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Lithium Aluminium Hydride Reduction of Pregnenolone under Conditions of Assumed Kinetic and Equilibrium Control

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Reduction of pregnenolone (*1*) with lithium aluminium hydride (LAH) yields pregn-5-ene-3β,20α-diol (*2a*) and its 20β epimer (*2b*) in a ratio of 1:3.8. Reduction with LAH/$AlCl_3$ under equilibrating conditions, using an excess of *1*, gave a complex product mixture containing *2a* and *2b* in a ratio of 1:9, pregna-3,5-dien-20-one (*3*), pregna-3,5-dien-20-ols (*4a* and *4b*), pregn-4-ene-20α-ol-3-one (*6a*), its 20β epimer (*6b*) as well as small amounts of other compounds, probably pregn-4-ene-3,20-diols. A reaction scheme is presented which accounts for the formation of the various reaction products.

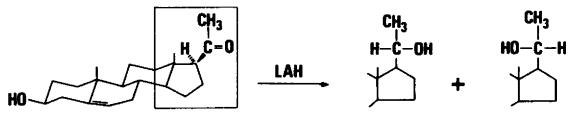
In connection with studies on certain enzymatic conversions of pregnenolone (*1*), tritium labelled pregn-5-ene-3β,20α-diol (*2a*) was synthesized by LAH reduction of the labelled ketone. This work initiated a more thorough investigation of the product composition obtained under different reaction conditions, assumed to represent kinetic and equilibrium control, respectively. The main purpose was to study the ratio of the epimeric diols (*2b*/*2a*) obtained in these reactions.

LAH reduction of ketones usually yields a product composition reflecting kinetic control of the reaction due to hydride attack from the sterically most favoured side of the plane of the carbonyl group. However, reduction by LAH in the presence of aluminium chloride and excess of ketone has been demonstrated to promote conditions of equilibrium control, in many cases highly affecting and even reverting the stereochemical outcome.[1] In this paper the results from LAH as compared to LAH/$AlCl_3$-reduction of pregnenolone are reported.

RESULTS

LAH reduction of pregnenolone gives almost quantitatively the diol mixture in which the 3β,20β-form (*2b*) predominates (ratio *2b*/*2a* = 3.8) (Fig. 1a). The results of LAH reduction in the presence of $AlCl_3$ and an excess of ketone were more difficult to analyze. In this case the product mixture was found to be composed of groups of compounds differing strongly in their elution behaviour from a C_{18} bonded phase analytical HPLC column as shown in Fig. 1b. The three more hydrophobic compounds were separated by straight phase column chromatography on silica gel and shown to be identical with pregn-3,5-dien-20-one (*3*), pregna-3,5-dien-20β-ol (*4b*) and pregna-3,5-dien-20α-ol (*4a*) in order of elution.

Analytical HPLC of the LAH/$AlCl_3$-reduction product mixture with UV detection at 235 or 247 nm revealed the presence of three compounds possessing a conjucated carbonyl chromophore. Co-chromatography of authentic samples showed



1 *2a* *2b*

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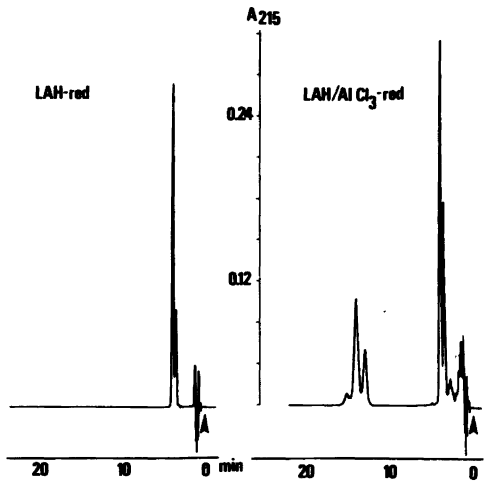


Fig. 1. Product composition obtained by LAH-reduction of pregnenolone as shown by reversed-phase HPLC in 75 % CH_3CN. Flow rate 2.0 ml/min, UV 215 nm. (a) reduction by LAH; (b) reduction by $LAH/AlCl_3$.

these compounds to be progesterone (pregn-4-ene-3,20-dione) (*5*), 20α-dihydroprogesterone (pregn-4-ene-20α-ol-3-one) (*6a*) and 20β-dihydroprogesterone (pregn-4-ene-20β-ol-3-one) (*6b*). These compounds interfered partly with the reduction products *2a* and *2b* at 215 nm (Fig. 2).

The complete disappearance of absorption from the non-conjugated compounds at 247 nm, however, made it possible to calculate the ratio *2b*/*2a* from the chromatograms monitored at 214 nm despite partial overlap. The ratio found in this way was 9.1, suggesting that the β-epimer (20R) should be thermodynamically favoured.

DISCUSSION

The results obtained are consistent with the reaction scheme outlined below, which accounts for all interconversions observed under the conditions of $AlCl_3$-mediated equilibration. We have no evidence for the formation of any 3α-epimers under these conditions, which also should be rather unlikely in view of the thermodynamically unfavourable axial position of the hydroxyl group in this case. We have not, however, been able to show whether a pregn-4-ene-3-ol system may be present or not; this is a possibility that cannot be ruled out *a priori*.

The epimer ratio *6b*/*6a* = 7.2, obtained upon $NaBH_4$-reduction of progesterone, is quite consistent with the favoured 20R-configuration obtained on kinetically controlled reduction, the higher ratio in this case being indicative of a more selective reaction taking place under milder reaction conditions.

Contrary to what has been claimed in the literature,[2] no isomerization of pregnenolone to its 17-epimer could be produced, despite attempts under a variety of conditions. The configuration at C-20 is remarkably well correlated to the NMR chemical shift of the methyl protons designated H-

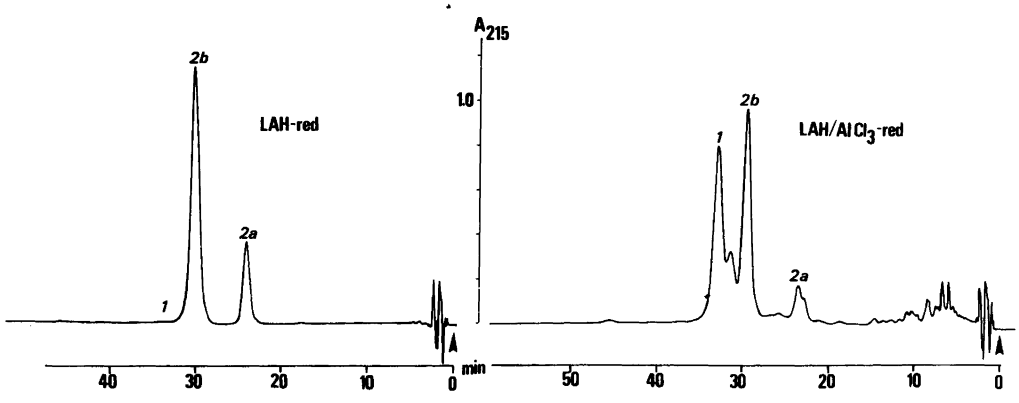
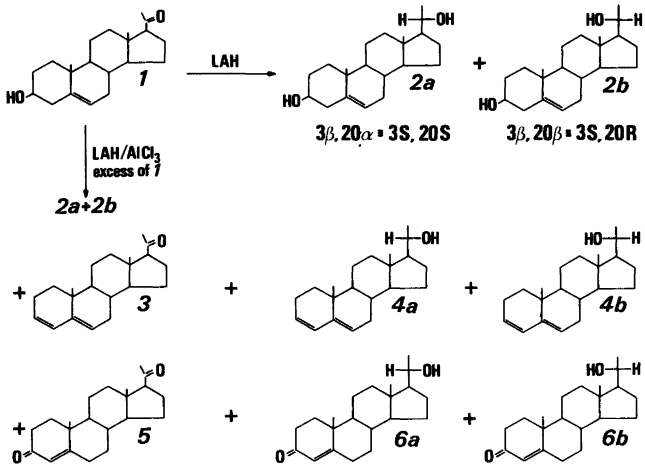
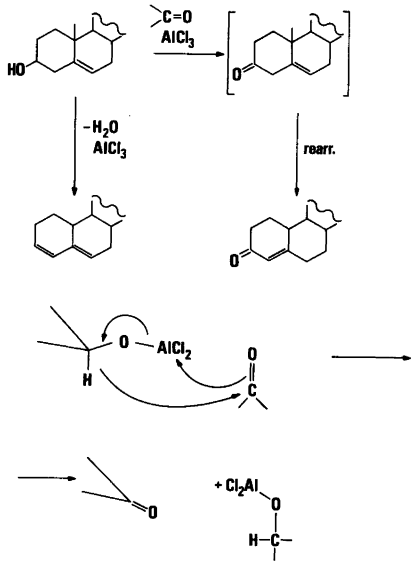


Fig. 2. Chromatograms showing (a) the epimer ratio obtained upon LAH-reduction, (b) the less retained products obtained upon $LAH/AlCl_3$-reduction. Reversed-phase HPLC in 50 % CH_3CN, flow rate 1.0 ml/min, UV 215 nm.



21 and H-18 as previously observed by Lee and Wolff for a series of C-20-oxygenated pregnane derivatives.[3] Our results give (in $CDCl_3$) for all 20α-epimers a downfield shift of 0.09 ±0.01 ppm for the H-21 signal and an upfield shift of 0.09±0.01 ppm for the H-18 signal, relative to the corresponding 20β-epimers. These findings are quite consistent with the closer proximity between the C-20 hydroxyl group and the protons at C-18 in the most stable conformation of the 20β-epimer.



Scheme 1.

EXPERIMENTAL

Reduction of pregnenolone. (a) Pregnenolone (500 mg, 1.58 mmol), dissolved in tetrahydrofuran (THF) distilled over lithium aluminium hydride (LAH), was added to a slurry of LAH (360 mg, 9.49 mmol) in THF (20 ml). The mixture was refluxed for 1 h. The excess of LAH was destroyed by successive addition of ethanol and water and the product was worked up in the usual way *via* extraction with chloroform yielding 464 mg (92%) of the isomeric diols.

The reaction was conveniently followed by TLC using silica gel-covered aluminium foil and elution with toluene/ethyl acetate (1/1). Visualization of the spots was effected by spraying with an ethanolic solution of 2,4-dinitrophenylhydrazine in sulfuric acid followed by gentle warming.

(b) Reduction with $LAH/AlCl_3$ (to cause equilibration) was carried out analogously to the method of Eliel *et al.*:[4] The ketone (1.0 g, 3.16 mmol) was added to a mixture of dry aluminium chloride (842 mg, 6.32 mmol) and LAH (66 mg, 1.74 mmol) in 25 ml of dry THF which had been refluxed for 30 min. After the addition the mixture was refluxed for 1.5 h, 0.25 ml of t-butanol added and reflux continued for an additional 2 h, the last 30 min after the addition of 50 mg of pregnenolone in order to maintain an excess of ketone. Then the mixture was stirred overnight. In both methods a conventional work-up procedure was performed, yielding a product mixture which was immediately taken for analytical and preparative HPLC.

Reduction of progesterone. Progesterone (200 mg, 640 μmol) was dissolved in 4 ml of methanol and 24 mg (640 μmol) of sodium borohydride added. After 1 h at room temperature under stirring the mixture was worked up in the usual way. The epimeric diols

formed were separated and isolated by preparative reversed-phase HPLC.

Chromatography. HPLC. A 4.6 × 200 mm stainless steel column, slurry-packed with Nucleosil C_{18} 5 μ (Macherey and Nagel, Düren, GFR) was used for analytical purposes and a 10.0 × 250 mm column containing the same material for the preparative work. The chromatograph consisted of an Altex mod. 100 constant flow solvent pump, a Rheodyne mod. 7120 injector valve, an LDC Spectromonitor III variable wavelength UV-VIS detector and a Hitachi model 561 potentiometric recorder. The analytical column was used with acetonitrile – or methanol – water mixtures as eluents under isocratic conditions throughout and flow rates between 1.0 and 3.5 ml/min. The chromatograms, resulting from 20 μl loop injections, were obtained by UV-monitoring of the effluent at selected analytical wavelengths. For the preparative column 70% acetonitrile was used, for reasons of solubility, at flow rates of 2.0 – 3.5 ml/min; the injection volume was 50 μl.

TLC. Straight-phase TLC was performed using silica gel 60 pre-coated TLC aluminium sheets with a layer thickness of 0.2 mm (DC-Alufolien, Merck) and reversed-phase TLC with pre-coated HPTLC plates (RP-8, Merck).

Attempted isomerization of pregnenolone to 17-isopregnenolone. Reflux of a solution of pregnenolone in methanol[2] overnight did not cause any observable transformation, but a complete recovery of the starting material. Upon addition of hydrochloric acid in acetic acid – water solvents a water elimination with the formation of *3* was observed. This reaction was readily followed by TLC as described above.

Product identification and characterization. Peak identification in the chromatograms was partially effected by preparative HPLC and isolation of compounds of interest, which were then characterized by various physico-chemical methods.

Optical rotations were measured with a Perkin-Elmer mod. 141 photoelectric polarimeter. NMR-spectra were recorded in $CDCl_3$ with a JEOL mod. FX 100 spectrometer and the mass spectra with a Hewlett-Packard mod. 5981/5933 GC-MS. Trimethylsilylation was performed by reaction with trimethylchlorosilane and hexamethyldisilazane in pyridine.[5] The GC-separation was carried out on an OV-117 column at 230 °C.

Pregn-5-ene-3β,20α-diol: ^{1}H NMR: H-6 5.33 (1H, d, br.), H-18 0.68 (3H, s), H-19 1.01 (3H, s), H-21 1.23 (3H, d, J = 6.1 Hz). MS: 462 (M^+, 1.9%), 372 (M – TMSOH, 2.8%), 282 (M – 2TMSOH, 2.6%), 117 ($TMSOCHCH_3^+$, 100%), 73 (TMS, 32%). $[\alpha]_D^{25}$ = −47° ($CHCl_3$), Ref. 6 = −59°. M.p. 176 – 177 °C, Ref. 6, 177 – 178 °C, Ref. 7, 180 – 183 °C.

Pregn-5-ene-3β,20β-diol: ^{1}H NMR, H-6 5.36 (1H, d, br.), H-18 0.77 (3H, s), H-19 1.02 (3H, s), H-21 1.14 (3H, d, J = 6.1 Hz). MS: 462 (M^+, 1.8%), 372 (M – TMSOH, 3.7%), 282 (M – 2TMSOH, 1.9%), 117 ($TMSOCHCH_3^+$, 100%), 73 (TMS 31%). $[\alpha]_D^{25}$ = −65° ($CHCl_3$), Ref. 6 = −64°. M.p. 206 – 207 °C, Ref. 6 200.5 – 201.5 °C, Ref. 8 201.1 – 203.5 °C.

Pregna-3,5-dien-20α-ol: ^{1}H NMR: H-3 5.57 (1H, m), H-4 5.93 (1H, d, br.), H-6 5.38 (1H, m), H-18 0.71 (3H, s), H-19 0.96 (3H, s), H-20 3.68 (1H, m), H-21 1.24 (3H, d, J = 6.1 Hz). MS: 372 (M^+, 2.5%), 282 (M – TMSOH, 0.9%), 117 ($TMSOCHCH_3^+$, 100%), 73 (TMS 40%). $[\alpha]_D^{25}$ = −44° ($CHCl_3$).

Pregna-3,5-dien-20β-ol: ^{1}H NMR: H-3 5.59 (1H, m), H-4 5.92 (1H, d, br), H-6 5.36 (1H, m), H-18 0.80 (3H, s), H-19 0.96 (3H, s), H-20 3.76 (1H, m), H-21 1.14 (3H, d, J = 6.1 Hz). MS: 372 (M^+, 4.3%), 117 ($TMSOCHCH_3^+$, 100%), 73 (TMS, 32%). $[\alpha]_D^{25}$ = −158° ($CHCl_3$). M.p. 95 – 124 °C, Ref. 9 134 – 136 °C.

Pregna-3,5-dien-20-one: ^{1}H NMR: H-3 5.58 (1H, m), H-4 5.92 (1H, d, br.), H-6 5.38 (1H, m), H-18 0.66 (3H, s), H-19 0.95 (3H, s), H-21 2.13 (3H, s). MS: 298 (M^+, 57%), 283 (M – CH_3, 16%), 255 (M – CH_3CO, 10%), 107 (69%), 105 (85%), 91 (100%), 43 (CH_3CO, 94%). $[\alpha]_D^{25}$ = −45° ($CHCl_3$), Ref. 10 = −52.4°. M.p. 130 – 136 °C, Ref. 10 139 – 142 °C.

Pregn-4-ene-20α-ol-3-one: ^{1}H NMR: H-4 5.71 (1H, s), H-18 0.72 (3H, s), H-19 1.19 (3H, s), H-20 3.54 (1H, m), H-21 1.22 (3H, d, J = 6.1 Hz).

Pregn-4-ene-20β-ol-3-one: ^{1}H NMR: H-4 5.72 (1H, s), H-18 0.80 (3H, s), H-19 1.19 (3H, s), H-20 3.72 (1H, m), H-21 1.14 (3H, d, J = 6.1 Hz). $[\alpha]_D^{25}$ = +92° ($CHCl_3$), Ref. 6 +84°, Ref. 11 +90.5°. M.p. 165 – 168 °C, Ref. 6 171 – 172 °C, Ref. 11 174 – 175 °C.

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