DETERMINATION OF IMMOBILIZED DIGITONIN WITH THE AID OF AN ANTHRONE REAGENT

I. V. Berezin, I. P. Andrianova, and Ya. I. Lapuk

UDC 547.918

A colorimetric method using an anthrone reagent has been developed for the direct quantitative determination of digitonin oxidized with $NaIO_4$ in solution and after immobilization on Aminosilochrome. The amounts of immobilized digitonin found agree with the results obtained by the sorption of cholesterol from blood serum.

In the creation of an affinity sorbent for extracting cholesterol from blood serum it is possible to use steroid glycosides as affinity ligands and Aminosilochrome as solid support. To evaluate the efficacy of the sorbent synthesized a method is required which permits the amount of steroid glycoside on the support to be determined. A method of determining digitonin with the aid of an anthrone reagent, based on the reaction of the carbohydrate moiety of the digitonin molecule with the anthrone reagent, is known [1, 2]. However, it has been used to determine free digitonin, while for the immobilization of digitonin we have used the periodate oxidation of the glycoside molecule, leading to the partial decomposition of its oligosaccharide moiety.

The aim of the present work was to study the possibility of using the anthrone method for determining oxidized digitonin immobilized on a support.

Determination of Free Digitonin Sorbed on a Support

Before proceeding to the immobilization of digitonin, we studied the sorption of free digitonin on Aminosilochrome. Samples of Aminosilochrome were stirred in solutions of digitonin of different concentrations in order to find the optimum concentrations at which the greatest sorption took place. As solvents we used water and a 50% aqueous solution of dioxane. The amounts of digitonin in the solutions above the precipitate were determined before and after sorption.

The degree of sorption of digitonin on a solid support was estimated from the concentration of digitonin in the solution and was expressed in milligrams per gram of sorbent.

The free digitonin was determined spectrophotometrically after the reaction with the anthrone reagent under the standard conditions used for determining neutral carbohydrates [3]. It is known that in the reaction of digitonin with the anthrone reagent a green coloration develops. The spectrum of the reaction mixture has two maxima — at 495 and 625 nm [4] — the latter being characteristic for the products of the reaction of the anthrone reagent with neutral sugars [5]. The optical density measured at 625 nm is directly proportional to the amount of free digitonin in the range of 10-100 μg (Fig. 1, curve 1). In the measurements of the sorption of digitonin (Fig. 2) it was found that the maximum sorption of digitonin on Aminosilochrome amounts to 8 mg/kg at a concentration of digitonin in either of the solvents used of 8-10 mg/ml. However, digitonin sorbed on Aminosilochrome is readily washed off by a tenfold amount of water with respect to the sorbent, and the support ceases to absorb cholesterol from blood serum.

Determination of Oxidized Digitonin

For the chemical binding of digitonin with Aminosilochrome, the steroid glycoside was oxidized with sodium periodate, as a result of which the α-glycol groupings of the carbohydrate residues of the digitonin were partially converted in aldehyde groups, which reacted with the amino groups of the Aminosilochrome. It was necessary to ascertain the possibility

M. V. Lomonosov Moscow State University. N. I. Pirogov Second Moscow State Medical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 453-457, July-August, 1981. Original article submitted January 14, 1981.

 0009-3130/81/1704-0334$07.50

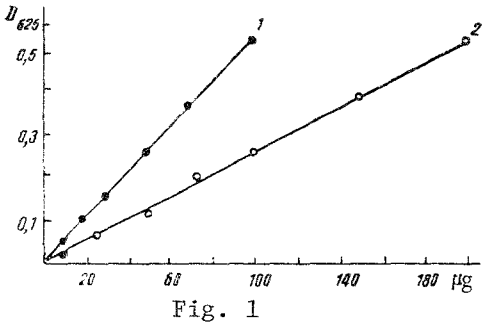

Fig. 1

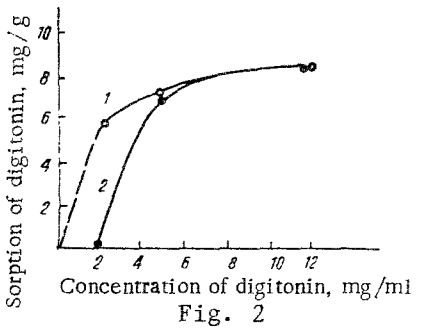

Fig. 2

Fig. 1. Calibration curves obtained in the reaction of the anthrone reagent 1) with digitonin (time of heating 5 min); 2) with oxidized digitonin (time of heating 10 min). The weight ratio of digitonin to sodium periodate was 1:2.

Fig. 2. Dependence of the sorption of digitonin on Aminosilochrome on the concentration of digitonin in water (1) and in 50% aqueous dioxane (2).

of the quantitative determination of the oxidized digitonin with the aid of the anthrone reagent. The digitonin was oxidized with sodium periodate in 50% aqueous dioxane solution for different times and with different weight ratios of digitonin and oxidizing agent. Samples corresponding to 10-200 μg of oxidized digitonin were taken from the reaction mixture and the anthrone reaction was performed. It was found that it was possible to follow the oxidation process from the change of the spectrum of the reaction mixture formed as the result of the reaction of the oxidized digitonin with the anthrone reagent. Depending on the amount of oxidizing agent, the spectrum underwent various changes: Thus, at an equimolar ratio of digitonin and oxidizing agent (weight ratio 1:1) in place of the maximum at 625 nm maxima appeared at 615, 555, and 510 nm. With a twofold molar excess of oxidizing agent (weight ratio of digitonin to oxidizing agent 1:2), the spectrum had no well-defined maximum in the 510-625 nm region, and in both cases the nature of the spectra did not change in a time of 3 h. Thus, the course of the process is determined by the amount of oxidizing agent.

For the quantitative estimation of the oxidation process, aliquots containing 10-200 μg of oxidized digitonin were taken from the reaction mixture, the reaction with the anthrone reagent was performed, the optical densities of the solutions at 625 nm were determined, and a calibration curve was plotted. It was found that in this case, in spite of the absence of a characteristic maximum at this wavelength a linear relationship between the optical density and the amount of oxidized digitonin was observed just as in the case of the native digitonin, which makes a quantitative determination of the oxidized digitonin possible. However, as could have been expected, the optical density for the oxidized, and, therefore, partially decomposed digitonin was lower than for the same concentration of native digitonin (Fig. 1, curve 2).

Determination of Immobilized Digitonin

Since a quantitative determination of oxidized digitonin with the aid of the anthrone reagent had proved to be possible, attempts were made to use it for the quantitative determination of immobilized digitonin. For immobilization, the reaction mixture after the oxidation of the digitonin was brought into contact with Aminosilochrome, and the low-molecular-weight reaction products were washed out with a tenfold amount of water in relation to the support. The digitonin-Aminosilochrome was analyzed for its digitonin content. For this purpose, a weighed sample of sorbent was treated with 4 ml of anthrone reagent and 1 ml of 50% aqueous dioxane and the mixture was heated on the water bath. The coloration that developed was measured at 625 nm against a control. It was found that the optical density of the reaction mixture was directly proportional to the weight of the sample of sorbent for a given time of heating (Fig. 3). With an increase in the time of heating of equal-sized samples the optical density rose and reached a maximum after 15 min. However, heating should not be carried out for longer than 10 min, since after this the optical density of the blank experiment rises sharply (Fig. 4). Consequently, for immobilized digitonin we used heating for 10 min. In this case, after reaction with the anthrone reagent 10 mg of sorbent exhibited an optical density of 0.760, which according to the calibration curve 2 of Fig. 1 corresponds

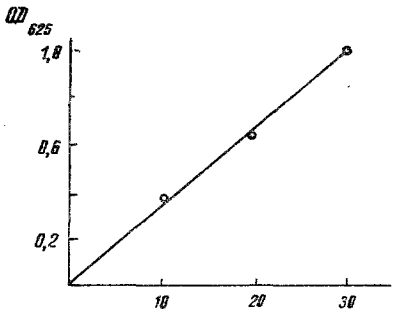

Fig. 3

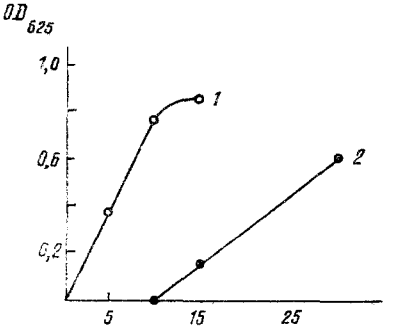

Fig. 4

Fig. 3. Dependence of the optical density on the weight of the sample in the reaction of the anthrone reagent with various amounts of digitonin-Aminosilochrome. Time of heating 5 min.

Fig. 4. Influence of the time of heating on the optical density of the reaction mixture obtained in the reaction of the anthrone reagent with 10 mg of digitonin-Aminosilochrome (1) and on the heating of 10 mg of Aminosilochrome with the anthrone reagent for different intervals of time (2).

to 30 mg of digitonin calculated to 1 g of sorbent. Since digitonin binds cholesterol in equimolar ratio [6], 1 g of such sorbent should bind 9 mg of cholesterol. Results obtained by Webster's method [7] in a determination of the amount of cholesterol in blood serum before and after sorption of the cholesterol by the affinity sorbent confirmed this.

EXPERIMENTAL

The anthrone reagent was prepared as described by Sudhof et al. [3].

The control solution of digitonin was obtained by dissolving digitonin in 50% aqueous dioxane to a concentration of 100 μg/ml. To plot the calibration curve, 0.1-, 0.2-, 0.3-, 0.5-, 0.7-, and 1.0-ml samples of this solution were taken.

Reaction with the Anthrone Reagent. Each of a number of test tubes with ground-in stoppers was charged with 4 ml of anthrone reagent, an aliquot of the control digitonin solution (0.1-1 ml), and 50% dioxane to a total volume of 5 ml. The contents of the test tubes were shaken vigorously, and they were placed in the boiling water bath (for 5, 10, and 15 min). A green coloration developed which was measured at a wavelength of 625 nm against a control — 4 ml of the anthrone reagent and 1 ml of 50% aqueous dioxane.

Sorption of Digitonin on Aminosilochrome in Water and 50% Aqueous Dioxane Solution. Six 0.025-g samples of digitonin were dissolved with heating on the water bath in 2, 5, and 10 ml of water and the same volumes of 50% aqueous dioxane. To each of the resulting solutions was added 0.5 g of Aminosilochrome and the mixtures were shaken at room temperature for 6 h and left for 12 h, after which the supernatant liquid was poured off and the amount of digitonin in it was determined by heating an aliquot with the anthrone reagent for 5 min. The amount of digitonin was determined from the calibration curve 1 of Fig. 1. Sorption was calculated in milligrams per 1 g. The results obtained are given in Fig. 2.

Oxidation of Digitonin with Sodium Periodate. To 0.05 g of digitonin was added 0.05 or 0.1 g of sodium periodate dissolved in 5 ml of 50% aqueous dioxane and the mixture was stirred in the dark for various intervals of time (30 min, 2, 3, and 3 h), the reaction with the anthrone reagent was performed for 10 min, and then the absorption spectrum was recorded in the 350-750 nm region.

Control Solution of Oxidized Digitonin. To 0.05 g of digitonin was added 0.1 g of sodium periodate dissolved in 5 ml of 50% aqueous dioxane the mixture was stirred in the dark for 1 h, aliquots were taken, the reaction with the anthrone reagent was performed for 10 min, and calibration curve 2 of Fig. 1 was plotted.

Immobilization of the Oxidized Digitonin on Aminosilochrome. To the oxidation mixture (see the control solution of oxidized digitonin) was added 1 g of Aminosilochrome and the resulting mixture was stirred for another 6 h and was then kept at room temperature for 12 h and the supernatant liquid was poured off, after which the sorbent was washed with 20 ml of water and was dried in the air. It was stored in the refrigerator in a dark vessel.

Determination of Digitonin Immobilized on Aminosilochrome. In a test-tube, 10 mg of digitonin-Aminosilochrome was covered with 4 ml of the anthrone reagent and 1 ml of 50% aqueous dioxane, the mixture was heated in a boiling water bath for 10 min, and the amount of immobilized digitonin was determined in milligrams per 1 g of sorbent from the control curve for oxidized digitonin.

CONCLUSION

1. A colorimetric method has been developed for determining oxidized digitonin in the free and immobilized states.

2. A correspondence of the amount of immobilized digitonin on the sorbent and of the sorption of cholesterol from blood serum has been shown.

LITERATURE CITED

1. P. J. J. van Manster, Pharm. Weekblad, 87, 790 (1952).
2. M. A. Copello, Anales Direc. Nacl. Quim., 6, 22 (1953).
3. H. Sudhof, H. Kellner, and N. Schulte, Z. Physiol. Chem., 300, 68 (1955).
4. T. Nakata, Tokyo Jikeikai Ika Daigaku Zasshi, 72, 472 (1957); Chem. Abstr., 52, 15835 (1958).
5. J. A. Kowald and R. B. McCormack, Anal. Chem., 21, 1384 (1949).
6. G. V. Vahouny, C. R. Borja, R. M. Mayer, and C. R. Treadwell, Anal. Biochem., 1, 3719 (1960).
7. D. Webster, Clin. Chim. Acta, 7, 277 (1962).

NEW SYNTHESIS OF (±)-O-METHYLDAURICINE

E. P. Nakova, O. N. Tolkachev, and R. P. Evstigneeva

UDC 547.944/945

A new synthesis of O-methyldauricine has been effected through an intermediate bisthioamide obtained by means of the Wilgerodt–Kindler reaction from 2-acetyl-5-methoxyphenyl 4'-acetylphenyl ether and homoveratrylamine. Subsequent Bischler–Napieralski cyclization, methylation, and reduction yielded racemic O-methyldauricine.

O-Methyldauricine, originally known as an unnatural derivative of dauricine obtained by methylating the alkaloid with diazomethane, was first detected in plant sources (*Colubrina asiatica* and others) only in 1970 [1-3]. The synthetic racemic compound obtained exhibited antitumoral activity [4]. The first attempt to synthesize this alkaloid dates back to 1935 [5]. Several schemes of synthesis of (±)-O-methyldauricine (V) including the stage of obtaining a bisamide and differing only by the method of obtaining the initial diphenyl ether have been published [5-7]. We have previously described a method of obtaining the intermediate bisamide from a bis(aminovinyl sulfide) [8]. Another method for synthesizing the alkaloid is based on the use of a Reissert compound [9, [0] and the Ullmann condensation of benzylisoquinoline fragments [11, [2]. However, the low overall yield of the desired compound is stimulating the search for new synthetic routes. We have also previously described a simple method for obtaining thioamides by the Wilgerodt–Kindler method which has been used for the synthesis of thioamides derived from 2-phenylethylamine and, from these, derivatives of N-methyl-

M. V. Lomonosov Moscow Institute of Fine Chemical Technology. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 457 460, July-August, 1981. Original article submitted January 26, 1981.

0009-3130/81/1704-0337$07.50