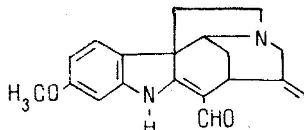
The IR spectrum has absorption bands at 830–860 cm^{-1} (1,2,4-trisubstituted benzene ring), 1560, 1650 cm^{-1} (carbonyl group conjugated with a double bond), and 3400 cm^{-1} (NH).

On comparing the mass spectrum of vincanicine with the spectrum of vincanine (see table) it can be seen that the values of the peaks of the nonindole ions in them are similar while the indole peaks differ by thirty mass units, which corresponds to the presence of a methoxyl group in the benzene part of the molecule. The NMR spectrum (taken on a JNM-4H-100/100 MHz instrument in $CDCl_3$) of vincanicine differs from that of vincanine by a three-proton singlet (methoxyl group) at δ 3.80 ppm. The mass spectra were taken on a MKh-1303 instrument with an ionizing potential of 32 eV at 105, 95, and 100° C, respectively.

When an ethanolic solution of vincanicine was boiled with methyl iodide, vincanicine methiodide was obtained. It proved to be identical (R_f, mp, IR and UV spectra) with the methiodide of the O-methyl ether of vincanidine [4].

On the basis of what has been presented above, it has been established that vincanicine is the O-methyl ether of vincanidine and has the structural formula



REFERENCES

1. S. Yu. Yunusov, Alkaloids [in Russian], Tashkent, p. 137, 1968.
2. P. Kh. Yuldashev and S. Yu. Yunusov, DAN UzSSR, no. 3, 28, 1960.
3. Kh. Ubaev, P. Kh. Yuldashev, and S. Yu. Yunusov, DAN UzSSR, no. 10, 34, 1964.
4. P. Kh. Yuldashev, Kh. Ubaev, M. A. Kuchenkova, and S. Yu. Yunusov, KhPS [Chemistry of Natural Compounds], 1, 34, 1965.
5. H. Budzikiewicz et al. Tetrah., 19, 1265, 1963

10 June 1969

Institute of the Chemistry of Plant Substances AS UzSSR

UDC 547.96

STRUCTURE OF THE PEPTIDES OF THE C-TERMINAL FRAGMENT OF PEPSIN

L. G. Muratova, E. D. Levin, and V. M. Stepanov

Khimiya Prirodnykh Soedinenii, Vol. 5, No. 5, pp. 462–463, 1969

It has been shown previously that the specific cleavage of reduced carboxymethylated pepsin with cyanogen bromide forms peptide fragments one of which (B-1) occupies the C-terminal position in the molecule of pepsin and contains 47–49 amino acids [1].

The chymotrypsin hydrolysis of B-1 at pH 10 (4 hr; 37° C; enzyme–substrate ratio 1 : 30) and the fractionation of the hydrolysate on Dowex 1 × 2 enabled a number of peptides to be obtained and the amino acid sequence in some of them to be determined. The peptide "9-2" contains Ile_1, Leu_1, Gly_1, Asp_1, Val_1, and Phe_1. Carboxypeptidase A successively split off from this peptide phenylalanine, valine, and aspartic acid, and by a combination of Edman's method and dansylation the N-terminal sequence of "9-2" was established as Ile-Leu-Gly. Thus, the peptide "9-2" has the structure Ile-Leu-Gly-Asp-Val-Phe, which is identical with the structure of the hexapeptide occupying the N-terminal position in a pepsin fragment containing 27 amino acids and found at the carboxyl end of the molecule of the enzyme [2].

The "12-3" decapeptide was subjected to hydrolysis with oryzin (pH 5.6; 1 hr; 37° C; enzyme–substrate ratio 1 : 10) and paper chromatography yielded two tripeptides having the compositions Ser_2, Gly_1 and Ser_1, Gly_1, Glu_1. Both tripeptides had serine at the N-end and therefore in the "12-3" peptide they are present in the composition of the sequence Ser-Ser-Gly-Glu. Carboxypeptidase A splits out tryptophan and leucine from the "12-3" peptide. In view of the sequence established previously for the pentapeptide present at the N-terminal part of B-1, Asp-Val-Pro-Thr-Ser [1], the structure of the "12-3" peptide may be given in the following way: Asp-Val-Pro-Thr-Ser-Ser-Gly-Glu-Leu-Try.

In addition to the "9-2" and "12-3" peptides, a "10" peptide with the composition Thr_1, Ile_1, Asp_1, Ser_2, Pro_1, Gly_1, Val_1, Leu_1, Glu_1, Ala_1, Try_1 was isolated from the chymotrypsin hydrolysate. We may note that the presence of tryptophan, which is split off in the course of acid hydrolysis, may distort the results of amino acid analysis and therefore the composition of the peptide "10" will be refined in the course of the structural investigations.

Taking into account the results obtained previously in a study of the structure of the fragment B-1 [3], it may be considered that porcine pepsin has the C-terminal sequence Asp-Val-Pro-Thr-Ser-Ser-Gly-Glu-Leu-Tri-(Thr_1, Ile_1, Asp_1, Ser_2, Pro_1, Gly_1, Val_1, Leu_1, Glu_1, Ala_1, Try_1)-Ile-Leu-Gly-Asp-Val-Phe-Ile-Arg-Gln-Tyr-Tyr-Thr-Val-Phe-Asp-Arg-Ala-Asn-Asn-Lys-Val-Gly-Leu-Ala-Pro-Val-Ala.

REFERENCES

1. V. I. Ostoslavskaya, I. B. Pugacheva, E. A. Vakhitova, V. F. Krivtsov, G. L. Muratova, E. D. Levin, and V. M. Stepanov, Biokhim., 33, 331, 1968.
2. T. A. A. Dopheide, S. Moore, W. H. Stein. J. Biol. Chem., 242, 1833, 1967.
3. R. A. Matveeva, R. F. Krivtsov, and V. M. Stepanov, Biokhim., 33, 167, 1968.

23 May 1969

Institute of the Chemistry of Natural Compounds AS USSR

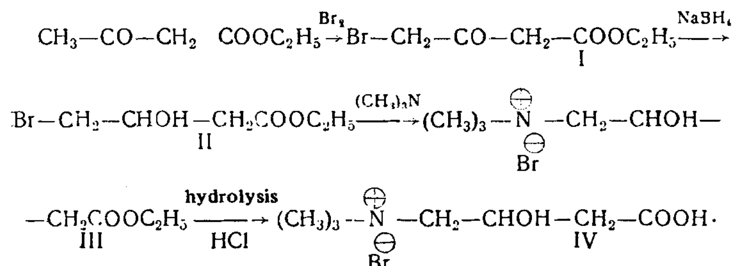
UDC 615.577.164.18-01

SYNTHESIS OF d,l-CARNITINE (VITAMIN B_T)

E. D. Vasil'eva

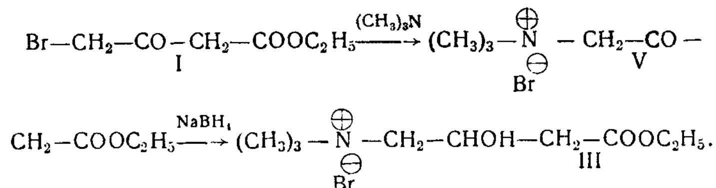
Khimiya Prirodnykh Soedinenii, Vol. 5, No. 5, p. 463, 1969

We have synthesized d,l-carnitine by the method of D'alo and Messerini [1] from acetoacetic ester by the following scheme:



To simplify the synthesis, the intermediate products, γ-bromoacetoacetic ester (I) and ethyl γ-bromo-β-hydroxybutyrate (II), were subjected to further reactions without preliminary purification. Carnitine was isolated in the pure form from the final mixture of products by means of ion-exchange chromatography on the cation-exchanger Dowex 50W × 8. The yield of carnitine chloride was 7% calculated on the initial acetoacetic ester.

The yield of carnitine increased to 10% if the scheme of synthesis was varied in such a way that I was subjected to amination instead of II, with subsequent reduction of the resulting γ-trimethylaminoacetoacetic ester salt V:



The d,l-carnitine chloride that we obtained was identical with the l-carnitine chloride isolated from rat muscle with respect to its mobility on paper chromatograms; it gave the characteristic coloration with Dragendorff's reagent and reacted with bromophenol blue, forming the corresponding complex [2]. Because of the exceptional hygroscopicity of carnitine chloride [3], the product obtained was not subjected to recrystallization: it had mp 190–192° C (decomp.). According to the literature [4], mp 195–196° C.