangement of 3α - and 3β -acetamido- 5α -androstan-17-one oximes gives homo-aza compounds and the corresponding "second order" Beckmann cleavage ω -cyanoolefin. The mixture of the compounds produced, was separated by column chromatography. The structure of the lactams and the exocyclic nitriles was apparent from ir and nmr spectra.

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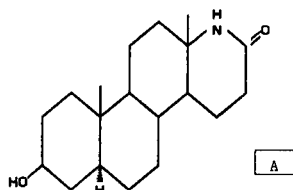
Introduction.

The concept in designing steroidal lactam amides arises from two main reasons:

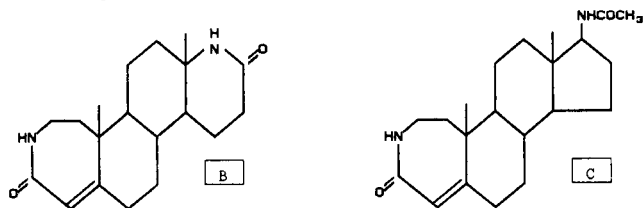
a. The biological action of lactams (which are characterized by the $-\text{NH}-\text{CO}$ group) may be structural specific and therefore prolonged as a result of the multiple interactions of this group with similar groups that exist in proteins and nucleic acids.

b. The $-\text{NH}-\text{CO}-$ group of the lactam molecule may be essential for antitumor activity and it may open enzymatically, forming species which could attack the contents of cancer cells [1-4].

In our efforts to study the action of the lactam molecule without alkylating agent, we found that lactam A was slightly below the limits required for further testing.



Lactam A and bilactam B were active in Ehrlich Ascites Tumor [5].



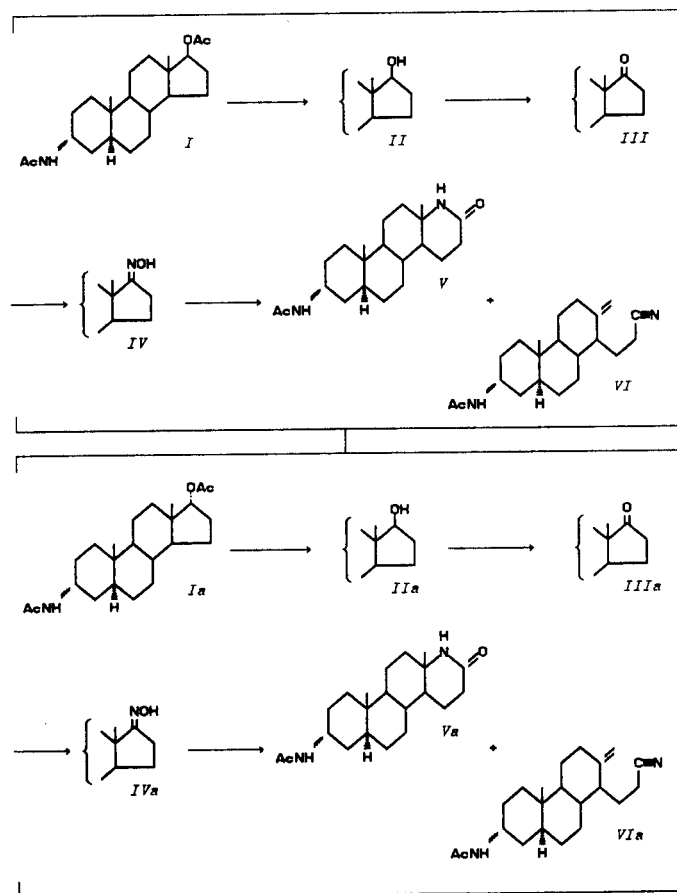
17β -Acetamido-3-aza-A-homo- 4α -androst-4-one (C), has cytostatic activity against Ehrlich Ascites Tumor, L1210 and P388 Leukemias in mice [6].

These satisfactory results, prompted us to continue our research on the synthesis of homo-aza-steroids containing a second amide group out of the steroidal nucleus and study their antitumor properties.

Chemistry.

The interesting biological properties of the compounds **A** and **B** prompted us to design a compound which bears a second $-\text{NH}-\text{CO}-$ group out of the steroidal skeleton.

As starting materials we used the two already reported isomers: 3β -acetamido- 5α -androstan-17 β -ol-acetate (**I**) and 3α -acetamido- 5α -androstan-17 β -ol-acetate (**Ia**) [7-9]. The latter compounds, produced the corresponding ketones **III** and **IIIa** after hydrolysis and Jones oxidation. Treatment of the compounds **III** and **IIIa** with hydroxylamine hydrochloride in pyridine-ethanol produces the oximes **IV**



and **IVa** respectively. Beckmann rearrangement of the 3β -acetamido- 5α -androstan-17-one-oxime (**IV**), produces the 3β -acetamido- 13α -amino- $13,17$ -seco- 5α -androstan-17-*oic*- $13,17$ lactam (**V**) and the "second order" Beckmann cleavage product, 3β -acetamido- $13,17$ -seco- 5α -androstan- $13,18$ -en-17-nitrile (**VI**). The two main products **V** and **VI** were separated by column chromatography. Under the same reaction conditions the oxime **IVa** was rearranged to the lactam **Va** and the exocyclic nitrile **VIa**.

In the formation of the ω -cyanoolefin the hydroxyl-group of the ketoxime should be *trans*-coplanar and have a proton antiparallel to one of the 18-CH_3 protons [10].

The orientation of the acetamido-group, which was assigned from the mode of reduction, was confirmed by nuclear magnetic resonance spectrometry of the final products. The equatorial proton at C_3 in the 3α -acetamido lactam and nitrile, **Va** and **VIa**, gave a broad signal centered at $\delta = 3.9$ ppm, whereas the signal for the axial 3α -proton in the 3β -acetamido lactam and nitrile, **V** and **VI**, was centered at $\delta = 3.6$ ppm. We have also noticed that the methyl signal of the equatorial 3β -acetamido group was observed at a higher field (1.97 ppm) than the corresponding signal of the 3α -epimer (2.01 ppm). These results agree with those previously found by other researchers [8-9].

The chemical shifts of the $-\text{NH}-\text{CO}-$ protons appeared as singlets at $\delta = 5.78$ ppm for the cyclic $-\text{NH}-\text{CO}-$ of both lactams, **V** and **Va**, whereas the signals for the $-\text{NH}-\text{CO}-$ protons of the acetamido-group at 3 position appeared as doublets at $\delta 5.26$ ppm for the 3β -acetamido compounds **V** and **VI** and at $\delta = 5.36$ ppm for the 3α -acetamido compounds **Va** and **VIa**.

EXPERIMENTAL

Melting points were determined on a Fisher Johns melting point apparatus and are uncorrected. The ir spectra were recorded with a Perkin-Elmer 521 in solid phase potassium bromide. The nmr spectra were determined with a Varian Associates A-60 and XL-100 instrument using deuteriochloroform as a solvent and tetramethylsilane as the internal standard. Elemental analyses were performed by the Analytical Laboratory of the Nuclear Research Center "Demokritos".

3β -Amino- 5α -androstan-17 β -ol.

3β -Amino- 5α -androstan-17 β -ol has been obtained in 92% yield by reduction of 17 β -hydroxy- 5α -androstan-3-one oxime with *n*-butyl alcohol and sodium [7].

3β -Acetamido- 5α -androstan-17-one (**III**).

3β -Amino- 5α -androstan-17 β -ol (3.5 g) was dissolved in 10 ml of pyridine and 10 ml of acetic anhydride, left at room temperature overnight, and then was poured into ice-water. The precipitate was collected by filtration, producing compound **I** in 95% yield, mp 274-276° (ethyl acetate) lit [7] 278°.

Two g of the compound **I** was dissolved in 50 ml of 10% methanolic potassium hydroxide solution (selective hydrolysis of the

C_3 group). The mixture was refluxed for 2 hours, poured into ice-cold water and the precipitate was collected by filtration to give 3β -acetamido- 3α -androstan-17 β -ol (**II**) in 90% yield (homogeneous as judged by tlc).

To a cold suspension of **II** in 300 ml of acetone, Jones reagent (8 ml) was added dropwise with stirring. After 24 hours the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water, dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The residue was crystallized from ethyl acetate, 1.7 g, mp 240°; ir: ν max 3450, 3280, 3080 cm^{-1} (NH), 1735 cm^{-1} (C=O), 1630, 1560 cm^{-1} (NHCO).

Anal. Calcd. for $\text{C}_{21}\text{H}_{33}\text{NO}_2$: C, 76.13; H, 9.97; N, 4.23. Found: C, 76.38; H, 9.81; N, 4.11.

Beckmann Rearrangement of 3β -Acetamido- 5α -androstan-17-one Oxime (**IV**).

3β -Acetamido- 5α -androstan-17-one (**III** 1.5 g) was dissolved in a mixture of 10 ml ethanol and 10 ml pyridine. Hydroxylamine hydrochloride (2.5 g) was added to the solution and the mixture was refluxed for 2 hours. The mixture was poured into cold water and the white precipitate collected by filtration was washed and dried. Recrystallization from ethyl acetate yields 1.2 g, mp 215°; ir: ν max 3300-3200 cm^{-1} (OH), 3080 cm^{-1} (NH), 1635, 1555 cm^{-1} (NHCO).

The above oxime was dissolved in 30 ml of purified dioxane. One ml of freshly distilled thionyl chloride in 15 ml of purified dioxane was added dropwise with constant stirring at room temperature. The mixture was stirred for an additional period of 24 hours and then was poured into ice-water, neutralized with ammonium hydroxide and extracted with chloroform. The organic layer was washed with water, dried over sodium sulfate and after evaporation of the solvent under reduced pressure, the resulting residue was chromatographed on a column of silica gel (80 g).

Elution with chloroform gave 350 mg of ω -cyanoolefin **VI**, mp 109-110° (ether); ir: ν max 3450, 3290, 3070 cm^{-1} (NH), 2245 cm^{-1} (C \equiv N), 1645, 1540 cm^{-1} (NHCO); ^1H nmr: δ 1.15 (19- CH_3), 1.97 ($\text{CH}_3\text{CONH}-$), 3.60 ($3\alpha\text{-H}$), 4.80, 4.65 (C=CH $_2$), 5.29 ($-\text{NHCO}$).

Anal. Calcd. for $\text{C}_{21}\text{H}_{32}\text{N}_2\text{O}$: C, 76.83; H, 9.75; N, 8.53. Found: C, 76.97; H, 9.55; N, 8.51.

Further elution with chloroform-methanol (98:2) gave 450 mg of 3β -acetamido- 13α -amino- $13,17$ -seco- 5α -androstan-17-*oic*- $13,17$ -lactam (**V**). Crystallization from ethyl acetate gave a mp above 300°; ir: ν max 3450, 3280, 3180, 3070 cm^{-1} (NH), 1680, 1635, 1545 cm^{-1} (NHCO); ^1H nmr: δ 0.78 (18- CH_3), 1.16 (19- CH_3), 1.97 ($\text{CH}_3\text{CONH}-$), 3.60 ($3\alpha\text{-H}$), 5.26 (NHCO-acetamide), 5.78 (NHCO-lactam).

Anal. Calcd. for $\text{C}_{21}\text{H}_{34}\text{N}_2\text{O}_2$: C, 72.83; H, 9.82; N, 8.09. Found: C, 72.58; H, 9.70; N, 8.21.

3α -Amino- 5α -androstan-17 β -ol.

3α -Amino- 5α -androstan-17 β -ol has been obtained in 92% yield by catalytic reduction of 17 β -hydroxy- 5α -androstan-3-one oxime [7].

3α -Acetamido- 5α -androstan-17-one (**IIIa**).

Under the same reaction conditions as for compound **III** the 3α -acetamido- 5α -androstan-17-one (**IIIa**) was obtained in 77% yield, mp 210-212° (ethyl acetate); ir: ν max 3440, 3270, 3080 cm^{-1} (NH), 1745 cm^{-1} (C=O), 1640, 1560 cm^{-1} (NHCO).

Anal. Calcd. for $\text{C}_{21}\text{H}_{33}\text{NO}_2$: C, 76.13; H, 9.97; N, 4.23. Found:

C, 75.92; H, 9.85; N, 4.22.

Beckmann Rearrangement of 3 α -Acetamido-5 α -androstan-17-one Oxime (**IVa**).

Under the same reaction and chromatographical conditions as for compound **IV**, the ω -cyanoolefin **VIa** was obtained in 28% yield, mp 115°; ir: ν max 3400, 3280, 3080 cm^{-1} (NH), 3250 cm^{-1} (C \equiv N), 1640, 1550 cm^{-1} (NHCO); ^1H nmr: δ 1.18 (19-CH₃), 2.00 (CH₃CONH), 3.90 (3 β -H), 4.78, 4.65 (C=CH₂), 5.39 (-NHCO).

Anal. Calcd. for C₂₁H₃₂N₂O: C, 76.83; H, 9.75; N, 8.53. Found: C, 76.88; H, 9.99; N, 8.59.

Further elution of the column with chloroform-methanol (98:2) gave 52% of 3 α -acetamido-13 α -amino-13,17-seco-5 α -androstan-17-*oic*-13,17-lactam (**Va**). Crystallization from ethyl acetate gave a mp above 300°; ir: ν max 3450, 3270, 3080 cm^{-1} (NH), 1670, 1630, 1570 cm^{-1} (NHCO); nmr: δ 0.82 (18-CH₃), 1.20 (19-CH₃), 2.01 (CH₃CONH-), 3.90 (3 β -H), 5.36 (NHCO-acetmide), 5.78 (NHCO-lactam).

Anal. Calcd. for C₂₁H₃₄N₂O₂: C, 72.83; H, 9.82; N, 8.09. Found:

C, 72.72; H, 9.69; N, 8.00.

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