UDC 547.918 : 582.938

58. **Hiroshi Mitsuhashi and Yuzuru Shimizu**: Studies on the Constituents of Asclepiadaceae Plants. II.[1] On the Structure of Cynanchogenin from *Cynanchum caudatum* Max.*[2]

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It was reported in the preceding paper[1] that the root of *Cynanchum caudatum* Max. (Asclepiadaceae) contained a glycoside mixture from which D-cymarose was identified as the sugar component and cynanchogenin (I) was crystallized as one of the aglycones.

Cynanchogenin forms long fine needles, m.p. 167°, $[\alpha]_D$ −39.5°(c=1.24, ethanol), for which the molecular formula $C_{28}H_{42}O_6$ was proposed from its elemental analysis and molecular weight determination (438, Rast). It is soluble in almost all organic solvents except light petroleum and gives a yellow color with tetranitromethane. The ultraviolet absorption maximum at 218 mμ implies α,β-unsaturated carbonyl (carboxyl) group and the infrared peaks at 3460, 1700, and 1640 cm^{-1} (Fig. 1) show the presence of O-H, C=O, and C=C (conjugated with C=O group), respectively. It was hydrogenated with palladised charcoal to a dihydro compound (II), m.p. 187°, $C_{28}H_{44}O_6$, $[\alpha]_D^{22}$ −21.7°(c=1.15, ethanol), which has infrared absorption maxima (Fig. 1) at 1727 and 1670 cm^{-1}. Subsequently, the absorption of the double bond conjugated with C=O group disappeared and the presence of two carbonyl groups could be confirmed.

Acetylation of (I) with acetic anhydride in pyridine yielded a monoacetyl derivative (III), m.p. 150°, which showed absorption of a free O-H group at 3470 cm^{-1}. Acetylation of (II) under the same condition as (I) gave a monoacetate (IV), m.p. 80∼100°, which was also formed by the hydrogenation of (III). (IV) also exhibited the free O-H absorption at 3470 cm^{-1}. Dihydrocynanchogenin (II) formed a monosemicarbazone (V), m.p. 230°(decomp.), but cynanchogenin itself gave a compound which had a formula $C_{29}H_{47}O_7N_3$ and was very probably a semicarbazide adduct at $>C=\overset{|}{C}-CO-$ double bond.

When (I) was treated with 5% methanolic potassium hydroxide solution, it gave a neutral compound (VII), m.p. 242°, and a liquid acid (VIII). The dihydro compound (II) also gave (VII) and a liquid acid (IX) on treating in the same way.

The acid (IX) had an unpleasant, irritating odor and was considered to be a saturated, branched fatty acid from its infrared spectrum (Fig. 2). It gave an anilide (XI), m.p. 105°, and an amide (X), m.p. 138.5°, and their elemental analyses suggested that the acid was a C_7 acid. Attempts were made to confirm the structure of this acid by synthesis. Among the possible C_7 acids, 3,4-dimethylpentanoic acid, synthesized as shown in Chart 1,[2] proved to be the same as that obtained from natural sources by the mixed melting point of the amide and anilide, and their infrared spectra.

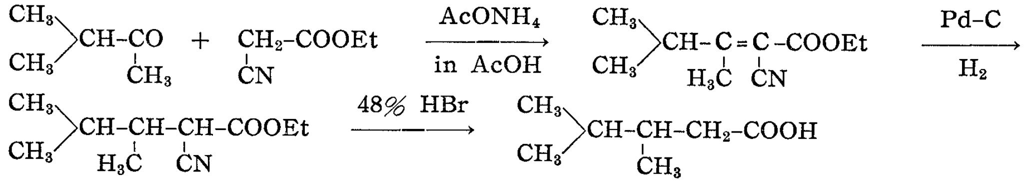

Chart 1.

(IX) b.p. 207° (Amide, m.p. 138°; Anilide, m.p. 110°)

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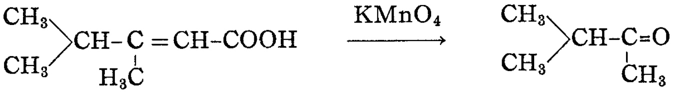
*[1] Kita-12-jo, Nishi-5-chome, Sapporo, Hokkaido (三橋 博, 清水 譲).

*[2] Part of this work was reported at the 3rd Hokkaido Local Meeting of the Pharmaceutical Society of Japan, June 27, 1959.

1) Part I: This Bulletin, 8, 313(1960).

2) Brit. Pat. 613,705 (December 2, 1948); F. F. Blicke, H. Zinnes: J. Am. Chem. Soc., **77**, 5400(1955).

The acid (VIII) also had an unpleasant odor and its infrared spectrum (Fig. 2), which had $\nu_{C=O}$ 1700, $\nu_{C=C}$ 1640 (conjugated with –COOH group), and $\delta_{>C=CH}$ 870 cm^{-1}, was much the same as that of 3-methylnonenoic acid,[3] from which it was concluded that (VIII) was the α,β-unsaturated acid of (IX). This conclusion was confirmed by its oxidation with potassium permanganate to methyl isopropyl ketone which was identified by the mixed melting point of its 2,4-dinitrophenylhydrazone, m.p. 121°, with the authentic sample.*3



Methyl isopropyl ketone
(2,4-Dinitrophenylhydrazone, m.p. 121°)

Chart 2.

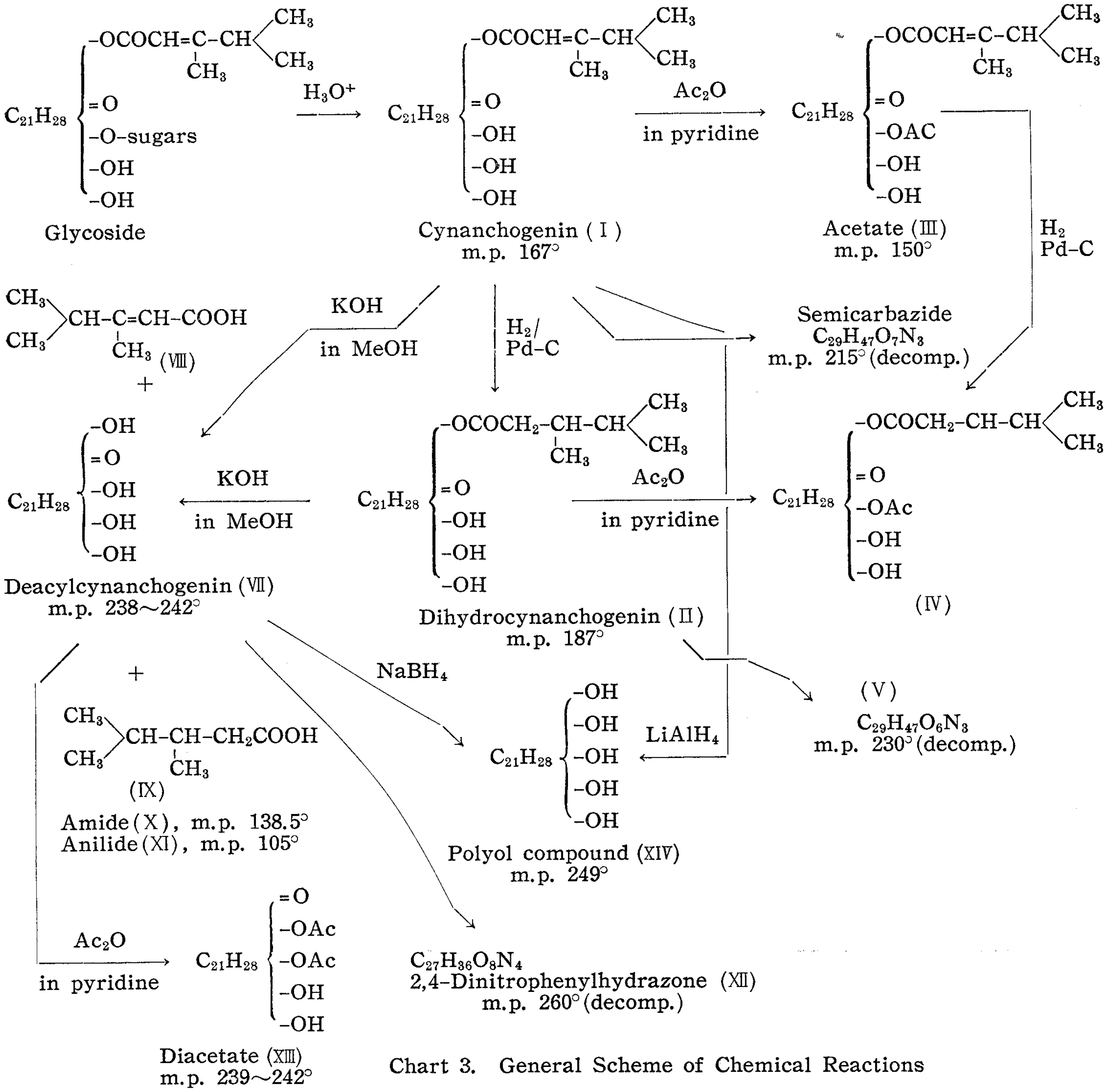


Chart 3. General Scheme of Chemical Reactions

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*3 The m.p. of 2,4-dinitrophenylhydrazone of methyl isopropyl ketone was reported as 117°, but the present sample (both natural and authentic) melted at 121°.

3) N. K. Freeman: J. Am. Chem. Soc., **75**, 1859 (1953).

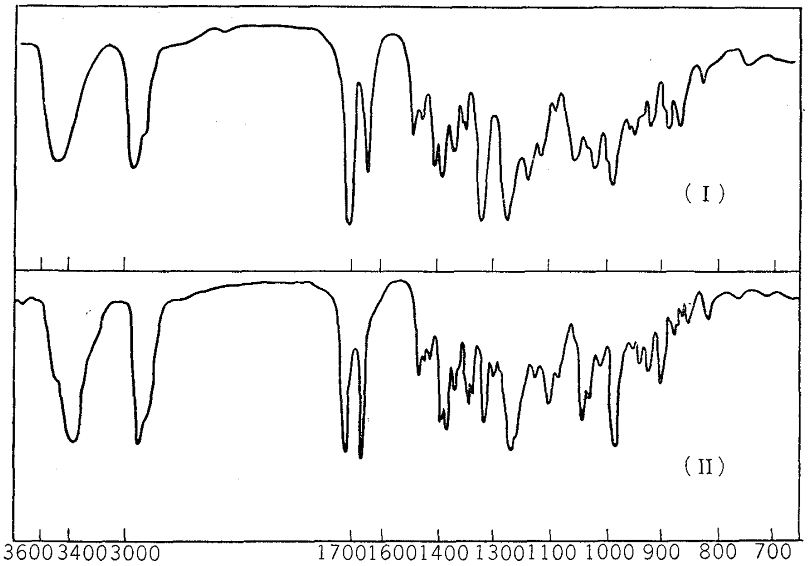
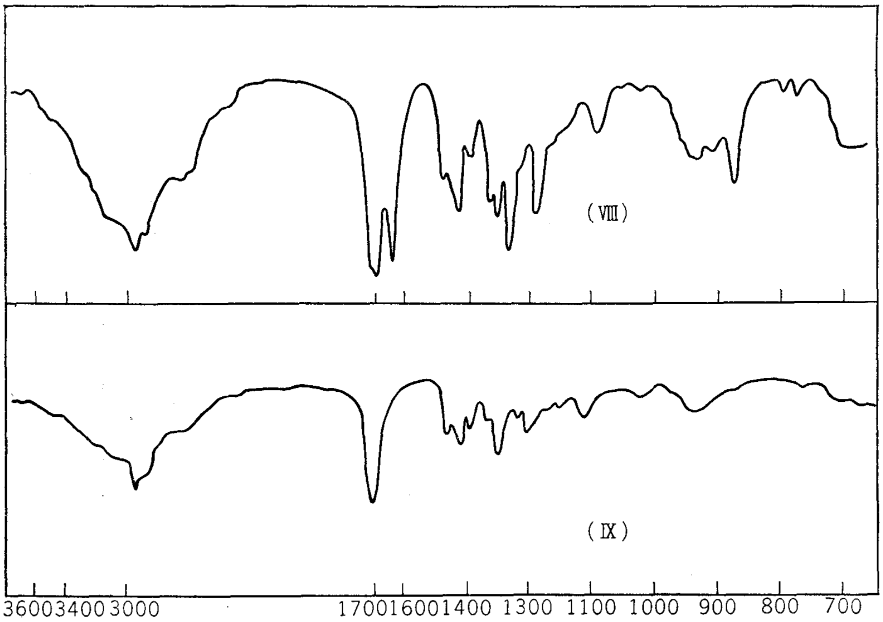


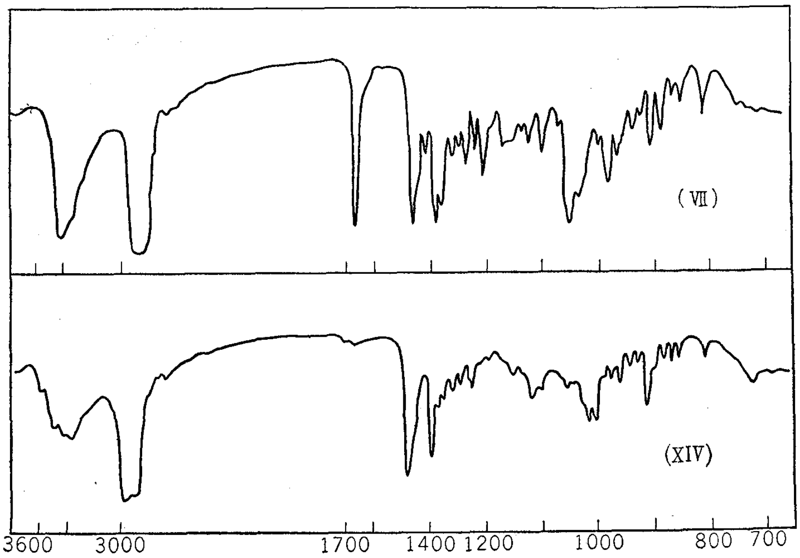
Fig. 1.

Infrared Absorption Spectra of Cynanchogenin (I) and Dihydrocynanchogenin (II) (KBr)



↖ Fig. 2.

Infrared Absorption Spectra of Unsaturated acid (VIII) and Saturated acid (IX) (Liq.)



← Fig. 3.

Infrared Absorption Spectra of Deacylcynanchogenin (VII) and Polyol Compound (XIV) (Nujol)

From the above facts, it is obvious that the proposed α,β-unsaturated carbonyl group in (I) was present in the attached acid portion.

The alcohol part (VII) was formulated as $C_{21}H_{32}O_5$ from its elemental analysis. It showed one C=O stretching band at 1670 cm^{-1} (open-chain ketone) and readily gave a mono-2,4-dinitrophenylhydrazone (XII), m.p. 260°(decomp.). Acetylation of (VII) with acetic anhydride in pyridine gave a diacetate (XIII), m.p. 239~242°, which has O-H stretching absorption at 3400 cm^{-1}, suggesting that at least one O-H group remained free. A positive tetranitromethane test showed the presence of a double bond. The reaction of sodium hypoiodite on (VII) gave iodoform which was characterized from its melting point (m.p. 120°). From this observation and infrared spectrum, an acetoxyl group (CH_3-CO-C) seems to be present in (VII).

Lithium aluminum hydride reduction of (I) in ether gave a polyol compound (XIV), m.p. 249° (from acetone-water), $C_{21}H_{34}O_5$,*4 in which no carbonyl absorption could be observed (Fig. 3), showing that cleavage of the ester linkage and reduction of the ketone had occurred. The polyol compound (XIV) was also gained by the reduction of deacylcynanchogenin (VII) with sodium borohydride. All these observations are summarized in Chart 3.

Experimental

Cynanchogenin (I)—Cynanchogenin (I) was isolated as described in Part I of this series[1)] and recrystallized from Et_2O-petr. ether to fine needles, m.p. 165~167°; $[\alpha]_D^{22}$ −39.5° (c=1.24, 90% EtOH). Lieberman-Burchard reaction, pink to yellow; 84% H_2SO_4, brown→violet; $SbCl_3$, green; tetranitromethane, yellow. UV λ_{max}^{EtOH} 218 mμ (log ε 4); IR λ_{max}^{KBr} cm^{-1} : ν_{OH} 3480, $\nu_{C=O}$ 1700, $\nu_{C=C}$ 1638. *Anal.* Calcd. for $C_{28}H_{42}O_6$: C, 70.85; H, 8.92; mol. wt., 462.2. Found : C, 70.67; 70.33; H, 8.90, 8.90, mol. wt. (Rast), 438.

Dihydrocynanchogenin (II)—A solution of 200 mg. of (I) dissolved in 11 cc. of EtOH was shaken with 200 mg. of 10% Pd-C in H_2 atmosphere for 100 min. H_2 uptake : 140 cc. (20°, 755.7 mm. Hg) (1.5 moles). After the catalyst was filtered off, the solution was evaporated to dryness and the residue was crystallized from Me_2CO-H_2O to fine needles, m.p. 187°. Color with tetranitromethane, slightly yellow. *Anal.* Calcd. for $C_{28}H_{44}O_6$: C, 70.55; H, 9.31. Found : C, 70.77; H; 9.21. IR λ_{max}^{KBr} cm^{-1} : 3420, 1730, 1672.

Cynanchogenin Monoacetate (III)—One hundred mg. of (I) was dissolved in 2 cc. of pyridine and 1 cc. of Ac_2O was added. The mixture was allowed to stand for 2 hr. at room temperature and then refluxed for 1 hr. The mixture was poured on ice and a white powder which appeared was collected and washed several times with H_2O. Crystallized from petr. ether (b.p. 50~60°) and a small quantity of benzene, m.p. 150°. *Anal.* Calcd. for $C_{30}H_{44}O_7$: C, 69.74; H, 8.58. Found : C, 69.68; H, 8.71. IR λ_{max}^{Nujol} cm^{-1} : 3490, 1720, 1635, 1245.

Dihydrocynanchogenin Monoacetate (IV)—a) One hundred mg. of dihydrocynanchogenin (II) was treated as described for (III) and crystallized from Et_2O. This compound formed fine silky needles in Et_2O but on filtration, turned to a white crystalline powder, m.p. 80~100°. *Anal.* Calcd. for $C_{30}H_{46}O_7$: C, 69.47; H, 8.94. Found : C, 69.83; H, 8.77. IR λ_{max}^{Nujol} cm^{-1} : 3470 (O-H), 1740 (C=O) (ester), 1700 (C=O), 1240 (acetyl).

b) Two hundred mg. of cynanchogenin acetate (III) and 100 mg. of 10% Pd-C in 20 cc. EtOH was shaken in H_2 atmosphere. H_2 uptake : 15 cc. (9°) (1.5 moles). Evaporation of the solvent and crystallization from Et_2O gave silky needles. It was confirmed to be identical with (IV) by m.p., mixed m.p., and IR spectrum.

Dihydrocynanchogenin Semicarbazone (V)—To 100 mg. of dihydrocynanchogenin (II) dissolved in 0.5 cc. of EtOH, a solution of 100 mg. of semicarbazide, 120 mg. of $AcONa \cdot 2H_2O$, and 0.3 cc. of H_2O was added. After allowing the solution to stand on a boiling water-bath for 45 min., H_2O was added. An oily substance separated out which gradually turned into a white powder. Recrystallization of this product from benzene-MeOH afforded fine needles, m.p. about 230° (decomp.). *Anal.* Calcd. for $C_{29}H_{47}O_6N_3$: C, 65.26; H, 8.88; N, 7.87. Found : C, 64.88; H, 8.46; N, 7.80.

Application of semicarbazide to (I) as above gave a crystalline powder from benzene, m.p. 215° (decomp.). The analytical sample was obtained as the semicarbazide instead of the semicarbazone.

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*4 This compound tends to contain water of crystallization or acetone (or both) and the analysis was carried out after exhaustive drying.

Anal. Calcd. for $C_{29}H_{47}O_7N_3$: C, 63.55; H, 8.61 : N, 7.6. Found : C, 63.83; H, 7.68; N, 7.74.

Hydrolysis of (I)—One hundred mg. of (I) was refluxed in 4 cc. of 5% methanolic KOH for 5 hr. After adding 2 cc. of H_2O, MeOH was removed under a reduced pressure. Crystals that separated out were collected and the filtrate was extracted with $CHCl_3$-EtOH. The extract was washed with H_2O and dried over Na_2SO_4. Evaporation of the solvent gave a minute quantity of crystals. Repeated recrystallization from Me_2CO-MeOH gave fine prisms (VII), m.p. 238~242°. Color reactions : Tetranitromethane, yellow; Lieberman-Burchard, red-violet→brown; conc. H_2SO_4, vermilion→dark green. *Anal.* Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.80. Found : C, 69.12; H, 8.83. IR λ_{max}^{Nujol} cm^{-1} : 3400, 1675, 1047. This compound is apt to contain water of crystallization when recrystallized from hydrous solvent.

The aqueous solution was acidified with conc. H_3PO_4, separating an oily acid. The emulsion was extracted thoroughly with Et_2O. The Et_2O solution was washed with H_2O, dried over Na_2SO_4, and evaporation of the solvent gave 22 mg. of an acid (VIII). It had an unpleasant odor similar to caproic acid. IR λ_{max}^{liquid} cm^{-1} : 1680 (C=O), 1640 (C=C, conj.).

Hydrolysis of (II)—A solution of the dihydro compound (II) in 50 cc. of 5% methanolic KOH was refluxed for 3 hr. To this solution 15 cc. of H_2O was added and MeOH was evaporated under a reduced pressure. The crystals that separated were collected, washed with H_2O, and recrystallized from Me_2CO, giving 240 mg. of micro-crystals, m.p. 238~242°, which proved to be (VII) by admixture and from IR spectrum.

The aqueous solution was treated as described above, affording 120 mg. of a liquid acid (IX) of unpleasant odor. IR : λ_{max}^{liquid} 1700 cm^{-1}.

The Amide of Saturated Acid (X)—A mixture of 60 mg. of the acid (IX) and 250 mg. of $SOCl_2$ was heated on a steam bath for 45 min. The reaction mixture was poured into conc. NH_3 water and the solution was extracted several times with Et_2O. The Et_2O solution was dried over Na_2SO_4 and evaporated to give crystals, which were recrystallized several times from hexane to plates, m.p. 138~138.5°. *Anal.* Calcd. for $C_7H_{15}ON$: C, 65.07; H, 11.70; N, 10.84. Found : C, 65.12; H, 10.70; N, 10.60.

The Anilide of Saturated Acid (XI)—A drop of the acid (IX) was heated with 3 drops of aniline in a sealed tube at 180° for 3 hr. The mixture was treated with dil. HCl to afford needles, which were recrystallized from EtOH-H_2O, m.p. 105°, which showed depression on admixture with the anilide of caproic, isocaproic, isobutyric, or isovaleric acid. *Anal.* Calcd. for $C_{13}H_{19}ON$: C, 76.05; H, 9.33; N, 6.82. Found : C, 75.81; H, 8.70; N, 7.33.

Synthesis of 3,4-Dimethylpentanoic Acid[2] **(IX)**—a) A solution of 15 g. of methyl isopropyl ketone, 19.8 g. of cyanoacetic ester, 1.2 g. of $AcONH_4$, and 0.9 g. of AcOH dissolved in 30 g. of benzene was refluxed for 12 hr. During the reaction, H_2O was removed as an azeotropic mixture (about 3 cc.). After cool, the solution was washed with 2% NaOH and H_2O, dried, and distilled. Yield, 15 g.

b) Hydrogenation of the cyanoacetic ester: 8 g. of the ester was hydrogenated with 1 g. of Pd-C in 20 cc. EtOH. The reaction was stopped after absorption of 1.4 L. of H_2 (15°) (about 1 hr.). The catalyst was filtered off and EtOH was evaporated. During this process, the ammonia odor remained and hence the nitrile might have been partially reduced. The residue was directly hydrolyzed by adding 65 cc. of 48% HBr and refluxed for 24 hr. The reaction mixture was poured on H_2O and extracted with Et_2O. The Et_2O extract was washed with H_2O and extracted with $NaHCO_3$. The $NaHCO_3$ layer was acidified with HCl and extracted with Et_2O. The Et_2O solution gave 4 g. of an acid, which distilled at b.p. 207°. The anilide and amide were prepared by the same method as described for (X) and (XI). The data for the mixed m.p.s were as follows :

	From cynanchogenin	Synthesized	Mixed
Amide, m.p. (°C)	138.5	138	138.5
Anilide, m.p. (°C)	105	110	108~110

The IR spectra of the two acids were the same.

c) Paper chromatographic comparison of the acids : 3*N* NH_4OH-BuOH (1:1), 15° for 12 hr. Paper: Toyo Roshi No. 50, detected by bromothymol blue. Rf of the acid synthesized, 0.67; natural acid, 0.67, caproic acid, 0.66, isovaleric acid, 0.50.

Permanganate Oxidation of Unsaturated Acid (VIII)—To 180 mg. of (VIII) suspended in 3 cc. of H_2O, saturated solution of 300 mg. of $KMnO_4$ was gradually added with cooling. The color of $KMnO_4$ vanished quickly. The reaction mixture was immediately steam-distilled and the distillate was collected in 2,4-dinitrophenylhydrazine reaction mixture. A good amount of yellow crystals separated. Recrystallization from EtOH gave needles, m.p. 121°, which showed no depression on admixture with 2,4-dinitrophenylhydrazone of methyl isopropyl ketone.

Deacylcynanchogenin 2,4-Dinitrophenylhydrazone (XII)—Deacylcynanchogenin was treated as usual. Recrystallization from AcOEt gave prisms, m.p. 260° (decomp.). *Anal.* Calcd. for $C_{27}H_{36}O_8N_4$: C, 59.54; H, 6.66; N, 10.29. Found : C, 59.50; H, 6.78; N, 9.69. IR λ_{max}^{Nujol} cm^{-1} : 3490, 1620, 1595.

Diacetyldeacylcynanchogenin (XIII)—To a solution of 100 mg. of deacylcynanchogenin (VII) from (I) dissolved in 2 cc. of pyridine, 1 cc. of Ac_2O was added. The mixture was refluxed for 1 hr. and then treated with ice water. Crystals that separated were collected, washed, and recrystallized from benzene to prisms, m.p. 238∼241°. *Anal.* Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09. Found : C, 66.97; H, 8.07. IR λ_{max}^{Nujol} cm^{-1} : 3500, 1720, 1690, 1260, 1240.

Deacylated compound from dihydrocynanchogenin gave the same result.

$LiAlH_4$ Reduction of Cynanchogenin—To 500 mg. of $LiAlH_4$ suspended in 20 cc. of dehyd. Et_2O, a solution of 500 mg. of cynanchogenin (I) in 20 cc. of Et_2O was added over a period of 30 min. After refluxing for 3 hr., the mixture was cooled and excess $LiAlH_4$ was decomposed with Et_2O saturated with H_2O. The Et_2O layer was separated and inorganic residue was washed with Et_2O. The combined Et_2O extract was treated as usual to give 30 mg. of polyol (XIV). The inorganic residue was dissolved in dil. HCl and on standing afforded cubic crystals, which were recrystallized from Me_2CO-H_2O to needles (XIV), m.p. 145∼151°. Yield, 100 mg. *Anal.* Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.63; H, 9.31. IR λ_{max}^{Nujol} cm^{-1} : 3270, 1665 (weak).

$NaBH_4$ Reduction of Deacylcynanchogenin—To a solution of 100 mg. of deacylcynanchogenin in 3 cc. of dioxane a solution of 30 mg. of $NaBH_4$ in 1 cc. each of dioxane and H_2O were added. After standing for 2 hr., the solution was acidified to pH 1 with 0.5*N* H_2SO_4 and extracted with $CHCl_3$-EtOH (3:2). The extract was washed successively with H_2O, Na_2CO_3 solution, and H_2O, and dried over Na_2SO_4. Evaporation of the solvent gave crystals, which were recrystallized from Me_2CO to needles, m.p. 237°. This m.p. is far lower than that of (XIV) but this substance easily takes up crystal solvent and after exhaustive drying, the two compounds showed the same IR spectrum. *Anal.* Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found (after thorough drying) : C, 68.99; H, 9.20. *Anal.* Calcd. for $C_{21}H_{34}O_5 \cdot 2C_3H_6O$: C, 67.19; H, 9.61. Found (dried at room temperature) : C, 67.19; H, 9.41.

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Summary

Hydrogenation of cynanchogenin (I), m.p. 167°, $C_{28}H_{42}O_6$, gave a dihydro compound (II). Alkaline hydrolysis of (I) gave an unsaturated acid (VIII) and deacylcynanchogenin (VII), $C_{21}H_{32}O_5$. The same treatment of (II) gave (VII) and a saturated acid (IX). The saturated acid was proved to be 3,4-dimethylpentanoic acid by synthesis. By the permanganate oxidation, the unsaturated acid was shown to be 3,4-dimethyl-2-pentenoic acid.

Deacylcynanchogenin has one ketone group, one double bond, and four hydroxyl groups, two of which easily undergo acetylation. Reduction of cynanchogenin with lithium aluminum hydride gave a polyol compound (XIV), which was also obtained by the reduction of deacylcynanchogenin with sodium borohydride.

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