Summary

Purpnigenin (I), $C_{21}H_{32}O_4$, is positive to the Liebermann reaction and has three C-CH_3 groups. It was assumed to have a pregnenolone skeleton from the presence of an absorption for 20-ketone in its infrared spectrum and formation of acetic acid and propionic acid by the decomposition of the Wolff-Kishner reduction product (II).

(I) forms a diaceate and a monoxime, and the infrared spectrum of the diacetate contains the absorption of a hydroxyl. Consequently, three of the four oxygens in (I) would belong to hydroxyls and the remaining one to the carbonyl. Oppenauer oxidation of (I) gives a 4-en-3-one compound (III) and (I) is therefore assumed as 5-en-3-ol. Since (I) consumes periodic acid and chromic acid oxidation of (III) produces five-membered ring ketone (IV), the two hydroxyls, other than that in 3-position, are likely to be secondary and tertiary, and in adjacent positions in the D-ring.

In spite of the Oppenauer oxidation, (III) retains the five-membered D-ring and, therefore, (I) is likely to be 14,15-diol, rather than 16,17-diol. From these experimental evidences, 3,14,15-trihydroxypregn-5-en-20-one is proposed as the structure for (I).

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55. Hiroshi Ishii : Studies on Digitalis Glycosides. XV.[1] The Structure of Purpnigenin. (2).

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In the preceding paper,[1] 3,14,15-trihydroxypregn-5-en-20-one was proposed as the planar structure of purpnigenin (I). Consequently, the 4-en-3-one compound (II) formed by the Oppenauer oxidation of (I) would correspond to 14,15-dihydroxyprogesterone. In order to prove this steroidal skeleton and the position of functional groups, derivation of (II) from progesterone (III) was attempted.

In recent years, it has become possible to introduce a hydroxyl into 14α-position in progesterone (III) by microbiological oxidation[2] and its dehydration should introduce a double bond, into 14~15 carbons[3] in (III) to give Δ^{14}-progesterone (V). Oxidation of this double bond with osmium tetroxide would give a *cis*-diol (VI) and cleavage of the epoxide (VII), obtained by treatment of (V) with perbenzoic acid, with perchloric acid should give a *trans*-diol (VIII). Comparison of these two substances with (II) showed that (VIII) is entirely identical with (II) and the objective of this work was successfully attained.

Microbiological oxidation of progesterone (III) with *Mucor parasiticus*, according to the method of Eppstein and others,[2] afforded 14α-hydroxyprogesterone (IV), $C_{21}H_{30}O_3$, in ca. 20% yield. This was identified with the authentic specimen kindly supplied by Dr. Peterson,[*2] through mixed melting point and comparison of their infrared absorption spectra.

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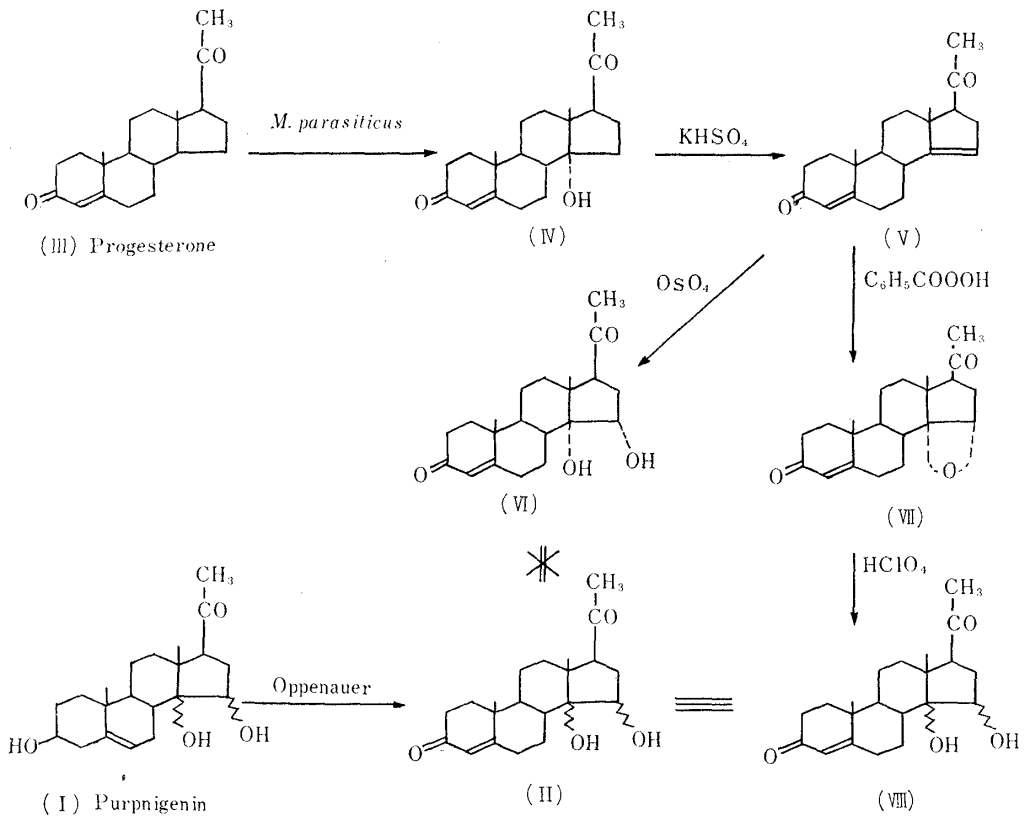
*1 Fukushima-ku, Osaka (石井 宏).

*2 Grateful acknowledgement is made to Dr. D. H. Peterson for his supply of the valuable sample.

1) Part XIV. H. Ishii : This Bulletin, **10**, 351 (1962).

2) S. H. Eppstein, *et al.* : J. Am. Chem. Soc., **80**, 3382 (1958).

3) B. M. Bloom, *et al.* : Experientia, **12**, 27 (1956).



Dehydration of (IV) with $KHSO_4$ by the method of Meister and others[4] preferentially formed Δ^{14}-progesterone (V), $C_{21}H_{28}O_2$, whose melting point and optical rotation values agreed with those listed in the literature.[4] Oxidation of (V) with osmium tetroxide gave a substance (VI), $C_{21}H_{30}O_4$, m.p. 195~200°, which was assumed to be 14α,15α-dihydroxyprogesterone, formed by the attack of the reagent from the α side. The melting point of this product was similar to that (m.p. 203~205°) of (II) but their admixture clearly showed depression of the melting point.

Oxidation of (V) with perbenzoic acid afforded 14α,15α-epoxyprogesterone (VII), $C_{21}H_{28}O_3$, and its decomposition with perchloric acid gave 14,15-dihydroxyprogesterone (VIII), $C_{21}H_{30}O_4$, m.p. 203~205°, $[\alpha]_D$ +126.1°. Physical constants of (VIII) were in good agreement with those[1] of purpnigenin derivative (II), their admixture showing no depression of the melting point and their infrared spectra showing perfect coincidence (Fig. 1). This has

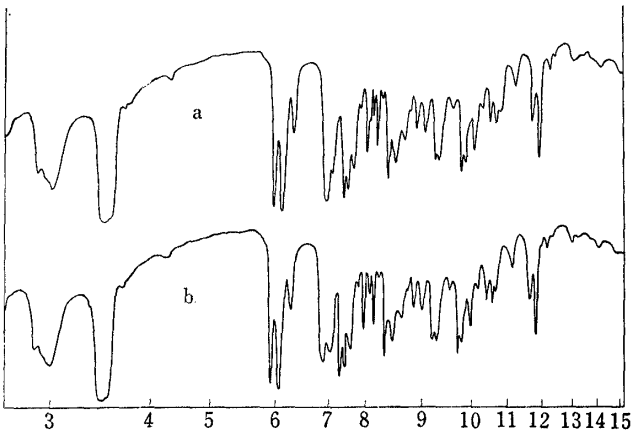


Fig. 1. Infrared Spectra (Nujol)

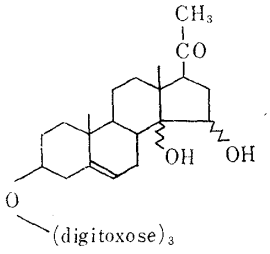
a. Δ^4-3-keto compound (II) derived from purpnigenin (I)
b. 14,15-Dihydroxyprogesterone (VIII) derived from progesterone (III)

4) P. D. Meister, H. C. Murray: U. S. Pat. 2,930,791 (1960) (C. A., **54**, 17471 (1960)).

proved the skeleton and position of functional groups in (II), and the assumed planar structure of 3,14,15-trihydroxypregn-5-en-20-one for purpnigenin (I) seems to be correct.

The oxidation with peracid is considered to have been made from the α side, as in the case of osmium tetroxide oxidation, and the α-epoxide thereby formed is considered to be cleaved to *trans*-diaxially by perchloric acid. According to this theory, the glycol hereby formed is likely to take the $14\alpha,15\beta$-configuration but other configurations cannot be entirely excluded since the conformation of the substituents in the D-ring does not differ greatly, compared to the axial and equatorial conformation of a six-membered ring. Moreover, digitalis leaves contain cardiotonic genins and diginigenin, which are steroids with a 14β-configuration. Therefore, there is a possibility that (I) takes a 14β-configuration, even from the consideration of biosynthetic route. Decisive conclusion on the determination of this diol conformation will be left for further examination.

It has already been confirmed from colorimetric determination of sugars[5] that the original glycoside, purpnin, is composed of one mole of purpnigenin and 3 moles of digitoxose, and the structure of purpnin is therefore considered to be represented as (IX).



(IX) purpnin

The present series of work has proved the presence of pregnenolone derivatives as a glycoside in plants, following the discovery of digipronin.[6] It is pharmacologically interesting that a substance which had hitherto been known as animal constituent has now been found in digitalis leaves.

Experimental

14α-Hydroxyprogesterone (IV)——A total of 7 L. of culture medium, containing 4% of glucose, 2% of peptone, and 0.3% of corn steep liquor, was placed in 500-cc. shake flasks, 100 cc. to each flask, sterilized, and inoculated with one platinum loop each of *Mucor parasiticus* Bain (ATCC 6476), cultured for 2 weeks on an oatmeal slant medium. The flasks were shaken at 26° for 20 hr. A solution of 3.9 g. of progesterone dissolved in 70 cc. of MeOH was added to the flasks, 1 cc. to each flask, and the flasks were shaken for further 25 hr. The culture liquid was treated in the usual manner and 4.69 g. of $CHCl_3$ extract was obtained. This extract was dissolved in benzene, the solution was passed through a column of 80 g. of alumina, and the column was eluted consecutively with $CHCl_3$ and $CHCl_3$-MeOH (99:1) mixture. Recrystallization of 2.41 g. of residue from the $CHCl_3$ eluate from MeOH-Et_2O gave 786 mg. of (IV), m.p. 184~195°. $[\alpha]_D^{21}$ +200.0° (c=1.043, $CHCl_3$). IR $\lambda_{max}^{CHCl_3}$ μ: 2.83, 5.88, 6.00, 6.18. *Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.52; H, 9.23.

Δ^{14}-Progesterone (V)——A solution of 1.124 g. of (IV) dissolved in 15 cc. of Ac_2O, added with 1.6 g. of freshly fused and powdered $KHSO_4$, was heated on a boiling water bath for 25 min., cooled, and $KHSO_4$ was filtered off. The filtrate was concentrated in a reduced pressure at room temperature, the residue was diluted with H_2O, and extracted with Et_2O. The extract was washed with H_2O, $NaHCO_3$ solution, and H_2O, dried over Na_2SO_4, and Et_2O was evaporated. Recrystallization of its residue from MeOH-Et_2O gave 230 mg. of (V) as plates, m.p. 143~146°. $[\alpha]_D^{23}$ +134.1° (c=1.034, $CHCl_3$). IR λ_{max}^{Nujol} μ: 3.24 (Δ^{14}), 5.85, 5.98, 6.18. *Anal.* Calcd. for $C_{21}H_{28}O_2$: C, 80.73; H, 9.03. Found: C, 79.96; H, 9.18.

5) Part XI. D. Satoh, H. Ishii, Y. Oyama, T. Okumura: This Bulletin, **10**, 37 (1962).
6) Part XII. D. Satoh: *Ibid.*, **10**, 43 (1962).

OsO_4 Oxidation of Δ^{14}-Progesterone (V)——A solution of 97 mg. of (V) dissolved in a mixture of 2 cc. of pyridine and 7 cc. of dehyd. Et_2O, added with 3 cc. of Et_2O solution of 100 mg. of OsO_4, was allowed to stand at room temperature for 38 hr. Et_2O was evaporated, 10 cc. of EtOH and 10 cc. of aqueous solution of 0.5 g. of Na_2SO_3 were added to the residue, and the mixture was refluxed for 3 hr. The black precipitate was filtered off, EtOH was evaporated from the filtrate, and the residue was extracted with $CHCl_3$. The extract was washed with water, dried, and $CHCl_3$ was evaporated. The residue (93 mg.) was dissolved in benzene and the solution was passed through an alumina column. Recrystallization of 25 mg. of residue obtained from $CHCl_3$-MeOH (9:1) eluate from MeOH-Et_2O afforded prismatic crystals, m.p. 195~200°, which showed depression on admixture with 4-ene-3-one compound (II), m.p. 203~205°, melting at 168~182°. *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found : C, 72.70 : H, 8.72.

Perbenzoic Acid Oxidation of Δ^{14}-Progesterone (V)——A solution of 220 mg. of (V) dissolved in 8 cc. of $CHCl_3$ was cooled in ice, 1.2 cc. of $CHCl_3$ solution of perbenzoic acid (90 mg./cc.) was added, and the mixture was allowed to stand at room temperature. Et_2O was added to this mixture, which was washed consecutively with aqueous solutions of KI, $Na_2S_2O_3$, $NaHCO_3$, and H_2O, Et_2O layer was dried over Na_2SO_4, and the solvent was evaporated. Recrystallization of 210 mg. of the residue so obtained from Me_2CO-Et_2O afforded 94 mg. of (VII) as plates, m.p. 175~180°. IR λ_{max}^{Nujol} μ : 5.88, 6.02, 6.18. *Anal.* Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found : C, 76.62; H, 8.60.

14,15-Dihydroxyprogesterone (VIII)——A solution of 60 mg. of (VII) dissolved in 15 cc. of Me_2CO, added with 2 cc. of H_2O and then 2 cc. of 2% $HClO_4$ solution, was allowed to stand at room temperature for 6 days. The mixture was diluted with H_2O, extracted with Et_2O, and the extract layer was washed with H_2O, $NaHCO_3$ solution, and H_2O. After drying over Na_2SO_4, Et_2O was evaporated and 51 mg. of the residue so obtained was recrystallized from MeOH-Et_2O to 18 mg. of (VIII) as thin scales, m.p. 203~205°. Alumina chromatography of the recrystallization mother liquor gave 14 mg. of the starting material (VII) and 15 mg. of (VIII). $[\alpha]_D^{25}$ +126.1° (c=0.866, MeOH). IR λ_{max}^{Nujol} μ : 2.94, 5.91, 6.01, 6.20 (Fig. 1). *Anal.* Calcd. for $C_{21}H_{30}O_4$: C, 72.80, H, 8.73. Found : C, 73.21, H, 8.77.

(VIII) showed no depression of the melting point on admixture with 4-ene-3-one (II), m.p. 203~205°, and the two showed the same Rf value of 0.71 on paper chromatography with the solvent system of BuOH-toluene (1:3)/$HCONH_2$.

The author expresses his deep gratitude to Dr. K. Takeda, the Director, and Dr. D. Satoh, the Assistant Director, of this Laboratory for their kind and unfailing guidance throughout the course of the present work, and to Prof. K. Tsuda of the University of Tokyo for valuable advices. He is indebted to Dr. Y. Nozaki and Mr. T. Okumura for their help in the microbiological oxidation experiments, to the members of the Analysis Room for elemental analytical data, and to the members of the Section of Physical Chemistry for the measurement of optical rotation and infrared spectra.

Summary

The Oppenauer oxidation product (II) of purpnigenin (I) was assumed to be 14,15-dihydroxyprogesterone and, therefore, two kinds of 14,15-dihydroxy compound (VI and VIII) were synthesized from progesterone (III) to compare with (II).

Oxidation of Δ^{14}-progesterone (V) with osmium tetroxide produced 14α,15α-dihydroxyprogesterone (VI) which did not agree with (II). Oxidation of (V) with peracid and cleavage of the epoxide thereby obtained with perchloric acid produced 14,15-dihydroxypregn-4-ene-3,20-dione (VIII) which was identified with (II). This has proved that (II) is 4-pregnene-14,15-Consequently, the original purpnigenin is considered to be 3,14,15-trihydroxypregn-5-en-20-one. The two hydroxyls at 14- and 15-positions are considered to be *trans*-diols but further examination will have to be made as to their exact configuration.

It is interesting that pregnenolone, hitherto considered as animal constituent, has now been found in a plant as an aglycone.

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