Formation of Bishomoazasteroids by the Beckmann Rearrangement

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Beckmann rearrangement of the geometrical isomer of 5α-androstanolone oximes gives the 3-aza-17β-hydroxy-A-homo-5α-androstan-4-one and the 4-aza-17β-hydroxy-A-homo-5α-androstan-3-one. These lactams were converted to the corresponding ketones by Jones reagent oxidation. Treatment with hydroxylamine produced the corresponding ketoximes. Beckmann rearrangement of the ketoximes generated the diaza 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 compounds was apparent from spectral data.

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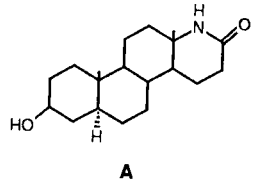
Introduction.

Studies have been recently made on the methods of synthesis of steroidal bislactams or acetamidohomoazasteroids, containing the -NHCO- group in the cyclopentanoperhydrophenanthrene or in the molecular side chain.

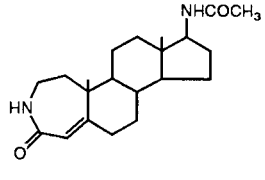
The biological action of steroidal lactams characterized by the -CONH- group, may be structurally specific and therefore more prolonged. Such action may be a result of the multiple interaction of the -NHCO- groups with similar groups present in the proteins and nucleic acids.

When cultures of human leukemia cells from untreated patients with acute leukemia, were treated with 3β-hydroxy-13α-amino-13,17-*seco*-5α-androstan-17-oic-13,17-lactam (**A**), they exhibited an increased proliferation activity. The leukemic cells were cultured for 48 hours and amount of the lactam (4, 12, 24 ng/ml) were added at zero time in the continuous presence of [^{3}H]-thymidine. The data suggested that, at high concentration, the lactam acts as a toxic agent while at the lower ones it seems to stimulate proliferation of the blast cell [1].



A

The acetamidolactam, namely 17β-acetamido-3-aza-A-homo-4α-androsten-4-one (**B**) [2], was found to have some cytostatic activity against Ehrlich Ascite Tumor cells, L1210 and P388 Leukemias.

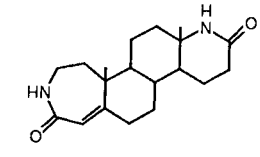


B

The mechanism of action of the acetamidolactam on L1210 leukemia cells and especially on nucleic acids and proteins was investigated and it was found that it inhibits the incorporation of thymidine in the DNA of L1210 cells. Inhibition is not stable and appears to get reduced when S-9 mix is added in the cell culture. On the other hand, compound **B** does not inhibit RNA and protein synthesis significantly. With TA98 Salmonella typhimurium tester strain, the acetamidolactam increases slightly the number of revertant colonies.

The same compound does not induce SCEs in CHO cells in culture, but it induces a significant number of chromosome aberrations in the same cells [3-4].

On the other hand, 3β-hydroxy-13α-amino-13,17-*seco*-5α-androstan-17-oic-13,17-lactam (**A**) and 3,17α-diaza-A,D-bishomoandrostan-4α-ene-4,17-dione (**C**) [5], are active in Ehrlich Ascites Tumor (EAT) and L1210 leukemias, causing a significant though temporary, suppression of the growth of EAT cells and a slight increase in the life-span of the treated animals whereas there is no increase in the life-span of the animals bearing leukemias [6].



C

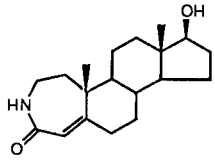
The satisfactory results of modified steroids, prompted us to synthesize isomer dilactams and to study their cytostatic activity.

Chemistry.

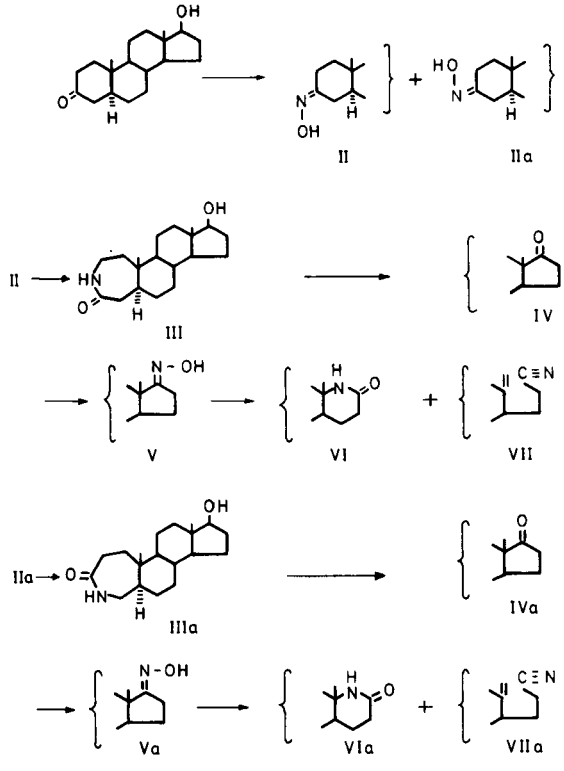
In the study of azasteroid synthesis, Beckmann rearrangement of 3-ketosteroid oximes to produce either inseparable mixtures of the two lactam isomers or only one of them in pure state has been already reported by many

workers.

However, during column chromatography of 5α-androstanolone oximes, the compounds resolve into *syn*- and *anti*-isomers [7]. 17β-Hydroxy-5α-androstan-3-one oximes (**II, IIa**) give 3-aza- and 4-aza-ϵ-lactams **III** and **IIIa** respectively by Beckmann rearrangement [8]. Hydrogenation of 3-aza-17β-hydroxy-A-homo-4-androsten-4-one [9-10] with palladium on charcoal gives the lactam **III**.



The 3-aza- and 4-aza-ϵ-lactams, **III** and **IIIa**, produce by oxidation the 3-aza-A-homo-5α-androstan-4,17-dione (**IV**) and 4-aza-A-homo-5α-androstan-3,17-dione (**IVa**). Treatment of **IV** and **IVa** with hydroxylamine hydrochloride in a mixture of pyridine-ethanol produce the corresponding ketoximes **V** and **Va** respectively. Beckmann rearrangement of 3-aza-A-homo-5α-androstan-4,17-dione oxime (**V**) in purified dioxane with fresh distilled thionyl chloride produces the diaza compound **VI** and the "second order" Beckmann cleavage product, 3-aza-A-homo-13,17-*seco*-5α-androstan-13,18-en-17-nitrile (**VII**). In the formation of ψ-cyanoolefin the hydroxy group of the ketoxime should be *trans*-coplanar and have antiparallel



to one of the 18-CH_3 protons [11], ν max 2240 cm^{-1} (C≡N), nmr at δ 0.88 (19-CH_3) and 4.10, 4.80 (C=CH_2).

Under the same reaction conditions the oxime **Va** is rearranged to the lactam **VIa** and the exocyclic nitrile **VIIa**, which shows ν max 2240 cm^{-1} (C≡N), nmr at δ 0.78 (19-CH_3) and 4.45, 4.76 (C=CH_2).

The C-19 methyl resonance of the isomer **VII** appears at higher field than that of the isomer **VIIa**. It is possible, however, that the unshared electron pair on the nitrogen atom may have some effect on the C-19 methyl resonance.

Nace and Watterson [12] and Oka and Hara [8] have shown for the 19-CH_3 resonances for the lactam isomers of 2- and 3-azapregnane derivatives, the following results: 2-aza-5α-pregnane-3,20-dione (59 cps) and 3-aza-5α-pregnane-2,20-dione (54 cps).

The lactams **III** and **IIIa** and the dilactams **VI** and **VIa** have high melting points and are barely soluble in organic solvents.

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. Ultraviolet spectra, were measured in ethanol solution on a Cary Model II instrument. Elemental analyses were performed by the Analytical Laboratory of the Nuclear Research Center "Demokritos".

5α-Androstanolone Oximes **II** and **IIa**.

17β-Hydroxy-5α-androstan-3-one oximes, were obtained by the reaction of hydroxylamine hydrochloride in a mixture of pyridine-ethanol and they have been separated according to the procedure of Oka and Hara [7].

3-Aza-17β-hydroxy-A-homo-5α-androstan-4-one (**III**) and 4-aza-17β-hydroxy-A-homo-5α-androstan-3-one (**IIIa**).

The 3-aza- and 4-aza-ϵ-lactams have been prepared from the corresponding oximes **II** and **IIa**, according to the procedures of Oka and Hara [8].

3-Aza-A-homo-5α-androstan-4,17-dione (**IV**).

Jones reagent (1 ml) was added dropwise with stirring to a cold suspension of 3-aza-17β-hydroxy-A-homo-5α-androstan-4-one (309 mg) in 20 ml of acetone. After 24 hours the reaction mixture was diluted with water and extracted with chloroform. The chloroform extract was washed with water, dried and the solvent was removed under reduced pressure. The residue was taken up in chloroform and passed through a column of silica gel. The product so obtained was crystallized from ethyl acetate, 220 mg, mp 293-295°; ir: ν max 1730 cm^{-1} (C=O).

Anal. Calcd. for $C_{19}H_{29}NO_2$: C, 75.24; H, 9.75; N, 4.62. Found: C, 75.53; H, 9.79; N, 4.52.

4-Aza-A-homo-5α-androstan-3,17-dione (**IVa**).

Under the same reaction conditions for **IV** the 4-aza-A-homo-5α-androstan-3,17-dione was obtained in 60% yield, mp 305° (ethyl acetate); ir: ν max 1730 cm^{-1} (C=O).

Anal. Calcd. for $C_{19}H_{29}NO_2$: C, 75.24; H, 9.75; N, 4.62. Found: C, 75.63; H, 9.76; N, 4.42.

Beckmann Rearrangement of 3-Aza-A-homo-5α-androstan-4,17-dione Oxime.

3-Aza-A-homo-5α-androstan-4,17-dione (**IV**) (0.350 g) was dissolved in a mixture of 15 ml of ethanol and 15 ml of pyridine. Hydroxylamine hydrochloride (0.350 g) was added to this solution and the mixture was heated under reflux for 2 hours. The solution was poured into ice-water and extracted with chloroform. The chloroform extract was washed with water, dried and the solvent removed under reduced pressure to yield 300 mg of oxime **V**. This oxime without further purification was dissolved in 150 ml of purified dioxane and 1.5 ml of fresh distilled thionyl chloride in 15 ml of purified dioxane was added dropwise under constant stirring at room temperature. This mixture was stirred for an additional period of 16 hours. It 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 (200 mg) was chromatographed on a column of silica gel (60 g). Elution with chloroform gave 30 mg of ω-cyanoolefin **VII**, mp 116-118°; ir: ν max 2240 cm^{-1} (C≡N); nmr: δ 0.88 (19-CH_3), 4.10 and 4.80 (C=CH_2), 5.75 (NH).

Anal. Calcd. for $C_{19}H_{25}N_2O$: C, 76.00; H, 9.33; N, 9.33. Found: C, 75.69; H, 9.70; N, 8.91.

With further elution of the column with chloroform-methanol (98:2), the 3,17α-diaza-A,D-bishomo-5α-androstan-4,17-dione (260 mg) was isolated. Crystallization from a mixture of chloroform-methanol gave a mp above 300°; ir: ν max 3350, 3200 (NH), 1680, 1650 cm^{-1} (NHCO).

Anal. Calcd. for $C_{19}H_{30}N_2O_2$: C, 71.69; H, 9.43; N, 8.80. Found: C, 72.00; H, 9.80; N, 8.90.

Beckmann Rearrangement of 4-Aza-A-homo-5α-androstan-3,17-dione Oxime.

Under the same reaction conditions and chromatographic conditions as for compound **V**, the ω-cyanoolefin **VIIa** was obtained in 16% yield, mp 173-175° (methanol); ir: ν max 2230 cm^{-1} (C≡N); nmr: δ 0.78 (19-CH_3), 4.45, 4.76 (C=CH_2).

Anal. Calcd. for $C_{19}H_{28}N_2O$: C, 76.00; H, 9.33; N, 9.33. Found: C, 75.83; H, 9.49; N, 8.97.

With further elution of the column with chloroform-methanol (95:5), the 4,17α-diazo-A,D-bishomo-5α-androstan-3,17-dione was isolated in 40% yield. Crystallization from chloroform-methanol gave a mp above 300°; ir: ν max 3320, 3100, 3040 (NH) and 1640 cm^{-1} (NHCO).

Anal. Calcd. for $C_{19}H_{30}N_2O_2$: C, 71.69; H, 9.43; N, 8.80. Found: C, 71.79; H, 9.65; N, 8.53.

Preparation of 3-Aza-17β-hydroxy-A-homo-5α-androstan-4-one (**III**) from 3-Aza-17β-hydroxy-A-homo-4α-androsten-4-one.

3-Aza-17β-hydroxy-A-homo-4α-androsten-4-one (1 g) in methanol (100 ml), was shaken with hydrogen under pressure for 24 hours with 10% palladium-charcoal (1 g). The catalyst was removed by filtration and the filtrate was worked up in the usual way, to yield the lactam **III** (400 mg) with mp and ir identical to the compound prepared from the *syn*-oxime **II** by the Beckmann rearrangement.

REFERENCES AND NOTES

[1] G. Politis, Ch. Camoutsis and P. Catsoulacos, *Meth. Find. Exptl. Clin. Pharm.*, **4**, 403 (1983).

[2] C. Athanasiou and P. Catsoulacos, *J. Heterocyclic Chem.*, **21**, 927 (1984).

[3] C. Athanasiou, P. Catsoulacos, A. Papageorgiou and K. Athanasiou, *Cancer Invest.*, **5**, 301 (1987).

[4] C. Athanasiou, P. Catsoulacos and K. Athanasiou, *Cancer Invest.*, **5**, 301 (in press).

[5] Ch. Camoutsis and P. Catsoulacos, *J. Heterocyclic Chem.*, **20**, 1093 (1983).

[6] Unpublished results from this laboratory.

[7] K. Oka and S. Hara, *Chem. Ind.*, 911 (1968).

[8] K. Oka and S. Hara, *Chem. Ind.*, 168 (1969).

[9] R. H. Mazur, *J. Org. Chem.*, **28**, 248 (1963).

[10] P. Catsoulacos, *Chim. Chron.*, New Ser., **1**, 147 (1972).

[11] C. W. Shoppee and S. K. Roy, *J. Chem. Soc.*, 1514 (1970).

[12] H. R. Nace and A. C. Watterson, Jr., *J. Org. Chem.*, **31**, 2109 (1966).