ON STEROIDS. CXXXX.*

B-NORSTEROID ANALOGUES OF CORTICOIDS

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The synthesis of the B-norsteroid analogue of Reichstein's substance S (17α,21-dihydroxy-4-pregnen-3,20-dione) is described.

In our papers on B-norsteroids we dealt with B-noranalogues of androgens[1-3], gestagens[4] and estrogens[5]. In addition also the microbial hydroxylation of the B-norsteroid nucleus has been studied[6] in our laboratory. Following these studies on the relationship between structure and biological activity of steroids we decided to prepare the B-noranalogues of corticoids. In this paper we describe the synthesis of the B-noranalogue of Reichstein's substance S (*X*) which represents the logical starting compound for these studies. Two alternative routes were apparent for the preparation of the key substance – the triol *VII*: Either to start with a simple B-norsteroid and to build up the side chain with one of the well developed methods or, to contract the B-ring in the compound already carrying the whole grouping at $C_{(17)}$. In our previous work we have shown[4] that the transformation of the 17-oxo group to the Δ^{16}-17-acetyl grouping *via* the cyanohydrine proceeds in the B-norsteroid series with very poor yields. We therefore studied the possibility of the ring contraction in the pregnene derivatives *I* and *VIII* using the general procedure for contraction of the steroid B-ring developed by Šorm[7,8].

The ketol-acetate *I* was oxidised with chromic acid in acetic acid to the corresponding keto acid which was lactonised in crude state to the lactone *II*. Subsequent thermal decarboxylation then afforded the desired B-norderivative *III*. We expected to apply the method described by Julian[9] and Ringold[10] for introduction of the acetoxy group into 21 position and subsequent oxidation at $C_{(3)}$. In this method the 3β-formyloxy derivative is transformed directly by modified Oppenauer oxidation into the 3-oxo compound. We therefore partially hydrolysed the diacetate *III* to the monoacetate *IV* and this in turn esterified to the formate *V*. But unexpectedly our attempts to introduce the 21-acetoxy group into this compound using the method

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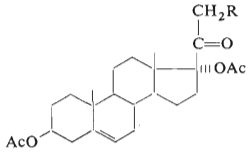
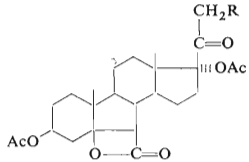
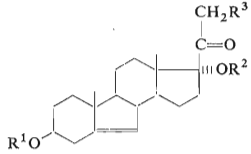
mentioned above were unsuccessful and we were not able to detect the desired 21-acetate in the complex reaction mixture. When the diacetate *III* was submitted to the analogous reaction sequence the 21-acetoxy derivative *VI* was isolated but in a very low yield. On the other hand, the alternative route based on the contraction of the B-ring in the 21-acetoxy derivative *VIII* gave triacetate *VI* in excellent yields. The hydrolysis to the triol *VII* was carried out with hydrochloric acid which gave better results than the alkaline hydrolysis. The final step – partial oxidation to the desired B-noranalogue of Reichstein's substance S (*X*) was carried out by *Flavobacterium buccalis*[11] in about 70% yield.



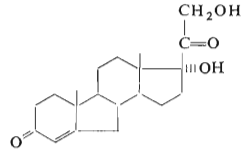
I, R = H
VIII, R = OAc



II, R = H
IX, R = OAc



III, $R^1 = R^2 = Ac$, $R^3 = H$
IV, $R^1 = R^3 = H$, $R^2 = Ac$
V, $R^1 = HCO$, $R^2 = Ac$, $R^3 = H$
VI, $R^1 = R^2 = Ac$, $R^3 = OAc$
VII, $R^1 = R^2 = H$, $R^3 = OH$



X

EXPERIMENTAL

Melting points were determined on a Kofler block. Analytical samples were dried at 80°C/0·2 Torr. Optical measurements were carried out in chloroform with an error of ±1°. The infrared spectra were recorded on the Zeiss UR 10 spectrometer. The NMR spectra were recorded on Varian HA-100 instrument in deuteriochloroform with tetramethylsilane as internal reference. The chemical shift is given in p.p.m. The identity of samples prepared by different routes was checked by mixture melting point determination, by thin-layer chromatography and by IR spectra.

3β,17α-Diacetoxy-B-norpregn-5-en-20-one (*III*)

A solution of the diacetate *I* (5 g) in acetic acid (50 ml) was treated dropwise (2 hours) with a solution of chromic acid (4 g) in 50% acetic acid (20 ml) the temperature being kept at 55°C. The stir-

ring was continued for additional 2 h at the same temperature. The excess oxidising agent was then removed with methanol (5 ml), solvents distilled off under reduced pressure and the residue dissolved in ether. The ethereal solution was extracted with a sodium carbonate solution (10%) the alkaline extract was cooled to 0°C, acidified with conc. hydrochloric acid and the keto acid taken into ether. The ethereal solution was washed with water, dried, and evaporated. The residual oil (2 g) was dissolved in pyridine (6 ml) treated with benzoyl chloride (2 ml) and allowed to stand at room temperature for 48 h. The reaction mixture was decomposed with water and ice and the product extracted with ether. The extract was washed with dilute hydrochloric acid, a sodium hydrogen carbonate solution, water, dried, and evaporated giving the lactone *II* as an oil. The crude lactone was decarboxylated by heating to 190°C for 10 minutes and the crystalline residue after cooling off was crystallised from methanol to yield 1 g of the B-nor-derivative *III*, m.p. 220–221°C, $[\alpha]_D^{20}$ −98° (*c* 1·84). For $C_{24}H_{34}O_5$ (402·5) calculated: 71·61% C, 8·51% H; found: 71·67% C, 8·39% H.

3β-Hydroxy-17α-acetoxy-B-norpregn-5-en-20-one (*IV*)

The diacetate *III* (500 mg in chloroform (3 ml) and methanol (3 ml) was treated with conc. hydrochloric acid (0·5 ml) and allowed to stand at 37°C for 20 h. The reaction mixture was diluted with water and the product taken into ether. The ethereal solution was washed with sodium hydrogen carbonate and worked up. The residue was crystallised from acetone–light petroleum (b.p. 40–60°C) to afford 300 mg of the alcohol *IV*, m.p. 247–248°C, $[\alpha]_D^{20}$ −89° (*c* 1·95). For $C_{22}H_{32}O_4$ (360·5) calculated: 73·30% C, 8·95% H; found: 72·94% C, 9·24% H.

3β-Formyloxy-17α-acetoxy-5-norpregn-5-en-20-one (*V*)

A solution of the alcohol *IV* (200 mg) in 98% formic acid (3 ml) was heated to 60°C for 2 hours in a nitrogen atmosphere. The reaction mixture was diluted with water (15 ml) and kept for 1 h at 0°C. The product was extracted with chloroform, washed with a sodium hydrogen carbonate solution, water, dried, and evaporated. The residue was chromatographed on a silica gel column (20 g) in light petroleum (b.p. 40–60°C)–ether (1 : 1) and the purified product crystallised from methanol. Yield 150 mg of the diester *V*, m.p. 187–188°C, $[\alpha]_D^{20}$ −108° (*c* 2·84). For $C_{23}H_{32}O_5$ (388·5) calculated: 71·10% C, 8·30% H; found: 71·14% C, 8·48% H.

3β,17α,21-Triacetoxy-B-norpregn-5-en-20-one (*VI*)

a) A stirred solution of the triacetate *VIII* (3·6 g) in acetic acid (35 ml) was treated at 55°C dropwise with a solution of chromic acid (2·8 g) in 50% acetic acid (15 ml) within 2 hours. After stirring for additional 2 h methanol was added (5 ml) and organic solvents were removed under reduced pressure. The residue was dissolved in ether and the keto acid taken into 3% sodium carbonate (200 ml). The alkaline extract was cooled to 0°C acidified with conc. HCl and the acid extracted with ether. The ethereal solution was washed with water, dried, and evaporated to dryness. The residue (2 g) was dissolved in pyridine (6 ml) treated with benzoyl chloride (2 ml) and set aside for 65 h. The reaction mixture was decomposed with ice and the lactone *IX* was taken into ether. The ethereal solution was worked up, dried, and evaporated. The oily residue was heated to 190°C for 15 minutes and the solid product was crystallised from methanol to yield 800 mg of the triacetate *VI*. The mother liquors gave after chromatography over silica gel and crystallisation from methanol second crop of the triacetate *VI* (450 mg), m.p. 208°C, $[\alpha]_D^{20}$ −82° (*c* 0·82). IR 1 730 cm^{-1}; NMR: 0·72 (s, 18-H), 0·90 (s, 19-H), 2·02, 2·08 and 2·15(3 s, ester CH_3), 4·15 (m, 3β-H), 5·40 (m, 6-H). For $C_{26}H_{36}O_7$ (460·6) calculated: 67·80% C, 7·88% H; found: 68·10% C, 7·67% H.

b) A solution of the diacetate *III* (360 mg) in acetic acid (7 ml) was treated at 18°C successively with solutions of bromine (220 mg) in acetic acid (0·5 ml) and 32% hydrogen bromide in acetic acid (0·75 ml) and stirred at room temperature for 1 1/2 hour. The solution was then treated with tetrachloromethane (7 ml) and bromine (220 mg) in acetic acid (0·5 ml) and stirred for additional 45 minutes at room temperature. The most part of the solvents was removed under reduced pressure at 40°C, water was added the precipitate collected by succion and washed with water. It was dissolved in benzene (20 ml) dried with magnesium sulphate and evaporated to dryness. The residue was dissolved in benzene (15 ml) treated with a solution of sodium iodide (2 g) in ethanol (15 ml) and set aside for 20 hours. The reaction mixture was then diluted with water and extracted with ether. The ethereal solution was washed with 3% sodium thiosulphate, water, dried, and evaporated. The solid residue was dissolved in acetone (15 ml) and refluxed with freshly fused potassium acete (1·5 g) for 3 h. The reaction mixture was diluted with water the product taken into ether and the ethereal solution worked up. The oily residue (360 mg) consists according to the thin-layer chromatography mainly from very polar components and only traces of compounds of the expected polarity of the acetate *VI* are present. Repeated chromatography on silica gel and crystallisation from methanol gave 1 mg of the triacetate *VI*, m.p. 206°C, identical with the compound prepared under *a*).

c) The triol *VII* (200 mg) was acetylated with acetic anhydride (2 ml) in pyridine (2·5 ml) at room temperature for 20 h. Usual working up afforded a residue (170 mg) which was dissolved in acetic anhydride (2 ml) treated with *p*-toluenesulphonic acid (8 mg) and heated to 70°C for 3 h. The mixture was diluted with pyridine (5 ml) and water, the product extracted with chloroform and washed with dilute hydrochloric acid, a sodium hydrogen carbonate solution, water, dried, and evaporated. Crystallisation from methanol afforded 90 mg of the triacetate *VI*, m.p. 208°C.

3β,17α,21-Trihydroxy-B-norpregn-5-en-20-one (*VII*)

The triacteate *VI* (1·5 g) in chloroform (11 ml) and methanol (11 ml) was treated with conc. HCl (1·2 ml) and allowed to stand at 30°C for 40 hours. The reaction mixture was then diluted with water, the product taken into ether and the ethereal solution washed with a sodium hydrogen carbonate solution, water, dried, and evaporated. The oily residue (1·1 g) was chromatographed on a silica gel column (100 g) in benzene–ether (1 : 1) to yield 550 mg of the crude triol. Crystallisation from ethyl acetate afforded 500 mg of the triol *VII*, m.p. 238—239°C $[\alpha]_D^{20}$ −45° (*c* 1·1 in ethanol). IR (KBr): 3420 cm^{-1}, 3025 cm^{-1}, 1706 cm^{-1}; NMR: 0·62 (s, 18-H), 0·87 (s, 19-H), 5·35 (m, 6-H). For $C_{20}H_{30}O_4$ (334·4) calculated: 71·82% C, 9·04% H; found: 71·93% C, 9·21% H.

17α,21-Dihydroxy-B-norpregn-4-en-3,20-dione (*X*)

The medium (1 200 ml) containing 0·8% glucose, 0·4% corn-steep extract (50% dry residue), 0·02% K_2HPO_4 and 0·03% NaCl was adjusted to pH 7·0 and sterilised at 120°C for 1 h. It was then inoculated with 100 ml of a submerged culture of *Flavobacterium buccalis* grown at 30°C in a medium of the same composition for 24 h. Cultivation was carried out at 30°C for 10 h (reciprocal shaker). The culture was additioned with triol *VII* (360 mg) dissolved in dimethylformamide (2 ml) and ethanol (3 ml) under warming. Transformation was carried out under identical conditions (30°C, shaking) for 16 h. The reaction mixture was then extracted with three 500 ml portions of butyl acetate, the combined extracts were washed with a sodium hydrogen carbonate solution, water, dried, and evaporated at 50°C. The residue was washed with two 100 ml portions of light petroleum (b.p. 40—60°C) leaving 280 mg of the crude dione. It was chromatographed on a silica gel column (30 g) in chloroform. Working up and crystallisation from acetone afforded 230 mg

of the dione *X*, m.p. 226–227°C, $[\alpha]_D^{20}$ +4° (*c* 0·83 in ethanol). IR: 3600 cm^{-1}, 1709 cm^{-1}, 1657 cm^{-1}, 1089 cm^{-1}; UV (ethanol): λ_{max} 241 nm, log ε 4·02; NMR: 0·66 (s, 18-H), 1·07 (s, 19-H), 5·78 (s, 4-H). For $C_{20}H_{28}O_4$ (332·4) calculated: 72·26% C, 8·49% H; found: 72·53% C, 8·65% H.

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