TRITERPENE SAPONINS FROM *Thalictrum minus*.

IV. THE STRUCTURE OF THALICOSIDE A

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The predominating saponin, which has been called thalicoside A, has been isolated from low meadow rue (*Thalictrum minus*). It has been established by chemical and spectral methods that thalicoside A is 3β,16β,22(S),29-tetrahydroxy-9,19-cyclo-20(S)-lanost-24-ene 3-O-β-D-galactopyranoside 29-O-β-D-glucopyranoside.

The plant *Thalictrum minus* L. (low meadow rue) contains several triterpene saponins [1]. The amount of the predominating saponin, which we have called thalicoside A (I) is 0.5% on the mass of the raw material. The genin of thalicoside A is the new tetracyclic triterpenoid thalicogenin — 3β,16β,22(S),29-tetrahydroxy-9,19-cyclo-20(S)-lanost-24-ene (II) [2]. The acid hydrolysis of (I) (scheme 1) gave, in addition to the artefact (III) [3], glucose and galactose in a ratio of 1:1 (GLC of the acetates of the corresponding aldonitriles [1] and of the acetates of the corresponding polyols [4]).

The Kuhn methylation of thalicoside A with repeated further methylation by Purdie's method gave a product (V) containing a free OH group bound by an intermolecular hydrogen bond (IR spectrum).

A comparison of the ^{13}C NMR spectra of the glycoside and of its methyl ether (V) (Table 1) showed that the sterically hindered hydroxy group at C-16 had remained unmethylated and the hydroxy groups of the carbohydrate residues had been methylated completely.

The methanolysis of (V) followed by the determination of the carbohydrate ethers by the GLC-MS method gave methyl 2,3,4,6-tetra-O-methylglucopyranoside and methyl 2,3,4,6-tetra-O-methylgalactopyranoside. This showed that thalicoside A is a bisdesmoside containing the residues of only two carbohydrate molecules.

It can be seen from a comparison of the ^{13}C NMR spectra of the genin (II) and the glycoside (I) that there is an appreciable difference in the chemical shifts (CSs) only for the signals of the C-3 and C-29 atoms (Table 1), which again shows the position of the glycosidic bonds at these atoms.

The nature of the carbohydrate units was confirmed by PMR spectra of the peracetate of the saponin of the acetate (IV) with the aid of INDOR experiments. The positions of the signals of the carbohydrate protons in the spectrum of (IV) and the values of their spin—spin coupling constants (SSCCs) show that the carbohydrate moiety of the molecule of (IV) consists of two β-pyranoside units — glucose and galactose tetraacetates (Table 2). The ^{13}C CSs of the carbohydrate moieties in the spectra of (I) and (IV) agree well with literature figures for β-gluco- and β-galactopyranosides and their acetates [5, 6].

The partial hydrolysis of thalicoside A gave a mixture of four products. Of the conditions for partial hydrolysis that were tested (sulfuric, hydrochloric, acetic, and oxalic acids in various concentrations), the best for the isolation of the predominating product proved to be hydrolysis with 80% acetic acid. This gave the progenin (VI). The further hydrolysis of (VI) (scheme 1) took place under severe conditions (10-20% H_2SO_4, 7-10 h) and led to glucose and to the artefact (III). There is one report in the literature on the study of a triterpene saponin glycosylated in the C-29 position with the simultaneous presence of a 9,19-cyclopropane fragment. The authors did not use acid hydrolysis to isolate the genin but only

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TABLE 1. Chemical Shifts in the ^{13}C Spectra (C_5D_5N, δ, ppm, TMS — 0)

C atom	I	II	IV	V	VI	VII	C atom	I	II	IV	V	VI	VII*
1	32,6	32,6	32,3	32,3	32,3	32,4	16	72,1	72,1	75,4	71,8	71,2	74,5
2	29,7	31,0	31,1	29,6	28,7	25,5	17	53,3	53,5	52,5	52,8	52,8	51,5
3	82,1	74,8	82,8	82,1	71,5	76,3	18	2 ,0	20,7	21,7	20,5	20,6	20,5
4	45,3	44,9	45,5	44,7	44,9	46,5	19	31,9	30,5	30,8	30,2	30,7	30,5
5	41,2	42,3	41,0	40,9	40,8	40,5	20	36,3	36,	32,5	34,4	38,6	38,7
6	21,1	21,4	21,8	20,5	21,1	20,3	21	14,9	14,8	12,7	13,1	14,9	11,6
7	26,8	26,9	27,0	26,3	26,4	26,3	22	75,6	75,8	76,0	79,7	82,5	79,2
8	48,8	48,6	48,7	48,3	48,3	48,3	23	34,0	34,0	34,0	30,8	30,7	29,7
9	20,3	20,3	21,0	20,0	19,6	20,0	24	123,8	123,9	120,2	122,6	33,0	31,4
10	26,3	26,0	26,7	25,8	26,0	25,8	25	131,9	132,4	134,0	132,0	80,5	80,3
11	26,6	26,5	27,0	26,3	26,4	26,3	26	26,2	26,2	26,7	25,8	27,7†	27,8 †
12	33,8	33,7	33,6	33,5	33,5	33,9	27	18,4	18,2	18,7	18,0	27,4†	28,6 †
13	46,3	46,3	46,7	45,8	45,9	45,9	28	19,9	19,7	19,6	19,5	19,6	18,6
14	47,7	47,6	48,4	47,1	47,0	47,2	29	71,5	68,5	70,2	70,9	72,7	70,3
15	49,1	49,0	47,3	49,3	48,7	48,0	30	12,1	11,4	12,0	11,8	11,4	11,4
1′	105,2	—	101,4	103,9	105,5	100,8	1″	106,2	—	103,1	105,2	—	—
2′	75,9	—	71,1	84,7	75,4	71,1	2″	75,9	—	71,5	81,5	—	—
3′	79,0	—	73,4	86,9	78,5	73,0	3″	73,8	—	72,2†	84,7	—	—
4′	72,5	—	69,0	80,1	71,6	68,9	4″	70,8	—	68,1	75,8	—	—
5′	78,2	—	71,7	75,1	78,5	71,7	5″	76,5	—	72,5†	73,4	—	—
6′	62,9	—	62,0	71,8	62,8	62,0	6″	63,5	—	62,5	71,8	—	—

*Solvent $CDCl_3$.
†Assignment ambiguous within a column.

enzymatic hydrolysis [7]. It is possible that, as in our case, this was connected with the difficulty of hydrolyzing such glycosides.

A comparison of the ^{13}C NMR spectra of the glycoside acetate (IV) and the progenin acetate (VII) showed that the latter lacked a carbohydrate substituent at C-3, and a comparison of the ^{13}C NMR spectra of (VII) and (VI) showed that in (VII) the substituent at C-3 was an acetyl group (Table 1). The ^1H and ^{13}C NMR spectra of (VII) showed that the carbohydrate component in this compound was a glucose tetraacetate residue. Consequently, in the progenin glucose was attached in the C-29 position.

The ^{13}C NMR results for the glycoside (I) and the progenin (VI) show good agreement of the CS values for the corresponding carbon atoms of the polycyclic moiety of the genin when the effects of glycosylation at C-3 are taken into account for (I) (Table 1). An appreciable difference in the CSs was observed only for the carbon atoms of the side chains. The absence of the signals of carbon atoms at a double bond in the spectrum of (VI), the appearance of an additional signal of carbon bound to oxygen (80.5 ppm, s), and the presence in the ^1H and ^{13}C spectra of new signals of CH_3 groups with the simultaneous disappearance of the signals of methyl groups at a double bond show that when (I) was hydrolyzed the cyclization of the side chain took place simultaneously with the cleavage of the glycosidic bond at C-3.

The mass spectrum of the progenin confirmed that the side chain of (VI) was closed, as in the artefact (III) (m/z, %: 99 (100), 126 (30) [3]), and at the same time there was a cyclopropane ring, as in the native genin (II) (m/z, %: 159 (20), 173 (16) [2]).

The progenin (VI) was 3β,16β,29-trihydroxy-22,25-epoxy-9,19-cyclolanostane 29-O-β-D-glucopyranoside. Consequently, in the glycoside (I) a D-galactose residue was attached to the C-3 position.

Thus, thalicoside A has the structure 3β,16β,22(S),29-tetrahydroxy-9,19-cyclo-20(S)-lanost-24-ene.

Having available the results of an x-ray structural analysis for (III) and performing the assignment of all the signals* in the ^{13}C NMR spectra of thalicogenin, the progenin,

*The assignment of the signals in the ^{13}C NMR spectra of compounds (I), (II), and (IV-VII) was carried out by comparing their spectra with one another and with that of cycloartanol [8, 9], and also on the basis of ^{13}C experiments with off-resonance and selective decoupling from interaction with protons for thalicogenin [2].

TABLE 2. Chemical Shifts and Spin–Spin Coupling Constants of the Protons in the PMR Spectra of Compounds (IV) and (VII) (δ, ppm; J, Hz; C_5D_5N, TMS — 0)*

Proton of the genin	Thalicoside A acetate (IV)	Progenin acetate (VII)
2H-19	0,37; 0,54; d, J=4,5	0,39; 0,56; d, J=4,6
H-3	3,63, m	4,82; q, $J_{3,2}$ = 4,9; $J_{3,2}$ = 11,0
H-16	5,04–5,10, m	5,31; sx, $J_{16,15}$=4,3; $J_{16,15}$=8,0; $J_{16,17}$=8,2
H-22	5,04–5.10 m,	3,86; t, J=7,5
2H-29	3,52; 3,37, d, J=10.5	3,62; 3,07; d, J=12,0
C=CH	4,80; t, J=7,5	—
CH_3COO	1,98; 1,99; 2,02 (6H); 2,04; 2,05; 2,07; 2,08; 2,12; 2,15	2,0; 2,02; 2,03; 2,04; 2,10; 2,13
CH_3	0,68; 0,88; 0,97, d., J=7,5; 1,13; 1,63; 1,69	0,82; 0,88, d, J=7,5; 0,89; 1,15; 1,7; 1,22

Carbohydrate protons	IV		(VII) Glucose
	glucose	galactose	
H-1	4,51; d, J=8,0	4,59; d, J=8,0	4,39; d, J=8,0
H-2	5,04	5,23	5,01
H-3	5,22	5,04	5,21
H-4	5,10	5,39; d, J=4,0	5,09
H-5	3,65	3,93	3,67
2H-6	4,24; 4,10	4,19; 4,08	4,22; 4,16

*d — doublet; t — triplet; q — quartet; m — multiplet; sx — sextet. The signals of the protons of the CH_3COO and CH_3 groups have a singlet nature. The experimental axial–axial SSCCs of glucose and galactose were 10.5 ± 0.5 Hz.

thalicoside A, and their acetates, we attempted to determine the conformations of the side chains of these compounds.

It followed from the results of the x-ray structural analysis of (III) [3], that an intramolecular hydrogen bond (inter-HB) between the 16-hydroxy group and the oxygen atom of the tetrahydrofuran ring stabilized the conformation of the side chain. This conformation is illustrated, as conformation *a*, in scheme 2 in the form of a Newman projection along the 20-17 bond for compounds (III) and (VI).

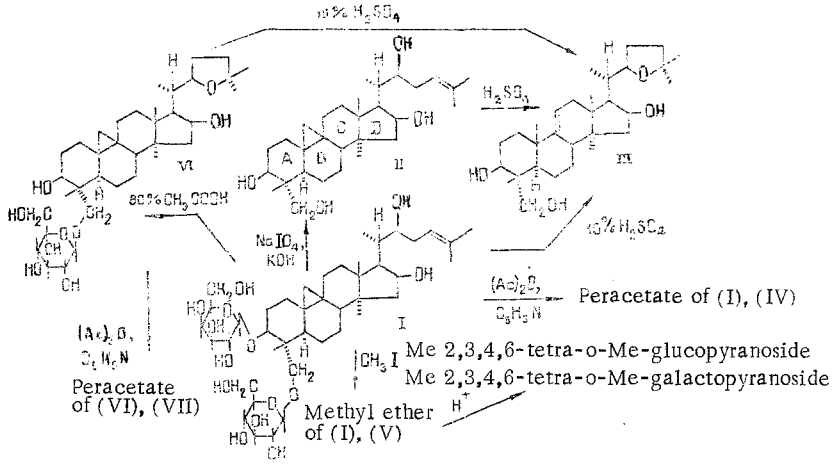
The upfield shift of the C-21 signal in the ^{13}C spectrum of compound (IV) by 2.2 ppm as compared with the position of this signal in the spectrum of (I) cannot be explained by the effects of the acetylation of the OH groups at C-16 and C-22. A similar shift was observed in the ^{13}C spectra of (V) and (VII). Consequently, the side chain in compound (I), as in (VI), is stabilized by an intra-HB between the OH groups at C-16 and C-22, and it has a different conformation in (IV), (V), and (VII), where an intra-HB is impossible.

A study of the conformations of the side chains of the glycoside acetate (IV) and the progenin acetate (VII), containing no intra-HB, from Drieding molecular models led to the most probable conformations b in the case of (VII) and d in the case of (IV) (scheme 2).

An analysis of the CSs of the C-20, C-21, C-22 atoms in the ^{13}C NMR spectra of compounds (I), (IV), (VI), and (VII) by the method of Beierbeck et al. [10] confirmed conformations c and d for the side chains of thalicoside A (I) and its acetate (IV), respectively (Table 3).

An evaluation of the difference in the CSs of the C-22 atom [10] in the ^{13}C spectra of compounds (VI) and (VII) without taking the influence of an intra-HB into account for conformations *a* and b, respectively, showed that in the case of conformation b of the side chain this atom must be screened by 4.4 ppm in comparison with conformation *a*. The difference in the experimental values of the ^{13}C CSs for C-22 agree well with the calculated values, amounting to 3.3 ppm.

A similar procedure for evaluating the difference in the CSs [10] of the C-22 atom in the ^{13}C spectra of (I) and (IV) with allowance for the effect of the replacement of the OH



Scheme 1

group by an OAc group did not give good agreement with experiment, which is probably connected with rotation around the 22-23 bond in (IV).

EXPERIMENTAL

The GLC analysis of carbohydrates in the form of polyol acetates was carried out on a Gas Chrom Q Chromatograph. A column 1500 × 4 mm filled with 3% QF-1 was used. The column temperature was programmed from 150°C to 225°C at the rate of 5°C/min, and the rate of flow of the carrier gas (argon) was 20 ml/min. We have described the GLC analysis of the carbohydrates in the form of the corresponding aldonitrile acetates previously [1].

All the other physical constants and spectral characteristics were obtained on instruments under the conditions that we have described in [1, 2].

The low meadow rue was collected in Irkutsk province in the flowering phase.

The thalicoside A (I) was isolated by a method described previously [1]. $C_{42}H_{70}O_{14}$, mp 255-258°C (methanol), $[\alpha]_{546}^{22}$ + 8.94° (c 2.0, pyridine).

Partial Hydrolysis of Thalicoside A. A solution of 2.6 g of glycoside (I) in 64.2 ml of 80% CH_3COOH was heated at 90°C for 6 h. The reaction mixture was evaporated in vacuum, the residue was dissolved in chloroform–ethanol (1:1), and this solution was reevaporated. This operation was repeated until the acetic acid had been completely eliminated. The reaction product was then chromatographed on a column of silica gel in the ethyl acetate–methanol–water (10:1:3) system. In this way, 138 mg of progenin (VI) was isolated. $C_{36}H_{50}O_9$, mp 148-150°C (ethyl acetate–methanol–water (10:1:3)). $[\alpha]_{546}^{22}$ 0° (c 1.83, pyridine). Mass spectrum, m/z (%): 636 (1), 618 (1), 474 (5), 456 (7), 329 (5), 313 (12), 311 (5), 261 (16), 187 (10), 175 (18), 173 (16), 161 (16), 159 (20), 126 (40), 121 (30), 109 (32), 99 (100), 85 (33), 69 (30).

Acid Hydrolysis of Thalicoside A (I) and of the Progenin (VI). The thalicoside A (200 mg) of the progenin (180 mg) was heated in 100 ml of 10% sulfuric acid at 100°C for 6 h. The hydrolysate was diluted with water, and the resulting precipitate was separated off, washed with water, and chromatographed on a column of silica gel in the ethyl acetate–methanol–water (10:1:3) system.

Compounds (I) and (VI) gave 12 and 16 mg of (III) respectively. $C_{30}H_{50}O_4$, mp 248-253°C (chloroform–methanol). The compound obtained gave no depression of the melting point with an authentic sample of (III) and had similar mass-spectrometric fragmentation [3].

After the removal of the precipitate, the hydrolysate was neutralized with AV-17 anion-exchange resin and was evaporated in vacuum.

This gave 80 and 60 mg of dry residue for (I) and (VI), respectively. Half of each of these residues was dissolved in 2 ml of pyridine, and then 6 mg of $NH_2OH \cdot HCl$ was added and the mixture was heated at 100°C for 1 h. Then 1 ml of acetic anhydride was added and the mixture was heated at 90°C for another 1 h. The product was concentrated in vacuum to dryness. The mixture was analyzed by GLC as described previously [1]. The peracetates of the aldono-

TABLE 3. Comparison of the Experimental and Calculated ^{13}C CSs of the C-20 and C-21 Atoms for Various Conformations of the Side Chain (δ, ppm)

C atom	Compound/conformation (scheme 2)							
	Ic		IV/*d*		VI•/*a*		VII•/b	
	calc.	expt.	calc.	expt.	calc.	expt.	calc.	expt.
C-20	38,0	36,3	32,4†	32,5	38,0	38,6	38,6	38,7
C-21	16,6	14,9	12,0	12,7	16,6	14,9	12,0	11,6

*The parameters of the OH group are taken as the additive parameters of the oxygen atom of the THF ring [10].
†The difference in the β effects of OH and OAc groups was taken as 2.0 ppm [11].

nitriles derived from glucose and galactose were identified for (I) and the peracetate of the aldononitrile derived from glucose for (VI).

The second half of each residue (40 and 30 mg for (I) and (VI), respectively) was dissolved in 4 ml of water, sodium tetrahydroborate (40 and 30 mg, respectively) was added, and the mixture was left at 22°C for 3 h. Then it was acidified to pH 5.0 and concentrated, and the boric acid was eliminated in the form of methyl borates by the addition of portions of methanol and their evaporation under vacuum. The resulting product was dissolved in 1 ml of pyridine, and then 0.5 ml of acetic anhydride was added and the mixture was left for 4 h. After the usual working up, the residue was analyzed by GLC. The acetates of the polyols dulcitol and sorbitol were identified for (I) and sorbitol acetate for (VI).

Periodate Oxidation of Thalicoside A (I). A solution of 10 mg of (I) in 10 ml of ethanol was treated with 20 ml of water, and then 30 mg of sodium periodate was added. The mixture was left at 5°C for 5 days. The reaction product was extracted with butanol, and the butanol layer was washed with water and evaporated. The residue was hydrolyzed with 10% H_2SO_4 at 90°C for 5 h. The reaction mixture was extracted with chloroform, and the aqueous layer was neutralized with AV-17 anion-exchange resin. In a chloroform extract, (III) was detected by thin-layer chromatography on silica gel in the hexane—chloroform—methanol (2:10:1) system. The aqueous layer contained no carbohydrates.

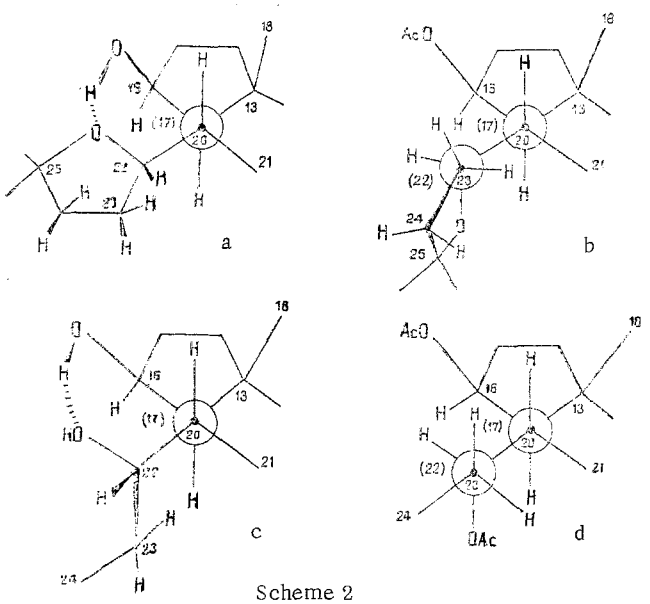
Production of Thalicogenin (II). Thalicogenin was obtained by the periodate oxidation of thalicoside A followed by alkaline hydrolysis, as described previously [2].

Acetylation of Thalicoside A (I). A solution of 150 mg of (I) in 3 ml of pyridine was treated with 1.5 ml of acetic anhydride and the mixture was left at 22°C for 12 h. Then it was worked up in the usual way and chromatographed on a column of silica gel. Elution with hexane—acetone (3:1) gave 100 mg of thalicoside A peracetate (IV). $C_{62}H_{90}O_{24}$, mp 114-115°C (hexane), $[\alpha]_{546}^{22}$ + 10.15° (c 1.28, pyridine). $\nu_{max}^{CCl_4}$: 1747 cm^{-1} (ester C=O).

Acetylation of Progenin (VI). A solution of 21 mg of (VI) in 0.4 ml of pyridine was treated with 0.2 ml of acetic anhydride and the mixture was heated at 40°C for 3 h. After the working up procedure, 25 mg of the peracetate (VII) was obtained. $C_{48}H_{72}O_{15}$, mp 174-175°C (hexane—acetone), $[\alpha]_{546}^{22}$ + 40.64 (c 0.31, pyridine). $\nu_{max}^{CCl_4}$: 1740 cm^{-1} (ester C=O).

Methylation of Thalicoside A. A solution of 1.6 g of (I) in 15 ml of dimethylformamide was treated with 15 ml of methyl iodide, 8 g of BaO and 4 g of $Ba(OH)_2 \cdot 8H_2O$. The reaction was performed at 0°C with stirring for 3 h and then at 50°C for another 24 h. During the reaction, two additional 1.5-ml portions of methyl iodide were added. The course of methylation was monitored by TLC on silica gel impregnated with NaH_2PO_4 in the benzene—acetone (2:1) system. The resulting mixture was diluted with water and was extracted with chloroform, and decoloration was carried out with sodium thiosulfate. The solid matter was separated off on a Schott filter and the filtrate was evaporated. The yield of partially methylated product was about 2 g.

Further Methylation by Purdie's Method. A solution of 2 g of the partially methylated glycoside in 10 ml of absolute methanol was treated with 10 ml of methyl iodide and 1 g of Ag_2O. The mixture was heated at 50°C with stirring under reflux and with the periodic addition of methyl iodide and silver oxide. The reaction was performed for 16 h. The solid matter was separated off by filtration. The reaction product was evaporated in vacuum at



Scheme 2

50°C. Purdie methylation in this way was carried out three times. The resulting mixture of methyl ethers was chromatographed twice on alumina in the benzene—acetone (9:1) system and then twice on impregnated silica gel in the benzene—acetone (4:1) system. This gave 300 mg of compound (V). Amorphous powder, $[\alpha]_{546}^{22}$ + 13.35° (c 1.33, pyridine). $\nu_{max}^{CCl_4}$, cm^{-1}: 3402; on dilution 3618 (OH).

Hydrolysis of the Methyl Ether of Thalicoside (V). A solution of 20 mg of (V) in 10 ml of 10% HCl in anhydrous methanol was boiled at 65°C for 4 h. The mixture was evaporated in vacuum, the residue was dissolved in 0.4 ml of pyridine, 0.2 ml of acetic anhydride was added, and the mixture was left for 12 h. Then it was evaporated and was analyzed by GLC-MS. Methyl 2,3,4,6-tetra-O-methylglucopyranoside and methyl 2,3,4,6-tetra-O-methylgalactopryanoside were identified.

SUMMARY

A new triterpene saponin has been isolated from low meadow rue — thalicoside A, which has the structure 3β,16β,22(S),29-tetrahydroxy-9,19-cyclo-20(S)-lanost-24-ene 3-O-β-galactopyranoside 29-O-β-D-glucopyranoside.

The structure of 3β,16β,29-trihydroxy-22,25-epoxy-9,19-cyclolanostane has been established for the progenin of thalicoside A.

The most probable conformations of the side chains of thalicoside A, of the progenin, and of their acetates have been determined on the basis of the results of an x-ray structural analysis and of ^{1}H and ^{13}C NMR spectra.

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PRIMARY STRUCTURE OF SUBUNIT B OF THE 11S GLOBULIN OF COTTON SEEDS OF VARIETY 108-F.

II. PEPTIDES OF TRYPTIC HYDROLYSIS AT LYSINE RESIDUES

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Six large lysine peptides have been isolated from a tryptic hydrolysate at lysine residues, making up all together 172 amino acid residues of the 180-190 amino acid residues of the whole polypeptide chain of the molecule.

In the study of the primary structure of subunit B of the main storage protein $His_{2\alpha}$ of cottonseed globulin we have performed several types of cleavages, including tryptic hydrolysis at lysine residues. Subunit B is readily soluble and therefore at the stage of the modification at arginine residues we used the protein maleylated at lysine NH_2 groups, which is soluble in borate buffer, this facilitating modification by cyclohexanedione. Since the initial protein was maleylated, before hydrolysis with trypsin the maleyl protection was removed from the lysine NH_2 groups. No cleavage of the polypeptide chain was observed under the conditions of eliminating the protection even when the time was increased to 64 h. This permitted the assumption that the molecule contained no acid-labile Asp-Pro bond.

The degree of cleavage and the nature of the peptides were confirmed with the aid of peptide maps [1]. The protein includes six lysine residues, and we assumed that 6-7 fragments would be obtained. Subunit B contains no disulfide bridges, and therefore preparation for digestion by trypsin was limited to thermal denaturation. The protein modified at the arginine residues was extremely poorly soluble in 0.2 M N-ethyl morpholine acetate buffer, and therefore digestion was performed in a suspension with magnetic stirring for 18 h.

A peptide map of the hydrolysate is shown in Fig. 1. With an increase in the time of hydrolysis to 40 h the number and distribution of the spots did not change, and we therefore limited ourselves to digestion for 18 h. The tryptophan-containing peptide 6 was detected after the plate has been sprayed with Ehrlich's reagent. After hydrolysis, the arginine was deblocked. To separate the peptides obtained, the dried mixture was dissolved in 30% acetic acid, but part of the hydrolysate did not dissolve.

The solution was centrifuged, the precipitate was separated off, and the supernatant liquid was deposited on a column of Sephadex G-50 fine equilibrated with 30% acetic acid. Elution was performed with 30% acetic acid. The results of the separation of the peptides on the column are shown in Fig. 2. The fractions obtained from the column were analyzed on the basis of their absorption at wavelengths 280 and 570 nm and, after alkaline hydrolysis, by the ninhydrin reaction. By analyzing the elution profile on the basis of absorption at a wavelength of 280 nm it was possible to obtain three large combined fractions, while combination with respect to the ninhydrin reaction and from the absorption at a wavelength of 570 nm gave 14 combined fractions, and we assumed that this combination permitted purer fractions to be obtained.

The purity of the combined fractions was checked by the TLC method on plates with a layer of cellulose and by determining the N-terminal amino acids by the dansyl method. According to their N-terminal amino acids and the results of TLC, of high-voltage paper electrophoresis and of disk electrophoresis, fractions 1, 2, 3, and 4 were homogeneous peptides. The amino acid compositions of these peptides were also identical, and they were therefore combined under the symbol $T_{Lys}1$. In addition, the peptide $T_{Lys}1$ corresponded to the first combined

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