516. *The Application of the Method of Molecular-rotation Differences to Steroids. Part XI. Wolff–Kishner Reduction of the Adreno-cortical Side Chain.*

By D. H. R. Barton, N. J. Holness, and W. Klyne.

Wolff–Kishner reduction of 21-acetoxypregn-5-en-3β-ol-20-one * gives *pregn-5-en-3β-ol* and, in smaller quantities, 3β-hydroxyætiochol-5-enic acid and pregna-5 : 20-dien-3β-ol. A mechanism for the formation of the last-mentioned compound is suggested.

Wolff–Kishner reduction of pregn-5-en-3β-ol-20-one also affords pregn-5-en-3β-ol; less than 1% of pregn-5-en-3α-ol, isolated as its *acetate*, is formed at the same time. The molecular-rotation data are briefly discussed.

In spite of the importance attached in steroid chemistry to the adreno-cortical side chain (·CO·CH_2·OH) we have found no reference to the degradation products formed therefrom under Wolff–Kishner reducing conditions. Through the courtesy of Professor T. Reichstein, of Basle, who very kindly provided us with a generous specimen of 21-acetoxypregn-5-en-3β-ol-20-one (I; R = ·CO·CH_2·OAc, R′ = H), we have now been able to rectify this omission.

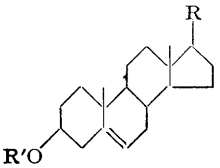
It is well known that Wolff–Kishner reduction of α-ketols often proceeds in a highly abnormal manner. This is illustrated, for example, by the behaviour of 2-acetoxycholestan-3-one and 3β-acetoxycholestan-2-one (Ruzicka, Plattner, and Furrer, *Helv. Chim. Acta*, 1944, **27**, 727). In both cases the main product of the reaction is cholestane. By analogy it would be expected that similar reduction of 21-acetoxypregn-5-en-3β-ol-20-one would furnish *pregn-5-en-3β-ol* (I; R = Et, R′ = H). For comparative purposes the latter was prepared by Wolff–Kishner reduction of pregn-5-en-3β-ol-20-one (kindly supplied by N. V. Organon, Oss, Holland) and characterised as the *acetate* and *benzoate*. Although Heard and McKay (*J. Biol. Chem.*, 1946, **165**, 677) had difficulty in obtaining pure androst-5-en-3β-ol (I; R = H, R′ = H) from androst-5-en-3β-ol-17-one semicarbazone (cf. Butenandt and Suranyi, *Ber.*, 1942, **75**, 591; Milas and Milone, *J. Amer. Chem. Soc.*, 1946, **68**, 738) by merely heating the latter with sodium ethoxide, we experienced no difficulty with the pregnene analogue when the reduction was effected with an excess of anhydrous hydrazine (cf. Dutcher and Wintersteiner, *ibid.*, 1939, **61**, 1992). The molecular-rotation difference of our pregn-5-en-3β-ol with respect to *allo*pregnan-3β-ol is +231°, which is in close agreement with the standard value of +243° found with cholesterol (Barton,

* The notation proposed by Fieser and Fieser (*Experientia*, 1948, **4**, 285; "Natural Products related to Phenanthrene," 3rd edn., Reinhold, New York, 1949) is adopted in this and succeeding papers of this series (cf. this vol., p. 1672, footnote).

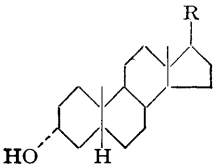
J., 1946, 1116; Barton and Klyne, *Chem. and Ind.*, 1948, 755; Barton, *Angew. Chem.*, **1949**, **61**, 57). For androst-5-en-3β-ol Heard and McKay (*loc. cit.*) recorded $[M]_D$ −186° (in dioxan) which gives a Δ value with respect to androstan-3β-ol ($[M]_D$ +18° (in dioxan); calculated according to Barton and Klyne, *loc. cit.*) of +204°. This is in fair agreement with the standard value of +228° found for cholesterol in the same solvent (Barton, *loc. cit.*). Norymberska, Norymberski, and Olalde (*J. Amer. Chem. Soc.*, 1948, **70**, 1256) reported for androst-5-en-3β-ol, prepared by treatment of dehydro*iso*androsterone dibenzyl mercaptal with Raney nickel, $[M]_D$ −208° (in dioxan). This gives a Δ value of +226°, in excellent agreement with the standard value. The molecular rotation recorded by Butenandt and Suranyi (*loc. cit.*) for their androst-5-en-3β-ol {$[M]_D$ −132° (in alcohol)} gives a Δ value with respect to androstan-3β-ol of only +165°.

Two by-products characterised by Heard and McKay from their reduction of androst-5-en-3β-ol-17-one were ætiocholan-3α-ol (II; R = H) and androstan-3β-ol (III; R = H). In our experiments less than 1% of a digitonin non-precipitable fraction was obtained; it was isolated as the acetate $C_{23}H_{36}O_2$, presumably *pregn-5-en-3α-yl acetate*. No saturated stanols could be detected, and there was no acid fraction.

The Wolff–Kishner reduction of 21-acetoxypregn-5-en-3β-ol-20-one furnished a neutral fraction and a relatively small acid fraction. The neutral fraction was acetylated and then chromatographed to give, as expected, pregn-5-en-3β-yl acetate, together with a second acetate, m. p. 132—133°, $[\alpha]_D$ −83°, which, according to analysis, was $C_{23}H_{34}O_2$ and thus contained two ethylenic linkages. This second compound was identified as pregna-5 : 20-dien-3β-yl acetate (I; R = CH:CH_2, R′ = Ac) by comparison with an authentic specimen



(I.)



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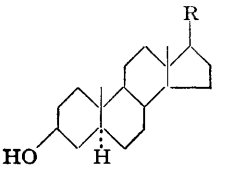
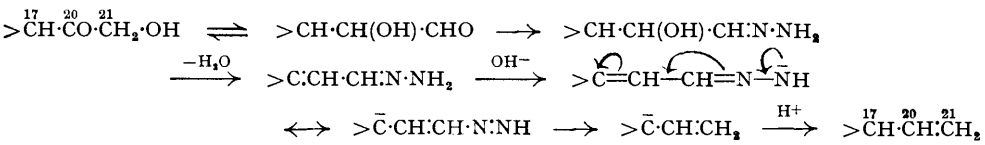


(III.)

kindly provided by Dr. P. L. Julian (Julian, Meyer, and Printy, *J. Amer. Chem. Soc.*, 1948, **70**, 887). The acid fraction from the reduction was methylated with diazomethane and then acetylated, yielding crystalline methyl 3β-acetoxyætiochol-5-enate (I; R = ·CO_2Me, R′ = Ac), identified by comparison with an authentic sample prepared from the corresponding hydroxy-acid methyl ester (for this and other compounds we are much indebted to Messrs. Ciba Ltd.).

When our experiments were carried out the formation of pregna-5 : 20-dien-3β-ol was unexpected. We explain the genesis of this, as indicated in the formulæ below, by an extension of the views of Seibert (*Chem. Ber.*, 1947, **80**, 494) on the mechanism of the Wolff–Kishner reaction :



There are in the steroid literature at least two analogies to the formation of this compound : first, the reduction of 17a-methyl-D-homoandrostane-3β : 17a-diol-17-one to 17a-methyl-D-homoandrost-17-en-3β-ol (Shoppee and Prins, *Helv. Chim. Acta*, 1943, **26**, 185), and, secondly, the formation of lithochol-11-enic acid, amongst other products, from 12-hydroxy-11-keto-lithocholic acids (Gallagher, *J. Biol. Chem.*, 1946, **162**, 539; Wintersteiner, Moore, and Reinhardt, *ibid.*, p. 707).*

It is of interest to compare the molecular-rotation differences for pregn-5-en-3β-ol with those for cholesterol. According to our previous work on the subject of " vicinal action " (Barton and Cox, *Nature*, 1947, **159**, 470; *J.*, 1948, 783) pregn-5-en-3β-ol and its simple derivatives

* Dr. O. Jeger (of Zürich) informs us that a migration of the ethylenic linkage, comparable to that postulated above, is of common occurrence in the reduction of αβ-unsaturated ketones by the Wolff–Kishner procedure. We are indebted to Dr. Jeger for this information.

should not show optical anomalies. The table shows that this is, indeed, the case. The absence of an optical anomaly in the ketone pregn-4-en-3-one is particularly worthy of comment, as supporting our previous classification of groups not liable to give " vicinal action " (cf. Barton and Cox, *loc. cit.*).

Substance.	$[M]_D$. Alcohol.	Acetate.	Benzoate.	Ketone.	Δ_1.	Δ_2.	Δ_3.
Cholest-5-en-3β-ol	−154°	−188°	− 74°	+357°	−34°	+80°	+511°
Pregn-5-en-3β-ol	−181	−214	−105	+331	−33	+76	+512
Δ values * for pregn-5-en-3β-ol	—	—	—	—	+ 1	− 4	+ 1

* Barton, *J.*, 1946, 1116; Barton and Cox, *J.*, 1948, 783; Barton, *Angew. Chem.*, 1949, **61**, 57.

Experimental.

(M. p.s are uncorrected.)

The substances whose rotations are listed below were dried, *in vacuo*, before weighing, at 20° below their m. p.s, or at 120°, whichever was the lower temperature. All rotations are for the Na_D line and in chloroform solution; they were measured in a 1-dm. micro-tube, of capacity about 1 ml.

Standard chemical operations were carried out as in Part IV (*J.*, 1948, 783) unless specified to the contrary. Light petroleum is of b. p. 40—60°.

Micro-analyses are by Drs. Weiler and Strauss, Oxford.

Wolff–Kishner Reduction of Pregn-5-en-3β-ol-20-one.—Pregn-5-en-3β-ol-20-one (150 mg.) was reduced by heating it with 150 mg. of sodium, dissolved in 3 ml. of ethanol, and 1·5 ml. of anhydrous hydrazine at 180° for 17 hours. After separation of the reaction product into acid (negligible) and neutral fractions, the latter fraction was acetylated. Chromatography over alumina furnished *pregn-5-en-3β-yl acetate*, crystallising from methanol in long needles, m. p. 147—148°, $[\alpha]_D$ −62° (*c*, 3·66), $[M]_D$ −214° (Found: C, 79·7, 80·6; H, 10·2, 10·8. $C_{23}H_{36}O_2$ requires C, 80·2; H, 10·55%). In a further experiment 550 mg. of pure acetate were obtained from 900 mg. of pregn-5-en-3β-ol-20-one. Alkaline hydrolysis gave *pregn-5-en-3β-ol*, crystallised from methanol, m. p. 134·5—135·5°, $[\alpha]_D$ −60° (*c*, 2·95), $[M]_D$ −181° (Found: C, 82·7; H, 11·2. $C_{21}H_{34}O$ requires C, 83·4; H, 11·35%). Benzoylation of this alcohol afforded *pregn-5-en-3β-yl benzoate*, crystallised from methanol, m. p. 154·5—155·5°, $[\alpha]_D$ −26° (*c*, 3·12), $[M]_D$ −105° (Found: C, 82·3; H, 9·2. $C_{28}H_{38}O_2$ requires C, 82·7; H, 9·4%). Oxidation of 100 mg. of pregn-5-en-3β-ol by the Oppenauer method and chromatography of the product over alumina furnished pregn-4-en-3-one, very soluble in all organic solvents but crystallising from light petroleum at −50°, and then having m. p. 91—92°, $[\alpha]_D$ +110° (*c*, 0·35), $[M]_D$ +331°; light absorption: Max., 241 mμ. (in alcohol); ε = 18,000. Marker and Lawson (*J. Amer. Chem. Soc.*, 1939, **61**, 586) gave m. p. 90—91°, for a specimen prepared by an alternative method, but did not record the rotation.

The mother-liquors from the crystallisation of the pregn-5-en-3β-yl acetate were hydrolysed with potassium hydroxide. The product was dissolved in the minimum of hot 95% alcohol and treated with digitonin (300 mg.) in 95% alcohol (16 ml.). After being kept overnight the precipitated digitonide was filtered off. The filtrate was evaporated to dryness and the residue extracted with ether. Evaporation of the ethereal extract, acetylation of the residue, and filtration in benzene solution through alumina furnished, after crystallisation from methanol, an acetate, m. p. 134—135°, which was presumably *pregn-5-en-3α-yl acetate* [8 mg. from the reduction of 900 mg. of pregn-5-en-3β-ol-20-one] (Found: C, 80·1; H, 10·9. $C_{23}H_{36}O_2$ requires C, 80·2; H, 10·55%). The acetate gave an immediate yellow colour with tetranitromethane and was unsaturated to the Liebermann–Burchard reagent.

The digitonides, precipitated as described above, were resolved in the usual way (Schönheimer, *Z. physiol. Chem.*, 1933, **215**, 59), and the slightly impure pregn-5-en-3β-ol was treated by the Anderson–Nabenhauer procedure (*J. Amer. Chem. Soc.*, 1924, **46**, 1957). Working up in the customary manner revealed no trace of saturated material.

On one occasion the Wolff–Kishner reduction of 300 mg. of pregn-5-en-3β-ol-20-one gave, as well as the products described above, about 50 mg. of highly crystalline material sparingly soluble in benzene. This had m. p. 264—266° (turning red) after several recrystallisations from chloroform–methanol and was identified as *pregn-5-en-3β-ol-20-one azine*. The authentic specimen of the latter was prepared by heating under reflux 50 mg. of pregn-5-en-3β-ol-20-one, 100 mg. of hydrazine hydrochloride, and 100 mg. of hydrated sodium acetate in aqueous methanol. After crystallisation from chloroform–methanol it had m. p. 266—269°, turning red, and gave no depression on admixture with the by-product of the Wolff–Kishner reduction (Found, for the latter compound: N, 4·6, 4·65. $C_{42}H_{64}O_2N_2$ requires N, 4·5%).

Wolff–Kishner Reduction of 21-Acetoxypregn-5-en-3β-ol-20-one.—600 Mg. of keto-alcohol were reduced as described above for pregn-5-en-3β-ol-20-one. Separation into acid and neutral fractions furnished, in the acid fraction, 60 mg. of a hydroxy-acid. This was identified as 3β-hydroxyætiochol-5-enic acid by conversion into the methyl ester (by diazomethane) and thence by acetylation into the methyl ester acetate, recrystallised from aqueous methanol, m. p. (after drying *in vacuo*) 151—152°, $[\alpha]_D$ −26° (*c*, 0·39). The latter derivative gave no depression in m. p. on admixture with authentic methyl 3β-acetoxyætiochol-5-enate, m. p. 152—153°, $[\alpha]_D$ −25° (*c*, 2·47), prepared (by C. J. W. Brooks) from a specimen of methyl 3β-hydroxyætiochol-5-enate.

The neutral fraction was acetylated and the product crystallised from acetone–methanol to give a substance, m. p. *ca.* 120°, $[\alpha]_D$ −74° (*c*, 1·51), −71° (*c*, 1·33). This was chromatographed over alumina (Savory and Moore); the following chromatogram is typical:

Fraction no.	Eluent.	M. p. of eluate (after one recrystn. from methanol).
1	100 ml. of 5% C_6H_6–95% light petroleum	trace, oily
2	50 ml. of 12% C_6H_6–88% ,, ,,	nil
3	30 ml. of 20% C_6H_6–80% ,, ,,	trace, oily
4	30 ml. ,, ,, ,, ,,	130—132°
5	30 ml. ,, ,, ,, ,,	132—137
6	50 ml. ,, ,, ,, ,,	137—138
7	20 ml. ,, ,, ,, ,,	124—128
8	30 ml. ,, ,, ,, ,,	115—118
9	20 ml. ,, ,, ,, ,,	117—119
10	30 ml. ,, ,, ,, ,,	114—117
11	30 ml. ,, ,, ,, ,,	125—126
12	30 ml. ,, ,, ,, ,,	122—125
13	60 ml. ,, ,, ,, ,,	129—130·5
14	100 ml. ,, ,, ,, ,,	129—130
15	50 ml. of 10% ether–90% C_6H_6	trace, 124—126
16	50 ml. of ether	nil

Fractions 4—7 inclusive were combined and recrystallised repeatedly from methanol to give pregn-5-en-3β-yl acetate (*ca.* 75 mg.), m. p. and mixed m. p. with an authentic specimen prepared as above, 147—148°. Hydrolysis and recrystallisation afforded pregn-5-en-3β-ol, m. p. and mixed m. p. with an authentic specimen prepared as above, 134·5—135·5°. Fractions 11—14 inclusive were combined and repeatedly recrystallised from methanol, to furnish pregna-5 : 20-dien-3β-yl acetate (*ca.* 20 mg.), m. p. and mixed m. p. with an authentic specimen (m. p. 132—133°, $[\alpha]_D$ reported as −77° (see text)), 132—133°, $[\alpha]_D$ −83° (*c*, 0·69), −81° (*c*, 0·50), $[M]_D$ −280° (Found : C, 80·5, 80·9, 81·0; H, 9·8, 10·4, 10·4. Calc. for $C_{23}H_{34}O_2$: C, 80·7; H, 10·1%).

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