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116. Hiroshi Mitsuhashi and Yuzuru Shimizu : Studies on the Constituents of Asclepiadaceae Plants. IV.[1] The Structure of Cynanchogenin. (3).*2

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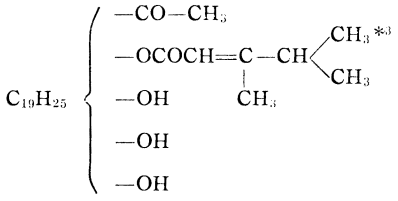
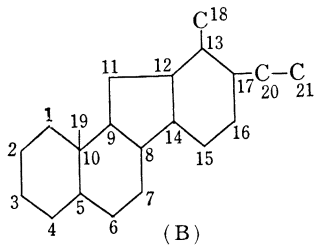
It was already shown that the partial structure of cynanchogenin (I) might be represented in the form of (A),[2] and C-nor-D-homopregnane skeleton (B) was postulated for the structure by the selenium dehydrogenation in the previous paper.[1,3]



(A)



(B)

The position of the ketone group, which is supposed to be present as CH_3CO–, is presumably at C-20, since any other appropriate interpretation could not be made. This assumption gives better coincidence with the data obtained.

One of the acetylatable hydroxyl groups in deacylcynanchogenin (II) would be restricted to C-3 from a possible analogy of this modified steroid to standard one, and it was proved by the coupling at the position of the double bond to form Δ^4-3-ketone compound (XI).

Deacylcynanchogenin (II) consumed two moles of lead tetraacetate rapidly*4 at room temperature to give the compound (III), m.p. 172~175°, which was formulated as $C_{21}H_{30}O_6$ from the elemental analysis. It reduces the Tollen's reagent, T. T. C. strongly, suggesting a presence of aldehyde group.

It is soluble in sodium hydroxide solution, but is not an enol compound, because it does not provide any characteristic ultraviolet absorption or color reaction.

Consuming one mole of lead tetraacetate, deacylcynanchogenin acetate (IV) was rapidly oxidized to seco-triketo-compound (V), m.p. 148~152°, having the formula of $C_{25}H_{34}O_7$. It also reduces the Tollen's reagent and alkaline tetrazolium strongly. The oxidation of (IV) with cromium trioxide in acetic acid gave the compound which was proved to be identical with the product (V). The preparation of the same compound with lead tetraacetate and cromium trioxide indicates a cleavage of a vicinal tertiary diol group, taken place during the oxidation and the reducing character could be attributed to the presence of an α-ketol acetate group.

*1 Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (三橋 博, 清水 譲).

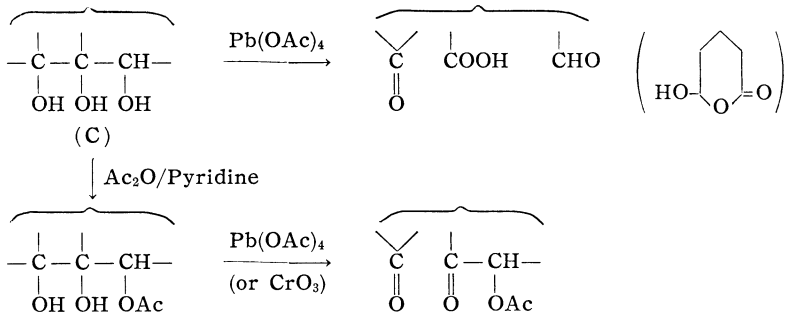
*2 A part of this work was reported at the 4 th Hokkaido Local Meeting of the Pharmaceutical Society of Japan, September 26, 1959, and published in this Bulletin, **7**, 949 (1959).

*3 The carbon skeleton of this acid is just the same as the side chain of ergostane, splitted at C_{20}–C_{22}, which presents biogenetic interests in this acid and C_{21} steroid.

*4 The compound (II) consumes periodic acid very slowly. The slowness might be due to the sterical problem.

1) Part III : This Bulletin, 8, 738 (1960).
2) H. Mitsuhashi, Y. Shimizu : *Ibid.*, 8, 318 (1960).
3) *Idem* : *Ibid.*, **7**, 749 (1959).

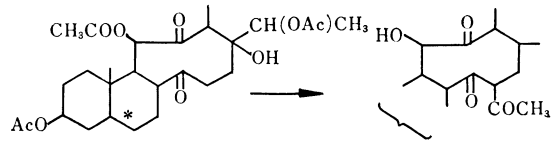
These observations on (V), coupled with those of (III), might interpret the partial structure (C).



The fact the ketone group of (II) is isolated and does not subject to the cleavage was evidenced by the determination of lead tetraacetate consumption by polyol compound (VI) and its triacetate (VII), the reduction of the carbonyl group to the corresponding alcohol, followed by the acetylation, gave (VII), which consumed one mole of lead tetraacetate as much the same amount as deacylcynanchogenin diacetate (IV), then the hydroxyl group at C-17 may be excluded.

The compound (III) should possess two ketonyl groups and one of each aldehyde, carboxyl and hydroxyl groups and but its infrared spectrum exhibits three maxima at C=O region and two maxima sharply defined at O-H region. To explain this inconsistency, a six membered lactonol structure was suggested; the carboxyl group cyclized with one of the carbonyls to produce a new hydroxyl group, whose infrared maximum should appear near 3400 cm^{-1}.

The seco-compound (V) converted to an enol compound (VIII) by treating with dilute sodium hydroxide solution. The enol properties were established by the color reaction with ferric chloride (intense violet) and the ultraviolet absorption at 290 mμ which shifted to 310 mμ in alkaline medium with increasing intensity. The compound has not been purified so far, but the subordinate ultraviolet absorption maximum near 230~240 mμ may suggest an occurance of aldol condensation. It is very difficult to explain this interesting change, but recently J. W. Cornforth[4] reported an analogous reaction on the oxidation produt of sarcostin triacetate.*[5] The explanation made to his compound is the transannular rearrangement of O-acetyl group to C-acetyl group favoured by 9-membered ring conformation.

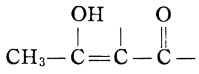
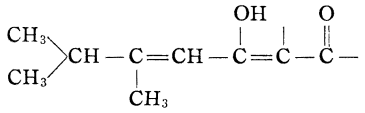
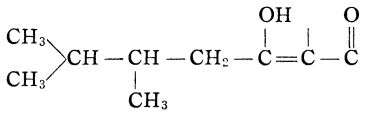


This explanation has been introduced to our compound and the acyl rearrangement was attempted. As described later, the 3,4-dimethyl-2-pentenoic residue in (I) is linked with the hydroxyl group which might be expected to be related to the rearrangement. If the enol structure is caused by O-acyl to C-acyl rearrangement, the lead tetraacetate oxidation product of cynanchogenin (I) will afford an enol compound whose ultraviolet

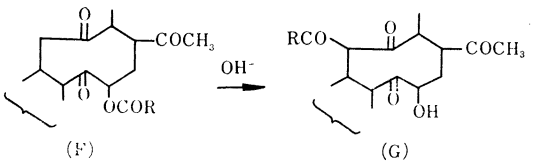
4) J. W. Cornforth : Chem. & Ind. (London), **1959**, 602, and a private communication.
5) Sarcostin was also isolated from our plant, *Cynanchum caudatum* Max., This Bulletin, **10**, 725(1962).

absorption shifts to longer wave length by as many as one conjugated double bond, but dihydrocynanchogenin, which is hydrogenated at the acid part, will give the compound having the similar spectrum to that of (VIII). If the acyl group did not participate in the reaction, the enol compounds derived should have the same properties. The experimental results shown in Table I indicate clearly that the acyl groups take part in the enol formation.

Table I,

	Enol structure conceivable	UV λ_{max} mμ in acidic medium	UV λ_{max} mμ in alkalin medium	$FeCl_3$
1.		290	309	violet
2.		336	343	green
3.		290	310	violet

This study, together with the foregoing experiments, would confirm a partial structure (F) for (V) and therefore (G) for the rearranged product.



(F) (G)

The double bond in deacylcynanchogenin (I) rather resists to hydrogenation, and so the hydrogenation with platinum oxide in acetic acid occurs at the cabonyl group in the initial stage to give a compound which proved to be identical with (VI) and at the double bond in the next stage. The thoroughly hydrogenated compound (IX), m.p. 222~226° is in accord with the elemental analysis for $C_{21}H_{36}O_5$ and lacks the ifrared maximum at 800 cm^{-1} in the original compound, which stands for the C-H out-of-plane deformation of trisubstituted double bond. Considering from the absence of conjugated system in either (III) or (V), the double bond might be located at Δ^5 which is usually known to be rather resistent to hydrogenation in normal steroids. The Oppenauer-oxidation of (I) gave the product (X), m.p. 224~225°, which afford a deacyl compound (XI), m.p. 135~140° by alkaline hydrolysis. The infrared spectra of both compounds (X) and (XI) suggest an appearance of α,β-unsaturated carbonyl system. Moreover, (XI) has the ultraviolet maximum at 241 mμ (log ε 4.15) which corresponds to a Δ^4-3-one system. The ultraviolet spectrum of (X) fits well to the one calculated from those of (I) and (XI).

Considering from the facts that cynanchogenin (I) gave a monoacetate and consumed one mole of lead tetraacetate, as well as the other results obtained, the ester linkage in (I) is limited to 15-OH. Thus the total structure of cynanchogenin would be represented as (H-1) and the reactions are summarized in chart I.

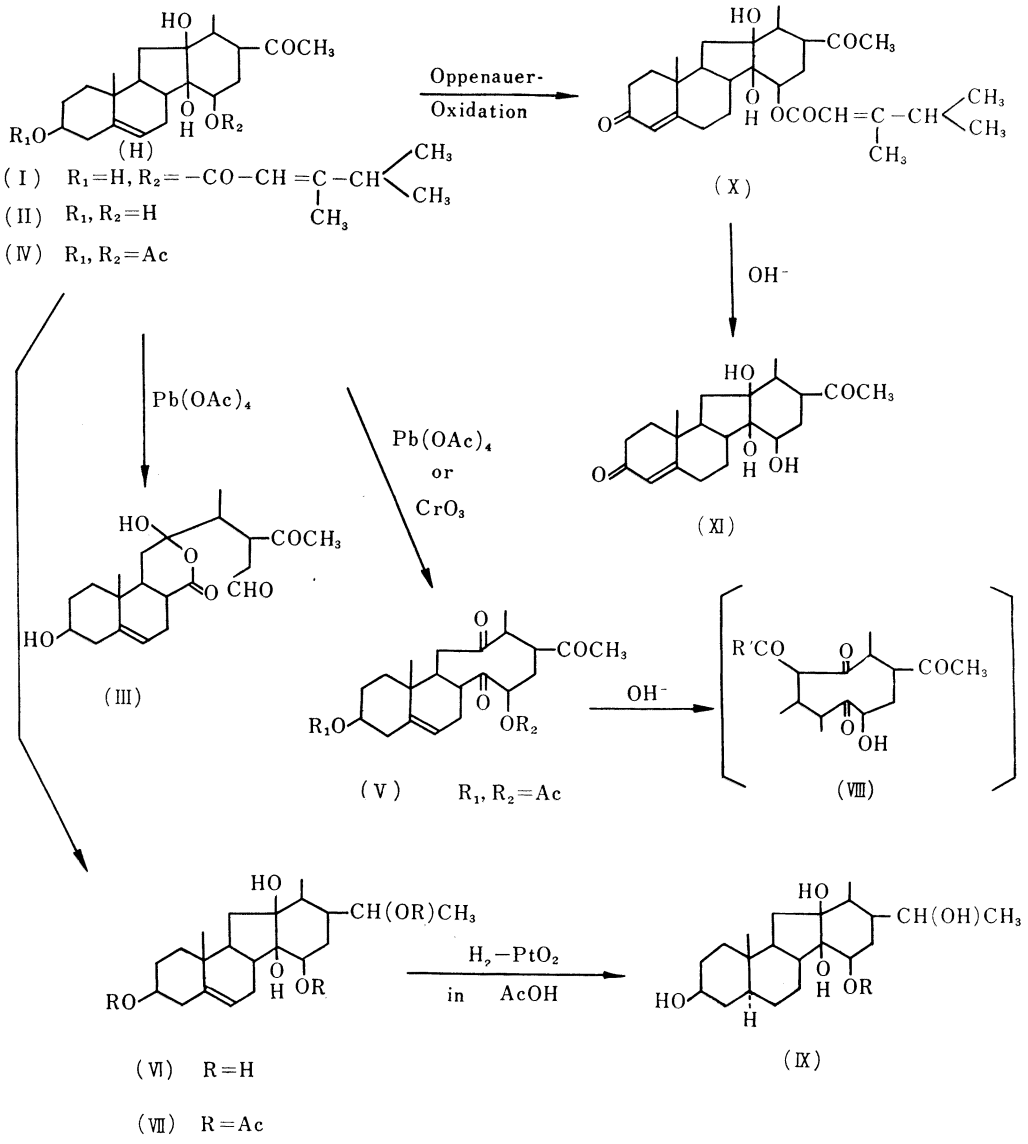


Chart 1.

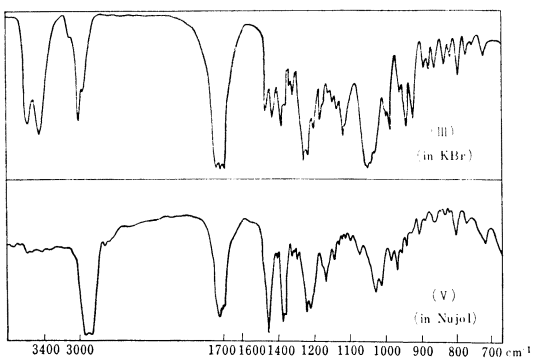


Fig. 1. Infrared Spectra of $Pb(OAc)_4$ Oxidation Products, (III) and (V)

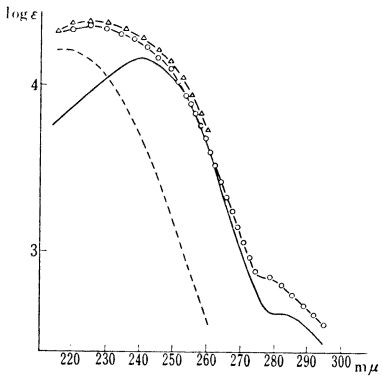


Fig. 2. Ultraviolet Spectra of (I), (X) and (XI)
..... (I)
○–○–○– (X)
——— (XI)
△–△–△– (I) + (XI)

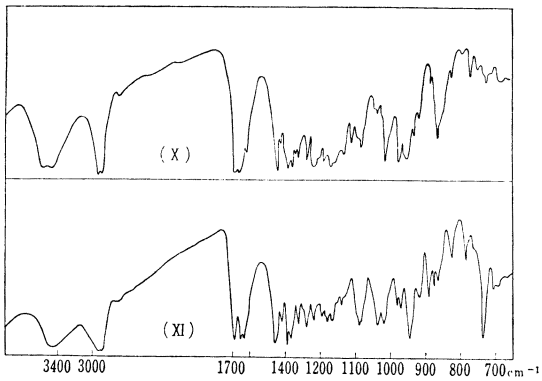


Fig. 3. Infrared Spectra of Δ^4-3-Ketone Compounds, (X) and (XI) (in Nujol)

Experimental

Estimation of $Pb(OAc)_4$ consumption——0.05 m. mole of each substance was dissolved in dioxane (2.5 cc.), and to this 10 cc. of $N/30$ $Pb(OAc)_4$ in AcOH was added and it was allowed to stand at room temperature (25~30°). 2 cc. each of the mixture was titrated iodometrically. The control was prepared and titrated similarly. The results are shown in the following Table.

Subtance	$Pb(OAc)_4$ moles (time : min.)		
Deacylcynanchogenin (II)	1.43(18)	1.90(70)	1.95(235)
Deacylcynanchogenin acetate (IV)	0.65(17)	0.8 (90)	0.9 (905)
Cynanchogenin (I)	0.65(15)	0.84(60)	0.94(250)
Dihydrocynanchogenin acetate	0.77(15)	0.90(30)	0.94(250)
Polyol-compound (VI)	1.90(170)		
Polyol-compound triacetate (VII)	1.03(170)		

Deacylcynanchogenin (II) consumed HIO_4 very slowly ($1.73M/21{,}000$ min.).

Seco-compound (III)——Deacylcynanchogenin (200 mg.) was oxidized with $Pb(OAc)_4$ (500 mg.) in AcOH (10 cc.) at room temperature for 4 hr. The reaction mixture was poured into H_2O and extracted with Et_2O. The Et_2O layer was washed with H_2O for several times, dried over Na_2SO_4 and evaporated. The residue gave prisms, m.p. 172~175° recrystallized from Et_2O (yield 150 mg.). *Anal.* Calcd. for $C_{21}H_{30}O_6$: C, 66.64; H, 7.99. Found : C, 66.33; H, 8.33. IR. see Fig. 1.

Seco-compound (V)——1) Deacylcynanchogenin diacetate (IV) (100 mg.) was dissolved in the saturated solution of $Pb(OAc)_4$ in AcOH (5 cc.) and allowed to stand at room temperature for 4 hr. The mixture was extracted with $CHCl_3$. It was washed with H_2O, dil. H_2SO_4 and dried over Na_2SO_4. The solvent was evaporated and the residue was crystallized to needles, m.p. 148~152°. *Anal.* Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.68. Found : C, 67.15; H, 7.72. IR. see Fig. 1.

2) Deacylcynanchogenin diacetate (IV) (100 mg.) was dissolved in AcOH (3 cc.) and 3 cc. of 2% CrO_3 in AcOH was added. Upon standing for 3 hr., the mixture was poured into H_2O and extracted

with $CHCl_3$. Usual treatment gave needles, m.p. 145~148°, which showed no depression on admixture with the specimen obtained from 1).

The Formation of Enol-compound from (V)——To the solution of Seco-compound (V) (100 mg.) in 4 cc. of MeOH was added 2 cc. of 5% NaOH solution. After 30 min., the solution was acidified with HCl and extracted with $CHCl_3$ and again the $CHCl_3$ solution was extracted with NaOH solution, which acidified with HCl and reextracted with $CHCl_3$. The $CHCl_3$ layer, following the usual treatment, gave enol fraction which shows deep violet coloration with $FeCl_3$.

Oxidation of Cynanchogenin with $Pb(OAc)_4$ and Enol-formation——Cynanchogenin (I) was oxidized as deacylcynanchogenin acetate, but the product could not be crystallized. The enol formation was carried out as same as (IV) by heating on a steam bath. The enol compound shows green coloration with $FeCl_3$.

Oxidation of Hydrocynanchogenin with $Pb(OAc)_4$ and Enol-formation——12 mg. of dihydrocynanchogenin was dissolved in 0.5 cc. of AcOH containing 12 mg. of $Pb(OAc)_4$ and maintained for 4 hr. Usual treatment gave colorless powder (9 mg.) which was not purified so far. The rearrangement was carried out by heating ca. 10 γ of the oxidation product with one drop each of EtOH and 10% NaOH solution in a steam bath for 5 min. Addition of two drops of 10% HCl and one drop of 5% $FeCl_3$ solution gave bright violet coloration.

Oppenauer-oxidation of Cynanchogenin (I)——Cynanchogenin (I) (500 mg.) was dissolved in toluene and 5 cc. of the solvent was distilled to remove moisture. To this solution, cyclohexanone was added and again 5 cc. was distilled off. This mixture was refluxed with 500 mg. of Al-isopropoxide for 2 hr. The cooled solution was acidified with $N/2$ H_2SO_4 and extracted with $CHCl_3$. The organic layer, when treated as usual, gave the residue which was crystallized from MeOH-Et_2O to fine needles (X) m.p. 224~225°. *Anal.* Calcd. for $C_{28}H_{40}O_6$: C, 71.16; H, 8.53. Found: C, 71.21: H, 7.62. UV. see Fig. 2. IR. see Fig. 3.

Deacyl-Δ^4-3-ketone compound (XI)——(X) (300 mg.) was refluxed for 3 hr. in 12 cc. of 5% methanolic KOH, and after evaporation of MeOH at room temperature, the reaction mixture was extracted with $CHCl_3$. The extract was washed with H_2O and dried. The product was crystallized from CH_2Cl_2-MeOH to prisms m.p. 150°(initial softening at 130°). Without CH_2Cl_2, crystallization did not occur. Beilstein test was positive. *Anal.* Calcd. for $C_{21}H_{30}O_5 \cdot \frac{1}{2}CH_2Cl_2$: C, 63.74; H, 7.72. Found: C, 63.62; H, 7.24. UV. see Fig. 2. IR. see Fig. 3.

Tetrahydrodeacylcynanchogenin (IX)——Deacylcynanchogenin (II) (200 mg.) was dissolved in AcOH (5 cc.) and shaken with PtO_2 in H_2 atomosphere. After 1.5 hr. about 1 mole of H_2 was absorbed. The catalyst was filtered off and the solution was evaporated to dryness. Repeated crystallization from Me_2CO gave needles which proved to be identical with polyol-compound (VI) obtained by the $LiAlH_4$ reduction of cynanchogenin (I). The mother liquor gave a small amount of needles which lacks in the absorption at 800 cm^{-1}. 100 mg. of (II) was treated as described above with 20 mg. PtO_2 for 4 hr. at 25°. In this case, (VI) could not be isolated but the solvated needles, m.p. 222~226° from Me_2CO-water were obtained. For elemental analysis and measurement IR sample was dried *in vacuo* at 150°. It has a typical IR spectrum for polyol compound and have no C=O and trisubstituted C=C-H absorption. *Anal.* Calcd. for $C_{21}H_{36}O_5$: C, 68.44; H, 9.85. Found: C, 68.34; H, 9.57.

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Summary

The structure of cynanchogenin was investigated. The locations of four hydroxyl groups, double bond and ester linkage were clarified, and total constitution was proposed as H-I (Chart I).

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