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CONFIGURATION OF SOME 17a-ALKYL-17a-HYDROXY-D-HOMOSTEROIDS

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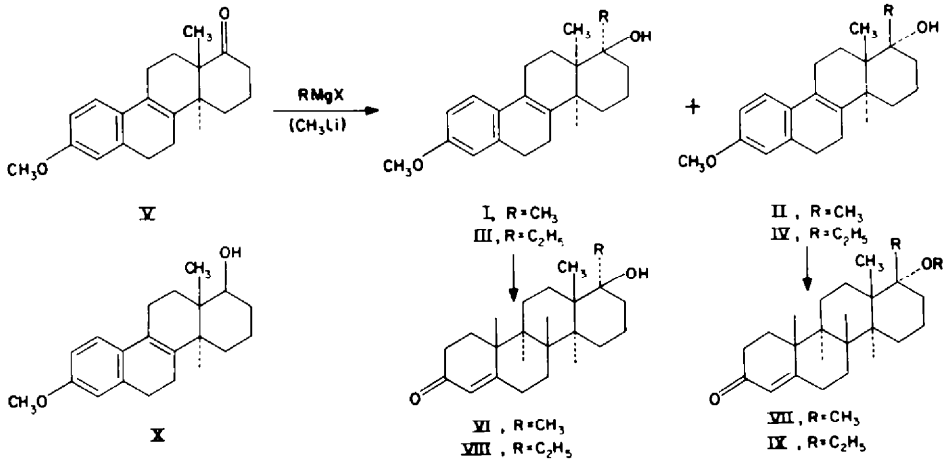
Abstract—The configurations of DL-17a-methyl- and 17a-ethyl-3-methoxy-$\Delta^{1,3,5(10),8}$-D-homoestratetraen-17a-ols (I–II and III–IV) and the corresponding DL-17a-methyl- and DL-17a-ethyl-19-nor-D-homotestosterones (VI–VII and VIII–XI) have been determined by three independent methods: chemical, NMR and fragment mass spectroscopy. The configuration of the hydroxyl group in the ketols (VI–IX) has been correlated with their anabolic activity.

THE preparation of DL-17a-alkyl-3-methoxy-$\Delta^{1,3,5(10),8}$-D-homoestratetraen-17a-ols (I, II, III and IV) by reaction of the corresponding Grignard reagent with 3-methoxy-$\Delta^{1,3,5(10),8}$-D-homoestratetraen-17a-one (V) and the conversion of I, II and III into the DL-17a-alkyl-19-nor-D-homotestosterones VI, VII and VIII which possess physiological activity, was described earlier.[1,2]

The configuration of the substituents at C-17a, however, still required clarification. The epimer, 17a-methyl-3-methoxy-$\Delta^{1,3,5(10),8}$-D-homoestraen-17a-ol (II) with m.p. 115°, was tentatively ascribed the 17a-β-OH configuration.

It is now established that compounds I (m.p.127–128°), III, (m.p.181–182°), VI (m.p.161–162°) and VIII (m.p.153–154°) have the hydroxyl in equatorial position, whereas in II (m.p.115–116°), IV (m.p.159–160°), VII (m.p.173–174°) and the recently synthesized IX (m.p.186–188°) the hydroxyl is axial (α-configuration).



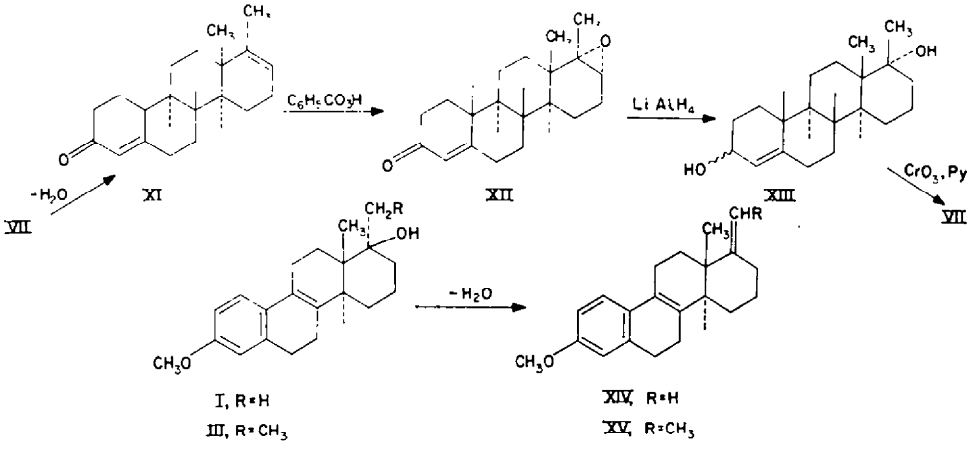
Reaction of the ketone (V) with methylmagnesium iodide predominantly yields II, the epimer I being produced in minor amounts together with a difficultly removable

[1] S. N. Ananchenko, V. M. Rzheznikov, V. N. Leonov and I. V. Torgov, *Izv. Acad. Nauk SSSR, Otd. Chim. Nauk* 1913 (1961).

[2] S. N. Ananchenko, V. E. Limanov, V. N. Leonov, V. M. Rzheznikov and I. V. Torgov, *Tetrahedron* **18**, 1355 (1962).

impurity, probably the secondary carbinol (X). Similarly, ethylmagnesium bromide and the ketone (V) give mainly IV, the epimer III being isolated only in 19% yield. In this connection, the Grignard reaction of 17a-keto-D-homosteroids of the androstane series yields exclusively tertiary carbinols with the hydroxyls axial,[3-5] but if methyllithium is substituted for methylmagnesium iodide in the reaction with the ketone (V), epimer I with the hydroxyl equatorial is the major product together with epimer II in yields of 60 and 22%, respectively, and, therefore, methylmagnesium iodide and methyllithium display different stereochemical selectivities.

Reduction of the carbinol IV with potassium and lithium in liquid ammonia in the presence of alcohol and subsequent acid hydrolysis affords DL-17aβ-ethyl-19-nor-D-homotestosterone (IX). The ketols V–VII, obtained in a similar way, have been described earlier.[1,2]



Dehydration of the ketol VII, by heating with acetic anhydride in pyridine, yields DL-17a-methyl-$\Delta^{4,17a}$-19-nor-D-homoandrostadien-3-one (XI), which may be converted to the epoxide XII. Reduction of the latter with lithium aluminium hydride and oxidation of the resultant product with chromic anhydride in pyridine yields the initial ketol (VII). Since the opening of the epoxide ring proceeds with the formation of an axial hydroxyl,[3,6,7] the ketol VII and its corresponding carbinol (II) should be ascribed the 17aα-OH-configuration and the epimer I and the ketol V, the 17aβ-OH-configuration.

Dehydration of carbinols I and III in pyridine with phosphorus oxychloride at room temperature gives good yields of 17a-methylene-3-methoxy-$\Delta^{1,3,5(10),8}$-D-homoestratetraene (XIV) and 17a-ethylidene-3-methoxy-$\Delta^{1,3,5(10),8}$-D-homoestratetraene (XV), the position of the double bonds being confirmed by NMR spectroscopy. No 17a-CH_3 peak was found in the spectrum of XIV but there is a two-proton peak ($\delta = 4{\cdot}65$) in the vinyl proton region. The spectrum of XV exhibits a

[3] L. Ruzicka, N. Wahba, P. T. Herzig and H. Heusser, *Chem. Ber.* **85**, 491 (1952).
[4] R. O. Clinton, R. G. Christiansen, H. C. Neumann and S. C. Lascowski, *J. Amer. Chem. Soc.* **80**, 3389 (1958).
[5] R. R. Burtner and R. E. Gentry, *J. Org. Chem.* **25**, 582 (1960).
[6] D. H. R. Barton, *J. Chem. Soc.* 1027 (1953).
[7] F. Sondheimer, O. Mancera, M. Urquiza and G. Rosenkranz, *J. Amer. Chem. Soc.* **77**, 4145 (1955).
[8] I. V. Berezin and J. Musher, Private communication.

one-proton peak in the same region (quadruplet with the main components at 5·17 and 5·29) and a three-proton peak split into two components of equal intensity (as a result of interaction between $C_{(20)}$ and $C_{(21)}$ protons) are located in the region of absorption of a conjugated methyl group (δ 1·56 and 1·68).

The carbinols II and IV do not undergo dehydration under these conditions which is in agreement with the literature,[4] and therefore serves as evidence of an axial hydroxyl at C-17a.

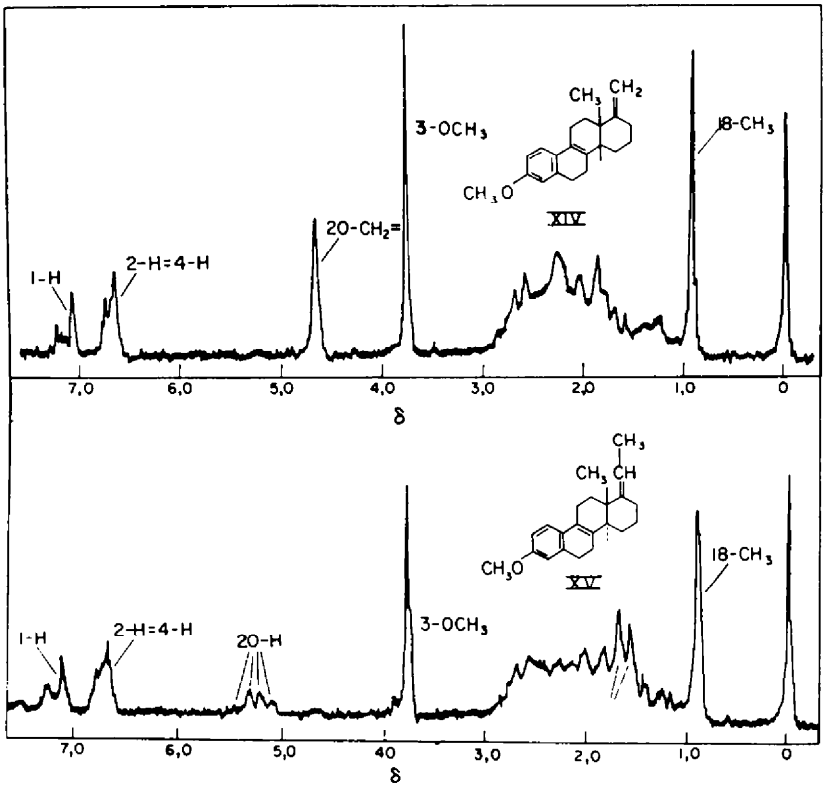
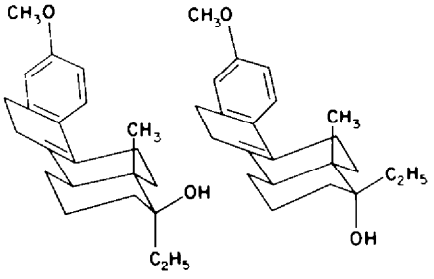


FIG. 1. NMR spectra of XIV and XV.

The difference in chemical behaviour of the epimeric carbinols I and II is manifest also on acetylation. Short time heating (2 hr) with acetic anhydride partially converts both isomers into the acetates (Ia and IIa); but if the time is increased to 6 hr carbinol I is dehydrated to the 17a-methylene derivative (XIV), whereas II is converted to the acetate.

The same conclusions regarding the configuration of the carbinols III, IV and VI–IX may be drawn from their NMR spectra. This analysis was based on the investigation by Berezin and Musher on the spectra of cyclic carbinols[8] in which they established that in cases where the hydroxyl oxygen is situated near a methyl group, the resonance line of the protons of the latter is shifted in the direction of weaker fields; the shorter the distance between the oxygen atom and the protons the greater the shift. Since in the epimer of 17a-ethyl-3-methoxy-$\Delta^{1,3,5(10),8}$-estratetraen-17a-ol with m.p.181–182°, the 18-CH_3 group absorbs at $\delta = 0{\cdot}95$, whereas in the other with

m.p.159–160° absorption is at $\delta = 0{\cdot}82$, the hydroxyl of the former must be closer to the 18-CH_3 group and should, therefore, possess an equatorial configuration. This is clearly seen on the molecular models and the second isomer must, therefore, possess an axial hydroxyl.



III IV

The chemical shifts of the 19-CH_3 protons of the epimeric ketols (VIII and IX) display the same relationship ($\delta = 1{\cdot}03$ and $0{\cdot}92$, respectively). Although the difference in chemical shifts are somewhat less in the ketols VI and VII ($\delta = 1{\cdot}00$ and $0{\cdot}92$), by analogy, the former was ascribed an equatorial configuration of the hydroxyl and the latter, an axial configuration.

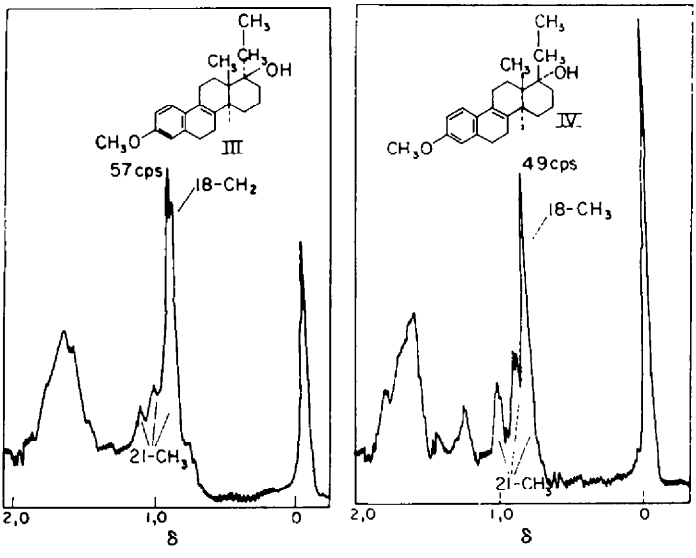


FIG. 2. NMR spectra of carbinols III and IV.

Independent confirmation of the configurations for compounds I–IV was obtained by mass spectrometric analysis.* Earlier Biemann,[9] by means of secondary and tertiary carbinols, showed that electron impact on the isomer with the more "crowded" configuration, forms a molecular ion of less intensity and, correspondingly, a more intense fragment with m/e M-18.

A comparison of the mass spectra (Figs. 5, 6 and Table 1) shows that the intensity

* This part of the work was carried out in collaboration with V. G. Zaikin.

[9] K. Biemann and J. Seibl, *J. Amer. Chem. Soc.* **81**, 3149 (1959).

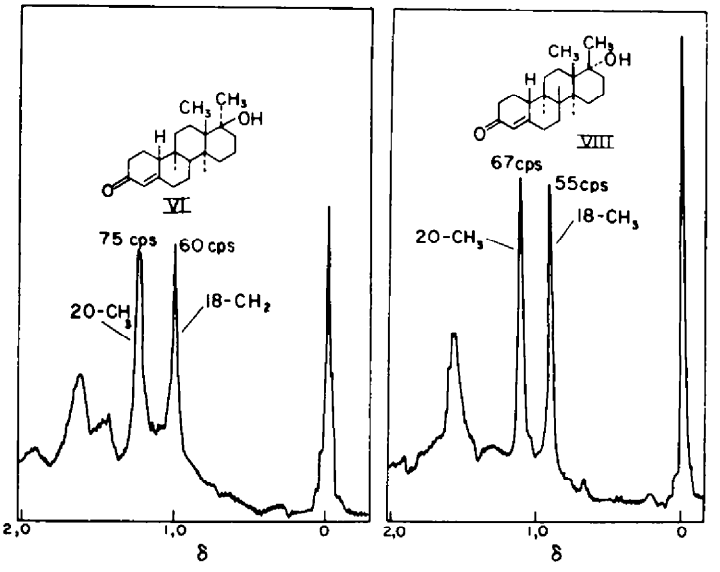


FIG. 3. NMR spectra of ketols VI and VII.

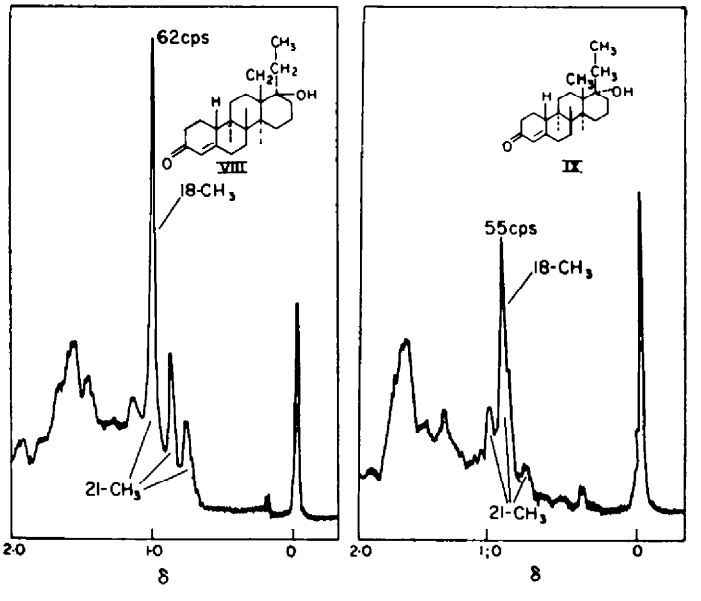


FIG. 4. NMR spectra of ketols VIII and IX.

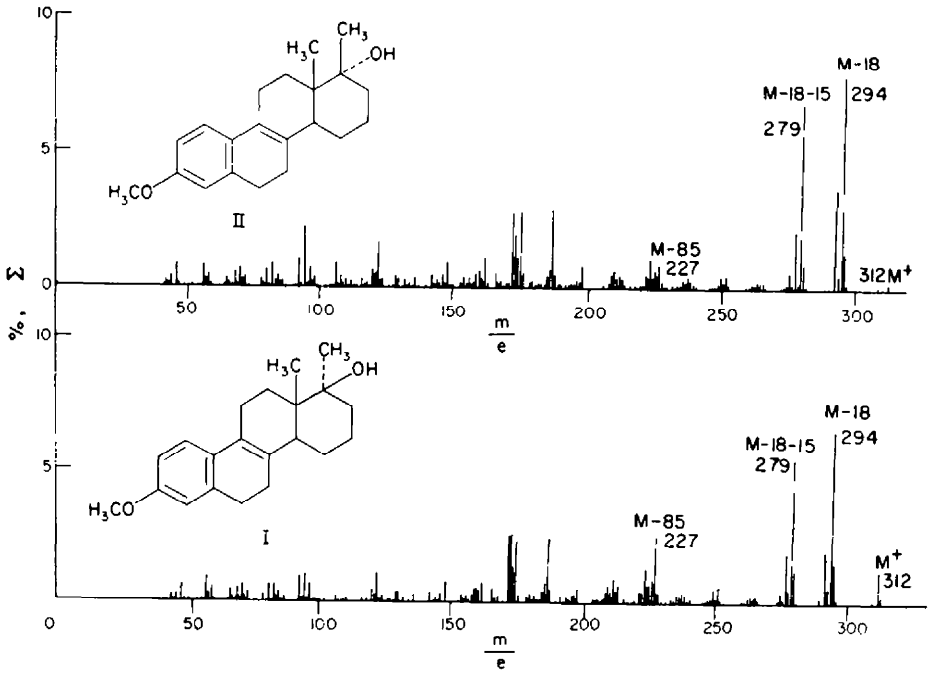


FIG. 5. Mass spectra of carbinols I and II.

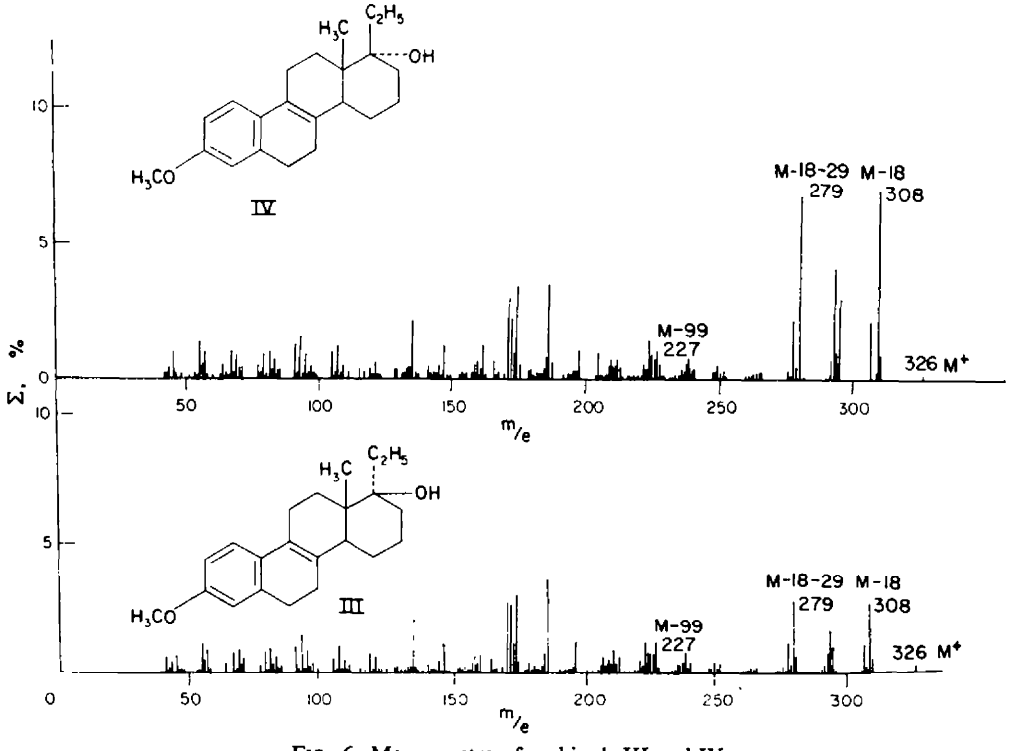


FIG. 6. Mass spectra of carbinols III and IV.

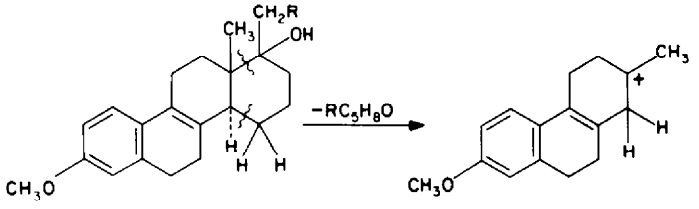
TABLE 1

Compound	% E					M^+-18	m/e 279	m/e 227	m/e 227/M (I, III)*
	M^+	M^+-18	m/e 279		m/e 227	M^+	M^+	M^+	m/e 227/M (II, IV)
			M-18-15	M-18-29					
II	0·25	7·85	6·9	—	0·35	31·2	27·8	1·4	1·61
I	1·2	6·6	5·5	—	2·7	5·5	4·6	2·25	
IV	0·2	7·1	—	6·8	0·70	35·5	34	3·5	1·71
III	0·2	2·8	—	2·9	1·2	14	14·5	6	

* % from M^+.

ratio of the dehydration peak (M-18) to the molecular ion (M^+) peak is much greater for the epimers II and IV than for I and III, and therefore, it follows that the first pair of epimers should possess a more "crowded" configuration. The molecular models of compounds I–IV show that the more "crowded" configuration is the one wherein the angular CH_3 and the 17a-alkyl are situated at the shortest distance from each other, so that in the epimers II and IV, the 17a-alkyl should be equatorial and the 17a-hydroxyl, axial, while epimers I and III should have equatorial hydroxyls. It is noteworthy that no peaks with m/e M-15 and M-29, are exhibited in the spectra, i.e. the respective CH_3 and C_2H_5 groups only appear after conversion of the molecular ion into the M-18 ion. Consequently, the fragment with m/e 279 is due to the elimination of 17a-CH_3 or 17a-C_2H_5 from the M-18 ion. In fact (Table 1) the intensity ratios of the m/e 279 fragment and the molecular ion are very close to the corresponding values for the peak with m/e M-18 which suggests that the peak M-(18 + 15 + 14n), where the number of CH_2 groups n = 0,1, is just as characteristic for the configuration of the epimers as is the peak of the M-18 ion.

Further study of the mass spectra, in spite of the similarity in the region from the m/e 279 peak and below, shows that for each pair of epimers the intensity of the m/e 227 peak is different. This fragment could form only from the molecular ion and not from the ion with m/e M-18, because the configuration of the latter should be the same for both epimers giving the same fragmentation products beginning with the peak m/e M-18.



I , R=H (M=312)
III , R=CH_3 (M=326)

m/e 227

The intensity of the peak with m/e 227 should be greater for the less "crowded" configuration; and the three-dimensional model shows that the C_{13}-C_{17a} bond in this case should be sterically more accessible. This conclusion is in good agreement with the data obtained for both pairs of epimers (I–II and III–IV) as can be seen by

a comparison of the dehydration peak (M-18) intensities and the peaks with m/e 279 given in the Table. The close values for the m/e 227 peak intensity ratios (expressed in percentage of M^+) for each pair of epimers (1·61 for I–II and 1·71 for III–IV) indicate identical mechanisms for the formation of this fragment in both cases (Table 1).

It has thus been established that there is a relationship between the structure of the epimeric 17a-alkyl-17a-hydroxy-D-homosteroids and their NMR and mass spectra, not only in the series of compounds with the aromatic ring A but also in the 19-nor-D-homoandrostane series. A similar relationship between the structure of these compounds and their IR spectra was sought but owing to the complexity of the spectra this could not be realized in the case of the carbinols (I–IV). In the case of ketols, the IR spectra of compounds VII and IX exhibit bands of medium intensity at 1120 and 1117 cm^{-1}, respectively, whereas ketols VI and VIII exhibit two characteristic bands of medium intensity at 1110 and 1137 cm^{-1} and 1109 and 1134 cm^{-1}, respectively. Further, the shape of the broad triplet band in the region of 1335–1380 cm^{-1} for VI and VIII differs greatly from that for VII and IX. In the first case the band at 1337–1340 cm^{-1} is fairly strong, whereas it is very weak in the second. The 1300–1305 cm^{-1} band present in the spectra of all four compounds is strong in the case of VII and IX and very weak in the case of VI and VIII.

We have also found a relationship between the configuration of the hydroxyl group in the ketols VII–IX and their physiological action. The ketols VI, VIII with an equatorial hydroxyl have an anabolic activity 2 to 4 times greater than that of the epimers VII, IX with an axial hydroxyl. A similar relationship has also been found in their androgenic activity (Table 2).

TABLE 2. PHYSIOLOGICAL ACTIVITY OF THE EPIMERIC DL-17a-ALKYL-19-NOR-D-HOMOTESTOSTERONES

Compound	Myotrophic activity	Androgenic activity	Index
Testosterone propionate	1	1	1
VI in dose 0·7 mg	0·9	0·58	1·5
3·5 mg	1·0	0·7	1·4
VII in dose 0·7 mg	0·4	0·06	6·6
3·5 mg	0·4	0·05	8·0
VIII in dose 0·7 mg	1·3	0·19	6·8
3·5 mg	0·95	0·29	3·2
IX in dose 0·7 mg	0·27	0·05	5·2
3·5 mg	0·24	0·04	6·0

Tests were carried out on rats according to a conventional procedure.[10]

EXPERIMENTAL

Prior to analysis, all substances were dried (80° at 0·5–1 mm Hg over P_2O_5).

For identification purposes, homogeneity tests and in order to follow the course of the reaction, use was made of thin layer chromatography on alumina (activity II–III according to Brockmann),

[10] J. G. Herschberger, *Proc. Soc. Exp. Biol. Med.* **83**, 175 (1957).

the thickness of the layer being 1 and sometimes 2 mm. The spots were developed in UV light or by iodine vapour.

The IR spectra were obtained on an UR-10 (Zeiss) spectrophotometer in Dr. Yu. N. Sheinker's laboratory. The NMR spectra were obtained on a Varian-A 60 instrument with tetramethylsilane as internal reference. Mass spectra were obtained on an instrument MX-1303 with a stainless steel injection system heated to 175° and an ionizing voltage of 28 eV.

The microanalysis were carried out in this Institute under the guidance of M. N. Chumachenko. Biological tests were carried out under the guidance of G. A. Zhdanov.

Dehydration of 17a*β-methyl*-19-*nor*-D-*homo*-17a-*isotestosterone* (VII). A solution of 1·89 g ketol (VII) in a mixture of 15 ml pyridine and 15 ml acetic anhydride was heated for 20 hr at 135°, cooled and poured into 100 ml HCl (1:7), allowed to stand 30 min and then extracted with chloroform, the extract washed with 5% NaOH aq. and water and dried over $MgSO_4$. After evaporation *in vacuo*, 1·9 g of a semicrystalline mass obtained was chromatographed on 100 g alumina. Elution with pet. ether–benzene mixtures (from 10:0 to 5:5) gave 0·24 g chromatographically pure XI which crystallized from cyclohexane–ethyl acetate (4:1), m.p. 123–125, 0·17 g (analytic specimen, m.p. 125–127°). IR spectrum: $\nu_{max}^{CHCl_3}$ cm^{-1} 1670, 1621 (COCH=C<); UV spectrum λ_{max}^{EtOH} 240 mμ (lgε 4, 11). (Found: C 84·45, H 10·07. $C_{20}H_{28}O$ requires: C 84·45; H 9·92%).

Subsequent elution with pet. ether–benzene mixtures, benzene and benzene–ether (9:1) afforded 0·46 g of a mixture of ketone XI and the initial ketone VII, which was separated on 2 mm alumina chromatoplates yielding another 0·18 g XI (after crystallization 0·12 g, m.p. 122–125°) and 0·25 g VII, the over-all yield of ketol based on VII was 52%.

The same ketone (XI) was isolated after hydrolysis of the methoxy derivative obtained by Birch reduction of the carbinol (II).

Conversion of ketone XI *into carbinol* VII*. A solution of 186 mg XI in 10 ml chloroform was mixed at −10° with a solution of perbenzoic acid in chloroform (1·55 ml, containing 11·5 mg active O_2) and after standing 1 hr at −10°, the reaction mixture was left for 48 hr at −2–0°, then treated with ice water and finally extracted with ether. The organic layer was washed with water, thiosulphate solution and again with water and dried over Na_2SO_4. After evaporation *in vacuo*, 200 mg of a semicrystalline product, separated on 2 mm alumina chromatoplates, yielded four bands (11, 129, 51 and 5 mg). The main fraction consisted of the crystalline epoxide (XII); the other fractions did not crystallize.

The epoxide XII (129 mg) in 5 ml tetrahydrofuran was added to a solution of 160 mg $LiAlH_4$ in 9 ml abs. tetrahydrofuran, the mixture left for 48 hr and then refluxed for 6 hr (the course of the reaction being followed chromatographically). The cooled mixture was decomposed with 10 ml ethyl acetate and then with HCl (1:9), the organic layer was separated and the aqueous solution extracted with ether. Evaporation of the extract afforded 102 mg of a mixture of the epimeric glycols (XIII) in the form of a yellow oil. This was dissolved in 3 ml dry pyridine and added to a suspension of Sarret's reagent (2 ml of pyridine and 90 mg of chromic anhydride). The mixture after standing at room temp was diluted with 7 ml water and 30 ml (1:1) benzene–ether solution and filtered. The organic layer was removed and the aqueous layer extracted with ether, the combined extracts after evaporation yielded 86 mg which crystallized from benzene–hexane (7:3), m.p. 172–173° (48 mg), and gave no m.p. depression with a known specimen of VII. The IR spectra of both specimens were found to be identical.

Dehydration of 17a*α-methyl- and* 17a*α-ethyl*-3-*methoxy*-$\Delta^{1,3,5(10),8}$-D-*homoestratetraenols*-17a*β* (I and III). To a solution of 624 mg carbinol (I, m.p. 127–128°) in 12 ml dry pyridine, 550 mg freshly distilled $POCl_3$ was added. The mixture was left 6 days and then poured into 75 ml ice water and extracted with chloroform. The extract was washed with a small amount of water and on evaporation yielded 589 mg of a semicrystalline, yellowish oil which was chromatographed on 25 g alumina. Elution with pet. ether gave 524 mg (88%) chromatographically pure XIV which crystallized from hexane, 424 mg, m.p. 112–113° (analytical specimen, m.p. of 115–116°). The IR spectrum: ν_{max}^{nujol} cm^{-1} 3090, 1642, 900 (>C=CH_2). (Found: C 86·25, H 8·97. $C_{21}H_{30}O$ requires: C 85·66, H 8·90%). Further elution with a pet. ether–benzene solution (2:3) gave 33 mg of the initial carbinol (I), m.p. 123–125°.

Similarly, from 810 mg III, 790 mg of a crystalline mass was obtained and chromatographed on

* This and the previous experiments were carried out by E. V. Shapkina.

alumina and eluted with pet. ether–benzene mixture (85:15) affording 608 mg (80%) chromatographically pure XV. Crystallization from cyclohexane gave 496 mg, m.p. 134–135° (analytical specimen, m.p. of 136–137°). IR spectrum: ν_{max}^{nujol} cm^{-1} 1630, 851 (>C=CHCH$_3$) (Found: 85·71; H 9·06. $C_{22}H_{28}O$ requires: C 85·66; H 9·15%). On further elution 99 mg of the initial carbinol (III) was isolated. Under these conditions the carbinols II (m.p. 115–116) and IV (m.p. 159–160°) undergo no change.

Acetylation of the epimeric 17a-*methyl*-3-*methoxy*-$\Delta^{1,3,5(10),8}$-D-*homoestratetraenols*-17a (I) *and* (II). A mixture of 150 mg carbinol (II) with 2 ml pyridine and 2 ml acetic anhydride was heated 2 hr at 115°, cooled, poured into dil. acetic acid (1:4), left for 30–50 min and extracted with chloroform. The extract was washed with 5% NaOH aq and water and dried over Na_2SO_4. After evaporation *in vacuo*, the residue was preparatively separated on 2 mm alumina chromatoplates or on a column with the same asdorbent. The acetate (IIa: 26 mg; 15%) was isolated and the analytical specimen, m.p. of 171–173°, crystallized from methanol. IR spectrum: ν_{max}^{nujol} cm^{-1} 1733, 1385, 1250 (OCOCH$_3$). (Found: C 77·59; H 8·66. $C_{23}H_{30}O_3$ requires: C 77·93, H 8·53%). A total of 113 mg of the initial carbinol (II) was recovered.

Acetylation of II (150 mg) for a period of 6 hr yielded 59 mg (35%) IIa and 93 mg of the initial carbinol (II).

Acetylation of 450 mg carbinol I for 2 hr and chromatography on a column afforded 87 mg (17%) chromatographically pure acetate (Ia). The analytical specimen, m.p. of 140–141°, was crystallized from methanol. IR spectrum: ν_{max}^{nujol} cm^{-1} 1715, 1367, 1262, 1241 (OCOCH$_3$). (Found: C 77·50, H 8·41. $C_{23}H_{30}O_3$ requires: C 77·93, H 8·53%).

Acetylation of 250 mg carbinol (I) for 6 hr afforded 279 mg brown oil which was chromatographed on 20 g alumina. Elution with (9:1) pet. ether–benzene mixture yielded 157 mg colourless oil which on grinding with a few drops methanol formed crystals, m.p. 95–101°. Three crystallizations from methanol yielded 18 mg, m.p. 111–113°, identical (with respect to m.p. and IR spectrum) with XIV. The acetate (Ia) could not be isolated, but its presence in the mother liquor after the first crystallization of XIV was proved spectroscopically (strong bands in the IR spectrum at 1715 and 1367 cm^{-1}). Further elution gave 87 mg of the initial carbinol.

Synthesis of 17aβ-*ethyl*-19-*nor*-D-*homo*-17a-*isotestosterone* (XI). A solution of 1·34 g carbinol (IV) in 300 ml ether–tetrahydrofuran (2:1) mixture was added to 200 ml liquid ammonia (dried by passing over KOH pellets) at −70° and to the resultant mixture, 2·46 g metallic K were added in small portions during 20 min. After standing for 4 min at −70°, 150 ml abs. alcohol were added dropwise (during 30 min) and then in the course of 1 hr, 5·15 g metallic Li were added. After 2 hr, the ammonia was driven off by slight heating and 750 ml water added to the remaining suspension. The organic layer was separated and the aqueous layer extracted with ether. After evaporating the combined extract *in vacuo*, the 1·45 g white crystals obtained, were dissolved by heating with 75 ml methanol and to the warm solution 5 ml glacial acetic acid and 15 ml HCl aq, (1:4) were added. The mixture was allowed to stand 2 hr, poured into 200 ml cold water and extracted with chloroform. The extract was washed with NaOH aq. and water and then dried over Na_2SO_4. After removal of the solvent under red. press., 1·34 g of a yellowish mass was chromatographed on 40 g alumina. On elution with pet. ether–benzene mixtures (from 10:0 to 7:3), 0·46 g of a thick, non-crystallizing oil was isolated, which according to thin layer chromatographic data was comprised of three substances. Further elution gave 0·37 g chromatographically pure ketol (IX) which after crystallization from cyclohexane–carbon tetrachloride (3:1) afforded 0·62 g (47%), m.p. 184–186° and an analytical specimen, m.p. 186–188°. IR spectrum: ν_{max}^{nujol} cm^{-1} 3500 (OH), 1665, 1621 (COCH=C<). (Found: C, 79·84; H, 10·26. $C_{21}H_{32}O_2$ requires: C, 79·70; H, 10·19%).

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