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# 54. Hiroshi Ishii : Studies on Digitalis Glycosides. XIV.*1 The Structure of Purpnigenin. (1).

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It has become known that glycosides having aglycone with pregnenolone skeleton are present in the non-cardiotonic component of digitalis leaves.[1] Three kinds of such glycosides, digipronin, purpnin, and purpronin, have already been isolated.[2] Satoh[3] examined the aglycone of digipronin, digiprogenin, and proposed 3$\beta$,17$\alpha$-dihydroxypregn-5-ene-11,15,20-trione as its structure.

In the present series of work, the Oppenauer oxidation product (III) of purpnigenin[2] (I), the aglycone of purpnin, was derived from progesterone and its structure was determined as 14,15-dihydroxypregn-4-ene-3,20-dione, proving that this substance possessed a pregnenolone skeleton like digiprogenin. As will be explained later, (III) has a 4-en-3-one structure but since the original purpnigenin (I) is negative to the Rosenheim reaction, 3,14,15-trihydroxypregn-5-en-20-one seems to be the most appropriate as its structure. In the present paper, courses leading to the proposal for the structure of purpnigenin (I) will be described.

Purpnigenin (I), $C_{21}H_{32}O_4$, is positive to the Liebermann reaction, has three C-$CH_3$ groups, and is assumed to be a digitanol-type[1] steroid. Acetylation with acetic anhydride and pyridine gives a diacetate and its infrared spectrum has an absorption at 2.88 $\mu$ for a hydroxyl. It forms a monoxime, $C_{21}H_{33}O_4N$. From these evidences, the four oxygen atoms in (I) can be accounted for by two in the acetylable hydroxyl, one in non-acetylable hydroxyl, and one in the carbonyl group. (I) is positive to the tetranitromethane test, showing the presence of a double bond.

Infrared spectrum of (I) exhibits absorptions at 5.96 and 7.32 $\mu$ suggesting that the side chain at 17-position is a methyl ketone. This is also supported by the fact that the Wolff-Kishner reduction of (I) and oxidation of its product, monodeoxopurpnigenin (II), $C_{21}H_{34}O_3$, by the Kuhn-Roth method gives propionic acid, besides acetic acid.[4]

Oppenauer oxidation of (I) gives a product (III), $C_{21}H_{30}O_4$, whose ultraviolet spectrum has an absorption at 241 m$\mu$, and its infrared spectrum has absorptions at 6.01 and 6.20 $\mu$, indicating the presence of a 4-en-3-one group. Oxidation of this 4-en-3-one compound (III) with chromic acid and acetic acid gives a triketone (IV), $C_{21}H_{28}O_4$, whose infrared spectrum has absorptions at 3.01 and 5.72 $\mu$, suggesting the presence of a tertiary hydroxyl and a five-membered ring ketone. Its ultraviolet spectrum and ferric chloride reaction do

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*1 Part XIII : Yakugaku Zasshi, **82**, 156 (1962).
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1) R. Tscheche : Proc. Fourth Intl. Congress Biochem., Vol. **IV**, 21 (1958).
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3) Part XII. D. Satoh : *Ibid.*, **10**, 43 (1962).
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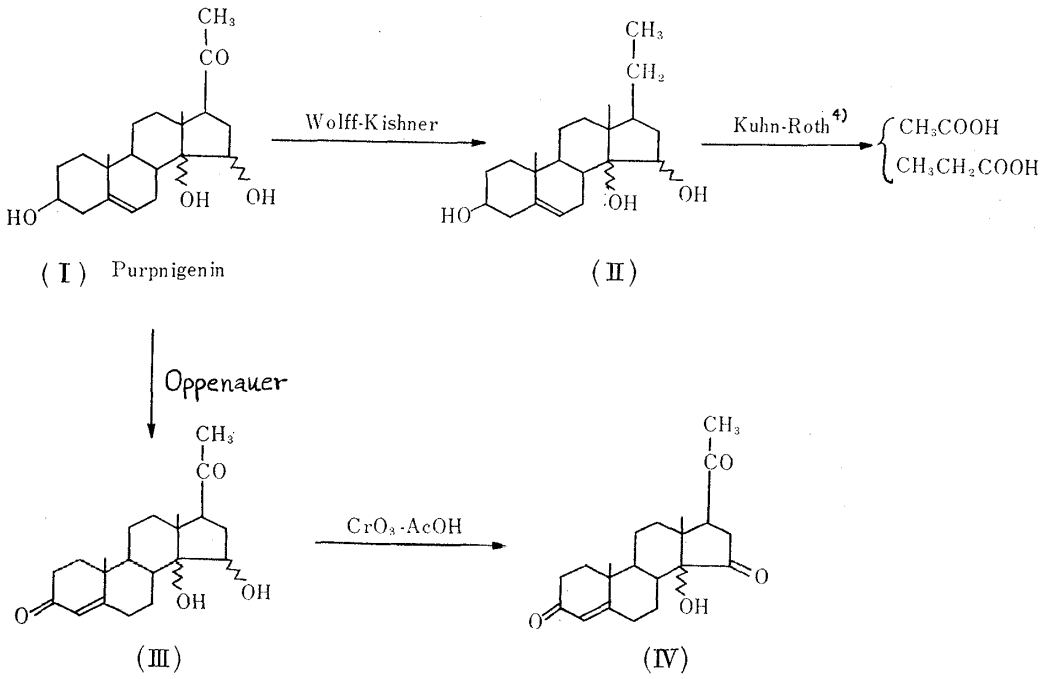


(I) Purpnigenin (II) (III) (IV)

not point to the presence of a $\beta$-diketone. Consequently, there is a secondary hydroxyl in the D-ring of (III). (I) consumes 0.58 mole of periodic acid in 26 hours and is negative to the $\alpha$-ketol reaction, so that the presence of $\alpha$-glycol may be presumed.

From the foregoing experimental evidences, it may be assumed that the one carbonyl in purpnigenin (I) is in the 20-ketone group, one hydroxyl and one double bond form the $\Delta^5$-3-ol group, and the other two hydroxyls are the secondary one in the D-ring and the tertiary one adjacent to it. Consequently, the planar position of the $\alpha$-glycol in the D-ring would be either 14,15-diol or 16,17-diol. The fact that D-home compound has not been obtained by passing the glycoside repeatedly through alumina chromatography[5] during the purification process or by Oppenauer oxidation[6] during the step of (I) to (IV), in both cases retaining the five-membered D-ring seems to support the 14,15-diol structure. It is therefore proposed that 3,14,15-trihydroxypregn-5-en-20-one is the most likely as the planar structure for purpnigenin (I).

## Experimental

**Purpnigenin**[2] **(I)**——Colorless plate crystals, m.p. 239~243°. $[\alpha]_D^{16}$ +21.1°(c=1.140, MeOH). UV: $\lambda_{max}^{EtOH}$ 279 mμ (log $\varepsilon$ 1.79). IR $\lambda_{max}^{Nujol}$ μ: 2.81, 5.96, 7.32. *Anal.* Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26; mol. wt., 348.5. Found: C, 72.26; H, 9.35; mol. wt., (Rast), 380.0; C-$CH_3$, 7.73. Control pregnenolone, 7.80.

**Purpnigenin Diacetate**——A solution of 30 mg. of (I) dissolved in 0.5 cc. of pyridine and added with $Ac_2O$ was allowed to stand over night at room temperature. The mixture was treated in a conventional way and the product was recrystallized from dil. MeOH to plates, m.p. 150~153°. IR $\lambda_{max}^{Nujol}$ μ: 2.88, 5.78, 5.91. *Anal.* Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39; $CH_3CO$, 19.90. Found: C, 69.17; H, 8.53; $CH_3CO$, 19.71.

**Purpnigenin Monoxime**——To a solution of 40 mg. of (I) dissolved in 2 cc. of MeOH, 0.2 cc. of aqueous solution of 100 mg. of $NH_2OH \cdot HCl$ and 140 mg. of $AcONa \cdot 3H_2O$ was added and the mixture was refluxed on a water bath for 3 hr. The solvent was distilled off, the residue was washed with $H_2O$, and recrystallized from dil. MeOH to 40 mg. of colorless needles, m.p. 276~279°. *Anal.* Calcd. for $C_{21}H_{33}O_4N$: C, 69.39; H, 9.15; N, 3.85. Found: C, 68.93; H, 9.42; N, 4.02.

5) N. L. Wendler, D. Taub: Chem. & Ind. (London), **1957**, 1237.
6) E. Batres, G. Rosenkranz, F. Sondheimer: J. Am. Chem. Soc. **76**, 5171 (1954).

**Monodeoxopurpnigenin (II)**——A solution of 50 mg. of (I) dissolved in 2 cc. of propylene glycol and added with 0.16 cc. of 80% $NH_2NH_2 \cdot H_2O$ was heated at 130~140° for 1 hr., cooled, and a solution of 0.18 g. of KOH dissolved in 0.2 cc. of $H_2O$ was added. The mixture was again heated at 130~140° for 2 hr. and the bath temperature was gradually raised until no more $H_2O$ came out. The mixture was then refluxed at 190~200° for 6 hr., diluted with $H_2O$, neutralized with HCl, and extracted with $CHCl_3$. The extract was washed with water, dried, and $CHCl_3$ was evaporated. The residue was purified by alumina chromatography and the residue obtained from $CHCl_3$ eluate was recrystallized from AcOEt to plates, m.p. 215~218°. *Anal.* Calcd. for $C_{21}H_{34}O_3$ : C, 75.40; H, 10.25. Found: C, 75.46; H, 10.23.

**Proof of $-CH_2CH_3$ Side Chain[4] in Monodeoxo-purpnigenin (II)**——A mixture of 6 mg. of (II) with 4 cc. of $CrO_3$-$H_2SO_4$ (20 cc. of 2*N* $CrO_3$ solution and 5 cc. of conc. $H_2SO_4$) and 6 cc. of $H_2O$ was heated until the distillate amounted to 2 cc., when 2 cc. of $H_2O$ was added and distillation was continued until the distillate amounted to 30 cc. The distillate so obtained, containing a volatile acid, was neutralized with 0.1*N* KOH and evaporated to dryness. The potassium salt so obtained was dissolved in 1 cc. of distilled water, passed through a column filled with 5 cc. of Amberlite IR-120 (H form) to remove $K^+$, and the column was washed several times with distilled water. Effluent and washings were combined, 2 drops of $Et_2NH$ were added, and the solution was concentrated to 0.1 cc. This was submitted to paper chromatography under the following conditions.

The test solution was spotted on a filter paper (Toyo Roshi No. 51) and the paper was hung in a developing vessel containing the developing solvent (0.025*N* aqueous solution of $Et_2NH$ saturated with BuOH) for 12 hr. to saturate the vapor. The paper was then dipped in the said solvent and developed for 14 hr. The paper was air-dried and sprayed with 0.04% EtOH solution of Bromocresol Violet. The spots colored bulish violet. Rf value of the control $CH_3COOH$ 0.24. $CH_3CH_2$-COOH 0.30. Reaction product of (II) 0.24, 0.30.

**Oppenauer Oxidation of Purpnigenin (I)**——A mixture of 640 mg. of (I), 30 cc. of toluene, and 8 cc. of cyclohexanone was warmed at 120~130° for 1 hr. to effect complete solution, during which ca. 2 cc. of toluene distilled out. To this solution, 700 mg. of Al (iso-PrO)$_3$ was added and the mixture was heated at 130~140°, during which ca. 10 cc. of toluene distilled out. When cooled, saturated aqueous solution of the Rochelle salt was added, stirred thoroughly, diluted with water, and concentrated in a reduced pressure to remove toluene and cyclohexanone by azeotropic distillation. The residue was extracted with $CHCl_3$, the solvent was evaporated from the extract, and the residue was recrystallized from $Me_2CO$ to 260 mg. of 4-en-3-one compound (III) as thin scales, m.p. 203~205 . $[\alpha]_D^{17}$ +130.6° (c=0.481, MeOH). UV : $\lambda_{max}^{EtOH}$ 241 mμ (log ε 4.20). IR $\lambda_{max}^{Nujol}$ μ : 2.94, 5.91, 6.01, 6.20. *Anal.* Calcd. for $C_{21}H_{30}O_4$ : C, 72.80; H, 8.73. Found : C, 72.63; H, 8.84.

Alumina chromatography of the recrystallization mother liquor afforded 25 mg. of triketone (IV) as thin scales, m.p. 235~238°, from the portion eluted with a mixture (1:1) of benzene and $CHCl_3$, and 79 mg. of (III) from the $CHCl_3$ eluate.

**$CrO_3$ Oxidation of (III)**——A solution of 120 mg. of (III) dissolved in 2 cc. of AcOH, added with 3 cc. of 2% AcOH solution of $CrO_3$ with ice-cooling, was allowed to stand at room temperature for 3.5 hr., MeOH was added to decompose excess $CrO_3$, and the mixture was concentrated in a reduced pressure. The residue was diluted with $H_2O$, extracted with $CHCl_3$, and the extract was washed with $NaHCO_3$ solution and $H_2O$. After drying over anhyd. $Na_2SO_4$, $CHCl_3$ was evaporated and 94 mg. of the residue so obtained was recrystallized from MeOH-$Et_2O$ to 64 mg. of triketone (IV) as thin scales, m.p. 235~238°. $[\alpha]_D^{21}$ +117.8° (c=0.768, MeOH). UV : $\lambda_{max}^{EtOH}$ 241 mμ (log ε 4.16). IR $\lambda_{max}^{Nujol}$ μ: 3.01, 5.72, 5.90, 6.01, 6.18. *Anal.* Calcd. for $C_{21}H_{28}O_4$ : C, 73.22; H, 8.19. Found : C, 73.19; H, 8.38.

**Periodate Consumption of Purpnigenin (I)**——In a 5-cc. measuring flask, 4.5 mg. of (I) was placed, dissolved in a small amount of MeOH, 1 cc. of 0.2*N* $NaIO_4$ solution was added, and the whole was brought to 5 cc. with MeOH. The mixture was shaken thoroughly and allowed to stand at room temperature for 26 hr. To 2 cc. of this solution, 2 cc. of 0.02*N* $Na_3AsO_3$ solution and 1 cc. of 20% KI solution were added, and the mixture was allowed to stand for 15 min. This was titrated with 0.02*N* $I_2$ solution, using a few drops of starch solution as an indicator. Consumption of 0.02*N* $I_2$ solution : 0.15 cc. This corresponded to 1.05 mg. of purpnigenin (0.58 mole of the sample taken).

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**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 14$\alpha$-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 $\Delta^{14}$-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 14$\alpha$-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).