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Scientific evaluation of Tian Hua Fen (THF)—history, chemistry and application

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Abstract - Tian Hua Fen (**THF**) was first recorded as a medicine for reestablishing menstruation in "Quian Jin Yi Fang" (about 682 A.D.) and as an abortifacient in "Tai Ping Sheng Hui Fang" (992 A.D.). **THF**, prepared from the root tuber of Trichosanthes kirilowii Maxim (Cucurbitaceae), had been used locally in admixture with several other herbal ingredients with severe side reactions.

Through series screening, an abortion-inducing protein, trichosanthin (**T**), of pI 9.4 has been isolated from the root tubers in pure crystals. It is composed of 234(3) amino acid residues: $Ala_{28}Arg_{13}Asn_{17}Asp_9Gln_9Glu_{10}Gly_{12}His_1Ile_{15}$-$Leu_{27}Lys_8Met_4Phe_9Pro_9Ser_{24}Thr_{13}Trp_1Tyr_{13}Val_{12}$; M.W.: found, 24,000; calculated, 25,682. **T** is obtained as a mixture of two homologous polypeptides differing only at their C-terminals. The C-terminal of one of them is -Met-OH, and that of the other, -Met-Ala-OH. Cyanogen bromide degradation of **T** gave 4 peptide fragments (CB1-4) and some free alanine. **T**, N-maleyltrichosanthin (MT) and CB1-4 were furthur subjected to enzymic degradations. The primary structure of **T** has been elucidated by sequencing **T** and all degradation peptide fragments from the N-terminals by usual Edman degradation methods and from the respective C-terminals by using carboxypeptidases A, B and Y. Trp was located in the T-200 position. X-ray crystallography revealed that **T** crystals from barbiturate buffer belong to monoclinic system and show 8 α-helices and 13 β-strands constituting 4 β- pleated sheets.

T is parenterally employed as a safe abortifacient in dose of 1.2 mg for woman of mid pregnancy and in combination with other medicines for early pregnancy. It is also effective in treating hydatidiform moles, ectopic pregnancy as well as choriocarcinoma.

HISTORY

Tian Hua Fen (**THF**) is a Chinese medicine prepared from the root tuber of the perennial plant Trichosanthes kirilowii Maxim (Cucurbitaseae). As early as in East Han Dynasty (at the beginning of the 3rd century), the famous physician Zhang Zhong-Jing had mentioned **THF** as traditional herbal medicine in his classical book "Synopsis of Prescriptions of the Golden Chamber". Later in Tang Dynasty (682 A. D.), the distinguished physician Sun Si-Miao wrote for the first time the successful application of **THF** to reset menstruation in obstetrical and gynecological cases in his famous medical book "Supplement to Prescriptions Worth a Thousand Gold". In "The Peaceful Holy Benevolent Prescriptions" compiled by Wang Huai-Yin in Song Dynasty (978-992), **THF** was recommended in a recipe "Gui Xin Scattered Prescriptions" for inducing abortion. In the late Ming Dynasty (1590) the great naturalist and physician Li Shi-Zhen had in his book "Compendium of Materia Medica" a very comprehensive and scientific compilation of the indication, and pharmacological properties of **THF** as an effective drug for "resetting menstruation" and "treating the detention of the after-birth".

In the past two to three decades, the Chinese practitioners and clinical

doctors in cooperation with pharmacologists, biologists and chemists of research institutes and pharmaceutical works had screened the composite **THF** prescriptions of seven or eight ingredients for inducing abortion down to two and finally to one principal ingredient **THF** alone (Table 1). In the composite prescription, the so-called Tian Ya San was composed of only two ingredients, of which the most important ingredient was **THF**, and the other ingredient, Ya-Zao, i.e., Gedditsia sinensis Lam. The latter acted only as a surfactant, and was proved to be the main toxic factor responsible for the necrosis of the tissue at the site of the external application and pyrogenic side action. Further studies on the isolation and purification of the active abortifacient factor led to the crystallization of the active protein trichosanthin (**T**) (Table 1).

Table 1 Properties of **THF** in some prescriptions

Prescription	THF composition (%)			Animal test (injn.) (mg/mouse)		Clinical application			
	Prot.	Ash	Sugar	Min. dose	LD_{50}	Admin.	Dosage (mg/cap)	Earlygestation	Midgestation
Tian-Zao composite recipe	30			0.5*	2.36*	extern.	90-500		96% effective; less bleeding; some fatal cases
THF for injn.	40-50	20	5-7	0.2	0.6	intramus. or intraamnion. injn.	5		successful; necrosis, highfever 39°C(13.6%)
Pure **T** for injn.	82	10		0.05	0.29	dito	2	112 cases, 86.6% success; fever 39.1°C(5.4%)	97% success; 39°C (9.7%), 38°C (80%)
Crystal **T** for injn.	95			0.03	0.236	dito	1.2	1042 cases, 92% success; no signif. side action 37.5°C(79%)	97% succuss; no signif. side action

* Only the whole lyophylizate of **THF** was accounted for.

CHEMISTRY

The root tubers are freshly collected, cut into pieces and pressed to obtain their juice. The juice is fractionally precipitated with acetone and purified by crystallization from barbiturate buffer (ref. 1). The yield of crystallized product was about 0.05% (on fresh root basis). The pure crystal is free from sugar and phosphate groups and homogeneous on agarose plate, polyacrylamide gel, SDS-polyacrylamide, gel-immuno eletrophoresis and gel-permeation and also by N-terminal amino acid determination. The active protein is named trichosanthin (**T**).

T is a basic protein of pI 9.4. Its molecular weight as determined by SDS-polyacrylamide gel electrophoresis is 24,000. It consists of one residue each of histidine and tryptophane but no cysteine or cystine (ref. 1-4). There are 4 methionine residues. The amino acid composition of trichosanthin is shown in Table 2.

The N-terminal amino acid is Asp as identified by chemical methods, and the C-terminal determined by carboxypeptidase A (CPA), hydrazinolysis and

thiohydantoin methods is Met, but always accompanied by Ala (ref. 5). The C-terminal Ala has also been isolated from cyanogen bromide (CNBr) degradation products of **T**.

T crystallizes from barbiturate buffer of pH 8.6 in the form of monoclinic system (ref. 6) and from citrate buffer of pH 4-6 in larger crystalline forms of orthogonal system (ref. 7) of the following parameters (Table 3):

Table 2 Amino acid composition of trichosanthin

Amino acid	A.a. anal.	From sequence
Ala	26.1	28(27)
Arg	13.2	13
Asp+Asn	25.6	26
Glu+Gln	20.8	19
Gly	11.8	12
His	0.9	1
Met	3.3	4
Ile	15.4	15
Leu	26.7	27
Lys	8.7	8
Phe	9.2	9
Pro	9.1	9
Ser	23.7	24
Thr	15.6	13
Trp*	1	1
Tyr	12.2	13
Val	11.8	12
Total	236	234(233)

*Separately determined by special method.

Table 3 Crystallographic data of trichosanthin

Crystal system Buffer solution	Monoclinic Barbiturate (0.01 M)	Orthogonal KCl(14%)-citrate (0.075 M)
pH	8.6	5.4
Z	2	1
Unit cell		
Parameters:		
a	75.64Å	38.2Å
b	75.52Å	76.8Å
c	88.85Å	79.4Å
	99.51°	
Space group	C2	$P2_12_12_1$
Density	1.19	

STRUCTURE OF TRICHOSANTHIN

Primary structure

T molecule does not consist of cysteine or cystine and has free N- and C-termini so that it is a polypeptide chain protein. The natural protein is quite resistent to enzymic hydrolysis, but the denatured ones are easily susceptible to the digestion by proteolytic enzymes. Because **T** is composed of 4 Met residues, it can be cleaved specifically by cyanogen bromide into five degration fragments or at least four fragments (CB1-4) if one of Met's is at the terminus (ref. 6) (Fig. 1).

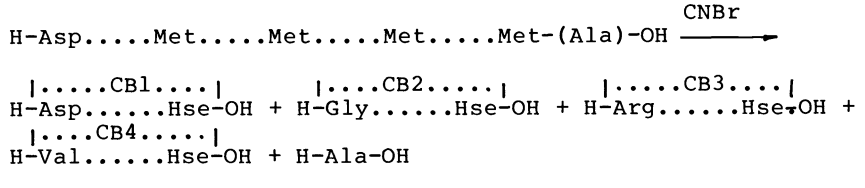

Fig. 1 Cyanogen bromide degradation of trichosanthin

The complete primary structure of **T** has been elucidated by sequencing the whole molecule of **T** and its N-maleyl or N-succinyl-derivative (**MT** or **ST**), and the fragments obtained from the combined chemical-enzymic degradations of **T** or **ST**.

The sequencing of the whole molecule was carried out by starting from N-terminal by Edman-Begg process using DMBA program (ref. 9) and also from C-terminal by carboxypeptidases A, B and Y (CPA, CPB and CPY). Both

automatic sequencer and manual method in solution and on solid phase were employed. For chemical degradations, cyanogen bromide was used for the specific cleavage at Met residue, and BNPS-sketole, for that at Trp site (ref. 10). Proteolytic enzymes used were trypsin, α-chymotrypsin, SV8-protease, thermolysin and pepsin.

Cyanogen bromide degradation of trichosanthin and N-succinyl-trichosanthin

Both **T** and **ST** have been subjected to cyanogen bromide degradation. The degradation products from **ST** (**ST-CNBr**) were separated on Sephadex G-75 into six fractions (Peak I-VI) (ref. 3), and, in addition, a fraction of free alanine. The N-terminal residues of Peaks III, V and VI were identified to be Val, Arg and Gly respectively. These fragments were later proved to be CB4, CB3 and CB2. The degradation produts from **T** (**T-CNBr** could also be separated into six fractions (Peaks I-VI) (ref. 4) on Sephacryl S200 column. The eluate of Peak I was fractionally collected and the first few fractions contained the unreacted **T** which was cut off. The remaining fractions were rechromatographed to remove any contaminating materials. The pure peptide fragment thus isolated was identified to be the N-terminal fragment of T-CNBr, i.e., CB1. The eluate of Peak III was also fractionally collected. The first collected fractions were identified to consist of the C-terminal fragment of **T**, i.e., CB4. The Peak II gave mixed peptide fragments occurring in adjacent Peaks I and III. Peak IV consisted of degradation fragements occurring in Peaks III and V. Peak V was found to contain CB3, and Peak VI, CB2.

Only Peaks III, V and VI of **ST**-CNBr-dagradation and part of Peaks I, III and V of **T**-CNBr degradation were used for sequencing.

The amino acid compositions of CB1, CB2, CB3 and CB4 were shown in Table 4.

Table 4 Amino acid compositions of **T**-CNBr fragments

Amino acid	CB1		CB2		CB3		CB4	
	A.a. anal.	From sequence	A.a. anal.	From sequence	A.a. anal.	From sequence	A.a. anal.	From sequence
Ala	6.9	7	6	6	8.9	8	6.8	6
Arg	6.1	6	1.0	1	3.0	3	3.0	3
Asp + Asn	10.4	10	3.0	3	5.3	5	8.4	8
Glu + Gln	3.2	3	2.0	2	3.2	3	11.2	11
Gly	4.0	4	2.0	2	4.0	4	2.0	2
His	1.0	1	-	-	-	-	-	-
Hse	1.0	1	1.0	1	1.0	1	1.0	1
Ile	4.7	5	-	-	2.0	2	8.0	8
Leu	10.6	11	-	-	8.9	8	7.6	8
Lys	2.1	2	2.0	2	1.2	1	3.2	3
Phe	2.2	2	3.0	3	1.0	1	3.1	3
Pro	3.8	4	-	-	3.0	3	2.0	2
Ser	8.4	9	2.0	2	4.6	4	8.1	9
Thr	2.8	3	2.0	2	4.5	4	3.6	4
Trp	-	-	-	-	-	-	1.0*	1
Tyr	4.2	5	3.0	3	3.2	4	1.4	1
Val	5.8	6	1.0	1	1.0	1	4.0	4
Total		79		28		52		74

*Tryptophane analysis according to special method.

Enzymic degradations of T and its fragments

MT has been hydrolyzed by trypsin, SV8-protease, α-chymotrypsin and thermolysin separately or successively, and **ST**, by α-chymotrypsin. The tryptic digestion mixture had only limited solubility in acidic solution. The acid-soluble portion gave a finger print of 13 spots on two dimensional cellulose electrophoresis-chromatography and could also be separated on HPLC into corresponding peaks. All of these spots have been sequenced. The spots 1 and 2 were found to be C-terminal fragments of trichosanthin H-Asn-Asn-Met-Ala-OH and H-Asn-Asn-Met-OH. The acid-insoluble portion of the tryptic

digests dissolved in TMK-buffer and 8M urea was separated on Sephadex G-75 column into four fractions (TIG 1-4). TIG1 was determined to be intact **MT**. TIG2 was further subjected to SV8-proteolytic and thermolytic digestions separately. The demaleyl-TIG2 was digested by trypsin. The digested produts were then separated by HPLC. All the isolated peptide fragments have been sequenced.

Sequencing

The direct sequencing of the N-terminal segment of the whole **T** molecule by Edman-Begg process using automatic sequencer (Beckman 890C) gave 38 a.a. residues (**T** 1-38). The C-terminal sequence of **T** was determined by subjecting **MT** to the action of CPA with the aid of computer simulation to be H-Asn-Asn-Met-OH in accompany with H-Asn-Asn-Met-Ala-OH (ref. 5). On further subjection to CPB action followed by CPY with the application of kinetic analysis of the a.a. liberation, a total of 9 a.a. residues of the C-terminal sequence were determined which has been verified by other ways (vide infra).

Most of the informations about **T** sequence was obtained by sequencing the peptide fragments of cyanogen bromide cleavage and enzymic degradations of **T**, **MT** and **ST**.

CB1 (Peak I) sequence

The sequence of N-terminal segment of CB1 (Peak I) was shown to be identical with that of **T**. Further information beyond **T**1-38 was provided by sequencing the peptide fragments obtained by tryptic, peptic and thermolytic digestions of CB1. The sequences of these fragments overlapped each other. The N-terminal sequence of CB1 was thus extended from **T**1-38 to **T**1-76 by the sequences (**T**39-46 and **T**47-73) of two more tryptic fragments and by the C-terminal sequence of CB1 (**T**73-79) determined by CPA to **T**1-79. The sequences of the above segments were joined together by the overlapping sequences of two peptic fragments (**T**32-39 and **T**38-50) and the N-termial sequence (**T**61-76) of a thermolytic fragment. The complete sequence of CB1 is shown to consist of 79 a.a. residues (Fig. 2). The only His residue was found at the position **T**51 of this fragment.

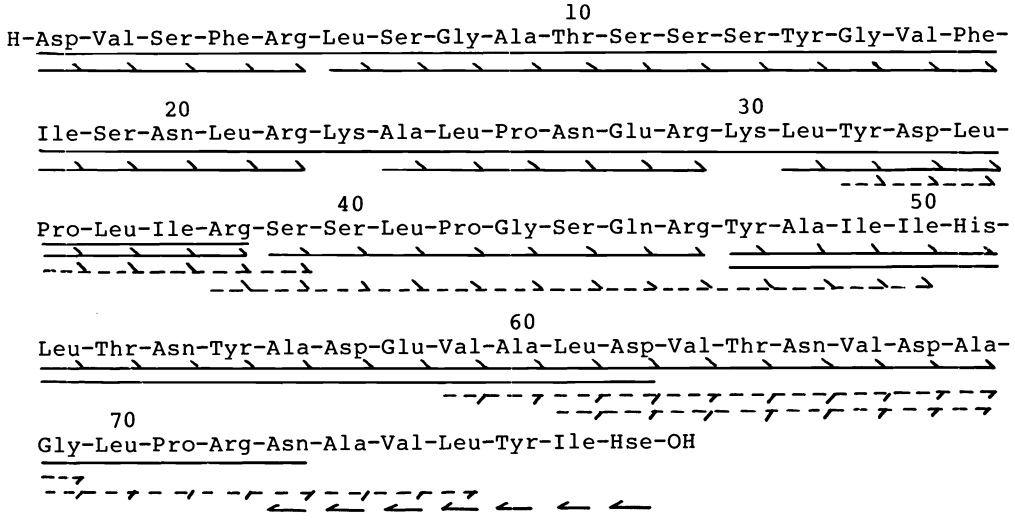

Fig. 2 Trichosanthin CB1 sequence

— automatic sequencing
<— CPA sequencing
--> peptic fragment
--> thermolytic fragment
—> tryptic fragment

CB2 (Peak VI) sequence

The sequence (**T**80-87) of Peak VI which was CB2 (vide infra) had been previously reported (ref. 3) as shown in Fig. 3. There are 28 a.a. residues.

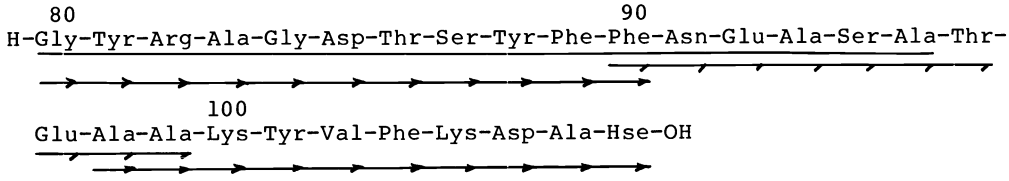

Fig. 3 Trichosanthin CB2 (Peak VI) sequence

—— automatic sequencing —→ SV8-proteolytic fragment
—→ α-chymotryptic fragment

CB3 (Peak V) sequence

The peptide fragment isolated from Peak V of **T-CNBr** or **ST-CNBr** with Arg as its N-terminus was directly sequenced from N-terminal as well as from C-terminal. Besides, it was first subjected to the separate digestions by trypsin, -chymotrypsin and SV8-protease. The various peptide fragments from these digestions were separated and also sequenced. The results were as follows:

The direct sequencing from the N-terminal was carried out up to 25 a.a. residues (**T**108-132). The sequence was extended by overlapping in succession by the 23 a.a. sequence (**T**130-152) of a tryptic digest fragment, the 14 a.a. sequence (**T**142-155) of a SV8-proteolytic fragment, the 10 a.a. sequence (**T**148-157) of an -chymotryptic fragment and the 7 a.a. C-terminal sequence (**T**153-159) of Peak V. The complete sequence (**T**108-159) of Peak V is composed of 52 a.a. residues (Fig. 4).

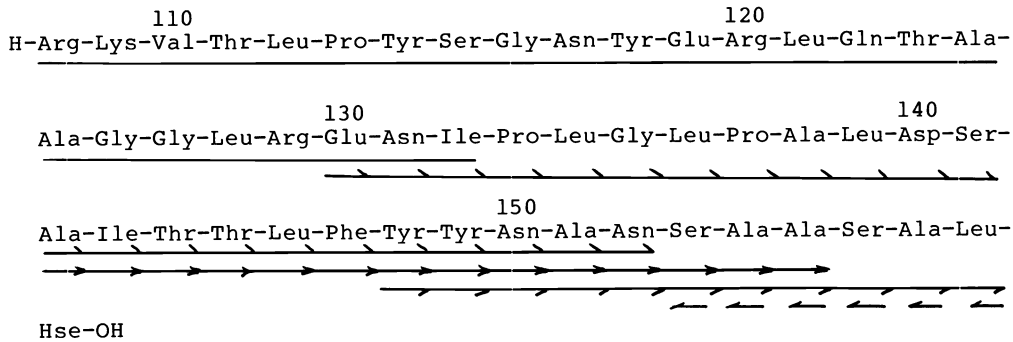

Fig. 4 Trichosanthin CB3 (Peak V) sequence

—— automatic sequencing —→ SV8-proteolytic fragment
←— CPA sequencing —→ tryptic fragment
—→ α-chymotryptic fragment

That Peak V is head (N-terminal of Peak V) to tail (C-terminal of Peak VI) linked to Peak VI is evident through the finding of the 11 a.a. residues segment (**T**106-116) of the Peak 4 fraction of the SV8-proteolytic digestion product of **MT**, which bridges over the C-terminal sequence (**T**106-107) of Peak VI and the N-terminal sequence (**T**108-116) of Peak V. Thus the linked polypeptide chain of Peak VI-Peak V is constituted of 80 a.a. residues and should correspond to CB2-CB3 (**T**80-159).

The linking between CB1 and CB2-CB3 was established by the finding of the key sequence (**T**78-82) of the 5 a.a. residues (H-Ile-Met-Gly-Tyr-Arg-OH) of the N-terminal sequence of an -chymotryptic fragment of **ST** which overlaps the C-terminal sequence (**T**78-79) of CB1 and the N-terminal sequence (**T**80-82) of CB2. The full sequence of CB1-CB2-CB3 is now extended to 159 a.a. residues (**T**1-159).

CB4 (Peak III) sequence

What was left not dealt with is Peak III, i.e., CB4. The characteristics of CB4 are that: (1) it consists of a tryptophane residue; (2)it has Val as N-terminus; (3) its C-terminal sequence should be the same as that of **T** except that the Met residue of **T** has been changed into homoserine in CB4;

and (4) according to amino acid analysis CB4 should be composed of about 75 amino acid residues.

The sequence of CB4 except that of the C-terminal segment was determined by the following ways: (1) directly sequencing its N-terminal segment, and (2) sequencing the peptide fragments obtained from tryptic and SV8-proteolytic digestions. The results were as follows:

On automatic sequencer, 24 a.a. residues of the N-terminal sequence (**T**160-183) of CB4 were determined, which were to the most part in common with the segment sequences (**T**160-170 and **T**173-181) of two tryptic digest fragments of CB4 and overlapped by the N-terminal sequence (**T**182-196) of a 15 a.a. segment of the above-mentioned TIG2 fragment. Thereby the N-terminal sequence (**T**160-196) was extended to 37 a.a. residues. On alignment of this resulting segment sequence with the N-terminal sequence (**T**185-201) of 17 a.a. residues of another tryptic fragment of CB4, the N-terminal sequence (**T**160-201) of CB4 was extended to 42 a.a. residues, except that the 41st residue (**T**200) was not identified at that time.

As mentioned above the C-terminal segment sequence of **MT** has been sequenced to 9(8) a.a. from the terminal Ala (Met). On aligment of this segment sequence with the sequence (**T**219-231) of 13 a.a. residues of a SV8-proteolytic fragment and that (**T**206-222) of 17 a.a. residues of a third tryptic fragment of CB4 in succession, the sequence (**T**206-231) of the C-terminal of CB4 was extended from C- toward N-terminal to 26 a.a. residues with 6 and 4 a.a. residues in overlapping respectively.

Now 69 out of 75 a.a. residues of CB4 have been accounted for, but the bridging sequence between the N- and C-terminals is still missing, and tryptophane residue should also be looked for.

Recently Trp residue has been located by subjecting TIG2 fragment to the cleavage by BNPS-skatole method. TIG2 was thereby splitted into two new fragments, the N-fragment (with the attached degraded tryptophane residue at its C-terminus) and the new C-fragment (with Leu at the N-terminus) (ref. 8). The C-fragment was sequenced by Edman method to give 9 a.a. residues (H-Leu-Ala-Leu-Lys-Gln-Ile-Gln-Ile-Ala-OH) of which the segment H-Gln-Ile-Gln-Ile-Ala-OH overlaps with the N-terminal segment of the C-terminal sequence (**T**206-233) of CB4, and Trp is thus located at the site

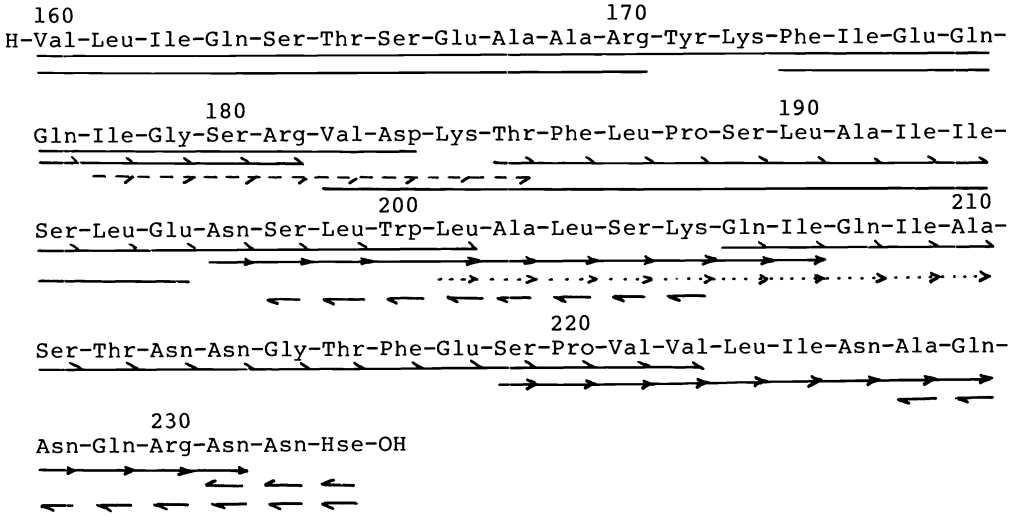

Fig. 5 Trichosanthin CB4 (Peak III) sequence

automatic sequencing
CPA sequencing
CPY sequencing
BNPS-skatol-cleaved fragment
SV8-proteolytic fragment
thermolytic fragment
tryptic fragment

of the previously unidentified 41st a.a. (i.e., T200), Its location was further confirmed by sequencing C-terminal segment (**T**198-205) by CPY of a tryptophane-constituted fragment of CB4 which was isolated from Peak 18 fraction obtained by HPLC of the tryptic digest of CB4. This segment

sequence H-Ser-Leu-Trp-Leu-Ala-Leu-Ser-Lys-OH together with the sequence (**T**197-207) of SV8-proteolytic fragment of CB4 also serve to bridge the N- and C-terminal sequences of CB4. Therefore, there are 74 a.a. residues in the complete CB4 sequence (**T**160-233) (Fig. 5).

Recent findings of the sequence of a pentadecapeptide fregment isolated from α-chymotryptic-tryptic digestion product of **ST** shows that it is identical with the a.a. sequence of trichosanthin seqment **T**148-162 and so overlaps the C-terminal segment of CB3 (**T**148-159) and N-terminal segment of CB4 (**T**160-162).

The primary structure of trichosanthin

In the preceding communication (ref. 4) we have proposed the main amino acid sequence of the principal primary structure of **T**. Since then the missing tryptophane residue has been located, and the missing amino acid sequence in CB1 has been found, and corrections have been made for some a.a. residues in the sequences of CB1, CB3 and CB4 (See Table 5). The **T** molecule is then composed of 234 (233) a.a. residues forming an open polypeptide chain. So far **T** appears always as a mixture of two homologous polypeptide chains differing only by one alanine residue at C-terminus. The calculated molecular weight should be 25,682 (25,610) (ref. 11).

On the basis of the above results the complete primary structure of trichosanthin is proposed as follows (Fig. 6):

```
                                        10
H-Asp-Val-Ser-Phe-Arg-Leu-Ser-Gly-Ala-Thr-Ser-Ser-Ser-Tyr-Gly-Val-Phe-

          20                                        30
Ile-Ser-Asn-Leu-Arg-Lys-Ala-Leu-Pro-Asn-Glu-Arg-Lys-Leu-Tyr-Asp-Leu-

                    40                                          50
Pro-Leu-Ile-Arg-Ser-Ser-Leu-Pro-Gly-Ser-Gln-Arg-Tyr-Ala-Ile-Ile-His-

                                  60
Leu-Thr-Asn-Tyr-Ala-Asp-Glu-Val-Ala-Leu-Asp-Val-Thr-Asn-Val-Asp-Ala-

     70                                        80
Gly-Leu-Pro-Arg-Asn-Ala-Val-Leu-Tyr-Ile-Met-Gly-Tyr-Arg-Ala-Gly-Asp-

                  90                                        100
Thr-Ser-Tyr-Phe-Phe-Asn-Glu-Ala-Ser-ala-Thr-Glu-Ala-Ala-Lys-Tyr-Val-

                           110
Phe-Lys-Asp-Ala-Met-Arg-Lys-Val-Thr-Leu-Pro-Tyr-Ser-Gly-Asn-Tyr-Glu-

120                                  130
Arg-Leu-Gln-Thr-Ala-Ala-Gly-Gly-Leu-Arg-Glu-Asn-Ile-Pro-Leu-Gly-Leu-

            140                                       150
Pro-Ala-Leu-Asp-Ser-Ala-Ile-Thr-Thr-Leu-Phe-Tyr-Tyr-Asn-Ala-Asn-Ser-

                       160                                        170
Ala-Ala-Ser-Ala-Leu-Met-Val-Leu-Ile-Gln-Ser-Thr-Ser-Glu-Ala-Ala-Arg-

                                180
Tyr-Lys-Phe-Ile-Glu-Gln-Gln-Ile-Gly-Ser-Arg-Val-Asp-Lys-Thr-Phe-Leu-

        190                                       200
Pro-Ser-Leu-Ala-Ile-Ile-Ser-Leu-Glu-Asn-Ser-Leu-Trp-Leu-Ala-Leu-Ser-

                     210                                         220
Lys-Gln-Ile-Gln-Ile-Ala-Ser-Thr-Asn-Asn-Gly-Thr-Phe-Glu-Ser-Pro-Val-

                                230
Val-Leu-Ile-Asn-Ala-Gln-Asn-Gln-Arg-Asn-Asn-Met-(Ala-)OH
```

Fig. 6 The primary structure of trichosanthin:

→ α-chymotryptic fragment → SV8-proteolytic fragment
← CPA sequencing ← CPY sequencing

Table 5 Corrections for the previously proposed sequence of the principal primary structure of trichosanthin

Location in ref. 4	Location in revised sequence	A. a. residues in ref.4	A. a. residues corrected
CB1 49		Leu	Ile
50		Leu	Ile
58		Ile	Glu
59		Gln	Val
60-70		missing	Ala-Leu-Asp-Val-Thr-Asn-Val-Asp-Ala-Gly-Leu-Pro-Arg-Asn-Ala-Val-Leu-Tyr-Ile
CB3 124	137	Ser	Asn
149	156	Leu	Ser-Ala-Leu
CB4 171	180	Lys	Ser
176	185	Gln	Thr
190-191	199-201	Leu-Leu	Leu-Trp-Leu
202	212	Gln	Thr

Three dimensional structure of trichosanthin

The crystallographic studies of trichosanthin have been carried out principally on crystals of monoclinic system by Crystallography Collaboration Group of the following Institutes of Academia Sinica: Fujian Institute of Research on the Structure of Matter, Institute of Biophysics and Shanghai Institute of Organic Chemistry.

X-ray crystallographic analysis reveals that the crystals of **T** from barbiturate buffer belong to monoclinic system (ref. 12). The parameters of the crystals are shown in Table 3. The tetraiodomercurate (HgI_4^{2-}), nitroplatinite ($Pt(NO2)_4^{2-}$) and uranyl (UO_2^{2+}) derivatives of **T** have been prepared for investigations. At 4Å resolution the electron density map shows clearly the course of the polypeptide chain of **T** (ref. 9). There are 8 α-helices (4 longer and 4 shorter) and 13 strands of β-structure, constituting 4 β-pleated sheets. They account for 39 and 32% of the amino acid residues of **T** respectively. In addition there are 8 β-turns in the polypeptide chains. The 8 -helices seem to be located relatively in the molecular center, surrounded by the 4 β-pleated sheets. The structure of **T** belongs to $\alpha + \beta$ type (ref. 11).

Recent information from the Crystalloyraphy Collaboration Group is that the positions of the most of the side chains of **T** molecule have been preliminarily determined from the molecular model constructed according to the electron density map of 3 and 2.6 Å resolutions (ref. 13).

The result from the preliminary study on the laser Raman spectrum of crystal of **T** in the powder form shows clearly the absorption bands of Phe, Trp, Tyr and the two amide groups (amides I and III) (ref. 14). The amide I and amide III bands are characteritic of β-pleated sheets and random structures. But the absorption band related to the random structure is very week.

CLINICAL APPLICATIONS

Trichosanthin used externally or parenterally in a single dose of 1.2 mg is able to induce abortion of a mid-gestation woman within 4-7 days without significant side reaction (ref. 15). In combination with reserpine and testosterone propionate, **T** is also effective in the termination of early pregnancy within 5-10 weeks (ref. 16).

In addition, **T** has been reported to have been used successfully in treating benign and malignant hydatidiform moles and ectopic pregnancy and also to be hopeful in curing choriocarcinoma cases.

MECHANISM OF TRICHOSANTHIN ACTION

The biologists of Shanghai Institute of Cell Biology, Academia Sinica and the medical practitioners of Ruijin Hospital of the Second Medical College of Shanghai and International Peace Maternal and Children's Health Hospital have reported the results of preliminary studies on abortifacient action of **T** in vivo and in vitro tests on mice. It shows that the direct, specific effect on the syncytiotrophoblast of the placental villi is the primary response and the circulation hindrance, secondary. Some changes of the hormonal levels were also observed (ref. 17).

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