MICROPHOTOMETRIC DETERMINATION OF 1-DIMETHYLAMINONAPHTHALENE-5-SULFONYLAMINO ACIDS WITH GLYCINE AS AN EXAMPLE

V. Kh. Lapuk, A. A. Strongina, and M. Z. Zalesskii

Khimiya Prirodnykh Soedinenii, Vol. 5, No. 6, pp. 531–535, 1969

UDC 547.466.22

The method of determining the content of N-terminal amino acids in peptides and proteins in the form of 1-dimethylaminonaphthalene-5-sulfonyl derivatives (dansyl derivatives)* [1] is being employed more and more widely because of its high sensitivity and simplicity in use. Thin-layer chromatography on silica gel is generally used to separate the dansyl amino acids. Up to the present time, the investigations of amino acids in the form of the dansyl derivatives have mainly been qualitative. Nevertheless, their quantitative evaluation on thin-layer chromatograms is of great interest. Little light has been thrown on this question in the literature [2–4].

By using the results of the work of Mardashev et al. [5] on the microphotometric determination of amino acids on thin-layer chromatograms, we have developed, using glycine as an example, a simple method for the quantitative determination of dansyl amino acids. It consists in photographing a thin-layer chromatogram of the dansyl amino acids on an ordinary "Foto-32" 35-mm film with a ZhS-12 filter and photometering the spots on the negative obtained with respect to their area in an MF-4 microphotometer under the conditions described by Mardashev et al. [5].

This method possesses a number of advantages. In the first place, the photometry of the negative can be carried out repeatedly at any intervals of time, in an attempt to obtain the best result. In the second place, the demands on the quality of the layer of silica gel fall markedly, its moisture content having no effect [2]. And, finally, in itself the negative is useful for documentation, since thin-layer chromatograms are generally cited in the form of schematic figures.

Under our conditions, the photographic film records fairly small amounts of dansyl amino acids (about $1 \cdot 10^{-10}$ mole per spot) on the chromatogram. Where it is necessary to increase the sensitivity of the method, a more sensitive film of Russian manufacture (about 130–180 GOST [State Standard] units) may be used. On photometry of the negative on the recording photographic plate of the MF-4 photometer, a curve is obtained which forms a series of peaks (Fig. 1). This curve is copied onto tracing cloth and the area of each peak is measured with a planimeter. As can be seen from Fig. 2, direct proportionality exists between the amount of dansylglycine in the spot and the area of the peak corresponding to this spot (from 10^{-10} to 10^{-9} mole). The time of exposure can be varied within wide limits without disturbing the direct proportionality. A total of 12 experiments was carried out. In these experiments the photographic exposure, the amount of glycine, the developers, etc., were varied. In all the experiments a satisfactory proportionality between the amount of dansylglycine in the spot and the area of the peak on the MF-4 was obtained over a wide range of amounts of dansylglycine. The greatest accuracy of the determination is obtained with exposures giving a light background on the negative, i. e., when the straight-line curve cuts the axis of ordinates above the origin. Thus, in Fig. 2 the arithmetic mean deviation of the points from the straight lines 1, 2, and 3 were, respectively, ±5.4%, ±0.8%, and ± 2.1%.

Since within the limits that we have mentioned the direct proportionality between the amount of dansylglycine and the area of the peak in the MF-4 is preserved satisfactorily, the practical determination of the amount of dansylglycine is carried out in the following way. The chromatography of the sample to be determined is carried out with the simultaneous deposition of a series of not less than three control spots containing accurately known amounts of dansylglycine. On analyzing the negative of the chromatogram in the MF-4, a calibration curve similar to those shown in Fig. 2 is constructed from the figures for these points. The amount of dansylglycine in the samples under investigation is determined from the graph. The method given has been used in practice for the determination of dansylglycine in amounts of the order of 10^{-10}–10^{-9} mole, i. e., the amounts that are usually found in thin-layer chromatographic practice, with an accuracy of ±3%.

*The following abbreviations are used: 1-dimethylaminonaphthalene-5-sulfonic acid (dansyl-OH); 1-dimethylaminonaphthalene-5-sulfonamide (dansyl-NH_2); 1-dimethylaminonaphthalene-5-sulfonyl chloride (dansyl-Cl).

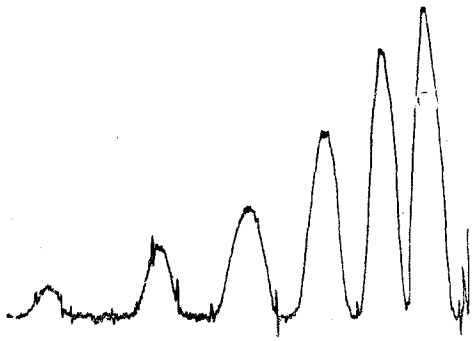

Fig. 1. Curve of the photometry, using a MF-4 instrument, of the spots on the negative obtained by photographing a thin-layer chromatogram of dansylglycine. The amounts of dansylglycine in order of increasing area of peaks were 1, 2, 3, 5, 6, and $8 \cdot 10^{-9}$ mole.

For quantitative evaluation it is important that the dansylation of the amino acids is carried out exhaustively. However, there is information that the dansylation of amino acids under the usual conditions (aqueous acetone) does not take place completely because of the parasitic formation of dansyl-NH_2 [6, 7]. When dansylation is carried out under the conditions that we have selected (in dioxane), no dansyl-NH_2 whatever is formed, either with glycine or with other amino acids, which may indicate the completeness of the dansylation process.

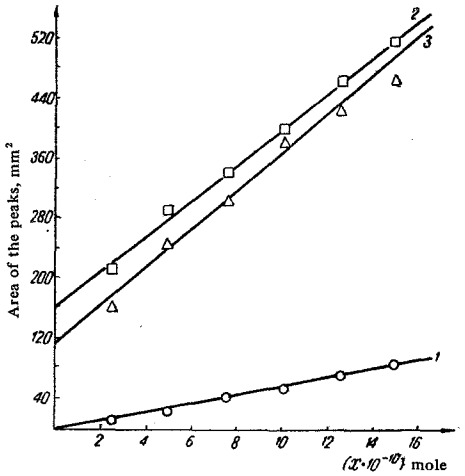

Fig. 2. Dependence of the areas of the peaks (Fig. 1) on the amount of dansylglycine in the spot on the thin-layer chromatogram corresponding to them: photographic exposures: 1) 3 min; 2) 5 min; 3) 15 min.

Preliminary experiments with the dansyl derivatives of six amino acids have shown that the areas of the peaks corresponding to similar amounts of different amino acids in the spots are similar, but not completely identical. Consequently, for the application of the proposed method to all dansyl amino acids it is obviously necessary first to determine correction factors for each of them in relation to one taken as a standard, after which it will be possible to use the calibration curve of this standard dansyl amino acid to determine the other dansyl amino acids.

The information given above shows that the proposed method for determining dansyl amino acids, as applied to dansylglycine as an example, is fairly simple, rapid, and accurate.

EXPERIMENTAL

Preparation of the silica gel plates and chromatography of the dansyl amino acids. The experiments were carried out with glass plates 13 × 18 cm upon each of which 1.25 g of KSK silica gel (150 mesh), 0.075 g of gypsum, and 10 ml of water were deposited. The layers were deposited on the plates manually by pouring. The plates were dried at room temperature for 12 hr and at 110° C for 30 min. Chromatography was carried out with the chloroform–benzyl alcohol–ethyl acetate–acetic acid (6 : 4 : 5 : 0.2) system [8]. The samples were deposited on the plates with a calibrated capillary having a scale value of 0.1 μl. The capillaries were calibrated with a mixture of dioxane and water (2 : 1). The volume deposited was 0.2–1 μl.

Preparation of the control solutions of dansyl amino acids (with dansylglycine as an example). A 0.1 ml sample was taken from a solution of 17.7 mg ($9.6 \cdot 10^{-5}$ mole) of glycine in a mixture of 4.8 ml of a 1% aqueous solution of $NaHCO_3$ and 4.3 ml of distilled dioxane (concentration $1 \cdot 10^{-5}$ mole/ml). To this sample was added 0.1 ml of a 0.25% solution of dansyl-Cl in dioxane and the mixture was kept in the dark for 2 hr; it was then evaporated to dryness in a rotary evaporator at a temperature not exceeding 30° C and was treated with 1 ml of dioxane–water, giving a solution of dansylglycine with a concentration of $1 \cdot 10^{-6}$ mole/ml, aliquots of which were deposited on the plates. Samples of the other dansyl amino acids were obtained similarly.

Photography of the thin-layer chromatograms of the dansyl amino acids. The chromatograms were photographed under UV radiation (λ = 360–390 mμ) by means of a portable luminescence irradiator with a UFO-15 lamp and Uviol glass filters. The irradiator was placed at a distance of 40 cm from the surface of the plate. "Foto-32" film and a "Zenit-S" camera with extension ring 2 were used. In the selection of the filter for photography, 19 different samples were tested, and a ZhS-12 filter was chosen. Using an aperture of 5.6, various exposures from 30 sec to 30 min were used, and it was found that under our conditions the optimum exposure was 5 min.

After the passage of the solvent, the chromatograms were dried in the air for 30 min and then at 100–110° C for 10 min, and they were photographed not later than 20 min after drying, since the fluorescence of the spots fades with time. The films were treated with DK-20 developer [9] and the spots were photometered in an MF-4 microphotometer [5] using a slit as narrow as possible with a height somewhat greater than the diameter of the largest spot on the chromatogram.

CONCLUSIONS

1. A method for the quantitative evaluation of thin-layer chromatograms of dansylglycine by photographing them and microphotometering the negative obtained has been proposed.
2. The method is applicable to amounts of $1 \cdot 10^{-10}$–$1 \cdot 10^{-9}$ mole of dansylglycine per spot with an accuracy of ±3%.
3. It is proposed to obtain dansyl amino acids by using dioxane as solvent, which completely prevents the parasitic formation of dansylamide.

REFERENCES

1. W. R. Gray and B. S. Hartley, Biochem. J., 89, 590, 1963.
2. N. Seiler and M. Wiechmann, Z. anal. Chem., 220, 109, 1966.
3. G. Pataki and E. Strasky, Chimia, 20, 361, 1966.
4. N. Seiler, G. Werner, and M. Wiechmann, Naturw., 50, 643, 1963.
5. S. R. Mardashev, M. Z. Zalesskii, S. I. Pekhtereva, and L. A. Semina, Biokhim., 32, 655, 1967.
6. D. J. Neadle and R. J. Pollitt, Biochim. J., 97, 607, 1965.
7. N. Seiler and M. Wiechmann, J. Chromatog., 28, 351, 1967.
8. B. G. Belen'kii, E. S. Gankina, and V. V. Nesterov, DAN SSSR, 172, 91, 1967.
9. V. P. Mikulin, Handbook of Photographic Formulations [in Russian], Moscow, 113, 1960.

16 April 1968

Zelinskii Institute of Organic Chemistry, AS USSR