41. **Ken'ichi Takeda, Tameto Okanishi, and Ariyoshi Shimaoka :** Studies on the Steroidal Components of Domestic Plants. V[1]. Constituents of Agave Species. (1).

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Agave sp. plants belong to the Amaryllidaceae of the tropical American origin. Most of the score or so of this species are cultivated as garden plants but some are cultivated in the tropical region as fiber material[2]. There are many plants of this species that contain steroidal saponins. Marker and others[3,4] isolated nine kinds of sapogenins from 45 kinds of plants in the United States and Mexico, such as manogenin (from 18 plants), hecogenin (from 16 plants), gitogenin (from 7 plants), smilagenin (from 7 plants), tigogenin (from 4 plants), sarsasapogenin (from 2 kinds), and agavogenin, rockogenin, and chlorogenin (from 1 plant each). Callow and others[5] and Spensley[6] reported that the waste obtained during the manufacture of fiber from *Agave sisalana* Perrine or its leaf juice is useful as the raw material for the preparation of hecogenin. It should be noted that the Agave spp., differing from the Yucca and Dioscorea spp., contain large amount of sapogenins containing a keto radical in C_{12}-position. However, it seemed possible that the kind and content of sapogenin would be different according to the age or portion of the plant, time of collection, and habitat that examinations were made as to the steroidal saponins contained in a few of the plants growing in Japan.

There are only a few kinds of Agave spp. in Japan including *Agave americana* L. and *A. americana* var. *variegata* Nichols which have already been naturalized, and some being cultivated in a greenhouse. The following three kinds of Agave were used for the present series of experiments.

TABLE I.

Plant	Part used	Location	Date Collected
I. *Agave rigida* Miller var. *sisalana* Engelm. (=*A. sisalana* Perrine)	(Leaves) Shoot	Takatsuki (Osaka Pref.)	June, 1952
	Leaves & Shoot	Tanegashima (Kagoshima Pref.)	September, 1952 August, 1953
	Leaves	Ohsumi Peninsula (Kagoshima Pref.)	December, 1952
	Shoot	〃	〃 〃
II. *Agave americana* L.	Leaves	Takatsuki (Osaka Pref)	March, 1953 August, 1953
III. *Agave salmiana* Jacobi	Leaves	〃	〃 〃

The method of experiments followed those described in the previous report[1]. The crude saponin was hydrolyzed with hydrochloric acid, the crude genin was separated and purified through chromatography using various organic solvents, and estimation was made through physicochemical properties, mixed fusion, and infrared absorption spectra of the genins and their derivatives.

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1) Part IV : This Bulletin, **2**, 178 (1954).

2) L. H. Bailey : The Standard Cyclopedia of Horticulture, **1**, 230 (1930) ; J. C. Medina : Bragantia, 8, 91 (1948) (cf. Brit. Abst. BIII-1, 198 (1952) ; *ibid.* : Cotton Fib. Trop., **6**, 44, 133 (1951) (cf. Brit. Abst., BIII-1, 515 (1952).

3) R. E. Marker, *et al.* : A. D. I. Document 23, 8, 4.

4) R. E. Marker, *et al.* : J. Am. Chem. Soc., **69**, 2220 (1947).

5) R. E. Callow, J. W. Connforth, P. C. Spensley : Chem. Ind., **33**, 699 (1951) (cf. J. Pharm. Pharmacol., **4**, 206 (1952)).

6) P. C. Spensley : J. Pharm. Pharmacol., **4**, 426 (1952) (cf. Brit. Abst., AII-4, 1231 (1952) ; C. A., **47**, 3324 (1952)).

Several series of experiments were carried out on *Agave rigida* var. *sisalana* with different materials, as listed above, but the chief component was found to be hecogenin in every case. The young plant yielded a substance assumed to be tigogenin. In majority of cases, hecogenin was isolated comparatively easily but a part of it was very difficult to purify and, during the course of experiments, was isolated as crystals identical with manogenin in melting point and analytical values. These were later found to be impure hecogenin[7].

Hecogenin was obtained as the chief component from *Agave americana* which also yielded a small amount of crystals of m.p. 230～240° and 120° which gave positive reaction for sterols. No further detailed studies on these were made due to the small amount available.

Agave salmiana only yielded hecogenin, the fact being different from the reports of Marker, *et al.* that manogenin had been obtained from this plant.

TABLE II.

Plant	Age	Sapogenin Found		Reported
		Identified	Unidentified	
Agave rigida var. *sisalana*	Young	Hecogenin	m.p. 193°	
〃	Old	Hecogenin		Hecogenin[5]
A. americana	Old	Hecogenin	m.p. 230～240°	Hecogenin[2,5]
A. salmiana	Old	Hecogenin		Manogenin[2,3]

Marker and others[8] state that the young Agave plants contain manogenin–saponin and as the plant grows it deteriorates to tigogenin–saponin through gitogenin– or hecogenin–saponin. However, manogenin and gitogenin were not isolated during the present series of experiments.

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Experimental

1) *Agave rigida* **Miller var.** *sisalana* **Engelm.: Isolation of Crude Sapogenin**—Coarse cut, half-dried sample (loss on drying, 36%) (3.4 kg.) of the leaves collected in Ohsumi was digested twice with warm methanol, the methanolic extract was concentrated under a reduced pressure, and ether-soluble portion was removed. The residual extract was dissolved in 50% alcohol, hydrochloric acid added to a 3% amount, and the mixture was heated for 4 hours on a water bath to effect hydrolysis. Alcohol was removed under a reduced pressure, water added to the residue, and the tar-like precipitate that formed on the addition of water was collected by filtration. This was washed with water, dried, and extracted 3 times with ether. Concentration of the ether extract yielded crystals (Ⅰ) of m.p. 230°. Yield, 3 g. (ca. 0.1%). The mother liquor obtained after the removal of (Ⅰ) yielded a small amount of crystals (Ⅱ) melting at around 120°.

Purification of the Sapogenin—Recrystallization of 3 g. of (Ⅰ) from acetone and chromatographic separation yielded three kinds of crystals: 1.5 g. of (Ⅲ), m.p. 230°, and (Ⅳ), m.p. 240°, and 0.2 g. of (Ⅴ), m.p. 253°.

Analytical results suggested that (Ⅲ) and (Ⅳ) were still impure hecogenin, and (Ⅴ), somewhat impure one that the combined (Ⅲ) and (Ⅳ) was again submitted to chromatography with the results shown in Table Ⅲ.

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7) For the isolation of hecogenin and tigogenin, cf. J. Gedeon, F. A. Kincl: Arch. Pharmazie, 286, 58 (7), 317 (1953).

8) Marker, *et al.*: J. Am. Chem. Soc., **69**, 2211 (1947).

TABLE III.

Solvent	Eluate (m.p., °C)
Chloroform : benzene (2 : 98)(i)	trace
Chloroform : benzene (2 : 8)(ii)	230~235(VI), 243(VII), 258~260(VIII), 260(IX)
Ethanol : benzene (2 : 8)(iii)	230~240(X)

Elementary analysis of each of these crystals indicated that (VIII) was pure hecogenin, while (VI) and (VII) were still impure, and (IX) and (X), somewhat impure, hecogenin. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.35; H, 9.77. Found: C, 75.55; H, 9.60.

Acetate of (VIII): m.p. 250°. Mixed fusion and infrared absorption spectrum agreed with those of hecogenin acetate. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.72; H, 9.32. Found: C, 73.82; H, 9.41.

2,4-Dinitrophenylhydrazone of (VIII): m.p. 279~280° (from EtOH). *Anal.* Calcd. for $C_{35}H_{48}O_8N_4$: C, 64.4; H, 7.4. Found: C, 63.9; H. 7.44.

Semicarbazone of (VIII): rectangular crystals (from EtOH), m.p. 195~200°.

Lower-melting portion: (II), (VI), and (VII) were combined and purified through chromatography, using the mixed solvents given in Table III. The mixed solvent (i) yielded needle crystals (XI), m.p. 193°, and the mixed solvent (ii), crystals (XII) of m.p. 258°, the latter being identical with hecogenin. (XI) showed no depression of the melting point on admixture with Tigogenin "Syntex," but the melting points of both the genin and its acetate (m.p. 198°) were somewhat lower than those of the sample. Since the analytical values were also slightly different, they were thought to be still not in perfectly pure state. *Anal.* Calcd. for $C_{27}H_{44}O_3$: C, 77.88; H, 10.58. Found: C, 76.96; H, 10.59.

Leaves from the Mature Plant from Tanegashima—The leaves were cut across into round slices, dried, and reduced to a coarse powder. This powder (900 g.) was digested twice with methanol and on cooling the extract, separated a large amount of precipitate containing a wax-like substance. The precipitate was collected by filtration, ether-soluble portion was removed, and submitted to hydrolysis with alcoholic hydrochloric acid. The precipitate was taken up in ether, washed consecutively with dil. alkali and water, and ether evaporated. Concentration of the residue yielded the crude genin as pale yellow needle crystals of m.p. 230~240°. Yield, 3.2 g. This crude genin was heated in about 5 volumes of acetic anhydride, with a small amount of pyridine, for 1 hour. A large amount of water was added to this mixture and the crystals that separated out were recrystallized from acetone. The slightly colored crystals hereby obtained were chromatographed as a solution in a mixture (99 : 1) of petroleum ether and benzene. The results of chromatography are shown in Table IV.

TABLE IV.

Solvent		Eluate (m.p., °C)
Petr. Ether : Benzene	(99 : 1)	60~62 (A)
〃 〃	(1 : 1)	230~241 (B)
〃 〃	(1 : 99)	241~243 (C)
Benzene : Acetone	(98 : 2)	243 (D)
〃 〃	(8 : 2)	trace
〃 〃	(1 : 1)	trace

The initial eluate gave a wax-like substance and the crystals, m.p. 230~243°, obtained from later eluates were submitted to chromatography as a solution in a mixture (99 : 1) of benzene and acetone.

The crystals melting at 243° obtained from the first eluate showed no depression on admixture with hecogenin acetate. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.72; H, 9.32. Found: C, 73.25; H, 9.47.

2) *Agave americana* L.—Two lowest leaves (4.015 kg.) from the mature plant were cut across into round slices, dried in air for 1 month, and 350 g. of the dried sample (loss on drying, 91.3%) was obtained. This dried sample was digested twice with 2 L. each of methanol, the extract was concentrated, and ether-soluble portion was removed. The portion insoluble in ether was adjusted to pH 4.8 with hydrochloric acid and the mixture was extracted 3 times with butanol. Butanol solution was added with the same volume of water, butanol was removed by azeotropic distillation, and the residual extract was dissolved in alcohol to make a 30% solution. To this was added hydrochloric acid in 5% amount and the mixture was heated for 4 hours to effect hydrolysis. The precipitate thereby formed was extracted with ether, the extract was evaporated, and the residue was saponified with 5% alcoholic potash. This was extracted with ether, the ether extract was washed with water and dried, and ether was removed. The residual syrup yielded 0.4 g. (ca. 0.1%) of crude crystals which were submitted to chromatography. The mixed solvent (i) yielded crystals (XIII) of m.p. 230~240° and of m.p. 120°, and the solvent (ii), crystals (XIV) of m.p. 258°. Detailed examination of (XIII) could not be carried out due to the small yield. (XIV) and its acetate showed no depression of the melting point on admixture respectively with hecogenin and its acetate. *Anal.* Calcd. for $C_{27}H_{42}O_4$: C, 75.35; H, 9.77. Found: C, 75.61; H, 9.54.

3) *Agave salmiana* **Jacobi**—The leaves from the mature plant were cut longitudinally, dried, and rendered into a coarse ground product. One kg. of the coarse ground was treated as in the foregoing and 5 g. (0.5%) of crude crystals were obtained. These were submitted to chromatography and a small amount of wax-like substance was obtaind from the mixed solvent (i). The mixed solvent (ii) yielded 0.4 g. of crystals (XV), m.p. 260°, and 0.2 g. of crystals (XVI), m.p. 250∼254°; and the mixed solvent (iii), 0.1 g. of crystals (XVII), m.p. 255∼260°. Analytical results and mixed fusion indicated all three to be hecogenin, with (XVII) alone being slightly less pure. *Anal.* Calcd for $C_{27}H_{42}O_4$: C, 75.35; H, 9.77. Found: C, 74.95; H, 9.61.

Acetate: m.p. 250°. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.72; H, 9.74. Found: C, 94.13; H, 9.51.

Summary

Hecogenin (I) alone was obtained from the leaves of a medium mature plant of *Agave rigida* var. *sisalana* collected in August and December, but a small amount of crystals of m.p. 193° were also obtained besides (I) from the leaves of a young plant collected in June and September. The crystals showed no depression on admixture with tigogenin. The leaves from *Agave americana*, collected in March, also yielded a small amount of crystals of m.p. 230∼240° besides (I). The leaves of *Agave salmiana*, collected in August, also yielded (I) alone. These results did not agree with the reports of Marker, *et al.* that manogenin had been obtained from the same plants growing in the United States.

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42. **Takuo Okuda**: Studies on the Components of *Coriaria japonica* A. Gray. XIII[1].

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In the preceding paper of this series Kariyone and Sato[2] reported that a toxic component contained in fruits of *Coriaria japonica* along with coriamyrtin was identified as tutin, which had been extracted by Easterfield and Aston[3] from leaves of three species of *Coriaria* found in New Zealand, by mixed melting point and by a comparison of crystal forms and color tests.

Kinoshita[4] had also treated tutin of *C. japonica* and obtained acetyl, dihydro, and some other derivatives. Later Slater[5] noted that acetyltutin and dihydrotutin produced by him from tutin from New Zealand sources are markedly different in melting point from Kinoshita's derivatives, and on account of these results Slater pointed out that the identity of tutin from Japanese sources with tutin from New Zealand sources was not complete.

In the present study, the reasons for the discrepancy of melting points of derivatives were investigated by checking the experiments of Kinoshita and Slater. Identification and further observation on the structure of tutin were carried out by the aid of infrared absorption spectra. The presence of pseudotutin in *C. japonica* was also ascertained.

Slater described that the m.p. of the dihydro derivative produced on catalytic reduction

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[1] Part XII: J. Pharm. Soc. Japan, **73**, 930(1953).

[2] T. Kariyone, T. Sato: *Ibid.*, **50**, 659(1930).

[3] T. H. Easterfield, B. C. Aston: J. Chem. Soc., **79**, 120(1901).

[4] K. Kinoshita: J. Chem. Soc. Japan, **51**, 99 (1930); *ibid.*, **52**, 171(1931).

[5] S. N. Slater: J. Chem. Soc., **1943**, 50.