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Studies of Mu-tong, Akebiae Caulis (2)[1] Outer and inner morphologies of woody stems of *Akebia* plants growing in Japan and the botanical origin of Mokutsu produced in Japan

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The Japanese Pharmacopoeia Fifteenth Edition prescribes Akebiae Caulis, Mokutsu in Japanese, should be the woody stem of *Akebia quinata* Decaisne and *A. trifoliata* Koidzumi of the family Lardizabalaceae. We examined morphologically the woody stems of 3 wild *Akebia* taxa growing in Japan, *A. quinata*, *A. trifoliata*, and *A.* × *pentaphylla* Makino, for the purpose of finding morphological criteria to discriminate them and for identifying Mokutsu in the market. We found that the 3 taxa could be distinguished by a combination of surface color of stem, inner morphologies of stone cells and sclerenchyma cells, *etc.* with an accuracy of more than 90%, though it had been reported that *A. quinata* and *A. trifoliata* could not be distinguished by only stem anatomy. Using these criteria, we found that the woody stems of all 3 *Akebia* taxa were circulated equally in the Japanese market. This is the first record that the stem of *A.* × *pentaphylla* is circulating in the market as Mokutsu.

Key words Akebiae caulis, Mu-tong, *Akebia* × *pentaphylla*, botanical origin, plant anatomy, colorimeter.

Introduction

The Chinese crude drug Mu-tong, Akebiae Caulis and "Mokutsu" in Japanese, is prescribed in The Japanese Pharmacopoeia Fifteenth Edition[2] as the woody stems of *AQ* (*Akebia quinata* Decaisne) and *AT* (*A. trifoliata* Koidzumi) of the family Lardizabalaceae. Recently Mokutsu used in Kampo medicine has been supplied from wild *Akebia* plants growing in Japan.[3] In addition to the above two species, *A*×*P* (*A.* × *pentaphylla* Makino), a hybrid of the two species,[4] also grows in Japan, and is probably collected as Mokutsu and circulated in the market.

Akebia plants growing in the wild can be identified based on the differences of the number of leaflets and the color and form of flowers.[4] However, since the crude drug, Mokutsu, is collected in the winter season when no leaves or flowers are attached to the plants,[5] it is very hard to identify the plant origin only by stem shape in the field. Therefore, *A*×*P* as well as *AQ* and *AT* may circulate on the Japanese market as Mokutsu. Though the plant origin is disregarded in the market for the therapeutic use, the appropriate use of each species is suggested because of chemical and pharmacological differences between the stems of *AQ* and *AT*.[6,7] Therefore, studies have been performed to find ways of identifying the plant origin of Mokutsu. Fujita[8] reported that no anatomical difference was recognized between the stems of *AQ* and *AT*, while Higashi[9] reported a capillary tube analysis could be used for distinguishing *AQ* and *AT*. In China, Lou *et al.*[6] proposed a diagnostic table for anatomically identifying *AQ* and *AT* grown in China, though it is unclear whether these diagnostic points are effective in the identification of Mokutsu produced in Japan. In addition to those findings, Mimaki *et al.*[10] reported that the cavity area size of vessels was different between *AQ* and *AT* grown in Japan. However, no method to identify Mokutsu derived from all 3 Japanese *Akebia* taxa including *A*×*P* has been elucidated so far. In this article, an identification method for Mokutsu produced in Japan by examination of outer and inner morphologies of the woody stem is demonstrated.

Materials

Voucher specimens and the remainder of all the experimental materials are kept in a herbarium of the Faculty of Pharmaceutical Sciences, Kanazawa University [KANP].

Wild *Akebia* plant. Woody stems taken from 63 wild *Akebia* plant specimens were used for the experiments (Table 1). They were collected in 1998-2006 at prefectures in Shikoku and Nagano Pref., the major production areas of Mokutsu in Japan, and their peripheries. Most of the samples were collected in the flowering season and identified by color and form of the flowers.[4] The plants without flower were identified by the number and form of leaflets[4]; *AQ* had 5 entire leaflets, *A*×*P* had 5 lobed leaflets and *AT* had 3 lobed leaflets.

Mokutsu samples on the Japanese market. Five samples each of long woody stems with different colors were intentionally chosen from Mokutsu produced in Tokushima Pref. (T1～5), and Nagano Pref. (N1～5) for the experiments (Table 2).

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Table 1. Collection data of plant materials and anatomical characteristics of the woody stems of 3 Akebia taxa growing in Japan

Botanical name	No	Date of Collection	Collection Place	Radius[1] (mm)	Anatomical data of cork					Peeling of cork layer
					Brown substance containing cork cell		Cork stone cells and Sclerenchyma cells			
					Amount	Distribution[2] : style[3]	Amount	Distribution[2] : style[3]	Lignification	
Akebia quinata	980909-1	1998.9.9	Hosoiri, Toyama, Toyama	9.5	+++	WR : l (1-5)	+++	WR : l (S:2-3, W:1-2)	strong	++
	980909-2	1998.9.9	Osawano, Toyama, Toyama	7.0	+++	WR : pl (1-4)	+	WR : l (S:1-3, W:1-2)	strong	++
	980923-3	1998.9.23	Mugikuci, Komatsu, Ishikawa	5.5	+++	WR : l (2-5)	+++	WR : l (S:1-2, W:1-2)	strong	++
	981009-8	1998.10.9	Tomuroshinbo, Kanazawa, Ishikawa	5.0	++	NC : sca, NO : l (1-3)	+++	NC : pl, NO : l (S:1-4, W:1-2)	strong	+
	981016-1	1998.10.16	Kariyahara, Matsumoto, Nagano	13.0	+++	WR : l (2-6)	+++	WR : l (S:1-2, W:1-2)	strong	++
	981016-2	1998.10.16	Kariyahara, Matsumoto, Nagano	11.0	···	···	···	···	···	···
	981016-5	1998.10.16	Kariyahara, Matsumoto, Nagano	15.5	···	···	···	···	···	···
	990501-1	1999.5.1	Nishiki, Kanazawa, Ishikawa	5.5	+++	WR : l (1-3)	+	WR : l (S:1-3, W:1-2)	strong	++
	990503-3	1999.5.3	Kotaki, Itoigawa, Niigata	4.5	+	WR : pl (1-3)	+	NC : pl (S:1, W:2-4)	strong	−
	990508-6	1999.5.8	Kasatori-touge, Sado, Niigata	4.3	···	···	···	···	···	···
	990509-4	1999.5.9	Washizaki, Sado, Niigata	5.5	+++	WR : l (1-3)	+++	WR : l (S:1-2, W:1-4)	strong	++
	990509-6	1999.5.9	Kasatori-touge, Sado, Niigata	6.0	+	WR : pl (1-3), sca	+	WR : pl (S:1-3, W:4-9)	strong	++
	050426-4	2005.4.26	Mt.Bizan, Tokushima	5.0	+++	WR : l (1-2)	+++	WR : l (S:1-2, W:1-2)	strong	++
	050426-9	2005.4.26	Mt.Bizan, Tokushima	8.4	+++	WR : l (1-2)	+++	WR : l (S:1-3, W:1-2)	strong	++
	050426-11	2005.4.26	Mino, Miyoshi, Tokushima	6.6	+++	WR : l (1-3)	+++	WR : l (S:1-2, W:1-2)	strong	++
	050426-14	2005.4.26	Ikeda, Miyoshi, Tokushima	5.3	+++	WR : l (1-5)	+++	WR : l (S:1-3, W:1-2)	strong	++
	050426-17	2005.4.26	Nishiiyayama, Miyoshi, Tokushima	8.3	+++	WR : l (1-2)	+++	WR : l (S:1-3, W:1-2)	strong	++
	050427-18	2005.4.27	Haruno, Agawa, Kouchi	6.3	−	···	+	WR : sl	weak	−
	050427-20	2005.4.27	Haruno, Agawa, Kouchi	6.3	+++	WR : l (1-3)	+	NC : pl (S:1-3, W:2-3)	strong	+
	050427-39	2005.4.27	Tsuno,Takaoka, Kouchi	6.5	+++	WR : l (1-2)	+++	WR : l (S:1-2, W:1-2)	strong	++
	050427-42	2005.4.27	Kouchi	9.2	+++	WR : l (1-3)	+++	WR : l (S:1-4, W:1-2)	strong	++
	050428-46	2005.4.28	Tamatani, Matsuyama, Ehime	7.3	+++	WR : l (2-3)	+	WR : l (S:1-2, W:1-2)	strong	++
	060423-9	2006.4.23	Mt.Hutatabi, Koube, Hyogo	4.2	+++	WR : l (2-3)	+++	WR : l (S:1-4, W:1-3)	strong	++
	060424-4-1	2006.4.24	Munakata, Hukuoka	5.9	+++	WR : l (1-3)	+++	WR : l (S:1-3, W:1-2)	strong	++
	60425-51	2006.4.25	Minamiawaji, Hyougo	4.6	+++	WR : l (1-3)	+++	WR : l (S:1-4, W:1-2)	strong	++
	060427-87	2006.4.27	Ougoshi, Sakaide, Kagawa	2.9	+++	WR : l (1-2)	+++	WR : l (S:1-2, W:1-3)	strong	++
	605032	2006.5	betw.Ganmon & Sekinohana, Ishikawa	9.9	+++	WR : l (1-3)	+++	WR : l (S:1-2, W:1-2)	strong	++
Akebia trifoliata	980502-1	1998.10.9	Mt.Iouzen, Kanazawa, Ishikawa	7.5	−	···	+	WR : sl	weak	−
	980920-5	1998.9.20	Sawa, Awara, Fukui	7.5	+	NC : l (3)	+	WR : sl	weak	−
	980923-1	1998.9.23	Sawa, Awara, Fukui	5.0	+	NC : l (2-3)	+	WR : sl	weak	−
	981006-1	1998.10.6	Notojimasuso, Nanao, Ishikawa	7.5	−	···	+	WR : sl	weak	−
	981009-2	1998.10.9	Mt.Iouzen, Kanazawa, Ishikawa	6.5	···	···	···	···	···	···
	981009-3	1998.10.9	Mt.Iouzen, Kanazawa, Ishikawa	6.0	+	NO : pl (2-3)	+	WR : sl	weak	−
	981009-6	1998.10.9	Tomuroshinbo, Kanazawa, Ishikawa	6.0	+	NC : l (1-3)	+	WR : sl	weak	−
	981011-5	1998.10.11	Yoshizaki, Awara, Fukui	6.5	···	···	···	···	···	···
	981011-6	1998.10.11	Yoshizaki, Awara, Fukui	6.0	+	NC : l (3-4), MP : sca	+	WR : sl	weak	−
	981016-3	1998.10.16	Kariyahara, Matsumoto, Nagano	10.0	+	NC : pl , OP : sca	+	WR : sl	weak	−
	981016-6	1998.10. l 6	Kariyahara, Matsumoto, Nagano	12.5	−	···	+	WR : sl	weak	−
	050427-37	2005.4.27	Kouchi	7.0	+	NC : sca	+	WR : sl	weak	−
	050428-48	2005.4.28	Mt.Takanawa, Matsuyama, Ehime	5.2	+	WR : pl (1)	+++	WR : al	weak	−
	050428-54	2005.4.28	Daieiyama, Niihama, Ehime	6.3	+	NC : pl (1)	++	WR : al	weak	−
				7.6	+	WR : l (1)	+++	WR : al	weak	−
	060424-14	2006.4.24	Nojimaezaki, Awaji, Hyougo	4.0	+	NC : pl (1)	+++	WR : al	weak	−
	53	2006.5.5	Shimane, Matsue, Shimane	4.1	+	WR : pl (1-3)	+++	WR : al	weak	−
	62	2006.5.5	Mihonoseki, Matsue, Shimane	4.9	+	NO : pl (1-3)	+++	WR : al	strong	−
A. ×pentaphylla	980502-2	1998.5.2	Mt.Iouzen, Kanazawa, Ishikawa	···	+	NC : l (1-2)	+	NC : pl (1-2), OP : sl	strong	−
	980920-2	1998.9.20	Kotaki, Itoigawa, Niigata	6.0	+	WR : sca	+	MP : pl, OP : sl	strong	−
	980920-3	1998.9.20	Kotaki, Itoigawa, Niigata	8.5	+	NC : l (1-2), OP : pl (1-2)	++	WR : pl (S:1-2, W:1-2)	strong	−
	980928-1	1998.9.28	Kasatori-touge, Sado, Niigata	7.0	···	···	···	···	···	···
	981016-4	1998.10.16	Kariyahara, Matsumoto, Nagano	11.0	++~+++	NC : l (1-3), OP : pl (1-3)	+++	WR : l (S:1-3, W:1)	strong	++
	990503-1	1999.5.3	Kotaki, Itoigawa, Niigata	8.0	+	WR : sca	+	WR : sl	weak	−
	990503-2	1999.5.3	Kotaki, Itoigawa, Niigata	6.0	+	MP : l (1-2)	+	WR : sl	strong	−
				8.0	+	WR : l (1)	+	WR : l (S:1-2, W:3-4)	strong	++
	990508-2	1999.5.8	Kasatori-touge, Sado, Niigata	6.5	+	WR : pl (1-3)	+	WR : l (S:1-3, W:4-6)	strong	++
	990508-4	1999.5.8	Kasatori-touge, Sado, Niigata	10.5	+	NC : l (2-4), OP : sca	+	NC : pl (S:1-4, W:1)	strong	−
	990508-7	1999.5.8	Kasatori-touge, Sado, Niigata	7.5	+	WR : pl (1-3)	+	WR : l (S:1-3, W:3-12)	strong	++
	990508-9	1999.5.8	Kasatori-touge, Sado, Niigata	7.0	+	NC : pl (1-3), OP : sca	+	WR : l (S:1-2, W:3-4)	strong	−
	990508-11	1999.5.8	Washizaki, Sado, Niigata	6.5	+	WR : pl (1-2)	+	WR : l (S:1-2, W:1-5)	strong	++
	990508-12	1999.5.8	Washizaki, Sado, Niigata	6.0	+	MP : l (1-2), OP : sca	+	WR : l (S:1-3, W:3-5)	strong	++
	050427-41	2005.4.27	Kouchi	5.7	+	NC : pl (1)	+	WR : l (S:1-2, W:3-4)	strong	−
	050427-43	2005.4.27	Kouchi	5.3	+	NC : l (1-3)	+	NC : l (S:1, W:1-2), OP : sl	strong	−
	060424-11	2006.4.24	Nojimaezaki, Awaji, Hyogo	3.7	++	WR : pl (1-3)	+++	WR : al	strong	+
	060425-32	2006.4.25	Nakatsugawagumi, Sumoto, Hyogo	4.1	++	WR : l (1-2)	+++	WR : l (S:1-2, W:3-5)	strong	+
	63	2006.5.5	Mihonoseki, Matsue, Shimane	4.5	+	NC : l (1-2)	+++	WR : pl (S:1-2, W:2-4)	strong	−
	605033	2006.5	Notojimakuki, Nanao, Ishikawa	9.0	+	NC : l (1-3)	+	NC : l (1), OP : sl	strong	+

1) Woody stems kept in 70% ethanol soln. were measured.
2) Distribution : 'WR' indicates whole region, 'NC' near cambium, 'NO' near outside, 'MP' middle part, 'OP' other part.
3) Style : 'l' means laminar structure, 'pl' partial laminar structure, 'sca' scattered, 'sl' some cork cells are lignified, 'al' all cork cells are lignified. Parenthesized numerals shows number of cell layers per laminar layer. 'S' : strongly lignified cork stone cells and sclerenchyma cells, 'W' : weakly lignified cork stone cells and cork cells. ··· : not examined or no correspondence

Table 2-1. Collection data of Mokutsu (Mu-tong) collected in Japan and its botanical origin from our results.

Sample No.[1]	Name	Date	KANP No.	Radius[2] (mm)	The color of the bark surface			Thickness of cork layer (μm)	Ratio of the thickness of the cork layer to the radius of woody stem (%)
					*L**	*a**	*b**		
N-1	Mokutsu	1998.4.5	3843	5.7	56.43	3.93	20.34	720.9	12.6
N-2	Mokutsu	1998.4.5	3844	10.3	47.54	2.03	14.12	600.0	5.8
N-3	Mokutsu	1998.4.5	3845	12.9	45.10	3.81	12.73	284.7	2.2
N-4	Mokutsu	1998.4.5	3846	9.3	56.71	2.73	19.06	598.3	6.4
N-5	Mokutsu	1998.4.5	3847	11.2	55.26	3.95	18.68	472.5	4.2
T-1	Mokutsu	1999.3.29	3989	11.7	44.77	5.35	14.57	265.1	2.3
T-2	Mokutsu	1999.3.29	4400	10.3	41.47	4.39	12.42	265.6	2.6
T-3	Mokutsu	1999.3.29	4401	14.1	43.88	4.07	13.09	314.6	2.2
T-4	Mokutsu	1999.3.29	4409	13.0	45.28	4.52	15.30	296.6	2.3
T-5	Mokutsu	1999.3.29	4731	7.5	42.95	3.70	11.72	225.9	3.0

1) Sample No. N-1~N-5 were offered from Takagi syokai (高木商会), Nagano.
Sample No. T-1~T-5 were offered from Ogawa syoyaku (小川生薬), Tokushima.
2) Woody stems kept in 70 % ethanol soln. were measured.

Table 2-2. Collection data of Mokutsu (Mu-tong) collected in Japan and its botanical origin from our results.

Sample No.	Anatomical date of cork							Botanical origin[5]
	Brown substance containing cork cell		Cork stone cells and Sclerenchyma cells				Peeling off of cork layer	
	Amount	Distribution[3] : style[4]	Amount	Distribution[3] : style[4]	Thickness of lignified part of cork stone cells (μm)	Lignified level of cork stone cells		
N-1	+	WR : sca	+	WR : pl (S : 1-2, W : 2-3)	1.8	weak	−	*AT*
N-2	+	WR : pl (1-2)	+++	WR : pl (S : 1-2, W : 1-2), sl	5.0	strong	−	*A×P*
N-3	+++	WR : l (1-4)	+++	WR : l (S : 1-3, W : 1-2)	4.4	strong	++	*AQ*
N-4	−	···	+	WR : sl	less than 1.0	weak	−	*AT*
N-5	+	NO : pl (1-2), OP : sca	+	NO : pl (S:1-2, W:2-3), OP : sl	3.8	strong	+	*A×P*
T-1	++	NO : l (1-2)	+++	WR : l (S : 1-2, W : 1-2)	6.2	strong	++	*A×P*
T-2	+++	WR : l (1-6)	+++	WR : pl (S : 1-5, W : 2-4)	4.5	weak	++	*AQ*
T-3	++	NO : l (1-2), NC : pl (1)	+++	WR : l (S : 1-2, W : 1-3)	6.9	strong	+	*AQ*
T-4	+	MP : pl (1), OP : sca	+	NO : pl (S:1, W:1)	5.7	strong	+	*A×P*
T-5	+	NC : l (1), OP : sca	+	NC : pl (1), OP : sl	4.0	weak	−	*AT*

3) Distribution : 'WR' indicates whole region, 'NC' near cambium, 'NO' near outside, 'MP' middle part, 'OP' other part.
4) Style : 'l' means laminar structure, 'pl' partial laminar structure, 'sca' scattered, 'sl' some cork cells are lignified. Parenthesized numerals shows the number of cell layers per laminar layer. 'S' : strongly lignified cork stone cells and sclerenchyma cells, 'W' : weakly lignified cork stone cells and cork cells.
5) *AQ*: *Akebia quinata, AT*: *A. trifoliata, A×P: A.×pentaphylla.* ··· : no correspondence

Methods

External morphology. The shape of the bark surface of the woody stem was observed with the naked eye. The color of 3 randomly chosen points on the bark surface of the dried stem with diameter of more than 8 mm (*AQ*:n=25, *AT*:n=18, *A×P*:n=17) were measured by means of a colorimeter: apparatus, Minolta CR-200; lamp, pulsed xenon lamp; light, standard illuminant C, measurement diameter of the colorimeter; 8 mm. The color expression method adopted was the *L*a*b** colorimetric system (JIS Z8729)[11] of the Japanese Industrial Standards. The data were expressed as mean of values of 3 randomly chosen points, and differences between groups were analyzed by Tukey-Kramer test.

Internal morphology. Lou *et al.*[6] reported that the existence of a brown substance in cork cells and the amount and position of crystal-containing stone-cells in the pericycle[12] of woody stems were useful to discriminate Chinese *AQ* and *AT*. We also observed these characters in Japanese materials as the first step. In addition other characteristics of the cork layers were observed.

Transverse sections of woody stems (*AQ*:n=24, *AT*:n=16, *A×P*:n=18) kept in a 70% ethanol soln. were made by means of a Minot microtome. We observed the sections, or those bleached by aude-Javel solution, using an optical microscope. Sections were stained with Sudan Ⅲ and acetic methyl-green for the observation of suberized and lignified cell walls, respectively. Dried samples of Mokutsu were sectioned after softening in 70% ethanol soln., and observed by the same method. The thickest part of a typical cork layer in a section was observed and measured with an eyepiece

micrometer or by digital image measurement software (Japan Pora-dedital Corporation: Micro-analyzer, Ver.1.1c). The thickest part of the lignified cell wall of three stone cells chosen arbitrarily were measured by the software, and the mean values of the thickness was calculated. The diameter of the stem was measured using a caliper before sectioning. The measured values and the numerical values calculated from them were processed by Tukey-Kramer test. Relationship between the radius of woody stem and the thickness of cork layer were analyzed by Spearman's correlation coefficient test.

Results

External morphology of Japanese wild *Akebia* plants

1) Appearance of the outer bark surface (Fig.1). In general, the bark surface of *AQ* was rough since it was partially peeled off, while the stem surface of *AT* was somewhat smooth and glossy with some exceptions. The outer appearance of the bark of *A*×*P* was varied, and about 50% of the collected *A*×*P* samples were similar in appearance to *AQ* and the others were similar to *AT*.

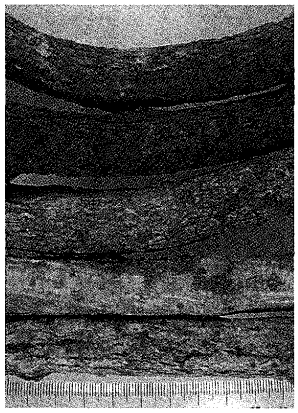
2) The color of the bark surface (Fig. 2). No significant difference was recognized between *AT* and *A*×*P* in all *L**, *a**, and *b** values. On the other hand, the *a** values of *AQ* were significantly higher than *AT* and *A*×*P* ($P<0.05$); the *a** values of more than half of the *AQ* samples indicated more than 2.6, while those of more than half of *AT* samples and *A*×*P* samples were less than 2.6. In addition to this, the *b** value for *AQ* was less than those for *AT* and *A*×*P* ($P<0.05$); about 95% of samples of *AQ* indicated less than 14.0, while about half samples of *AT* and *A*×*P* indicated less than 14.0. Moreover, the *L** value for *AQ* showed a smaller value than for *A*×*P* ($P<0.01$); about half of the samples of *AQ* indicated less than 40.0, while about 90% of samples of *AT* and *A*×*P* indicated more than 40.0.

Internal structure

1) Cork cell containing a brown substance (Table 1, Fig. 3). Lou *et al.*[6] reported that the appearance of a brown substance in the cork cell was characteristic of *AQ* in Chinese *Akebia* plants. However, we found that Japanese *AT* generally also had cork cells containing some brown substance scattered in the cork layer. In particular, the plants collected in Shimane Pref. had many cork cells containing a brown substance. While we found that Japanese *AQ* had one to three tangentially arranged continuous layers of cork cells with a brown substance much more than *AT*. In addition, the cork layer partially peeled off next to the position of the brown substance-free cell layer.

On the other hand, *A*×*P* generally had cork cells containing less brown substance, though there were some samples showing similar structure to *AT* or *AQ*.

2) Amount and position of stone cells containing crystals in the pericycle. A continuous ring consisting of alter-



Upper side:
Akebia quinata (050427-42, Kouchi)
A. quinata (060532, Ishikawa)
A. trifoliata (981016-3, Nagano)
A.×*pentaphylla* (0605033, Ishikawa)
A.×*pentaphylla* (990508-4, Niigata)

Fig. 1 The woody stems of 3 *Akebia* taxa growing in Japan
The bark surface of *Akebia quinata* is rough, while that of *A. trifoliata* is somewhat smooth and glossy with some exceptions. The outer appearance of the bark of *A.*×*pentaphylla* is widely varied, some are similar to *A. quinata* and the others to *A. trifoliata.*

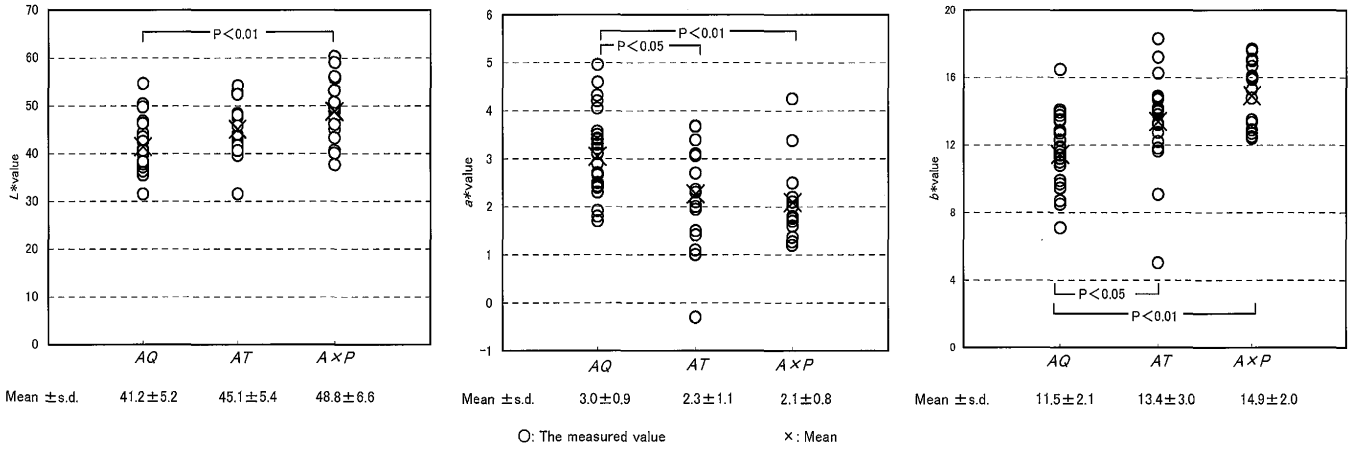


Fig. 2 Comparison of the color of the bark surface of 3 *Akebia* taxa growing in Japan
The *a** values of *Akebia quinata* are significantly higher than *A. trifoliata* and *A.*×*pentaphylla*; the *a** values of more than half of the *A. quinata* samples indicate more than 2.6, while those of more than half of *A. trifoliata* samples and *A.*×*pentaphylla* samples are less than 2.6. The *b** value for *A. quinata* is less than those for the other 2 taxa; about 95% of samples of *A. quinata* indicated less than 14.0 , while about half samples of *A. trifoliata* and *A.*×*pentaphylla* indicate less than 14.0. The *L** value for *A. quinata* shows a smaller value than for *A.*×*pentaphylla*; about half of the samples of *A. quinata* indicate less than 40.0, while about 90% of samples of *A. trifoliata* and *A.*×*pentaphylla* indicate more than 40.0. *AQ* : *A. quinata* (n=25), *AT* : *A. trifoliata* (n=18), *A*×*P* : *A.*×*pentaphylla* (n=17)

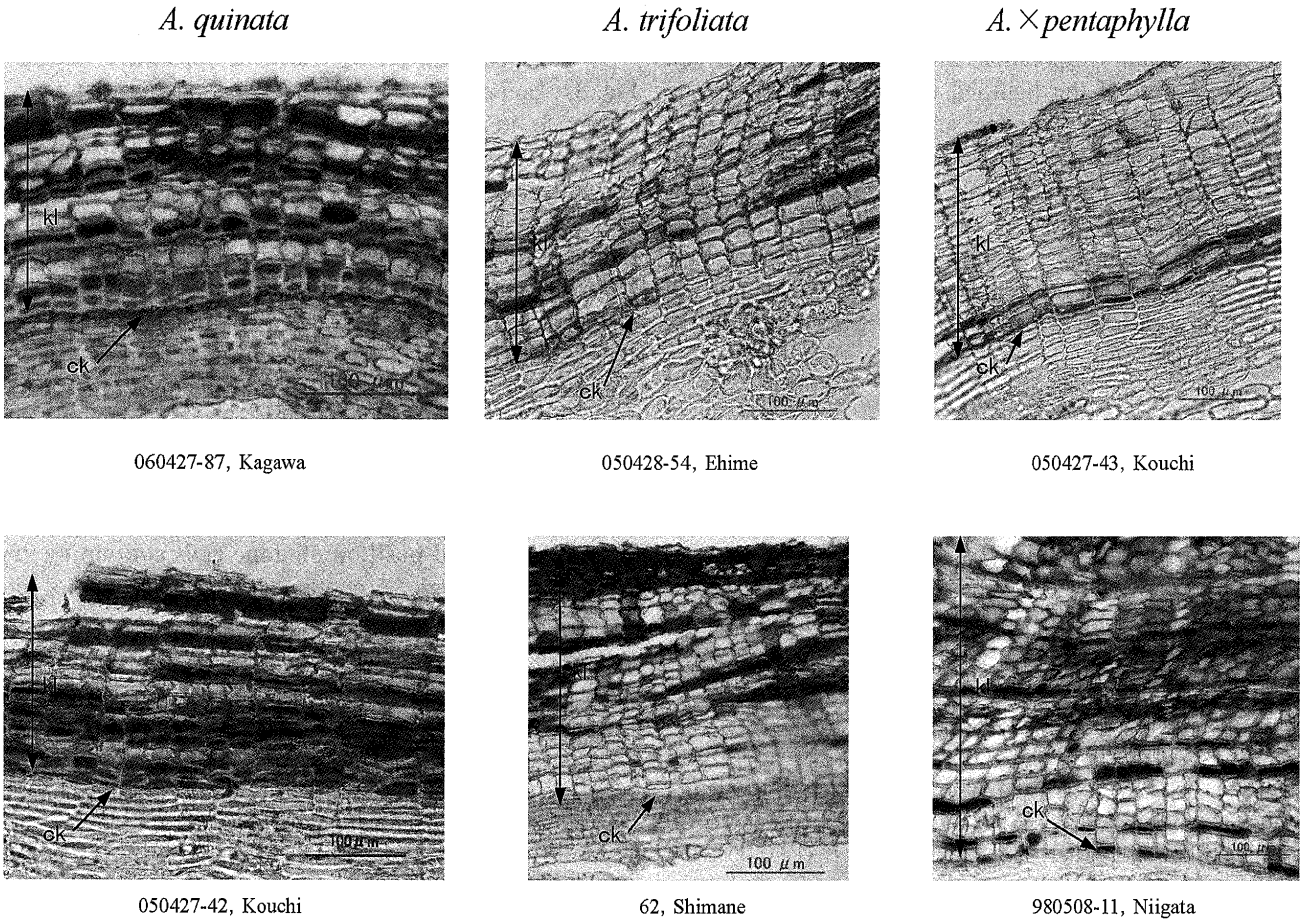


Fig. 3 Photomicrographs of transverse sections of the woody stems of 3 *Akebia* taxa growing in Japan
Akebia quinata have several tangentially arranged continuous layers of cork cells with much brown substance. On the other hand, *A. trifoliata* generally have cork cells containing fewer brown substance, though the plants collected in Shimane Pref. has exceptionally much brown substance. As to *A. ×pentaphylla*, some samples show similar structure to *A. quinata* and the other to *A. trifoliata.*
Abbreviations: ck (cork cambium), kl (cork layer)

natively arranged groups of crystal including fibers and crystal including stone cells has been recognized in the pericycle[6,12] of woody stem of *Akebia* plants. Lou *et al.*[6] reported that distribution pattern of these cell groups were specific characteristic to Chinese *Akebia* species. However, we could not find specific characteristics in Japanese species because the pattern varied widely.

3) Sclerenchyma cells and stone cells in the cork layer (Table 1, Figs. 4, 5). In the cork layer of the woody stem of *AQ*, a lamina structure was recognized. This structure was formed of layers consisting of 1-4 cells thick of sclerized and lignified cork stone cells[13] or sclerenchyma cells and 1-3 cells thick of normal cork cells or thin cell wall cork stone cells. The thickness of the lignified adaxial wall of the innermost cork stone cell in each layer was 5-8 μm, and the wall was conspicuously lignified in a fiber-like pattern, as seen in the pericycle.[6,12] The bark generally peeled off just inside of these conspicuously lignified cork stone cell layers.

On the other hand, in the case of *AT*, the cork stone cells and sclerenchyma cells were less lignified and the thickness of the lignified part of the cell wall was generally less than 3 μm, with some exceptions in the samples collected in Shimane Pref. whose lignified part of the cork stone cell wall was 5-6 μm thick. A significant difference was observed in the thickness of the lignified cell wall of *AT* and *AQ* ($P<0.01$), and the lamina structure and peeling of cork layer were not recognized in *AT*.

In the case of *A ×P*, the stone cells and sclerenchyma cells in the cork layer were rather small in number, and the stone cells were strongly lignified similar to those of *AQ*. The thickness of the lignified part of stone cell wall was about 5 μm, which was between those of *AQ* and *AT*, and significant difference was recognized between *A ×P* and the other two species ($P<0.01$). Almost all the samples showed a similar lamina structure to that of *AQ*. However, layers consisting of 3 to 6 cells thick of cork cells and weekly-lignified cork stone cells were recognized, and the lamina structure was not recognized in some samples, or appeared only near the cork cambium. No relationship was recognized between the distribution style and the thickness of the lignified wall of cork stone cells.

4) Relationship between the radius of woody stem and the thickness of cork layer (Figs. 6, 7). A positive correlation was recognized between the radius of the woody stem and the thickness of the cork layer in *AQ* and *AT* ($P<0.01$); coefficient of correlation: $r^{AQ}=0.55$, $r^{AT}=0.72$. Furthermore, the ratio of the thickness of the cork layer to the radius of

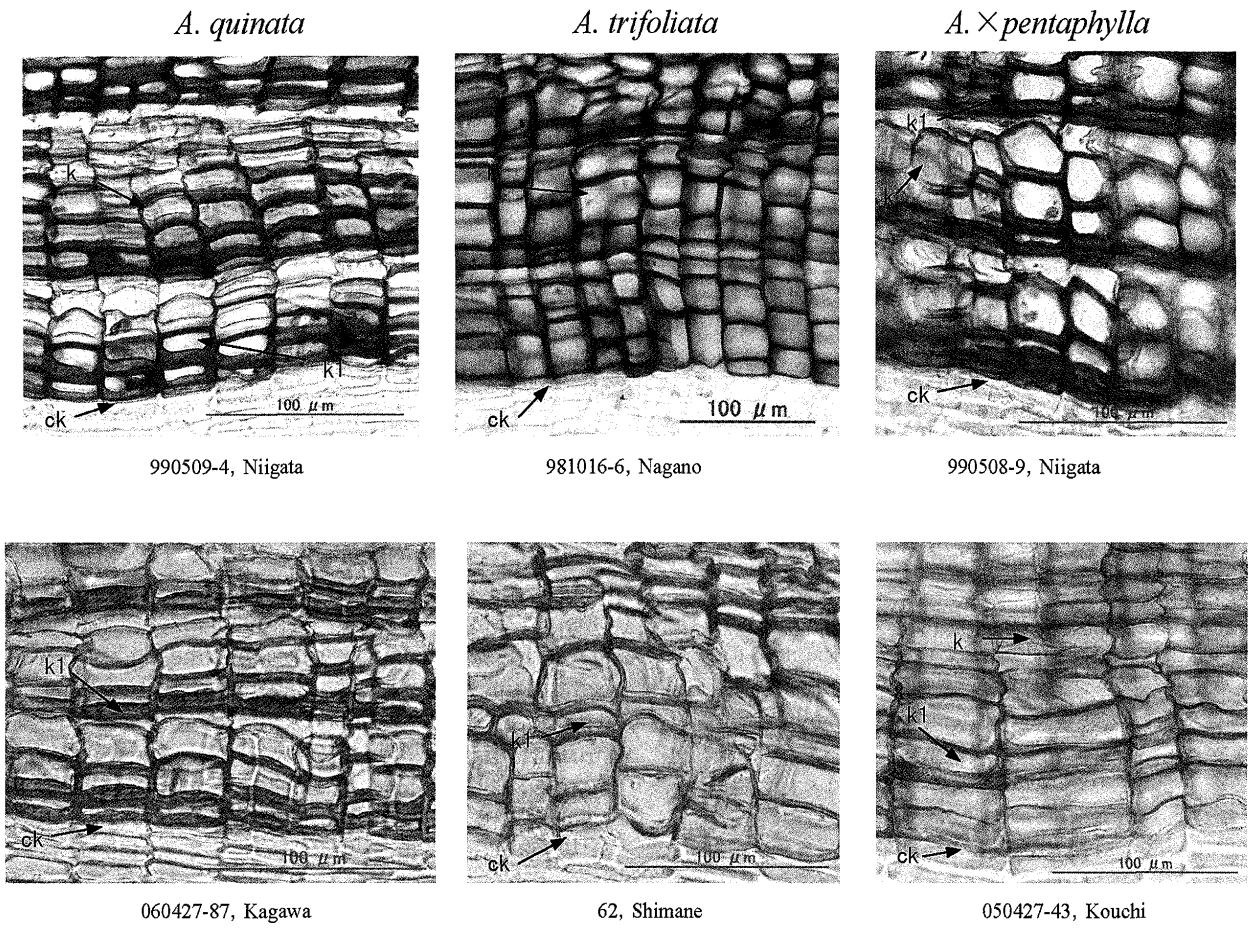


Fig. 4 Photomicrographs of dyed transverse sections of the woody stems of 3 *Akebia* taxa growing in Japan
In the cork layer of the woody stem of *Akebia quinata*, a lamina structure is recognized. This structure is formed of layers consisting of sclerized and lignified cork stone cells or sclerenchyma cells and normal cork cells or thin cell wall cork stone cells. The lamina structure is unclear in *A. trifoliata.* In the case of *A. ×pentaphylla*, almost all the samples show a similar lamina structure to that of *A. quinata.*
Abbreviations: ck (cork cambium), k1 (cork stone cell), k(cork cell)

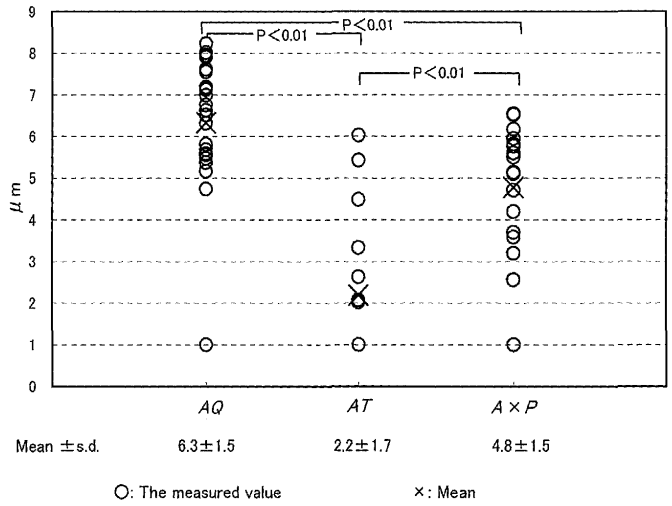


Fig. 5 Thickness of the lignified part of cork stone cell walls of woody stems of 3 *Akebia* taxa growing in Japan
The lignified cell wall of *Akebia quinata* is significantly thicker than of *A. trifoliata. A. ×pentaphylla* has the middle sized wall.
AQ : *A. quinata* (n=24), *AT* : *A. trifoliata* (n=16),
A×P : *A. ×pentaphylla* (n=18)

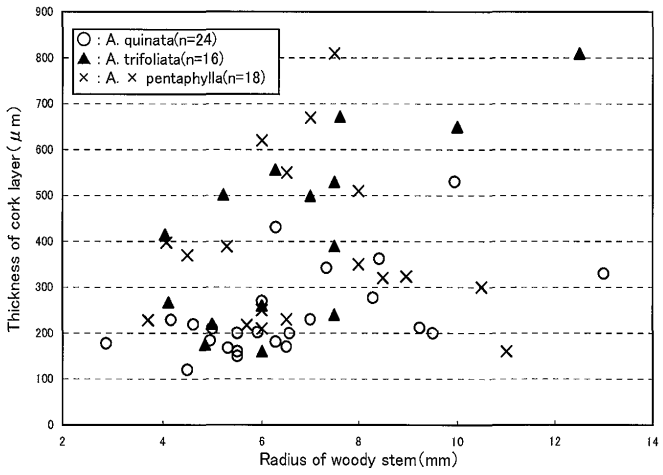


Fig. 6 Relationship between the radius of woody stems and the thickness of the cork layers of 3 *Akebia* taxa growing in Japan
A positive correlation is recognized between the radius of the woody stem and the thickness of the cork layer in *Akebia quinata* and *A. trifoliata*; coefficient of correlation: $r^{A.\ quinata}$=0.55, $r^{A.\ trifoliata}$=0.72. On the other hand, the same correlation was not recognized in *A. × pentaphylla*; coefficient of correlation: $r^{A.\times pentaphylla}$=-0.05.

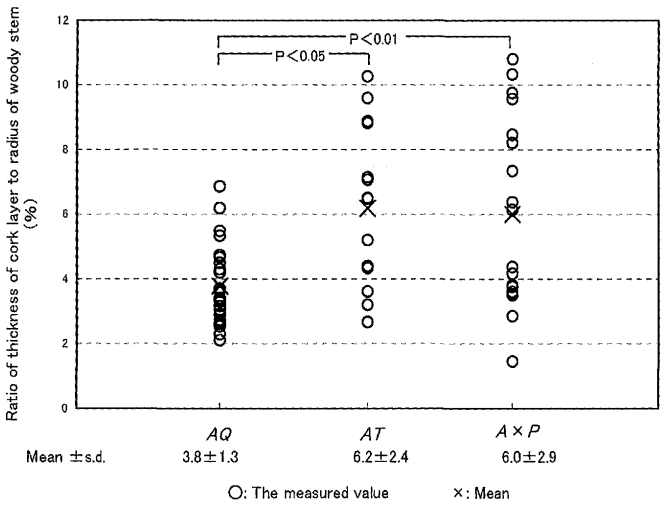


Fig. 7 Ratio of the thickness of the cork layer to the radius of woody stems of 3 *Akebia* taxa growing in Japan
The ratio of the thickness of the cork layer to the radius of the woody stem of *Akebia trifoliata* is significantly bigger than that of *A. quinata*. In addition, *A. quinata* tend to show a smaller ratio than that of *A.* × *pentaphylla*. This figure shows that all the materials showing ratios of more than 8.0 % are derived from *A. trifoliata* or *A.* ×*pentaphylla*.
AQ : *A. quinata* (n=24), *AT* : *A. trifoliata* (n=16),
A×*P* : *A.* ×*pentaphylla* (n=18)

the woody stem of *AT* was bigger than that in *AQ*; in cases where the stem had a radius of more than 9 mm, all the samples with a cork layer more than 0.6 mm thick corresponded to *AT*. On the other hand, *A*×*P* had variable cork layer thickness, and the same correlation was not recognized; coefficient of correlation: $r^{A\times P} = -0.05$. In addition, *AQ* tended to show a smaller ratio than those of either *AT* or *A*×*P* ($P<0.05$) . All the materials showing ratios of more than 8.0 % were derived from *AT* or *A*×*P*.

5) Key to the species. Based on the results described above, a key to the 3 taxon of Japanese *Akebia* plants was made (Key 1). Ninety four % of the wild *Akebia* plant examined in this study could be identified by this key.

Key 1. Key to the species of genus *Akebia*, Lardizabaraceae, growing in Japan on the basis of morphological characteristics of woody stems

A1. In the cork layer, cork stone cells and sclerenchyma cells are scarcely recognized, or a lamina structure formed by sclereid cell layers and cork parenchyma cell layers is not recognized. Cork stone cells are weakly lignified, and the thickness of the lignified part of the cell wall is ≦6.0 μm. The thickness of the cork layer is ≧0.6 mm in cases where the stem has a radius of more than 9.0 mm.
---------- *A. trifoliata*

A2. In the cork layer, a lamina structure formed by strongly lignified sclereid cells layers and cork parenchyma cells or weakly lignified cork stone cells layers is recognized. Lignified cell walls of cork stone cells are conspicuously thickened.

B1. If the sample meets more than two of the following requirements. Bark surface color: L^*≦40.0, a^*≧2.6, b^*≦14.0, and the ratio of the thickness of the cork layer to stem radius is <8.0%.
---------- *A. quinata*

B2. If the sample meets more than two of the following requirements. Bark surface color: L^*>40.0, a^*<2.6, b^*>14.0, and the ratio of the thickness of the cork layer to stem radius is ≧8.0%.
---------- *A.* ×*pentaphylla*

The morphological characteristics of Mokutsu on the market and their botanical origin (Table 2-1, 2)

By observing internal and external morphological characters of the Mokutsu produced in Japan, their botanical origins were identified based on Key 1, as follows.

Mokutsu produced in Nagano Pref.. Sample N-3, whose bark was partly peeled off and the surface was rough, had many cork cells containing a brown substance and cork stone cells in the cork layer. The lamina structure was formed by sclereid cell layers and normal cork cell layers. In addition, the cork stone cell was conspicuously lignified and had extremely thick cell wall. The a^* value of bark surface color was 3.81 and the b^* value was 12.73. The ratio of the thickness of the cork layer to the stem radius was 2.2%. These characteristics corresponded well with those of *AQ*.

On the other hand, of the 4 samples with smooth surfaces, N-1 and N-4 corresponded well with *AT*; cork stone cells and sclerenchyma cells in the cork layer were small, and almost no lamina structure was recognized. Lignification of the cork stone cell was weak and the lignified wall was below 2μm in thickness. N-2 and N-5 corresponded well with *A*×*P* with the cork layer showed a partial lamina structure, lignification of cork stone cells was strong and lignified cell walls were conspicuously thickened, the L^* value of bark surface color indicated more than 40.0 and the b^* value more than 14.0.

Mokutsu produced in Tokushima Pref.. The surfaces of all the samples from Tokushima Pref. were rather rough. Samples T-1, T-2, and T-3 had many cork stone cells and sclerenchyma cells in the cork layer, and the cork layer showed a lamina structure and the lignified cell walls of cork stone cell were conspicuously thickened. The b^* value of T-1 was more than 14.0, but those of T-2 and T-3 were below 14.0. Therefore, the former was identified as *A*×*P* and the latter as *AQ*, though the L^* values of all samples were more than 40.0 and the a^* values more than 2.6. Sample T-4 was identified as *A*×*P*, because the cork stone cells were extremely lignified and the L^* and a^* values of stem surface were more than 40.0 and 14.0, respectively, though the cork stone cells and sclerenchyma cells were small in number and a lamina structure were partly recognized in the outer part of the cork layer. On the other hand, sample T-5 was identified as *AT* based on the characteristics that its cork stone cells was lower in number and weakly lignified, though the cork stone cells showed a lamina structure near the cork cambium.

Conclusion and Discussion

1. We have identified the specific characteristics of the outer and inner morphologies of the woody stems of Japanese 3 *Akebia* taxa, *A. quinata* Decaisne, *A. trifoliata* Koidzumi and *A.* × *pentaphyla* Makino of the family Lardizabalaceae. Morphologically, the outer bark surface of *AQ* was dark reddish in color, rough, and partly peeled off. On the other hand, the bark surface of *AT* was bright and yellowish in color, and was somewhat smoother than *AQ*. The color of the bark surface of *A*×*P* was similar to of *AT*.

In terms of inner morphology, *AQ* has cork cells with substantial amounts of a brown substance, and a lamina structure formed with tangentially arranged cell layers of strongly lignified sclerenchyma cells and cork parenchyma cells or weakly lignified stone cells. *AT* had less of the brown substance and scarcely any cork stone cells and sclerenchyma cells, or the cork stone cells were weakly lignified, if any, and the lamination of the sclerenchyma cells layer and normal cork cells layer was unclear.

2. From molecular-biological studies *A*×*P* has been reported as a hybrid of *AQ* and *AT*.[14] Anatomical results in this study also agreed well with the assertion that *A*×*P* is a hybrid variety, having some intermediate structures between *AQ* and *AT* in the cork layer, though the color of the bark and the ratio of the thickness of the cork layer to the radius of the stem were rather similar to those of *AT*, and the cork stone cells were strongly lignified similar to those of *AQ*.

3. A key to the 3 taxa based on anatomical characteristics is shown in Key 1. More than 90% of samples examined in this study could be identified by this key. On the other hand, it was impossible to distinguish *AQ* and *AT* growing in Japan clearly by stem anatomy based on the characteristics reported by Lou et al.[6] for Chinese *Akebia* plants, such as the existence or not of a brown substance in cork cells and the amount and position of crystal-containing stone-cells in the pericycle.[12]

4. Mokutsu circulating in the Japanese market was identified by Key 1 as follows: of five samples from Nagano Pref., one was *AQ*, two were *AT*, and the other two were *A*×*P*; while of five from Tokushima Pref., two were *AQ*, two were *A*×*P*, and one was *AT*. This is the first report that the stem of *A*×*P* is circulating in the market as Mokutsu. In addition to this, *A*×*P* is thought to be commonly circulated in the market, because two samples of *A*×*P* were found in each lot of Mokutsu produced in Nagano and Tokushima Pref.

5. The function of cork layers, or periderm, is the protection of the inner part as a substitute for the epidermis,[15] and the brown substances, they are regarded as tannin,[13] in the cork cells observed frequently in the stem of *AQ* are regarded as enhancing this function.[16] In the case of *AT*, we think that the brown substance is not necessary because the thicker cork layer sufficiently protects the inner tissues from the damage caused by insects or microbes.

Acknowledgement

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Japanese abstract

漢薬「木通」は，日局でその基源がアケビ科のアケビ又はミツバアケビの蔓性の茎であると規定され，わが国での国内需要のすべては国内野生品でまかなわれている。日本に分布する本属植物は主にアケビ，ミツバアケビ及びこれらの雑種とされているゴヨウアケビの3分類群であるが，生薬について種の区別はなされておらず，ゴヨウアケビ由来のものも流通している可能性が高い。また，アケビとミツバアケビについて薬理作用や成分に差が認められ，同属植物内でも種による使い分けの必要性が示唆されている。しかし，日本産につ

いては生薬の形態から原植物の鑑別は不可能であるとされてきた。そこで，日本産本属植物3分類群の木質茎の形態学的特徴を検討し，市場品の基源鑑別法の確立を試みた。その結果，木質茎表面の色，コルク組織の構造及び茎の半径とコルク層の厚みの関係において相違が認められ，90％以上の精度で3分類群を識別できる検索表を作成した。これにより，日本産「木通」について原植物の識別がほぼ可能になり，日本産「木通」の主産地である信越及び四国産にゴヨウアケビ由来品が流通していることが示唆された。

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