The nature of the splitting of this signal (quartet, $J_1 = 11$ Hz, $J_2 = 4$ Hz) permits the assumption that the proton at C_8 is equatorial and, consequently, the methyl group is axial.

Analysis of the NMR spectra of nitramine and a number of its derivatives shows a similar orientation of the OH and CH_3 groups, and the difference between the alkaloids is apparently due to the method of linking the rings.

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PRIMARY STRUCTURE OF TRIACETINASE – AN ESTERASE FROM COTTON SEEDS. PEPTIDES FROM CYANOGEN BROMIDE HYDROLYSIS

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The isolation [1] and the quaternary structure [2, 3] of triacetinase – in esterase of cotton seeds [4] – have been reported previously. It has been shown that the esterase consists of four identical polypeptide chains with mol. wt. ~10,000 [1] and the amino-acid composition of a subunit has been determined (moles per mole): Asp, 13.5; Thr, 7.0; Ser, 9.8; Glu, 15; Pro, 4.2; Gly, 14; Ala, 11.1; Val, 3.2; Met, 3.8; Ileu, 3.0; Leu, 5.2; Phe, 3.8; His, 2.0; Lys, 4.2; Arg, 6.8; Tyr, 1.6; 1/2 Cys, 1.8. It has also been established that the N-terminal amino acid is methionine [1] and the C-terminal amino acid is tyrosine.

We now give the results of an investigation of the peptides from the cyanogen bromide hydrolysis of the triacetinase.

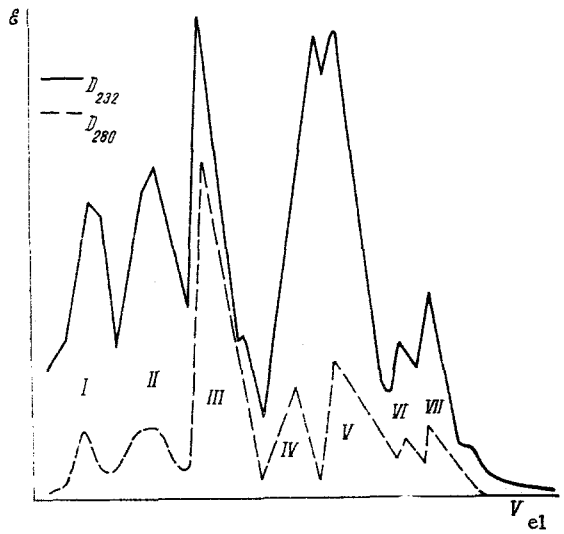

Fig. 1. Gel filtration of the peptides from the BrCN cleavage of triacetinase (column 180 × 1.5 cm equilibrated with 1 M ammonium acetate buffer, pH 7.4, $V_{el} = 3$ ml/h).

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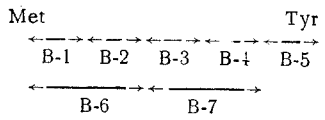
The reduced and carboxylated methylated protein was incubated with cyanogen bromide in a ratio of 1:100 (mole/mole) calculated as methionine, in 75% formic acid solution at room temperature for 30 h; the mixture of peptides was separated by gel filtration on Sephadex G-25 (superfine). Seven fractions non-homogeneous on TLC were obtained (Fig. 1), and these were purified by partition chromatography on Whatman 3 MM paper in the Bu–Py–AcOH–H_2O (15:10:3:12) system. This gave seven peptides of which the amino-acid compositions are given below:

Amino Acid	*B-1*	*B-2*	*B-3*	*B-4*	*B-5*	*B-6*	*B-7*
CM-Cys		0,7 (1)			0,9 (1)	0,8	
Aspartic acid	1,8 (2)	1,2 (1)	3,9 (4)	3,1 (3)	3,1 (3)	2,4 (3)	6,9 (7)
Threonine		0,9 (1)	1,7 (2)	1,6 (2)	1,7 (2)	0,7 (1)	3,7 (4)
Serine	1,1 (1)	1,2 (1)	2,8 (3)	3,2 (3)	2,2 (2)	2,3 (2)	5,4 (5)
Glutamic acid	1,2 (1)	2,2 (2)	5,2 (5)	3,9 (4)	3,1 (3)	2,3 (2)	9,3 (9)
Proline		0,6 (1)	0,9 (1)	1,7 (2)	1,3 (1)	0,7 (1)	2,4 (3)
Glycine	2,2 (2)		3,8 (4)	4,0 (4)	3,2 (3)	2,0 (2)	6,9 (7)
Alanine	1,3 (1)	1,2 (1)	3,2 (3)	3,6 (4)	2,5 (2)	2,1 (2)	7,9 (8)
Valine			1,2 (1)	1,4 (1)	1,1 (1)		2,4 (2)
Methionine							0,9 (1)
Leucine			2,0 (2)	2,3 (2)	2,0 (2)		4,1 (4)
Isoleucine			0,9 (1)	1,1 (1)	0,9 (1)		1,8 (2)
Tyrosine				0,8 (1)	0,7 (1)		1,0 (1)
Phenylalanine		1,2 (1)	0,9 (1)	1,4 (1)	1,1 (1)	1,3 (1)	2,2 (2)
Histidine			0,8 (1)		0,7 (1)		1,1 (1)
Lysine		1,0 (1)	1,5 (1)	1,0 (1)	1,0 (1)	1,1 (1)	2,8 (3)
Arginine	1,3 (1)		1,7 (2)	2,1 (2)	2,2 (2)	0,9 (1)	3,9 (4)
N-Terminal	Arg	Thr	Asp	Pro	His	Arg	Asp
Total	8	10	32	32	27	18	64

As can be seen from the figures given, peptide B-6 is a product of the incomplete hydrolysis of peptides B-1 and B-2 at the Met-Tre bond and peptide B-7 is the product of the incomplete hydrolysis of peptides B-3 and B-4 at Met-Pro bonds and, therefore, the cyanogen bromide peptides B-1, B-2, B-3, B-4, and B-5 make up the complete triacetinase molecule. On comparing the results on the amino-acid composition and the N-terminal sequence of the native enzyme and of the peptides isolated it is possible to propose the following scheme of reconstruction of triacetinase from the cyanogen bromide fragments:



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