UDC 547.92.02

23. Masao Uchibayashi: Studies on Steroids. XVI.*1 Structure of the $NaBH_4$-Reduction Products of Reichstein's Substance S and Related Compounds.

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In the course of investigations on the microbiological transformation of steroids, 17α,20β,21-trihydroxy-4-pregnen-3-one (II) was needed as a reference compound. The first synthesis of compound (II) was reported in 1939 from two independent laboratories. Ruzicka and Müller[1] prepared this compound by a series of reactions beginning with 17β-hydroxy-17-isopregna-4,20-dien-3-one (17-vinyltestosterone), and Logemann[2] presented a brief description of the identical method. Later, Julian and his co-workers[3] recorded the synthesis of compound (II) by the lithium aluminum hydride reduction of the 3-ethyl-enol ether of the 21-acetate of Reichstein's Substance S (I) (17α,21-dihydroxy-4-pregnene-3,20-dione) or 21-acetoxy-16,17-epoxy-4-pregnene-3,20-dione.

For the present purpose, the most suitable method seemed to be the application of the method of Norymberski and Woods[4] which involved selective reduction of the 20-keto group in 3,20-dioxosteroids by sodium borohydride under strictly controlled conditions. Thus, treatment of Reichstein's Substance S (I) with sodium borohydride, followed by acetylation and column chromatography, afforded two products; one was the acetate (III) of the desired compound*1 as expected and the other an unidentified compound (V) melting at 164~166°. This second product was assumed to have been formed by reduction of both the 3- and 20-keto groups of (I). Hydrolysis of (V) with potassium hydrogencarbonate yielded feathery crystals (IV) melting at 187~189°, whose analytical values and infrared spectrum indicated (IV) to be a 3,20-tetrahydro derivative of the starting material (I). The 20-hydroxyl group is undoubtedly in β-configuration. For assignment of configuration of the 3-hydroxyl group and the position of the double bond, the molecular rotation of (IV) was compared with those of related steroids in cholesterol and testosterone series (see Table I). Sondheimer and Klibansky[5] stated that in 4-en-3β-ol steroids, values of molecular rotation difference of hydroxyl and keto

Table I. Comparison of Molecular Rotations

	4-en-3-one	4-en-3β-ol	4-en-3α-ol	5-en-3β-ol	5-en-3α-ol
Cholesterol series[a)]	+341 E	+170 B	+467 B	−151 C	−145 C
Teststerone series[b)]	+314 E	+140 E	+543 P	−150 E	−156 E
Pregnene-3,17,20,21-tetrol series	+233 D[c)]	+ 80 M[d)]	—	−193 A[b)]	—

a) L. F. Fieser, M. Fieser: "Natural Products Related to Phenanthrene," 3rd Ed., 216(1949). Reinhold Publ. Corp., U.S.A.
b) "Elsevier's Encyclopaedia of Organic Chemistry," ed. F. Radt, Vol. 14-Suppl. (1956). Elsevier Publ. Co., The Netherlands.
c) This paper; also Footnotes 2) and 3).
d) This paper; taken in methanol because of low solubility in dioxane or acetone.
Solvent: A=acetone, B=benzene, C=chloroform, D=dioxane, E=ethanol, M=methanol, P=pyridine.

*1 This paper constitutes a part of a series entitled "Studies on Steroids" by Hayao Nawa. Part XV. M. Uchibayashi: This Bulletin, 8, 117(1960).

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1) L. Ruzicka, P. Müller: Helv. Chim. Acta, **22**, 755(1939).
2) W. Logemann: Naturwissenschaften, **27**, 196(1939).
3) P. L. Julian, E. W. Meyer, W. J. Karpel, W. Cole: J. Am. Chem. Soc., **73**, 1982(1951).
4) J. K. Norymberski, G. F. Woods: J. Chem. Soc., **1955**, 3426.
5) F. Sondheimer, Y. Klibansky: Tetrahedron, **5**, 15(1959). See also J. A. Mills: J. Chem. Soc., **1952**, 4976.

compounds (Δ_1) fall within the range of $-160°$ and $-200°$, and values of the difference of acetoxyl and keto compounds (Δ_2) are around $-300°$, whereas 4-en-3α-ol steroids show highly positive values both in Δ_1 and Δ_2. Δ_1 ($-153°$) and Δ_2 ($-343°$) of the present compounds are roughly accordant with their data. Further, sodium borohydride reduction of 4-en-3-one steroids is known to produce chiefly a 3β-hydroxyl group.[6] Thus, it may be concluded from the above discussion that the compound (IV) has a 4-en-3β-ol structure and consequently (IV) would be represented as 4-pregnene-3β,17α,20β,21-tetrol. This tetrol (IV) must have been formed by partial non-selective reduction, and (IV) was obtained in a good yield when (I) was treated with sodium borohydride under conventional conditions.

As is known, manganese dioxide[7] has been used as an effective agent for the selective oxidation of allylic alcohols in the presence of other hydroxyl functions. Recently, Morita[8] showed that active N-halogenated compounds, such as N-bromoacetamide, N-bromosuccinimide, and isocyanuryl bromide, were also usable for the same purpose. For confirmation of its structure, (IV) was treated with N-bromoacetamide. Column chromatography of the product led to the isolation of the desired compound (II), as a result of the expected selective oxidation of the 3-allylic hydroxyl group, albeit in a low yield.

Further support was supplied for the validity of the structure of (IV) and (V) when (V) was subjected to hydrogenation in the presence of platinum oxide in ethanol. The product obtained after chromatography was proved to be 3β,20β,21-triacetoxyallopregnan-17α-ol (VI; the triacetate of Reichstein's Substance K) by comparison of the physical constants with those found in the literature.[9] Rather unexpectedly this hydrogenation also gave another product (VIII) in almost the same yield as that of the desired (VI), and its analytical values indicated loss of two oxygen atoms.

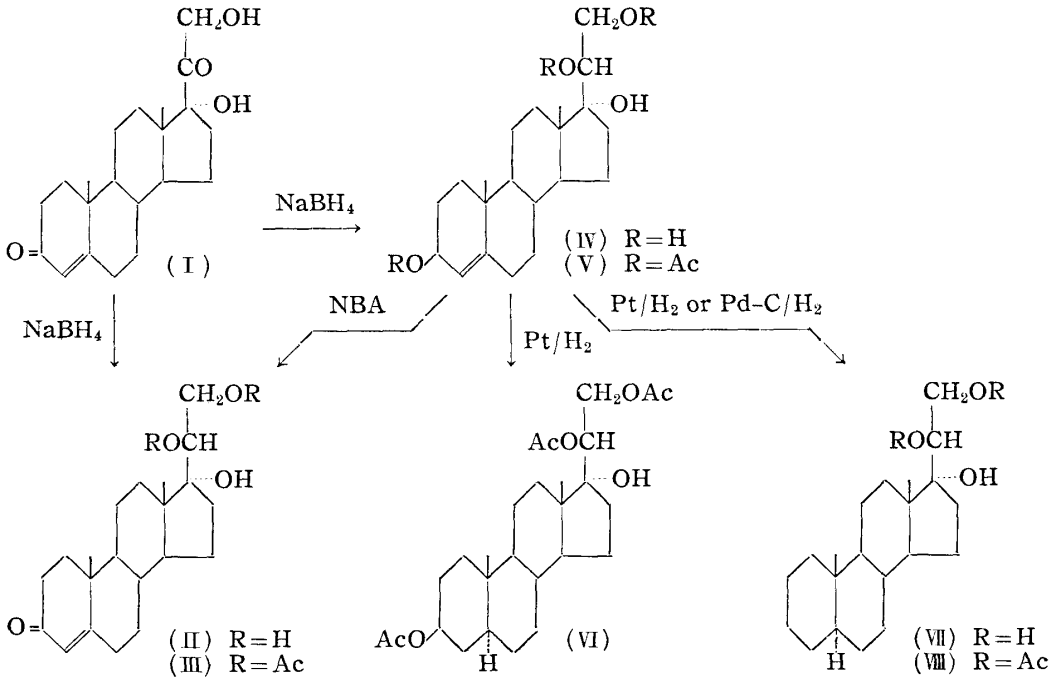
In their study of hydrogenation with platinum oxide of 4-cholesten-3α-ol, 4-cholesten-3β-ol, and 3-acetoxyl and 3-methoxyl derivatives, Shoppee and his collaborators[10] have found that partial loss of the oxygen function at the 3-position took place, giving saturated hydrocarbon compounds and this hydrogenolysis was accelerated by the addition of a small amount of acid (sulfuric or perchloric acid) to the reaction medium. Camerino and Alberti[11] obtained allopregnan-20β-ol by catalytic hydrogenation of the sodium borohydride reduction product of progesterone in the presence of platinum oxide in glacial acetic acid. Nishimura and Mori[12] also encountered the same type of hydrogenolysis in their neutral hydrogenation of 4-cholestene-3β,6β-diol and attributed this side reaction to the presence of traces of acidic contaminants in the catalyst.

In view of these findings, the product (VIII) was assumed to have been produced by removal of the 3-acetoxyl group by hydrogenolysis. In order to convert it to the known compound, (VIII) was set to hydrolysis with potassium hydrogencarbonate and compound (VII) obtained was ascertained to be allopregnane-17α,20β,21-triol by direct comparison (mixed melting point and infrared spectrum) with the same compound synthesized by

6) W.G. Dauben, R.A. Micheli, J.F. Eastham : J. Am. Chem. Soc., **75**, 6344(1953).
7) G. Rosenkranz, *et al.* : J. Chem. Soc., **1953**, 2189; J. Am. Chem. Soc., 75, 5390, 5932(1953); J. Chem. Soc., **1954**, 1226.
8) K. Morita : Bull. Chem. Soc. Japan, **31**, 450(1958); *ibid*, **32**, 227(1959). See also T.H. Kritchevsky, D.L. Garmaise, T.F. Gallagher : J. Am. Chem. Soc., **74**, 483(1952); R.E. Jones, F.W. Kocher : *Ibid.*, **76**, 3682(1954).
9) M. Steiger, T. Reichstein : Helv. Chim. Acta, **21**, 546(1938); T. Reichstein, K. Gätzi : *Ibid.*, **21**, 1195(1938); A. Serini, W. Logemann, W. Hildebrand : Chem. Ber., **72**, 391(1939).
10) C.W. Shoppee, B.D. Agashe, G.H.R. Summers : J. Chem. Soc., **1957**, 3107.
11) B. Camerino, C.G. Alberti : Gazz. chim. ital., **85**, 51(1955).
12) S. Nishimura, K. Mori : Bull. Chem. Soc. Japan, **32**, 103(1959); Abstracts of Papers, 12th Annual Meeting of the Chemical Society of Japan, 340, April, 1959.

Wagner and Moore[13] by other method. It became evident, therefore, that the hydrogenation was accompanied by hydrogenolysis of the 3-oxygen function, though conducted in the neutral medium. Hydrogenation of (IV) with palladium-carbon as a catalyst also led to the same hydrogenolysis. This hydrogenolysis takes place so smoothly in the presence of hydrochloric acid that it may constitutes a convenient method to obtain 3-deoxyallopregnane compounds which are, in many cases, difficult or laborious to prepare by other methods.

In this way, a secondary product of the sodium borohydride reduction of Reichstein's Substance S was structurally clarified as 4-pregnene-3β,17α,20β,21-tetrol (IV). For further confirmation, (IV) was subjected to the selective oxidation of allylic alcohols by N-bromoacetamide and to the catalytic hydrogenation, which furnished an interesting finding of hydrogenolysis.



Experimental[*2]

17α-Hydroxy-20β,21-diacetoxy-4-pregnen-3-one (III) and 3β,20β,21-Triacetoxy-4-pregnen-17α-ol (V) from (I)—Reduction of 1.00 g. of Reichstein's Substance S (I) with 126 mg. of $NaBH_4$ was carried out as described previously.[*1] The Et_2O eluates of the Florisil chromatography gave 700 mg. of colorless needles (V) (from AcOEt), m.p. 164~166°; $[\alpha]_D^{27}$ +55°(c=0.5 in Me_2CO); M_D +262°. IR ν_{max} cm^{-1} : 3436(OH), 1730, 1718, 1704(acetate), 1626(double bond). *Anal.* Calcd. for $C_{27}H_{40}O_7$: C, 68.04; H, 8.46. Found : C, 67.87; H, 8.22.

Further elution with Et_2O-Me_2CO(98:2) mixture afforded 200 mg. of colorless needles (III), m.p. 191~192°.

4-Pregnene-3β,17α,20β,21-tetrol (IV) from (V)—A solution of 390 mg. of (V) in 30 cc. of MeOH was heated under reflux for 1 hr. with a solution of 1.1 g. of $KHCO_3$ in 4.5 cc. of water. Evaporation of the reaction mixture gave a water-insoluble residue which on crystallization from MeOH yielded 210 mg. of colorless feathers, m.p. 187~189°; $[\alpha]_D^{27}$ +23°(c=1.0 in MeOH); M_D +80°. IR ν_{max} cm^{-1} : 3333, 3194(OH), 1647(double bond). *Anal.* Calcd. for $C_{21}H_{34}O_4 \cdot H_2O$: C, 68.44; H, 9.85. Found: C, 68.62; H, 9.78.

4-Pregnene-3β,17α,20β,21-tetrol (IV) from (I)—To a solution of 8.0 g. of (I) in 1 L. of MeOH was added 1.2 g. of $NaBH_4$ with stirring. After standing at room temperature overnight, the solution was

*3 All m.p.s are uncorrected and the infrared spectra were measured as Nujol mulls.

13) R. B. Wagner, J. A. Moore : J. Am. Chem. Soc., **72**, 5303(1950). Grateful acknowledgement is made to Dr. James A. Moore of the University of Delaware for generous supply of the compound (VII) prepared by him.

mixed with 19 cc. of glacial AcOH and 200 cc. of water, and concentrated to distill off MeOH. The substance isoluble in water was collected by filtration, dried *in vacuo*, and recrystallized repeatedly from MeOH to 5.5 g. of colorless fine needles, m.p. 187~189°.

17α,20β,21-Trihydroxy-4-pregnen-3-one (II) from (IV)—In a solution of 320 mg. of (IV) in 3 cc. of benzene and 9 cc. of pyridine 150 mg. of N-bromoacetamide was dissolved and the solution was stirred in the dark at room temperature for 3.5 hr. The faint yellow mixture was diluted with 30 cc. of Et_2O and 10 cc. of benzene, washed successively with 10% $NaHSO_3$ solution, 10% NaOH solution, water, dil. HCl, and water. After concentration of the organic layer under vacuum, the residue was recrystallized from Me_2CO to yield a crystalline material melting at 168~170°, which was dissolved in a small volume of MeOH-$CHCl_3$(1:1) mixture and subjedted to column chromatography over 10 g. of alumina.

Successive elution with Et_2O, Et_2O-Me_2CO(95:5), (90:10), (80:20), (50:50), and Me_2CO gave no substance. The fractions from the Me_2CO-MeOH(50:50) eluates were recrystallized from Me_2CO to give 40 mg. of colorless fine needles melting at 187~188°. The infrared spectrum of this compound was identical with an authentic spectrum of (II) and the mixed melting point with an authentic sample did not show any depression.

Further elution with MeOH afforded 50 mg. of a material which melted at 172~179° after repeated recrystallization from Me_2CO and was found by infrared spectrum to be a mixture of the starting material (IV) and the product (II).

The Triacetate (VI) of Reichstein's Substance K (3β,20β,21-Triacetoxyallopregnan-17α-ol) and 20β,21-Diacetoxyallopregnan-17α-ol (VIII) from (V)—A mixture of 380 mg. of (V) and a catalyst (prepared from 100 mg. of PtO_2) in 40 cc. of EtOH was shaken in H_2 atmosphere, absorbing 56 cc. of H_2 in 2 hr. After filration the solution was concentrated and the residue recrystallized from MeOH to afford a material, m.p. 130~132°. This was dissolved in a small volume of Me_2CO and added to a column of 50 g. of Florisil. Elution with petr. ether-Me_2CO(99:1), (98:2), (96:4), and (92:8) gave no substance. The eluates of petr. ether -Me_2CO(85:15) were combined and evaporated, and the residue after recrystallization from Me_2CO yielded 180 mg. of colorless feathers (VIII), m.p. 152~153°; $[\alpha]_D^{31}$ +35° (c=2.0 in Me_2CO). IR ν_{max} cm^{-1} : 3649(OH), 1733(acetate). *Anal.* Calcd. for $C_{25}H_{40}O_5$: C, 71.39; H, 9.59. Found : C, 71.63; H, 9.52.

The eluates of petr. ether-Me_2CO(50:50) gave crystals, which were recrystallized from Me_2CO to yield 130 mg. of colorless plates (VI), m.p. 172°; $[\alpha]_D^{31}$ +58°(c=2.0 in Me_2CO). IR ν_{max} cm^{-1} : 3413(OH), 1718(shoulder), 1704(acetate). *Anal.* Calcd. for $C_{27}H_{42}O_7$: C, 67.75; H, 8.85. Found : C, 67.81; H, 9.03. These physical constants agree with those of the triacetate of Reichstein's Substance K.[9]

Allopregnane-17α,20β,21-triol (VII) from (VIII)—To a solution of 94.7 mg. of (VIII) in 30 cc. of MeOH 450 mg. of $KHCO_3$ dissolved in 20 cc. of water was added. After being refluxed for 1 hr., the mixture was concentrated *in vacuo* and the crystals were collected. Recrystallization from MeOH furnished 60.5 mg. of colorless feathers, m.p. 175~176°; $[\alpha]_D^{21}$ −8°(c=1.0 in dioxane). *Anal.* Calcd. for $C_{21}H_{36}O_3$: C, 74.95; H, 10.78. Found : C, 74.84; H, 10.55.

The physical constants for this compound reported by Wagner and Moore[13] are : m.p. 173~175°; $[\alpha]_D^{29}$ −8.5°(c=3.31 in dioxane). The mixed melting point of the material obtained in this experiment and the preparation supplied by Dr. J. A. Moore was 174~175°, and the infrared spectra of the two samples were completely identical.

Allopregnane-17α,20β,21-triol (VII) from (IV)—Hydrogenation of 100 mg. of (IV) in 30 cc. of EtOH was performed in the presence of a Pd-C catalyst (prepared from 60 mg. of $PdCl_2$ and 250 mg. of Norit). The hydrogenated material was chromatographed over 25 g. of Florisil. Elution with petr. ether-MeOH(99:1) yielded crystals melting at 169~172°. Infrared spectrum and mixed melting point determination confirmed the identity of the product with (VII) obtained as above. No other products were detected from the eluates of the column.

The author expresses his gratitude to Dr. S. Kuwada and Dr. S. Tatsuoka for their encouragement, to Messrs. T. Shima and H. Nakamachi for spectral and optical data, and to Mr. M. Kan and his associates for microanalyses. The technical assistance of Mr. T. Takahashi is also gratefully acknowledged.

Summary

Sodium borohydride reduction of Reichstein's Substance S (I) furnished 17α,20β,21-trihydroxy-4-pregnen-3-one (II) and 4-pregnene-3β,17α,20β,21-tetrol (IV), and the structure of the latter was verified. Selective oxidation by N-bromoacetamide converted compound (IV) into (II) and catalytic hydrogenation of compound (V) was accompanied by hydrogenolysis to afford the triacetate (VI) of Reichstein's Substance K and 20β,21-diacetoxyallopregnan-17α-ol (VIII), which was led to the free steroid (VII) for identification.

(Received July 17, 1959)