STEROIDS XXV*

SEPARATION OF PREDNISOLONE-3,20-DIOXIME ISOMERS AND ATTEMPTS TO DETERMINE THEIR CONFIGURATIONS

By

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The four structural isomers of prednisolone dioximes (11-beta, 17-alpha, 21-trihydroxypregn-1,4-diene-3,20-dioxime) were separated, and some of their physicochemical characteristics were studied, in an attempt to determine their configurations.

The oxime-producing reaction of ketones can be utilized in many ways and for different purposes. We earlier examined and compared the mechanism of the Beckmann rearrangement of steroid monoximes [1]. The dehydroepiandrosterone-17--ketoxime were successfully separated by thin-layer chromatography (TCL) [2]. Additionally, we dealt with the formation of some dioximes [3], including prednisolone--3,20-dioximes, where the four *cis-trans* isomers involving the C=N bonds, were separated [4].

The structures of the stereoisomers are the following:

I. II. III. IV.

In the present publication some physicochemical characteristics of isomers I—IV and some methods for their separation are described.

Experimental and results

The prednisolone used was prepared according to the description of the 19th USP [5]. Reagents and solvents were obtained from "REANAL" Fine Chemical Works Budapest, Hungary. Melting points were determined with a Boetius instrument

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Table I

Chromatographic properties of the products

Compound	R_f values		Colour formed		
	solvent A	solvent B	reagent A	reagent B	reagent C
Compound 1	0.19	0.51	green	none	none
Compound 2	0.30	0.64	green	none	none
Compound 3	0.41	0.72	brownish--green	none	none
Compound 4	0.50	0.80	brownish--green	none	none

Solvent A: chloroform:ethanol (90:10)
Solvent B: benzene:dioxane:diethyl ether (100:65:65)
Reagent A: 2% $CuCl_2$ in water
Reagent B: 0.5% tetrazolium blue in 2.5 N NaOH [5]
Reagent C: 0.3% 2,4-dinitrophenylhydrazine in methanol
0.3% HCl [5]

Table II

Some spectral and other characteristics of the compounds

Compound	UV λ_{max} nm in ethanol	Ext. coeff. ε and ε	Colour formation with $CuCl_2$ λ_{max} (nm)	M.p. (°C)	Relative amount formed in reaction		
					A1	A2	B
Product A	256	12.500 1.500	343	174			
Product B	256	13.000 1.500	350	167—169			
Compound 1	264	—	350	142—144	46	53	55
Compound 2	245	—	350	150—152	28	19	45
Compound 3	250	—	334	172	14	18	—
Compound 4	240	—	334	171	12	10	—

and were not corrected. The IR spectra were determined with a UNICAM SP 200 spectrophotometer on KBr pellets. The UV spectra in ethanol obtained with a SPECORD UV VIS recording spectrophotometer.

The prednisolone-3,20-dioximes were basically prepared in two ways.

Reaction A. Prednisolone of the above mentioned quality (1 g) was dissolved in pyridine (20 ml) and dry hydroxylamine. HCl (4 g) was then added. The mixture was kept on a boilingwater bath for 3 h (reaction A1) for 6 h (reaction A2), followed by standing at room temperature for 18 h in both cases. Subsequently, an indentical

volume of ice-cold water was added to the solutions. The resulting precipitate was extracted with ether, the ether was evaporated off and the residue was dissolved in ethanol. Depending on the degree of purity, it was precipitated several times with water. After standing, the precipitate was filtered off, dissolved again and finally crystallized from ethanol-water. Reactions A1 and A2 gave similar yields and analyses. A typical result: Weight of dry substance: 0.86 g (yield: 85%). Analysis: $C_{21}H_{30}N_2O_5.H_2O$. Calc: C: 61.76%; H: 7.84%; N: 6.85%. Found: C: 61.40%; H: 8.10%; N: 6.93%.

Reaction B. The previously used prednisolone (1 g) was dissolved in ethanol (36 ml, 96%) and anhydrous sodium acetate (3.2 g) was added. Refluxing and filtration followed.

After a partial evaporation of the ethanol, the steroid oximes were treated with ice-cold water (appr. 5 ml). The precipitate was filtered off and purified by dissolution in ethanol and precipitation with water as before.

Weight of dried material: 0.95 g (yield: 91%). Analysis: $C_{21}H_{30}N_2O_5.H_2O$. Calc: as in the case of Reaction A. Found: C: 61.33%; H: 8.17%; N; 6.80%.

TLC chromatography

The TLC separation of the products (A1, A2 and B) was performed on Kieselgel *G* nach Stahl plates, with an adsorbent layer thickness of 250 μm.

For the preparative separation of the oxime isomers we used thicker Kieselgel *G* plates: layers of 0.5 or 1—2 mm. The mixture of isomers was applied in the form of bands, and after the running each band was eluted with anhydrous ethanol or ether. To isolate the materials, solvent was pumped off in vacuum at low temparature. The proportions of the components were determined by weight measurement [7].

Some solvents which led to successful separation:

a) $CHCl_3$:EtOH (9:1)

b) C_6H_6:dioxane:diethyl ether (100:65:65)

c) $CHCl_3$:MeOH:EtOH:propanol:butanol (90:2.5:2.5:2.6:2.5).

(We tried to carry out TLC separation on alumina (Al_2O_3) layers as well, but failed. The paper-chromatographic separation of the oxime isomers was also unsuccessful.)

In order to detect the TLC spots on the Kieselgel plates and in part to prove the presence of functional groups, the following developments and treatments were made:

1. CAMAG UV lamp. After the running, the TL plates were developed with 20—25% aqueous phosphoric acid, dried with an infrared lamp for about 20 min, and then examined under the UV lamp. Two oximes gave blue colours, and the other two gave brown colours.

2. Pretreatment was made with $CHCl_3$ saturated with $SbCl_3$ and the layers were treated according to point 1; the oxime isomers were lilac-blue to the naked eye, and vivid red in UV light.

3. I_2 vapour. This detected every steroid spot.

4. The layers were coated with a 2% aqueous solutions of $CuCl_2$ and dried. Only the spots of the oximes could be seen, but not those of the starting material.

5. An aqueous solution of $FeCl_3$ is as good a developer as the $CuCl_2$ but it gives half-tones.

6. Check on oxime production with 2,4-dinitrophenylhydrazine. Only the prednisolone reacted with this reagent, but not the oximes.

7. The tetrazolium blue reaction for detection of the 21-hydroxy-20-keto group was also negative [6].

Column chromatography

The oximes were separated by column chromatography on Brockman II Al_2O_3 or silica gel. The adsorbents were suspended in anhydrous benzene and the dioxime isomers were subjected to an increasing gradient of anhydrous ethanol. In general, the quantity of ethanol had to be increased from 1% to 10% in order to elute the oximes.

IR and UV spectra

The IR spectra of the individual compounds and mixtures were recorded in KBr in the interval 400—4000 cm^{-1}. The IR spectra of the products of reactions A and B and of the separated components were almost indentical. A broad, split OH band (3150—3550 cm^{-1}) was to be found in the spectrum of each product: this is indicative of strongly associated hydroxy groups. The band at 1655 cm^{-1} can be ascribed to the C=N bond. The bands at 800—1460 cm^{-1} are attributed to deformational β and γ OH vibrations (Table III). PALM and WERBIN [8] dealt with the analysis of these bands as regards the oximes of aromatic compounds, and in certain cases demonstrated the characteristic differences between the isomeric oximes.

Copper complexes

For characterization of the compounds, their copper complexes were also examined. The material was dissolved in ethanol and 0.1 ml 2% $CuCl_2$ solution in water was added at room temperature for each 2 ml of solution. The products of oxime-formation reaction gave green colours. The intensities of these colours and the spectra of the Cu complexes were established. The absorption maxima of compounds *1* and *2* differ from those of compounds 3 and 4.

Stability studies

In the solid phase, each of the products is stable at room temperature. The products of reactions A and B and the individual separated components were dissolved in pyridine, in ether or in pyridine-ether (1:1), and could be kept at room temperature for several weeks or refluxed for 24 h without decomposition or the transformation of

Table III

IR absorption bands of the products A1, A2, B and compounds 1—4

Frequencies in cm^{-1} in the interval 800—1700 cm^{-1}					
product	product	separated compounds			
A1, A2	B	1	2	3	4
795 m	795 m	795 m	800 m	793 w	795 w
875 s	875 s	877 s	865 s	878 m	873 m
890 m	890 s	890 m	893 s		
925 s	922 s	925 s	922 s	928 s	923 s
965 s	964 s	963 s	965 s		
				975 s	973 s
990 m	990 m	990 m	990 m	990 s	990 s
1035 s	1038 s	1038 s	1039 s	1030 m	1030 m
				1042 m	1042 m
1075 w	1070 w	1075 w	1078 w	1078 m	1078 m
1115 s	1115 s	1115 s	1116 s	1120 s	1120 s
1158 w	1160 w	1160 w	1160 w	1158 w	1160 w
1173 w	1170 w	1170 w	1170 w		
1245 w	1242 w	1245 w	1245 w		
1275 w	1270 w				
		1290 w	1293 m	1290 s	1285 s
1350 m	1345 m	1345 w	1348 m	1350 w	1350 w
1370 m	1370 m	1375 w	1375 m	1379 m	1378 m
1390 m	1390 m	1390 w	1390 w	1390 w	1390 w
1445 s	1440 s	1445 s	1453 s		
				1463 s	1463 s
1655 s	1655 s	1655 s	1655 s	1655 s	1655 s

s: strong
m: medium
w: weak

compounds *1* and *2* into compounds *3* and *4*. In the above solutions, chromatographically separated compunds *1* and *2* give a mixture of *1* and *2*, while separated compounds 3 and *4* give a mixture of *3* and *4*. In a water-saturated pyridine-ether (1:1) solution at room temperature, however, in 4 days the mixture of *3* and *4* was converted quantitatively to a mixture of *1* and *2*.

The results are given in Tables I—III and in Figures 1—2.

Discussion

The first thing to be said is that there is no connection between Nos. 1—4 in the Tables and Nos. I—IV representing the structures of the bis-oximes. In TLC No. 1 represented the material with the smallest R_f, etc. This transcription is consequent in the Tables. Table I gives the R_f data and the colour reactions of the bis-oximes. Fig. 1. shows the separated bis-oximes besides prednisolone in solvents *A* and *B*.

Here we do not deal with the connection between the TLC running distance (R_f values) and the structure, and this is why we do not go into details relating to the

connection of the structures I—IV and the TLC spots No 1—4 but rather make some general statements. In the oxime formation in pyridine solution, four bis-oxime isomers were formed (isomers 1—4).

The appearance of two geometric *syn* and two *anti* oxime isomers is to be expected in the formation of asymmetric ketoximes as was detailed above. Only a few examples are known of the separation of the two isomers for steroid monoximes: *e.g.* the *syn* and *anti* isomers of cholest-en-3-oximes [10]. We earlier separated the *syn* and *anti* isomers 3-beta-hydroxy-androst-5-en-17-oximes [2]. On the formation of the steroid--20-ketoximes, the occurrence of the isomer better stabilized by H-bond formation is preferred [8]. In general, previous experience has shown that only one of the isomers can be detected in the case of the 20-ketoximes [11]. However, the *syn* and *anti* isomers of pregnenolone-20-ketoxime were separated [12]. *Syn* and *anti* isomers of pregn-5-ene-3,20-bis-oxime, involving the C=N bond, are also known [13]. The isolated *syn* and *anti* steroid oximes differ in their physical and chromatographic characteristics. Aromatic oximes have been reported to give different colours with $CuCl_2$ after TLC separation in the case of *syn* and *anti* isomers [14].

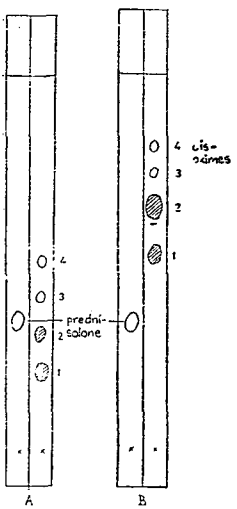

Fig. 1. TLC separation of products 1—4 into the system A and B

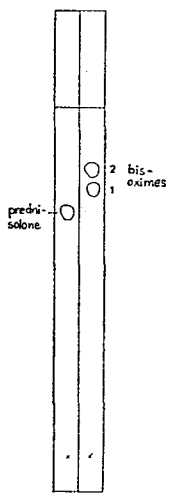

Fig. 2. TLC separation of the products 1 and 2 on Kieselgel *G* plate. Running solvent: chloroform:ethanol (90:10)

It is also well known that steroid oxime isomers can easily transform into a more stable form, *e.g.* in solvent, on the action of UV light, *etc;* in these cases only one isomer can be isolated. We also made some isomerization experiments as mentioned in the experimental part [9, 11].

It may be concluded that prednisolone proved to be a good model for the formation of bis-oximes. We succeeded in separating all four geometric isomers on the basis

of their TLC characteristics. Our experiments indicate that the $\Delta^{1,2}$ bond and the 17-alpha and 21-hydroxygroups have equal significance in the stabilization of the structures of the bis-oximes.

It remains a great task to determine the actual configuration and to assign a concrete structural formula.

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СТЕРОИДЫ, XX. ВЫДЕЛЕНИЕ ИЗОМЕРОВ ПРЕДНИЗОЛОН-3,20-ДИОКСИНА И ПОПЫТКА ОПРЕДЕЛЕНИЯ ИХ КОНФИГУРАЦИИ

М. Мариан и Б. Маткович

Выделено четыре структурных изомера 11-β,17-α,21-тригидроксипрегн-1,4-диено-3,20-диоксина и изучены некоторые физико-химические характеристики с попыткой определения их конфигураций.