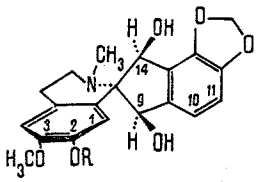
methanol), the properties of which agree well with those of d-raddeanine [2]. The hydroxy group in ledebouridine is present at C_2, since in its NMR spectrum the methoxy group gives a signal at 3.75 ppm, and in the product of its methylation signals at 3.35 and 3.78 ppm [1]. On the basis of the facts given, the following structure (II) may be proposed for ledebouridine:



I. R=CH_3
II. R=H
III. C_9=O, R=CH_3

LITERATURE CITED

1. I. A. Israilov, M. S. Yunusov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 268 (1975).
2. T. Kametani, M. Takemura, M. Ihara, and H. K. Fukumoto, Heterocycles, 4, 73 (1976).
3. C. K. Yu and D. B. MacLean, Can. J. Chem., 49, 3025 (1971).
4. S. -T. Lu, T. -L. Su, T. Kametani, A. Ujiie, M. Ihara, and K. Fukumoto, J. Chem. Soc., Perkin I, 63 (1976).

INDOLE ALKALOIDS OF A CALLUS CULTURE OF Vinca rosea

T. I. Andreeva and L. N. Bereznegovskaya

UDC 547.944/945

There is contradictory information concerning the capacity of callus tissue of Vinca rosea (Madagascar periwinkle) for synthesizing alkaloids. Some workers [1] have obtained tissue capable of biosynthesizing monomeric and dimeric alkaloids, while others [2] have found only monomeric bases in such tissues. This is the first time that information on the chemical composition of the tissue has been published.

Callus tissue which we obtained in 1974 was grown in modified Murashige-Skoog medium in the dark at −27°C and at a humidity of 70%.

By standard methods we showed the capacity of the tissues for synthesizing indole alkaloids. Depending on their origin and the times of their growth we isolated 0.10-0.20% of combined alkaloids. By chromatographic methods in extracts from the tissues we detected alkaloids with R_f 0.18, 0.29, 0.35, 0.51, 0.60, 0.64, 0.67, 0.76, and 0.90 (TLC on alumina, benzene–ethanol (9:1) system). The combined alkaloids consisted of a complex mixture of bases which it was difficult to separate by the usual methods.

We separated the combined material into several fractions [3]. By chromatographing them in various systems and treating the chromatograms with specific reagents (1% solution of cerium ammonium sulfate in 85% orthophosphoric acid) we were unable to achieve a good separation and staining of the alkaloids, and therefore some of them contained a considerable amount of pigments. By chromatographing these fractions on a column containing inactivated alumina [4] followed by preparative separation on "Silufol" plates we obtained five individual substances. Their melting points, R_f values in various systems, color reactions, UV spectra, and a comparison with literature information [5] permitted the conclusion that they were ajmalicine, catharanthine, vindoline, lochnericine, and vinblastine. The other alkaloids could not be obtained in the pure form and be identified. Apart from the known alkaloids, seven bases the R_f values and color reactions of which corresponded to no compounds given in the literature were isolated.

Tomsk Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 3, p. 429, May-June, 1977. Original article submitted February 22, 1977.

LITERATURE CITED

1. F. C. Czygan, Planta Med., Suppl., 169-185 (1975).
2. B. D. Patterson and D. P. Carew, Lloydia, 32, 2 (1969).
2. G. H. Svoboda and J. S. N. Neuss, J. Amer. Pharm. Assoc., Sci. Ed., 48, 11 (1959).
4. G. H. Svoboda and J. S. Johnson, J. Pharmac. Sci., 51, 8 (1962).
5. N. J. Cone, R. Miller, and N. Neuss, J. Pharm. Sci., 52, 7 (1963).

DETERMINATION OF THE NUMBER OF METHIONINE RESIDUES IN PEPTIDES BY THE METHOD OF PARTIAL SUBSTITUTION

A. B. Silaev, G. S. Katrukha, and S. N. Maevskaya

UDC 547.96

Methionine is one of the few amino acids the quantitative determination of which is not infrequently made difficult through its loss during the isolation and hydrolysis of peptides because of its ease of oxidation to the corresponding sulfoxide or sulfone [1-4].

In our opinion, it may be better to determine the number of methionine residues (or, in general, the number of sulfide groupings) in natural materials by a method based on partial substitution in combination with paper electrophoresis, the principle of which has been described in detail previously in papers dealing with the determination of the number of free NH_2, -SH, -COOH, and OH groups in polypeptides and aminocarbohydrates [5-9].

In contrast to residues of diamino or dicarboxylic amino acids, methionine residues present in a polypeptide chain are electroneutral, and therefore the electrophoretic variant of the method of partial substitution can be used only if the sulfide grouping of methionine is alkylated with a suitable alkyl halide. It is known [10] that the acylation reaction forms a sulfonium salt derivative of the sulfide bearing a charge of +1:

$$\begin{matrix}CH_3\\R^1\end{matrix}\!\!>\!S + X - R^2 \rightarrow \left[\begin{matrix}CH_3\\R^1\end{matrix}\!\!>\!\overset{+}{S} - R^2\right] X^-,$$

where X is I, Br, Cl.

Of the alkyl halides that we have tested, the most suitable proved to be iodoacetamide (IAA), which in the pH range below 4.0 reacts strictly specifically with methionine residues to form the S^+-carbamoylmethyl derivative of methione (S^+-CAM-Met), which has an additional positive charge (+1) in a wide pH range [11, 12]. Because of the acquisition of the additional charge, the S^+-CAM derivative of methionine (or a peptide containing a methionine residue or another compound with a sulfide group) will migrate to the cathode on paper electrophoresis at a greater rate than the initial compound. If the peptide analyzed contains one methionine residue (or one-SR group), the electrophoretogram should show one additional spot migrating to the cathode faster than the initial peptide, and if the peptide contains two methionine residues there should be two additional spots, and so on. The number of methionine residues in the peptide N=n-1, where n is the number of spots on the electrophoretogram including the spot of the initial peptide. The degree of substitution is regulated by the time of reaction of the IAA, taken in excess, with the peptide. The method has been checked on a number of model compounds and peptides (Fig. 1), and this has shown its reliability. The number of methionine residues in peptides can be determined with a high accuracy using microamounts (2-10 μmole) with molecular weights between 100 and 3000 daltons.

The reaction of 2-10 μmole of a peptide with 20-30 μmole of IAA is performed in 0.5 ml of 85% HCOOH-CH_3COOH-H_2O (28:20:52) [6] at 37°C for 3-5 h with the periodic removal (every 1 h) of aliquots and their de-

M. V. Lomonosov Moscow State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 429-431, May-June, 1977. Original article submitted January 12, 1977.