43.2, and 54.0 μM) of the starter substrate (cinnamoyl-CoA, cinnamoyl-NAC, or diketide NAC) in the assay mixture, containing 108 μM of malonyl-CoA, 5 μg of purified enzyme, 1 mM EDTA, in a final volume of 500 μL of 100 mM Tris–HCl buffer, pH 7.5. Incubations were carried out at 30 °C for 20 min. The reaction products were extracted and separated by TLC (Merck Art. 1.11798 Silica gel 60 F_{254}; ethyl acetate/hexane/AcOH = 63:27:5, v/v/v). Radioactivities were quantified by autoradiography using a bioimaging analyzer BAS-2000II (Fujifilm). Lineweaver–Burk plots of data were employed to derive the apparent K_M and k_{cat} values (average of triplicates ± standard deviation) using EnzFitter software (BIOSOFT).

HPLC and HPLC-ESIMS. The enzyme reaction products were separated by reverse-phase HPLC (JASCO 880, JASCO) on TSK-gel ODS-80Ts column (4.6 × 150 mm, TOSOH) with a flow rate of 0.8 ml/min. Elutions were monitored by a multichannel UV detector (MULTI 340, JASCO) at 290, 330, and 360 nm; UV spectra (198–400 nm) were recorded every 0.4 s. Gradient elution was performed with H_2O and MeOH, both containing 0.1% TFA: 0–5 min, 30% MeOH; 5–17 min, linear gradient from 30% to 60% MeOH; 17–25 min, 60% MeOH; 25–27 min, linear gradient from 60% to 70% MeOH.

On-line HPLC-ESIMS spectra were measured with a Hewlett–Packard HPLC 1100 series (Wilmington, DE) coupled to a Finnigan MAT LCQ ion trap mass spectrometer (San Jose, CA) fitted with an ESI source. HPLC separations were carried out under the same conditions as described above. The ESI capillary temperature and capillary voltage were 225 °C and 3.0 V, respectively. The tube lens offset was set at 20.0 V. All spectra were obtained in the negative and positive modes; over a mass range of *m/z* 100–500, at a range of one scan every 2 s. The collision gas was helium, and the relative collision energy scale was set at 30.0% (1.5 eV). Product analysis was carried out as described before by direct comparison with the authentic compound [17,18].

PKR enzyme reaction. The reaction mixture contained 108 μM of substrate (cinnamoyl-CoA, cinnamoyl-NAC, or diketide-NAC), 1 mM NADPH, and 6 μg of the purified enzyme in a final volume of 500 μL of 100 mM potassium phosphate buffer, pH 6.5, containing 1 mM EDTA. Incubations were carried out at 30 °C for 20 min to overnight. The products were then extracted with 1 mL of ethyl acetate (2×), concentrated by N_2 flow, and separated by reverse-phase HPLC (TSK-gel ODS-80Ts, 4.6 × 150 mm; 40% MeOH; 0.8 ml/min).

CHS/PKR enzyme reaction. The reaction mixture contained 54 μM of starter substrate (cinnamoyl-CoA, cinnamoyl-NAC, or diketide-NAC), 108 μM of malonyl-CoA, 1 mM NADPH, and 5 μg of *S. baicalensis* CHS, and 60 μg of *G. echinata* PKR in a final volume of 500 μL of 100 mM potassium phosphate buffer, pH 7.0, containing 1 mM EDTA. Incubations were carried out at 30 °C for 20 min to overnight and stopped by adding 50 μL of 20% HCl. The products were then extracted with 1 mL of ethyl acetate (2×), concentrated by N_2 flow, and analyzed by the reverse-phase HPLC as described above for the CHS enzyme reaction.

Results and discussion

Enzymatic formation of chalcone from NAC-thioesters by CHS

Although it has been reported that plant type III polyketide synthases including CHS from *Pinus sylvestris* accepted NAC-thioesters as a substrate [25], detailed analysis of enzyme kinetics as well as complete characterization of the enzyme reaction products have thus far not been carried out. Our TLC based assay (Fig. 3) and the LC-ESIMS analyses of the enzyme reaction products unambiguously demonstrated that purified recombinant *S. baicalensis* CHS [17,18] readily accepted

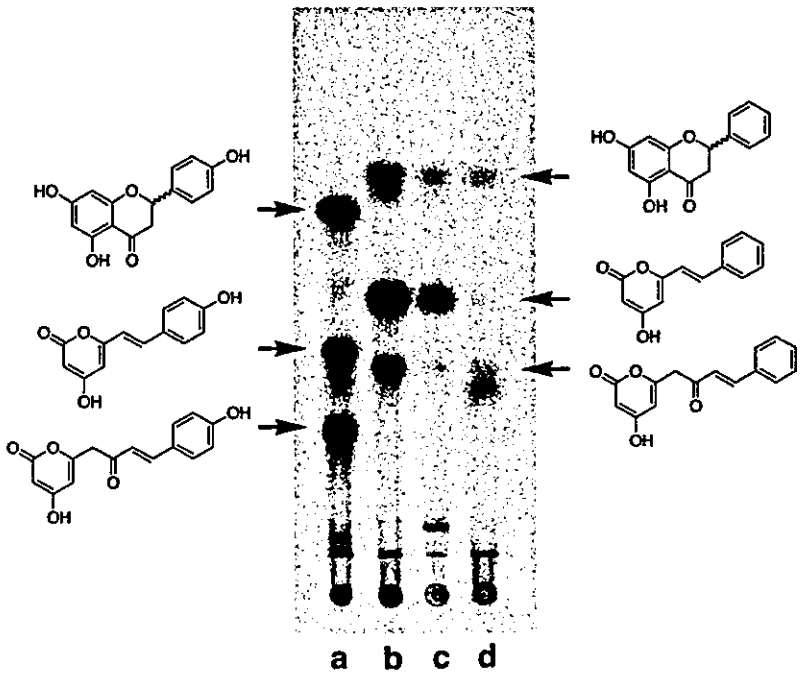

Fig. 3. TLC analysis (autoradiography) of the CHS enzyme reaction products from [2-^{14}C]malonyl-CoA and (a) 4-coumaroyl-CoA (**1a**); (b) cinnamoyl-CoA (**1b**); (c) cinnamoyl-NAC (**6**); and (d) cinnamoyl diketide-NAC (**7**). Note that by acid treatment chalcones are converted to racemic flavanones through a non-stereospecific ring-C closure.

both cinnamoyl-NAC (**6**) and cinnamoyl diketide-NAC (**7**) as a starter substrate, and carried out sequential decarboxylative condensations with malonyl-CoA to produce 2′,4′,6′-trihydroxychalcone (pinocembrin chalcone) (**3b**). In addition, as in the case of enzyme reaction with the regular starter CoA esters, formation of the early released derailment by-products, tetraketide and triketide α-pyrone (**4a** and **5b**) [3,4], was also detected in the assay mixture. However, interestingly, no evidence was obtained for the formation of 4-(phenyl)-but-3-en-2-one (benzalacetone), derived from decarboxylation of the diketide-NAC [14,15].

Table 1
Steady-state kinetic parameters for CHS enzyme reactions[a]

Starter substrate	k_{cat} (min^{-1})	K_M (μM)	k_{cat}/K_M ($s^{-1} M^{-1}$)
Cinnamoyl-CoA	0.72 ± 0.02	6.27 ± 1.02	1920
Cinnamoyl-NAC	0.53 ± 0.02	6.30 ± 1.26	1390
Cinnamoyl diketide-NAC	0.23 ± 0.00	9.28 ± 0.71	413

[a] Steady-state kinetic parameters were calculated for formation of pinocembrin chalcone (actually the flavanone rather than the chalcone was quantified). Lineweaver–Burk plots of data were employed to derive the apparent K_M and k_{cat} values (average of triplicates ± standard deviation) using EnzFitter software (BIOSOFT).

Steady-state kinetic analysis revealed that the recombinant *S. baicalensis* CHS accepted cinnamoyl diketide-NAC (**7**) with less efficiency than cinnamoyl-CoA (Table 1). The K_M value of **7** was 1.5-fold higher, while the k_{cat}/K_M value was only 21% of that of cinnamoyl-CoA. In an earlier report, it was roughly estimated that *P. sylvestris* CHS accepted cinnamoyl diketide-NAC with 60–70% efficiency when compared with cinnamoyl-CoA [25]. In contrast, it was remarkable that cinnamoyl-NAC (**6**), the simple NAC-thioester of cinnamic acid, primed the CHS enzyme reaction almost as efficiently as cinnamoyl-CoA; it showed the k_{cat}/K_M value as much as 72%, while the K_M value was almost as equal as that of cinnamoyl-CoA.

The unusually broad substrate specificities toward the NAC-thioesters provided further mechanistic and stereochemical insights into the polyketide formation reactions of the CHS enzyme. It appears that the 3′-phosphoadenosine diphosphate moiety of the CoA molecule (Fig. 2) is not essential for the substrate recognition. Instead, the NAC moiety seems to be just

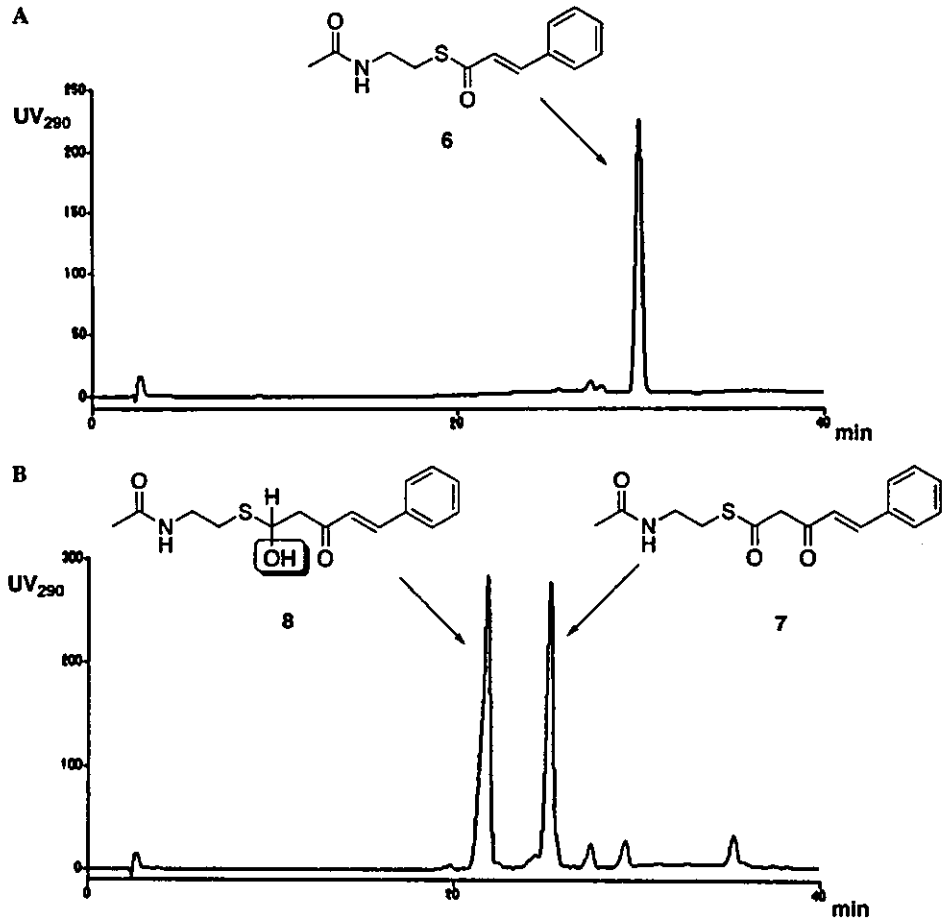

Fig. 4. HPLC profile of the enzyme reaction products from (A) cinnamoyl-NAC (**6**); (B) cinnamoyl diketide-NAC (**7**) by *G. echinata* PKR.

enough for the loading of the starter substrate to the active site of the enzyme, while recently reported crystal structure of CHS from *Medicago sativa* revealed a CoA binding tunnel at the substrate entrance of the active site of the enzyme [5]. This suggests that chemically synthesized NAC-thioesters, instead of CoA-ester analogues, would be excellent probes to further explore the catalytic potential of the enzyme to produce unnatural and novel polyketides.

Enzymatic formation of 6′-deoxychalcone from NAC-thioesters by co-action of CHS and PKR

It was for the first time demonstrated that the diketide-NAC (**7**), the NAC derivative of the polyketide intermediate of the CHS enzyme reaction, was also accepted as a substrate by recombinant *G. echinata* PKR [30,31] to produce the corresponding β-ketohemithioester (**8**) in the presence of NADPH (Fig. 4B). The structure of the reduced dikteide was confirmed by LC-ESIMS, which gave a parent ion peak $[M+H]^+$ at *m/z* 294, 2 Da higher than the unreduced form. In contrast, neither cinnamoyl-NAC (**6**) nor cinnamoyl-CoA (**1b**) was accepted as a substrate by PKR, suggesting that presence of the β-ketocarbonyl moiety is essential for the enzyme reaction, while, as in the case of the above-mentioned CHS, the 3′-phosphoadenosine diphosphate moiety of the CoA molecule is not required for the substrate recognition (Fig. 2). On the other hand, it was also confirmed that *G. echinata* PKR did not accept malonyl-CoA (**2**), 4,2′,4′,6′-tetrahydroxychalcone (**3a**), and 2′,4′,6′-trihydroxychalcone (**3b**) as a substrate.

By co-action of *S. baicalensis* CHS and *G. echinata* PKR, both cinnamoyl diketide-NAC and cinnamoyl-NAC were converted to 2′,4′-dihydroxychalcone (a 6′-deoxychalcone) (**3c**) in the presence of NADPH and

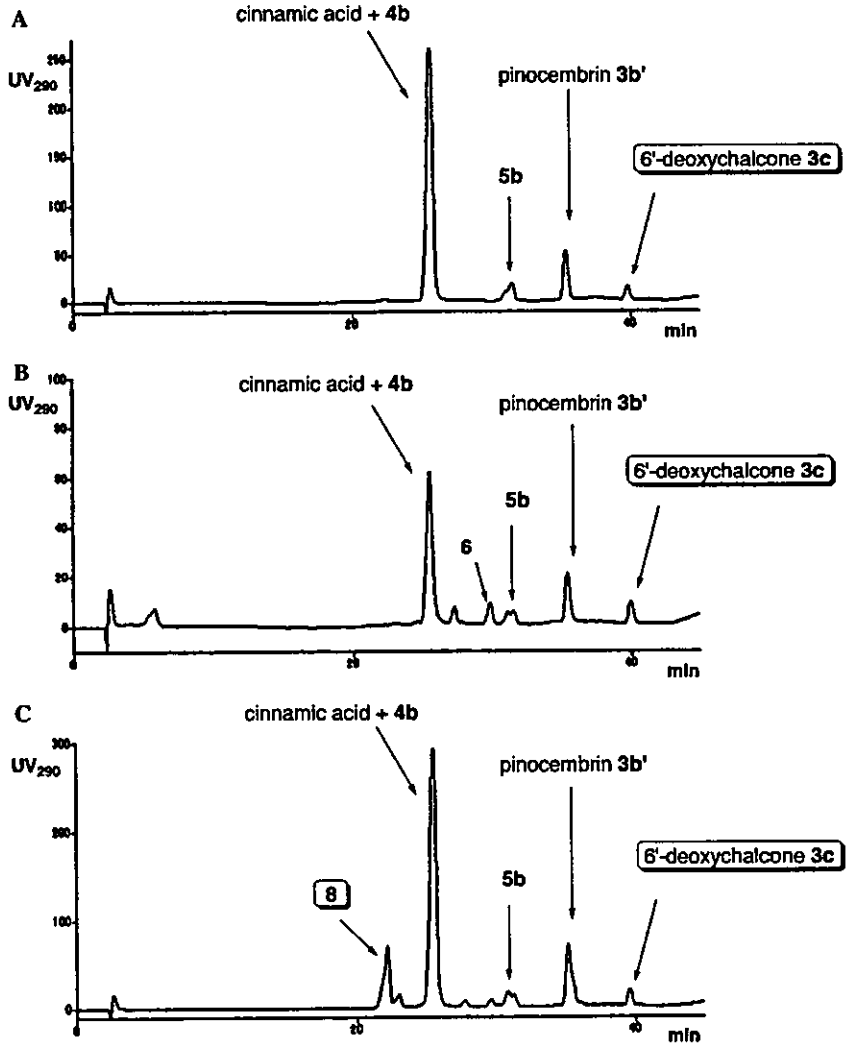

Fig. 5. HPLC profile of the CHS/PKR enzyme reaction products from malonyl-CoA and (A) cinnamoyl-CoA (**1b**); (B) cinnamoyl-NAC (**6**); and (C) cinnamoyl diketide-NAC (**7**).

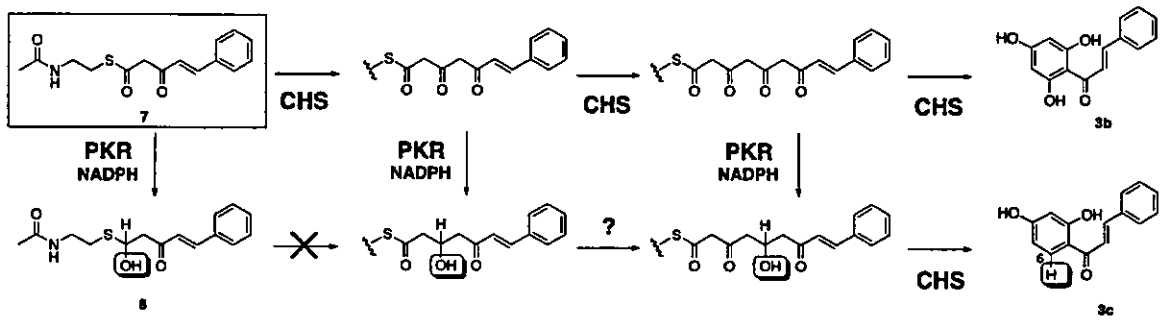

Fig. 6. Proposed mechanism for formation of a 2′,4′-dihydroxychalcone (a 6′-deoxychalcone) (3c) from cinnamoyl diketide-NAC (7) by the co-action of CHS and PKR. Note that reduction of the carbonyl group of the diketide intermediate would interrupt further polyketide chain elongation reactions catalyzed by CHS.

malonyl-CoA, just as in the case of cinnamoyl-CoA (Fig. 5). In addition, 2′,4′,6′-trihydroxychalcone (a 6′-hydroxychalcone) (**3b**) was also obtained as a major product along with tetraketide (**4b**) and triketide α-pyrone (**5b**), however, interestingly, formation of deoxy-type triketide and deoxy-type tetraketide α-pyrone was not detected by LC-ESIMS analysis (Fig. 5). It was thus demonstrated that the CHS/PKR enzyme reactions always produced both 6′-deoxy and hydroxy products, suggesting an intrinsic property of the interaction between the dimeric CHS and the monomeric PKR.

Interestingly, the once reduced cinnamoyl diketide-NAC (**8**), which was prepared by enzymatic conversion of the diketide-NAC by PKR (Fig. 4B), was found to be no longer a substrate for the CHS enzyme reaction; when incubated with CHS, **8** was recovered unchanged in the reaction mixture. It is thus likely that the reduction of a carbonyl group of the diketide or triketide intermediate would interrupt further polyketide chain elongation reactions catalyzed by CHS. The observations suggest that, during the 6′-deoxychalcone formation reaction, the PKR-catalyzed reduction of a linear polyketide intermediate may possibly take place at the tetraketide stage prior to the CHS-catalyzed cyclization reaction (Fig. 6).

Although precise molecular interactions for the formation of the 6′-deoxychalcone by the co-action of the CHS/PKR enzyme still remain to be elucidated, the buried nature of the CHS active site precludes PKR from accessing the CHS-enzyme-bound polyketide intermediate [2]. It is thus presumable that a portion of CoA-linked polyketide intermediates are once released from the active site of CHS, diffused into the surrounding solution, and get reduced to the corresponding β-ketoalcohol by PKR that catalyzes transfer of the *pro-R* hydrogen of NADPH [27]. The reduced linear tetraketide is then reloaded onto the cyclization pocket of the active site of CHS, where the Claisen-type cyclization leads to formation of the new aromatic ring system of the 6′-deoxychalcone. The mixed formation of the 6′-deoxy and hydroxy-type products in the assay mixture would support this hypothesis. To further elucidate the catalytic mechanism of the CHS/PKR enzyme reaction, structure–function analysis of *G. echinata* PKR is now in progress in our laboratories.

Acknowledgments

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A Plant Type III Polyketide Synthase that Produces Pentaketide Chromone

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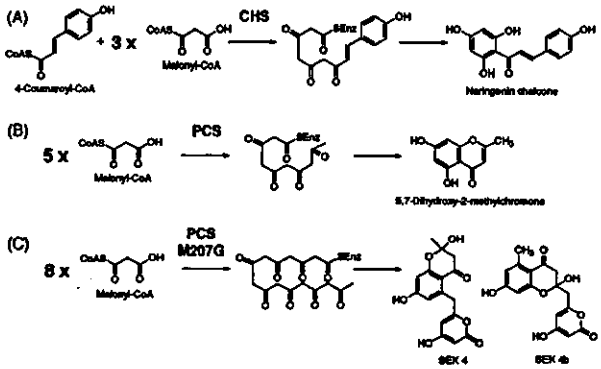
A growing number of functionally diverse type III polyketide synthases (PKSs), the chalcone synthase (CHS) (EC 2.3.1.74) superfamily enzymes, have been cloned and sequenced from various plants,[1] which include recently reported a diketide (benzalacetone)[2a,b] and a heptaketide (aloesone) synthase (ALS)[2c] from *Rheum palmatum*. In addition, bacterial type III PKSs, such as a pentaketide 1,3,6,8-tetrahydroxynaphthalene-producing enzyme, have been also reported.[3,4] The CHS-superfamily enzymes are structurally and mechanistically distinct from the type I (modular type) and type II (subunit type) PKSs, using free CoA thioesters as substrates without the involvement of acyl carrier protein, to carry out a complete series of decarboxylation, condensation, cyclization, and aromatization reactions with a single active site. The homodimer of 40–45 kDa proteins typically selects 4-coumaroyl-CoA as a starter and performs up to three condensations with malonyl-CoA to produce naringenin chalcone, (4,2′,4′,6′-tetrahydroxychalcone), which is the biosynthetic precursor of flavonoids (Scheme 1A). Recent crystallographic and site-directed mutagenesis studies have revealed structural and functional details of the plant and bacterial type III PKSs.[1–6]

Here we report a novel plant-specific type III PKS that catalyzes formation of a pentaketide chromone, 5,7-dihydroxy-2-methylchromone, from five molecules of malonyl-CoA (Scheme 1B). Remarkably, replacement of a single amino acid residue Met207 (corresponding to the *Medicago sativa* CHS active-site residue Thr197) yielded a mutant enzyme that efficiently produces aromatic octaketides, SEK4 and SEK4b, the products of the minimal PKS for the benzoisochromanequinone actinorhodin (*act* from *Streptomyces coelicolor*)[7] (Scheme 1C). A cDNA encoding the pentaketide chromone synthase (PCS) (the GenBank accession no. AY823626) was cloned and sequenced from young roots of aloe (*Aloe arborescens*), a medicinal plant rich in aromatic polyketides including chromones and anthraquinones, by RT-PCR using degenerate primers based on the conserved sequences of known CHSs as described before.[2] A 1212-bp open reading frame encoded a M_r 44,568 protein with 403 amino acids. The deduced amino acid sequence showed 50–60% identity to those of CHS-superfamily enzymes from other plants (58% identity (232/403) with *M. sativa* CHS,[5a] and 50% identity (206/403) with *R. palmatum* ALS[2c] that catalyzes formation of a heptaketide, aloesone (2-acetonyl-7-hydroxy-5-methylchromone), from acetyl-CoA and six molecules of malonyl-CoA). *A. arborescens* PCS maintains an almost identical CoA binding site, and the catalytic triad of Cys164, His303, and Asn336 (numbering in *M. sativa* CHS) is absolutely conserved in all type III PKSs. Furthermore, most of the active-site residues including Met137, Gly211, Gly216, Pro375, as well as Phe215, and Phe265,[1] are conserved in PCS (Figure 1). The CHS-based homology modeling predicted that *A. arborescens* PCS has the same three-dimensional overall fold as *M. sativa* CHS,[5a] with the total cavity volume (1124 Å^3) slightly larger than that of CHS (1019 Å^3) and almost as large as that of *R. palmatum* ALS (1173 Å^3).[2c]

Scheme 1. Formation of Polyketides by CHS-Superfamily Enzymes



Recombinant PCS was heterologously expressed in *Escherichia coli* BL21(DE3)pLysS as fusion protein with GST at the N-terminal (pET vector). After cleavage of the GST-tag, the purified enzyme gave a single band with molecular mass of 44 kDa on SDS-PAGE, while the native PCS appeared to be a homodimer since it had molecular mass of 88 kDa as determined by gel filtration. *A. arborescens* PCS efficiently accepted malonyl-CoA as a sole substrate to yield a single product with a parent ion peak $[M + H]^+$ at *m/z* 193 on LC-ESIMS. Spectroscopic data (^{1}H NMR, LC-MS, and UV) of the product obtained from a large-scale enzyme reaction (1.0 mg from 20 mg of malonyl-CoA) are completely identical with those of an authentic 5,7-dihydroxy-2-methylchromone. The aromatic pentaketide has been isolated from several plants and is known to be a biosynthetic precursor of khellin and visnagin, the anti-asthmatic furochromones found in *Ammi visnaga*.[8] Interestingly, acetyl-CoA, resulting from decarboxylation of malonyl-CoA, was also accepted as a starter substrate but not so efficiently as in the case of *R. palmatum* ALS.[2c] This was confirmed by the ^{14}C incorporation rate from [1-^{14}C]acetyl CoA in the presence of cold malonyl-CoA, while the yield of the pentaketide from [2-^{14}C]malonyl-CoA was almost at the same level in the presence or absence of cold acetyl-CoA in the reaction mixture. The recombinant PCS showed the K_M = 71.0 μM and k_{cat} = 445 × 10^{-3} min^{-1}, with a broad pH optimum within a range of 6.0–8.0. On the other hand, like other type III PKSs,[1,9] *A. arborescens* PCS showed the promiscuous substrate specificity; the enzyme also accepted aromatic (4-coumaroyl, cinnamoyl, and benzoyl) and aliphatic (*n*-hexanoyl, *n*-octanoyl, and *n*-decanoyl) CoA esters as a starter substrate; however, it yielded only triketide and teraketide α-pyrones.

One of the characteristic features of *A. arborescens* PCS is that the CHS active-site residues, Thr197, Gly256, and Ser338 (numbering in *M. sativa* CHS),[5a] are uniquely replaced with Met, Leu, and Val, respectively. Interestingly, the three residues are also missing in the heptaketide-forming *R. palmatum* ALS[2c] (T197A/G256L/S338T), and in *Gerbera hybrida* 2-pyrone synthase (2PS)[6] (T197L/

10.1021/ja0431206 CCC: $30.25

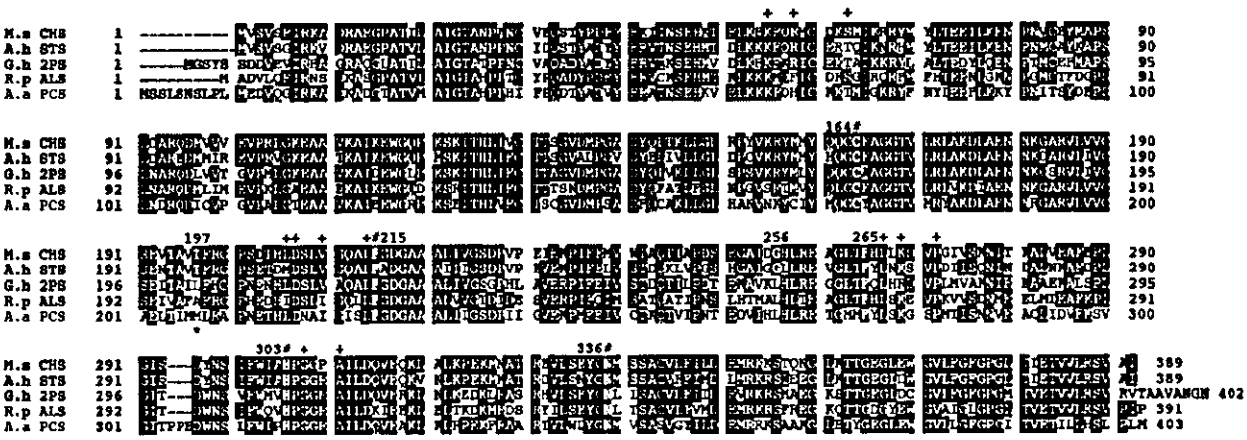


Figure 1. Comparison of primary sequences of *A. arborescens* PCS and other CHS-superfamily enzymes. M.s CHS, *M. sativa* CHS; A.h STS, *Arachis hypogaea* stilbene synthase; G.h 2PS, *G. hybrida* 2PS; R.p ALS, *R. palmatum* ALS. The active-site residues conserved in the CHS-superfamily enzymes (Cys164, Phe215, His303, and Asn336, numbering in *M. sativa* CHS) are marked with #, and residues for the CoA binding, with +.

G256L/S338I) that also selects acetyl-CoA as a starter to produce a triketide pyrone. A CHS triple mutant (T197L/G256L/S338I) has been shown to yield an enzyme that was functionally identical to 2PS, suggesting the substitutions are responsible for the starter substrate specificity of the enzymes.[6b] To test the hypothesis, a mutant enzyme was constructed in which Met207 (corresponding to Thr197 in CHS) was replaced by Thr. However, the point mutation did not significantly affect the enzyme activity; PCS M207T mutant was functionally almost identical to the wild-type PCS. In contrast, when Met207 was substituted with Gly, there was a dramatic change in the enzyme activity; PCS M207G mutant efficiently afforded two new products with a parent ion peak $[M + H]^+$ at *m/z* 319 on LC-ESIMS, which were identified as aromatic octaketides SEK4 and SEK4b (ratio 1:4), the shunt products of the minimal type II PKS for actinorhodin,[7] by direct comparison with authentic compounds. Here formation of only a trace amount of 5,7-dihydroxy-2-methylchromone was detected by LC-MS. The pentaketide-forming PCS was thus transformed into an octaketide-producing enzyme by the single amino acid mutation. This is the first demonstration of a type III PKS catalyzing seven successive polyketide chain elongation reactions.

In conclusion, *A. arborescens* PCS is a novel plant-specific type III PKS that produces an aromatic pentaketide from five molecules of malonyl-CoA. Site-directed mutagenesis revealed that Met207 determines the polyketide chain length and the product specificity; PCS M207G mutant yielded SEK4 and SEK4b from eight molecules of malonyl-CoA.[10] This provided new insights into the catalytic functions and specificities of type III PKSs. Further characterization of the enzymes including their three-dimensional structure will be reported in due course.

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Supporting Information Available: Materials and methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0431206

薬用植物

栽培と品質評価

Part 11

ウイキョウ

オオツヅラフジ

オミナエシ

カラスビシャク

ヨロイグサ

目　次

作成要領

各項の記載内容は下記の基本的考えに基づいて記載した.

1．植物名

和名を記載した．ただし，和名がなく生薬名が広く植物名として用いられている場合はその名前を記載した.

生薬名は漢字または片仮名で代表的な名称を用いた．学名は日本薬局方及び植物分類学の分野で広く使用されているものを用いた.

2．利用部位

生薬として用いるときの部位を記載した.

3．植物の性状

植物の特徴的な形態を記載した.

4．生薬の特徴及び産地

特徴は主として日本薬局方及び日本薬局方外生薬規格の記載内容並びに専門分野で広く認められている形態的特徴を記載した.

産地は主な生産地を記載した.

5．栽培種の特性

特性は形態，生態，成分，地域及び生育に分けて記載した.

地域適性及び生育特性は気候区分（気温を寒さの区分と暖かさの区分で分け，日照条件を1箇月の平均日照時間で区分），土壌区分（土壌分類と土壌への適性）及び遮光の必要性を記載した．気候区分の寒さの区分，暖かさの区分及び日照条件並びに土壌区分の土壌分類は区分地図に掲載した.

6．栽培法

栽培法は品種，繁殖法，栽培（適性，繁殖，播種，育苗，植え付け，肥料，管理，病虫害，収穫，調製，収量）の順に記載した.

肥料は窒素，燐酸，加里の成分量を記載した.

収量は特に記載がない限り10a当たりの乾燥収量を記載した.

7．生薬の品質評価

原則として，日本薬局方の規格に従った.

8．特性分類表

各種苗の特性を形質区分に従って表示した.栽培条件によって異なる形質があるので，これらの数値は参考として示した．なお，試験栽培地を付記した.

9．栽培暦

栽培法の記載内容を栽培手順に従って暦に表した．なお，標準的な適用地域を付記した.

10．資料

種苗の来歴，確認栽培（種苗の特性及び栽培適地の判断の参考とするもので，栽培法を参考にして行う），用途，配合漢方処方名を記載した.

ウイキョウ

1．植物名　　ウイキョウ　　セリ科

（生薬名）　茴香（ウイキョウ）

（学　名）　*Foeniculum vulgare* Miller

2．利用部位　　果実（分果）

3．植物の性状

ヨーロッパ原産のセリ科の多年生草本．茎は高さ 1～2 m，茎は直立し上部で分枝する．全草黄緑色，独特な香りがある．葉は互生．２回羽状複葉で裂片が深裂するため，葉身は糸状，葉柄は根生葉では長く，茎生葉では上部のものほど短くなり，基部はいずれも茎を抱込み，さや状となる．複散形花序を枝端に付け，多数の黄色の小花を開く．果実は卵状だ円形の双懸果で，香りが強い．花期は夏．ウイキョウには *Foeniculum vulgare* Miller var. *piperitum* Countinho と *F. vulgare* Miller　var. *dulece* (Mill.) Thell.の２変種があり，前者は苦茴香 bitter fennel と呼ばれ，後者は甘茴香 sweet fennel と呼ばれる．香りは甘茴香 sweet fennel が良いが，アネトールの含有量は苦茴香 bitter fennel の方が多い．

4．生薬の特徴及び産地

1）　特徴

長さ 3.5～8 mm，幅 1～2.5 mm で，外面は灰黄緑色～灰黄色で，互いに密接する２個の分果の各々に５本の隆起線がある．双懸果はしばしば 2～10 mm の果柄を付ける．

横断面を実体顕微鏡で観察するとき，横断面全体で６個，接合面に２個の油管が認められる．

2）　主な産地

（国内）長野県，鳥取県，北海道

（国外）中国（山西省，内蒙古自治区）

5．栽培種の特性

在来種が栽培されている．

1）　形態的特性

草丈は１年目で 1.2～1.8 m，２年目では２m 以上となる．生育の進行に伴って主茎の各節から分枝を伸ばし，下位節から伸びた分枝は茎となり，各分枝にはさらに２次分枝が発達する．北海道名寄市の事例では，１年生で 3～6 本，２年生以降では 15～20 本，多い株では 30 本以上の茎数となる．

2）　生態的特性

日本各地で広く栽培可能であるが，収量から判断すると暖地が適している．暖地では実生繁殖１年目で結実し収穫が可能であるが，寒冷地では結実しないか結実してもわずかで

あり，本格的な収穫は２年目以降となる．冬期に土壌が凍結する地域では株が枯死するため栽培は不可能である．

3） 成分的特性

精油3~８％．精油成分：フェニルプロパノイドとしてアネトール，エストラゴール，モノテルペンとしてフェンコン，リモネン，ピネンなどが含まれる．

4） 地域適性及び生育特性

a）気候区分

（1）気温	（ア）寒さの区分	II～V
	（イ）暖かさの区分	55～170
（2）日照条件		Ⅱ～V

b）土壌区分

（1） 土壌分類	I～III
（2） 土壌への適性	
（ア） 排水及び保水条件への適性	排水及び保水の良い場所に適する．
（イ） 土質及び土性	砂壌土～壌土に適する．
（ウ） 肥沃地への適性	肥沃地に適する．
c）遮光の必要性	不要

6．栽培法

1） 品種

在来種が用いられる．

2） 繁殖法

種子（植物学上は分果）を用いる．

3）栽培

a）適性

生育適応土壌の範囲は広い．排水良好で耕土の深い地に適する．春に圃場へ播種して間引を行って栽培する直播法と，ポットなどへ播種して苗を育て初夏に圃場へ定植する移植栽培法がある．

b）播種

種子の発芽適温は15～25℃である．直播栽培においては，暖地では３月下旬～４月上旬，寒冷地では５月上～下旬に行う．整地が終わった圃場に，条間80 cm～100 cm，株間75 cm～100 cmに点播し（10 a当たり1250～1333株），薄く覆土する．10日～２週間で発芽が始まる．

寒冷地では，４月上旬にポットなどへ播種して加温ハウスで苗を育成し，５月中旬～6月上旬に圃場に定植すれば１年目秋に収穫が可能になる．

c）肥料

基肥として，10 a 当たり堆肥 1000 kg，苦土石灰 60～100 kg，窒素 4 kg，燐酸 5～6 kg，加里 7 kg を全層に施す．追肥として，暖地では 7 月上～中旬，寒冷地では 7 月下旬～ 8 月上旬に 10 a 当たり窒素 4 kg，加里 7 kg を施用する．

2 年目以降は，春の萌芽期に 10 a 当たり苦土石灰 100 kg，窒素 5～6 kg，燐酸 8～10 kg，加里 7 kg，暖地では 6 月中～下旬，寒冷地では 7 月下旬～ 8 月上旬に 10 a 当たり窒素 5～6 kg，加里 7 kg を施用する．

d）管理

直播栽培においては，1 年目の発芽後，密生した所を適宜間引きし，6 月下旬～ 7 月上旬までに所定の株間にする．適宜，除草中耕を行う．

e）病虫害

生育期間を通じ，キアゲハの幼虫による葉の食害があるが，著しい被害とはならない．結実期にアカスジカメムシによる果実の吸汁害がみられる．病害については，特に重篤な病気は認められていない．

f）収穫・調製

寒冷地においては 1 年目では 10 月中旬～下旬，2 年目以降は 8 月下旬～9 月中旬から，緑色の果実が成熟し，黄緑色に変わり始めた頃に順次採取する．東京都小平市での事例では，1 年目では 9 月下旬～11 月上旬，2 年目以降は 7 月中旬～9 月中旬に順次採取する．採取した果実は天日乾燥するか風通しの良い日陰で速やかに乾燥する．

北海道名寄市の事例では，果実は登熟日数の経過とともに順次緑色から黄緑色，黄褐色に変わり，開花後 51 日から 55 日目では約 50%の果実（分果）が黄褐色となり，60 日目以降ほぼ完全に褐色となる．これに伴い，精油含量も緩やかに低下する傾向があり，従って，開花後 50 日目程度が好ましい．

生薬として，緑色を帯び香りが強く，やや甘味のあるものが良品とされる．

g）収量

北海道名寄市の事例では，乾燥果実の 10 a 当たり収量は 1 年生 10～25 kg，2～3 年生 80～100 kg 程度であるが，札幌市の事例では，1 年生 29.9 kg，2 年生 285.7 kg，東京都小平市での事例では，1 年生 61 kg，2 年生 320 kg，三重県の事例では 2 年生で 423 kg という報告もある．

種子の精油及びアネトール含量は，暖地ほど高くなる傾向が認められている．

株が古くなると収量が低下するので，6 年目を目安に株の更新を行う．

h）採種法

2 年目以降，採種用に未熟種子を収穫せずに株を残し，黄褐色を呈した完熟種子（植物学上は分果）を 9 月下旬～10 月中旬（寒冷地では 10 月上旬～11 月上旬）に採取する．

i）種子の保存

密封して吸湿を防ぎ，冷蔵庫か冷暗所で保存すれば少なくとも 5 年以上は発芽力は維持されるが，冷凍庫内保存では発芽力は低下する．

7．生薬の品質評価

日本薬局方の試験の適否

a）生薬の性状	4.1）に同じ.
b）灰　分	10.0%以下.
c）酸不溶性灰分	1.5%以下.
d）精油	0.7 ml/50 g 以上.

１０．資料

1）　種苗の来歴

本種は平安時代に日本に導入され，江戸時代には各地で栽培されたといわれている．特性分類調査に用いた *Foeniculum vulgare* Miller は，青森県で栽培していた系統を，国立医薬品食品衛生研究所北海道薬用植物栽培試験場に導入し，維持されているものに基づく．

2）　確認栽培

a）１区の面積	5.6 ㎡以上	条間 80 cm，株間 100 cm
b）供試個体数	5 個体以上	
c）反復数	2 反復以上	

3）　用途

粉末を芳香性健胃薬として配合剤（胃腸薬）の原料とする．１日最大分量３g（粉末として１g）．健胃消化薬，鎮痛・鎮けい薬とみなされる漢方処方に配合される．

4）　配合漢方処方

安中散，丁香柿蒂湯

8.特性分類表

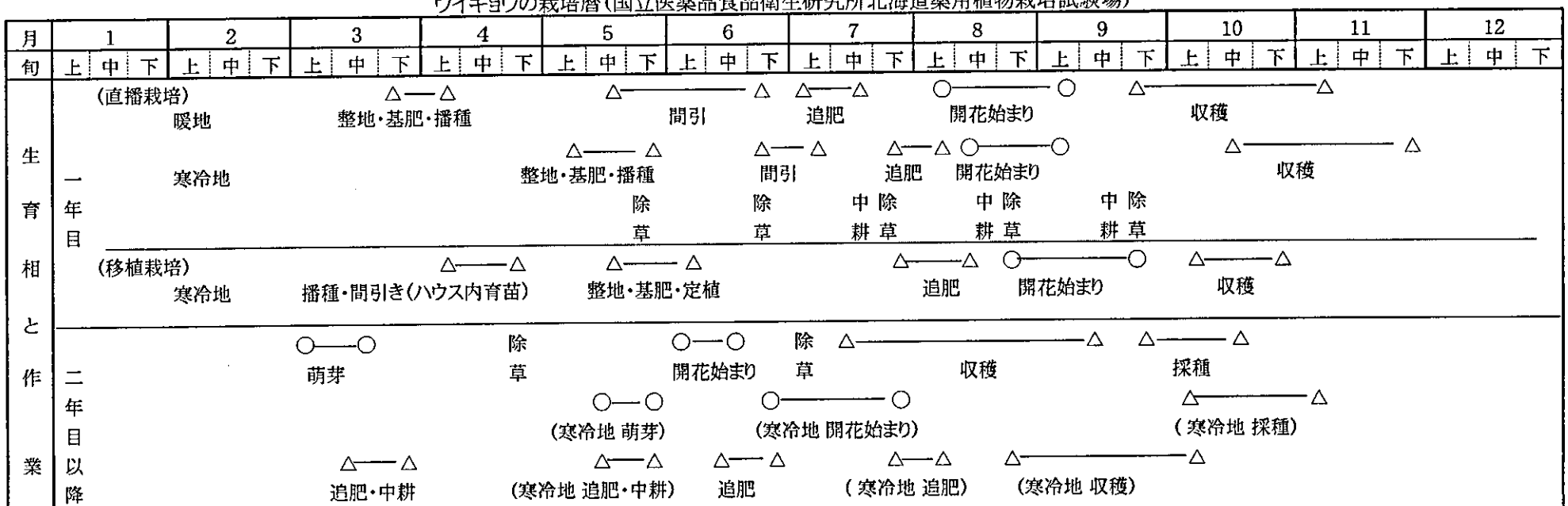
ウイキョウの特性分類表

特性		植物の品種名または系統名
区分	形質	在来種
草状	草姿(2年生開花期における草型)	直立～開帳
	草丈(2年生開花期における高さ)	中(150～220 cm)
	茎数(2年生開花期)	中(5～19)
	茎の色(2年生開花期)	粉白を帯びた緑色
葉の形状	根出葉の切れ込み(2年生初夏)	3～4回羽状分裂
	根出葉の裂片の形状(2年生初夏)	糸状
	茎葉の色(2年生初夏)	粉白を帯びた緑色
花の形状	花序の総苞	無し
	花序の小総苞の数	無し
	花の色	黄色
果実の形状	種子(植物学上は分果,以下同様)の形状(完熟種子)	長だ円形,偏平
	種子の長さ(完熟種子)	中(4.5～7.0 mm)
	種子の幅(完熟種子)	中(1.9～3.0 mm)
	種子の横断面全体にみられる油管数(完熟種子)	中(6～8)
	種子の接合面にみられる油管数(完熟種子)	中(2～4)
開花期	開花の早晩(2年生株における茎の頂花)	中 (7月中旬～8月上旬)
環境耐性	耐寒性(1年生冬期に枯死株を生ずる程度)	中
	耐倒伏性(2年生結実期に倒伏を生ずる程度)	中
収量性	2年生における10 a当たり黄緑色を呈した乾燥未熟果実重	中(80～280 kg)
成分特性	乾燥果実の精油含量(黄緑色を呈した果実50 g当たり)	0.7 ml 以上

(栽培地:国立医薬品食品衛生研究所北海道薬用植物栽培試験場)

9.栽培暦

ウイキョウの栽培暦(国立医薬品食品衛生研究所北海道薬用植物栽培試験場)



作業の内容

☆基肥(10 a当たり):
- 堆肥:1000 kg
- 苦土石灰:60~100 kg
- 窒素:4 kg
- 燐酸:5~6 kg
- 加里:7 kg

☆播種(直播栽培の場合):
- 条間80~100 cm
- 株間75~100 cm に点播

☆定植(移植栽培の場合):
- 条間80~100 cm
- 株間75~100 cm
- (1 a当たり125~133株)

☆追肥(10 a当たり):

1年目 7月上~中旬(寒冷地 7月下旬~8月上旬)
- 窒素:4 kg
- 加里:7 kg

2年目以降 3月中~下旬(寒冷地 5月中~下旬)
- 苦土石灰:100 kg
- 窒素:5~6 kg
- 燐酸:8~10 kg
- 加里:7 kg

同 6月中~下旬(寒冷地 7月下旬~8月上旬)
- 窒素:5~6 kg
- 加里:7 kg

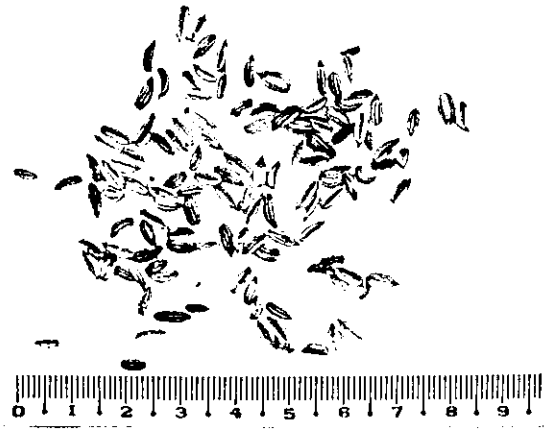
☆病虫害:生育期間を通じ,キアゲハの幼虫による葉の食害があるが,著しい被害とはならない。結実期にアカスジカメムシによる果実の吸汁害がみられる.

☆収穫・調製:緑色の果実が成熟し,黄緑色に変わり始めた頃に順次採取する.採取した果実は天日乾燥するか風通しの良い日陰で速やかに乾燥する.

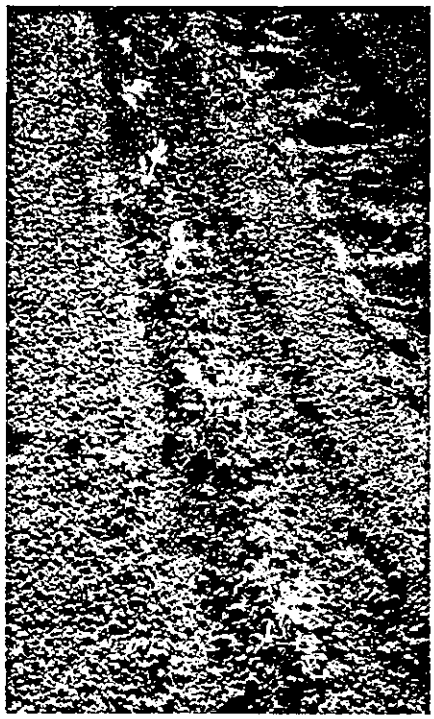
☆収量:北海道名寄市の事例では,乾燥果実の10 a当たり収量は1年生10~25 kg,2~3年生80~100 kgであるが,札幌市の事例では,1年生29.9 kg,2年生287.5 kg,東京都小平市での事例では,1年生61 kg,2年生320 kg,三重県の事例では,2年生で423 kgという報告もある.

☆採種:黄褐色を呈した完熟種子(植物学上は分果)を,9月下旬~10月中旬(寒冷地では10月上旬~11月上旬)に採取する.

☆種子の保存:密封して吸湿を防ぎ,冷蔵庫か冷暗所で保存すれば少なくとも5年以上は発芽力は維持されるが,冷凍庫保存では発芽力は低下する.



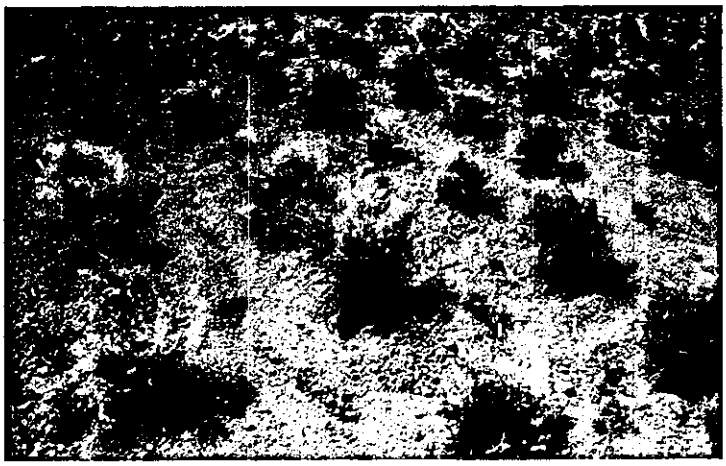
ウイキョウの種子(植物学上は分果)



ウイキョウの1年目生育初期
(5月下旬 圃場へ定植直後)

ウイキョウの1年目生育中期(7月上旬)

ウイキョウの1年目生育盛期(8月上旬)

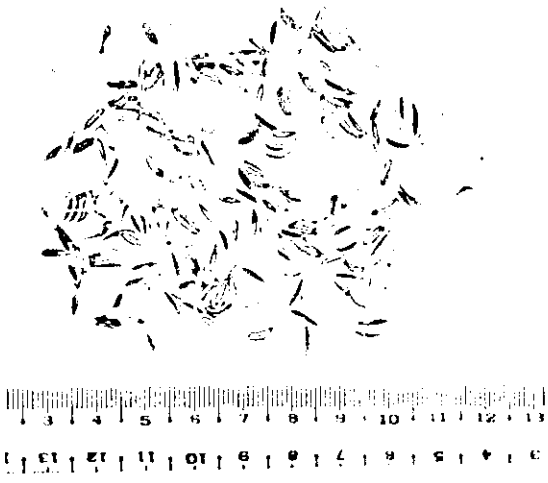


ウイキョウの2年目萌芽期(5月下旬)

ウイキョウの2年目生育盛期(7月上旬)

ウイキョウの2年目開花期(8月上旬)

ウイキョウの花



生薬ウイキョウ

オオツヅラフジ