A SPECTROPHOTOMETRIC METHOD FOR THE QUANTITATIVE DETERMINATION OF D-HOMOPANTOTHENIC ACID

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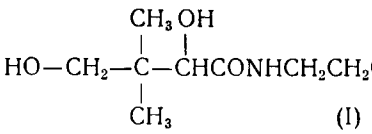
D-Homopantothenic acid is a natural homolog of D-pantothenic acid in which the β-alanine has been replaced by γ-aminobutyric acid (GAMBA). This compound is found in many natural tissues, including the brain of mammals [1, 2]. Chemically, homopantothenic acid is γ-(α,γ-dihydroxy-β,β-dimethylbutyryl-amino)butyric acid (I).



(I)

In recent years, the biochemical and pharmacological properties of homopantothenic acid have been widely studied, and it has been shown that it is an effective psychotropic preparation [3]. We have recently developed a new method of obtaining D-homopantothenic acid. At the present time, its physicochemical and pharmacological properties are being studied. One of the stages in these investigations is the development of a new quantitative method for determining homopantothenic acid. The existing colorimetric method of quantitative determination, consisting in the hydrolysis of homopantothenic acid and the formation of a colored complex of GAMBA with sodium 1,2-naphthaquinone-4-sulfunate [4], is unsatisfactory because of the instability of the complex formed and also the great laboriousness and low selectivity of the method. In developing the new method of analysis of homopantothenic acid, we made use of the property of sugars for forming complexes with concentrated sulfuric acid that absorb in the UV region [5, 7]. The samp principle has been used by Vachek for the quantitative determination of pantothenic acid and panthenol [8]. The UV spectrum of homopantothenic acid in aqueous solution has no appreciable absorption peaks in the 200-400 nm region; there are no maxima in this region for a solution in concentrated sulfuric acid, either. After a solution of homopantothenic acid in concentrated sulfuric acid has been heated, an intense maximum is found in the 260 nm region (Fig. 1), the appearance of which is apparently due to the fact that the D-pantolactone formed as a result of the hydrolysis of the homopantothenic acid gives a complex with concentrated sulfuric acid which absorbs at 260 nm.

TABLE 1

Weight of homopantothenic acid taken, g	D at λ 260 nm, amt. of soln., 0.5 ml	Homopantothenic acid found		Deviation from arithmetic mean
		g	%	
0,0125	0,70	0,0123	98,4	−1,02
0,0125	0,70	0,0123	98,4	−1,02
0,0128	0,72	0,0127	99,21	−0,21
0,0130	0,75	0,0132	101,53	+2,11
0,0134	0,78	0,0136	101,48	+2,06
0,0141	0,79	0,0139	98,58	−0,81
0,0145	0,81	0,0143	98,62	−0,80
0,0147	0,82	0,0145	98,63	−0,79
0,0150	0,85	0,0150	100,0	+0,58
0,0154	0,87	0,0153	99,35	−0,07

Mean at n = 10, σ = 1.459, S = 0.462, T_a = 2.262, E = 1.04 99.42.

In order to determine the optimum conditions for analysis with minimum consumption of time, we studied the dependence of the optical density of the product from homopantothenic acid on the time of heating at various temperatures (Fig. 2). When a solution of homopantothenic acid in conc. sulfuric acid was heated at 150°C, the maximum optical density was reached after 30 min. This time is the most suitable for practical purposes. The complex formed

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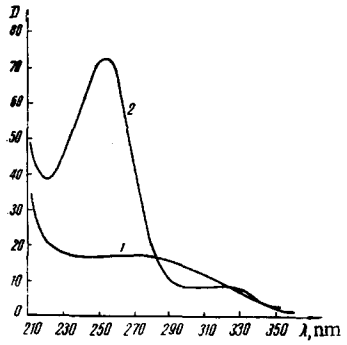

Fig. 1

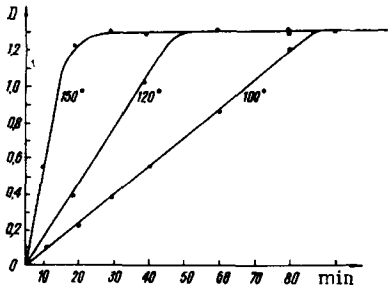

Fig. 2

Fig. 1. Absorption spectrum of D-homopantothenic acid: 1) without heating; 2) after heating at 150°C for 30 min.

Fig. 2. Dependence of the optical density of D-homopantothenic acid on the time of heating.

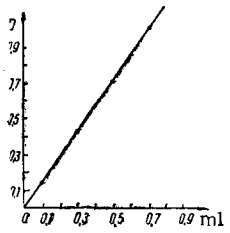

Fig. 3. Calibration graph for the spectrophotometric determination of D-homopantothenic acid.

is stable for days after heating; an important condition for the reproducibility of the method is the stability of the temperature (150 ± 5°C) and of the concentration of sulfuric acid (96-98%).

EXPERIMENTAL

The measurements were performed in a Hitachi M-124 automatic spectrometer in a cell with a layer thickness of 1.002 cm. The work was performed with samples of calcium homopantothenate having mp 198-200°C.

Construction of a Calibration Curve. An accurately weighed sample of 12.5 mg of D-homopantothenic acid, thrice recrystallized from 95% methanol and dried to constant weight, was dissolved in 25 ml of distilled water. Conical flasks with a capacity of 20 ml were charged with 0.1, 0.3, 0.5, 0.7, and 1.0 ml of the solution (50, 150, 250, 350, and 500 μg of homopantothenic acid, respectively), and in each flask the volume was made up to 1 ml with distilled water, and 6 ml of conc. sulfuric acid was added. The solution was stirred, heated in the thermostat at 150°C for 30 min, cooled for 10-15 min, and the optical density was measured. The comparison cell contained 1 ml of water and 6 ml of conc. sulfuric acid. It can be seen from the calibration curve constructed (Fig. 3) that the Bouguer–Lambert–Beer law is applicable in the range of concentrations from 50 to 350 μg. $E^{1\%}_{1\,cm}$ – the specific absorption index – is 284.

Determination of Homopantothenic Acid. An accurately weighed sample of the preparation was placed in a 25-ml measuring flask and dissolved in water, and the solution was made up to the mark. Depending on the weight of the sample, 0.1, 0.3, 0.5, or 0.7 ml of solution was transferred to a 10-ml conical flask. The volume of the solution in the flask was made up to 1 ml of distilled water, and 6 ml of sulfuric acid was added. The solution was stirred in a thermostat at 150°C for 30 min and was then cooled. The optical density of the solution was measured on an SF-4A spectrophotometer in a cell having a layer thickness of 1.002 cm. The concentration of the material (%) was calculated from the formula

$$X = \frac{D \cdot B}{E^{1\%}_{1\,cm} \cdot A},$$

where D is the optical density of the solution under investigation; B is the dilution; A is the weight of the sample, g; and $E^{1\%}_{1\,cm}$ is the specific absorption index.

The results of the spectrophotometric determination of homopantothenic acid treated statistically are given in Table 1.

The proposed method is characterized by rapidity, high sensitivity, and fairly good accuracy and reproducibility.

SUMMARY

A spectrophotometric method for the quantitative determination of homopantothenic acid at λ 260 nm has been developed.

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