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87. **Keiichi Takamura**: Studies on Saponins. VIII.* The Preparation of Oleanolic Acid Monoglucuronoside and Diglucuronoside.

(*Pharmaceutical Institute, Faculty of Technology, Nihon University***)

Recently, Hardegger and his co-workers reported on the synthesis of methyl oleanolate quinovoside[1] and glucoside.[2] The present author attempted the synthesis of oleanolic acid monoglucuronoside and diglucuronoside from oleanolic acid and glucuronic acid. Methyl α-bromotriacetylglucuronate (α-BGM) was prepared by the method of Goeber, *et al.*[3] from methyl α- and β-tetraacetylglucuronate and hydrogen bromide.

Monoglucuronoside

Methyl oleanolate was condensed with α-BGM, in the presence of mercuric cyanide, in anhydrous benzene for 10 hours. The reaction mixture was purified by chromatography and methyl oleanolate 2-methyltriacetylglucuronoside was obtained as colorless needles, m.p. 125°(decomp.), whose alkaline hydrolysis yielded methyl oleanolate 2-glucuronoside as needles, m.p. 241.5°(decomp.).

This substance is very slightly soluble in hot water, but it seems to possess a saponin-like properties as foaming when shaken. When compared with other saponins, the soulbility in water was, however, very low, probably becoming less hydrophilic as the result of its one molecule of sugar. It was found that methyl oleanolate 2-glucuronoside (I), 2-quinovoside (II), 2-glucoside (III), and sugar-beet saponin (IV) show approximately equal values in specific optical rotation, so that the configuration of sugar combination in those glucosides seems to be identical. Comparing (II) and (III) with (IV), Hardegger already determined their configuration to be in "β-form" according to Barton's molecular rotation method. In cosequence, (I) is considered to be in β-configuration.

Diglucuronoside

Silver 2-acetyloleanolate was reacted with α-BGM in benzene for 3 hours and 2-acetyloleanolic acid 28-methyltriacetylglucuronoside (V) was obtained as colorless needles, m.p. 225°(decomp.). On the other hand, silver oleanolate was reacted with α-BGM in benzene and oleanolic acid 28-methyltriacetylglucuronoside (VI) was obtained in amorphous form. Its acetylated compound of m.p. 225°(decomp.) was determined to be identical with that obtained from silver 2-acetyloleanolate. Oleanolic acid 28-methyltriacetylglucuronoside was prepared from silver oleanolate and it was condensed with α-BGM, in the presence of mercuric cyanide, in anhydrous xylene for 10 hours. The reaction mixture was purified by chromatography and oleanolic acid 2,28-bis(methyltriacetyl)-glucuronoside (VII) was obtained in an amorphous state. By alkaline hydrolysis of (VII), oleanolic acid 2,28-diglucuronoside (VIII) was produced as a crystalline powder, m.p.

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* Reports have already been published on a series of studies on Thea-saponin, camellia-saponin, etc. under the following titles; Seed Saponin of *Thea sinensis* L. I, J. Pharm. Soc. Japan., **72**, 1522(1952); Part. Ⅱ, *ibid.* **72**, 1525(1952); Studies on the Saponin of the Fruit of *Camellia japonica* L. I, **73**, 347(1953); Part Ⅱ, *ibid.*, **74**, 641(1954); Part Ⅲ, *ibid.*, **74**, 645(1954); Samen Saponin von *Thea sinensis* L. Ⅲ, this Bulletin, **2**, 175(1954); Studies on the Relation between Hemolytic Activity and Film Expanding Power of Saponins, this Bulletin **2**, 335(1954). Hereafter, above studies will be respectively referred to as follows: "Studies on Saponins. I, Ⅱ, Ⅲ, Ⅳ, V, VI, and Ⅶ" by Morizo Ishidate.

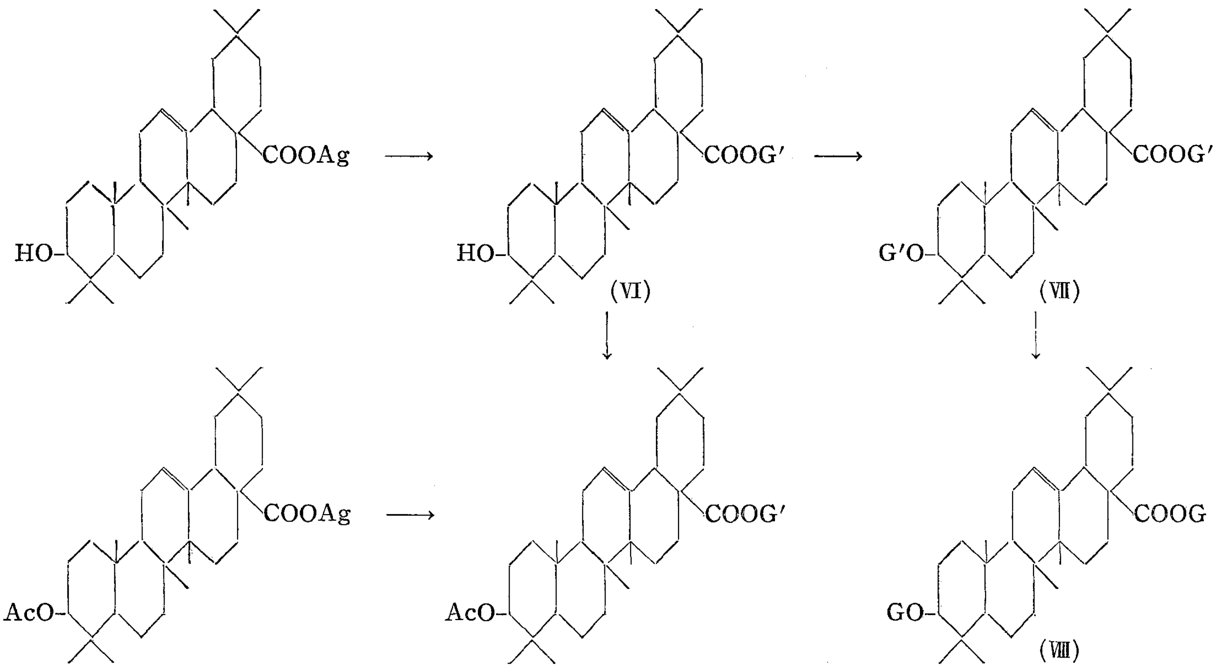
** Kanda-Surugadai, Chiyoda-ku, Tokyo (高村圭一).

[1] E. Hardegger, *et al.*: Helv. Chim. Acta, **33**, 1871(1950).

[2] E. Hardegger, *et al.*: *Ibid.*, **35**, 824(1952).

[3] W. F. Goebel, *et al.*: J. Biol. Chem., **111**, 348(1935).

Chart 1.



G = Glucuronic acid G' = Methyl triacetylglucuronate

187.5°(decomp.). These syntheses are summarized in Chart 1. This substance dissolves in hot water and produces continuous foaming which is characteristic of saponins. It forms an alkali salt which is easily soluble in water, in alcoholic alkali solution, and acidification of the solution with acetic acid again produces diglucuronoside.

Using the rabbit blood-corpuscle test, the hemolytic action of diglucuronoside was compared with those of camellia saponin and potassium glycyrrhate under the same conditions. The activity of this compound, as shown in Table I, was much stronger than that of potassium glycyrrhate and less than that of camellia saponin, so the diglucuronoside is believed to possess saponin-like properties of moderate activity.

Table I. Comparison of Hemolytic Activity
(Concn. of rabbit blood corpuscles, 2%. $NaHCO_3$-Na_2HPO_4 buffer (pH 6.91), determined 5 mins. after run. Temp., 25°)

Saponin : Buffer	Camellia saponin	Potassium glycyrrhate	Oleanolic acid 2,28-diglucuronoside
1 : 220	—	somewhat active	—
1 : 2,000	active	inactive	active
1 : 20,000	〃	〃	inactive

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Experimental

Methyl Oleanolate 2-Glucuronoside—A mixture of 4.7 g. of methyl oleanolate (0.01 mole) and 5.04 g. of $Hg(CN)_2$ (0.02 mole) was dissolved in 300 cc. of dehyd. benzene and 100 cc. of solvent was distilled off while stirring. A solution of 80 cc. of dehyd. benzene containing 3.9 g. (0.01 mole) of α-BGM was added to it, 80 cc. of benzene was distilled off, and the residual mixture was refluxed for 3 hrs. Further 3.9 g. (0.01 mole) of α-BGM dissolved in 80 cc. of dehyd. benzene was added to the former solution, then 80 cc. of benzene was removed, and the mixture refluxed for 7 hrs. After cooling, it was washed with 10% $Na_2S_2O_3$ solution, 10% NaOH solution, and water, dried over $CaCl_2$, and the benzene evaporated under a reduced pressure. An amorphous substance was obtained in a yield of 14 g. This was dissolved in dehyd. benzene (50 cc.), chromatographed over alumina (250 g., Brockmann's), eluted with benzene and a mixture of benzene : ether (1 : 1), and extracted with hot AcOH. From the AcOH layer, 3.9 g. of methyl oleanolate-methyl triacetylglucuronate, was obtained. Recrystallization from 70% EtOH gave m.p. 125°(decomp., corr.). *Anal.* Calcd. for $C_{44}H_{66}O_{12}$: C, 67.17; H, 8.53. Found: C, 67.20; H, 8.75.

Hydrolysis of this compound in 150 cc. EtOH and 10.5 cc. *N* NaOH solution was carried out on a water bath for 1 hr. Recrystallization from 70% EtOH gave (I), m.p. 241.5°(decomp., corr.); yield, 1.5 g. (25%). This is insoluble in EtOH, MeOH, benzene, and petroleum solvents, and soluble in hydrated alcohol, dioxane, and pyridine. $[\alpha]_D^{20}$ +20.1°(c=1.29, pyridine). *Anal.* Calcd. for $C_{37}H_{58}O_9$: C, 68.69; H, 9.04. Found : C, 68.23, 68.21; H, 9.05, 8.87.

2-Acetyloleanolic Acid 28-Methyltriacetylglucuronoside—i) A solution of 1 g. of silver 2-acetyloleanolate and 1 g. of α-BGM dissolved in dehyd. benzene was refluxed for 3 hrs., benzene was distilled off, and the residue was recrystallized from EtOH. Yield : 0.9 g. (80%)of m.p. 225°(decomp., corr.). It is soluble in $CHCl_3$, acetone, AcOH, AcOEt, and dioxane, and insoluble in petroleum solvents. $[\alpha]_D^{20}$ +43.9°(c=1.025, $CHCl_3$). *Anal.* Calcd. for $C_{45}H_{66}O_{13}$: C, 66.33; H, 9.04. Found : C, 66.20, 66.18; H, 8.01, 8.87.

ii) A mixture of 3.7 g. of silver oleanolate and 4 g. of α-BGM dissolved in 200 cc. of benzene was refluxed for 3 hrs. After evaporation of benzene, the residue was dissolved in EtOH and poured into water. The precipitate was collected, dried, and an amorphous substance was obtained. This was acetylated with Ac_2O and pyridine giving needles, m.p. 225°(decomp., corr.), as recrystallized from EtOH, no depression of m.p. on admixture with silver 2-acetyloleanolate.

Oleanolic Acid 2,28-Diglucuronoside—A mixture of 5.1 g. of silver oleanolate and 5.1 g. of α-BGM was reacted in 250 cc. of benzene, the solvent was removed, and the residue was dissolved in 300 cc. of xylene. To this, 7.8 g. of $Hg(CN)_2$ was added and 100 g. of xylene was evaporated while stirring. To this residue 80 cc. of benzene containing 3.9 g. of α-BGM (0.01 mole) was added at the same time when 80 cc. of xylene was removed. After 3 hrs., dehyd. xylene containing 3.9 g. of α-BGM was added and 80 cc. of xylene was removed. After 7 hrs.' refluxing and cooling, it was washed with 10% $Na_2S_2O_3$ solution, 10% NaOH solution, and water, dried, xylene distilled off under reduced pressure, and 12.5 g. of syrupy substance was obtained. This was purified over alumina (Brockmann), eluted with benzene and a mixture of benzene : ether (1 : 1), and extracted with hot AcOH. From the AcOH layer, 3.3 g. of amorphous (VII) was obtained. This was dissolved in benzene and chromatographed over $KHPO_4$ but it remained amorphous. Hydrolysis for 1 hr. with 70% EtOH containing 0.3% NaOH gave a crystalline powder, m.p. 187.5°(decomp., corr.); yield, 24%. This is soluble in hydrated alcohol and insoluble in other organic solvents. It dissolves in hot water and in 10% NaOH, which produced foaming when shaken and deposited by acidification with AcOH. $[\alpha]_D^{25}$ +19°(c=1, 70% EtOH). *Anal.* Calcd. for $C_{42}H_{64}O_{15}$: C, 62.21; H, 8.20. Found : C, 61.86; H, 7.69.

Summary

Methyl oleanolate 2-monoglucuronoside was prepared from methyl oleanolate and α-BGM in the presence of mercuric cyanide as a catalyst. Oleanolic acid 2,28-diglucuronoside was obtained from oleanolic acid 28-methyltriacetylglucuronoside with mercuric cyanide and α-BGM. This substance had a saponin-like properties and the hemolytic activity of diglucuronoside was compared with that of other saponins.

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