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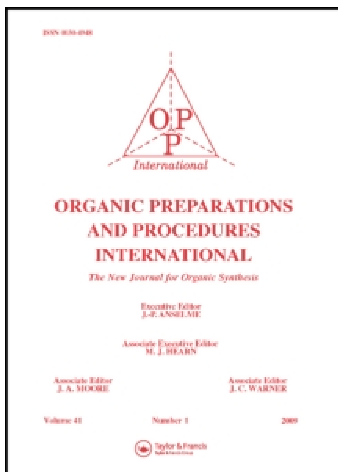

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GRADIENT ELUTION CHROMATOGRAPHY OF S-PALMITYL COENZYME A*

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GRADIENT ELUTION CHROMATOGRAPHY OF S-PALMITYL COENZYME A*

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While procedures are available for the synthesis of long-chain acyl coenzyme A derivatives,[1,2,3] a method for the purification of the synthesized material has not been fully described. We have found that gradient elution chromatography using silicic acid and increasing concentrations of methanol in dichloromethane, is a good method for the preparation of S-Palmityl-Coenzyme A (Pal-CoA) free from by-products and unreacted starting material. Dichloromethane is a better solvent than commercial chloroform as it permits monitoring of the column effluent for ultraviolet absorption at 260 mμ allowing detection of the adenine ring of Coenzyme A (CoASH).

Procedure:

Silicic acid (-325 mesh) prepared by the method of Hirsch and Ahrens[4] (Bio-Rad Laboratories) was partially activated by placing it in a vacuum desiccator containing P_2O_5 and KOH under the vacuum generated by a water aspirator. The material was allowed to remain at room temperature for 16 hours and gave partially activated silicic acid after this time.

The silicic acid so treated must be fresh and not previously activated at high temperatures. Highly activated silicic acid binds Pal-CoA strongly, and large volumes of absolute methanol are then required for

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elution of Pal-CoA. When the silicic acid was known or suspected to have been highly activated, it was first deactivated by placing it in a desiccator at room temperature and atmospheric pressure over a dish of water for 24 hours; it was then partially activated as described above.

Dichloromethane (Eastman Grade; Distillation Products Industries) was tested for ultraviolet absorption at 260 mμ against a methanol[8] blank before use. When significant amounts of absorption (more than 0.010 A_{260} units) were present, the dichloromethane was purified by distillation over calcium chloride.

Pal-CoA, synthesized and initially purified by Seubert's method,[2] was essentially free from unreacted CoASH and other material soluble in aqueous perchloric acid.

A 2 x 13 cm column of partially activated silicic acid was packed in the dry state and washed with chloroform[7] until the column was free from trapped air, and a yellow-brown contaminant was removed (this contaminant absorbed light at 260 mμ and was probably the Lubriseal stopcock grease used in sealing the vacuum desiccator during activation of the silicic acid). The column was then washed with dichloromethane and gave a flow rate of 1.5 ml/min with a 40 cm pressure head of dichloromethane.

A sample of synthetic Pal-CoA represented as 1035 A_{260} units in 20 ml of dichloromethane-methanol (3/1, V/V) was placed on the column and washed in with 10 ml of dichloromethane. The initial absorption of the methanol-containing sample caused a gas to be evolved and the column developed gas pockets, but this did not greatly affect the elution pattern. All air spaces between the top of the packed column and the constant volume mixing chamber were filled with dichloromethane before starting the gradient. If this was not done, bubbling would occur with the dichloromethane from the

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mixing chamber eventually filling these spaces causing an irregularity in the gradient being generated.

The gradient was started with 125 ml of dichloromethane in a constant volume lower mixing chamber fitted with a separatory funnel as an upper reservoir containing 250 ml of methanol.[8] Five ml fractions were collected and assayed for A_{260} after dilution with 1:1 (V/V) dichloromethane-methanol. Figure I shows the chromatogram obtained and the gradient used.

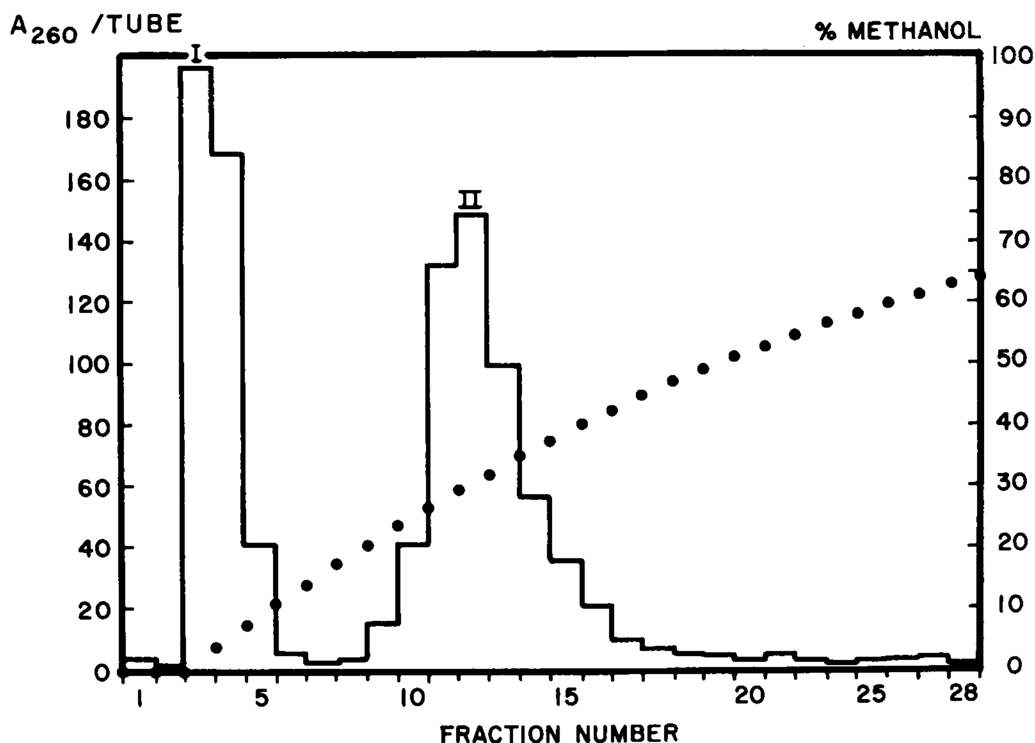

FIG. I

Chromatogram for Palmityl-CoA on silicic acid. Dotted line shows the exponential gradient generated during elution.

Fractions of 5 ml each were collected and A_{260} was determined using a solvent of 50% dichloromethane in methanol for dilutions when necessary.

Fractions 1 and 2 represent the forerun from the column.

Peak II contains Palmityl-CoA.

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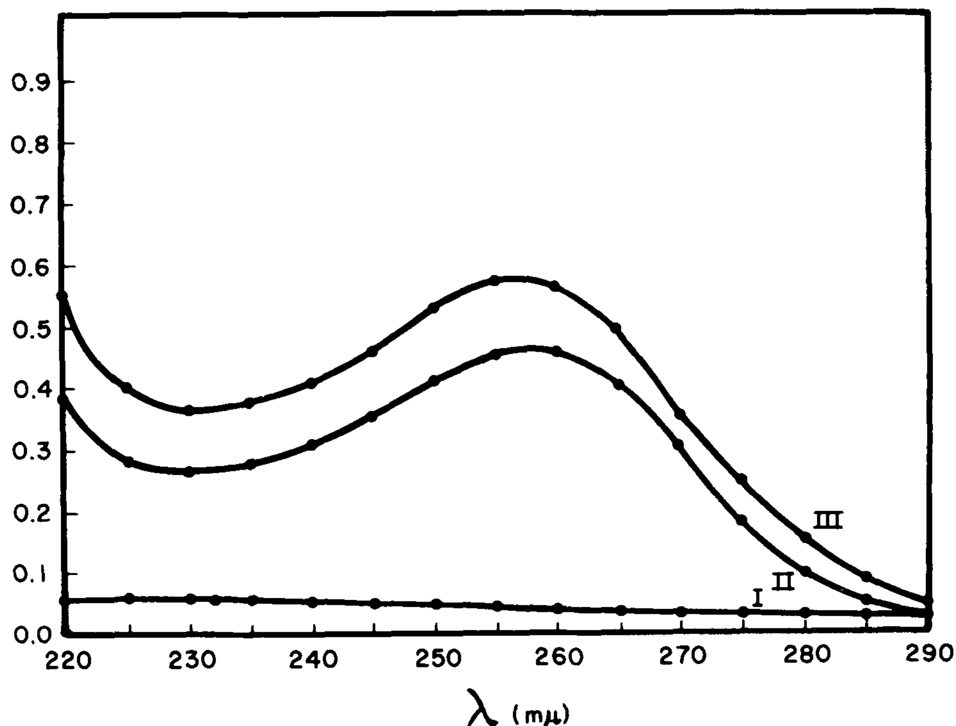

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FIG. II

Ultraviolet spectra of material eluted from the silicic acid column shown in FIG. I. Material from the individual peaks were collected in single fractions, and after removal of solvent the fractions were taken up in 50% dichloromethane in methanol. Small aliquots were placed in quartz cuvettes and the solvent removed with a stream of nitrogen. The dried material was taken up in 3 ml of the indicated buffer, and spectra were determined on a Beckman DU spectrometer against a buffer blank.

I. Spectrum of material from peak I dissolved in pH 5.0, 0.1 M sodium acetate buffer.

II. Spectra of material from peak II (Palmityl-CoA) dissolved in pH 7.5, 0.05 M Potassium phosphate buffer. Palmityl-CoA concentration is 28 μM.

III. Spectrum of Palmityl-CoA (35 μM) is pH 5.0, 0.1 M sodium acetate buffer. Material here is the same as that in peak II.

Figure II gives the ultraviolet spectra of the two peaks obtained. On the basis of the spectra peak II was identified as Pal-CoA.

Identification of the peaks and determination of the concentrations was based on the known molar absorption coefficients for Pal-CoA and CoASH in aqueous solutions.[5]

Pal-CoA $\varepsilon_{260} = 16.4 \times 10^3$ $\varepsilon_{232} = 9.4 \times 10^3$

CoASH $\varepsilon_{260} = 16.4 \times 10^3$ $\varepsilon_{232} = 5.0 \times 10^3$

The ratio $\frac{\varepsilon_{232}}{\varepsilon_{260}} = \frac{A_{232}}{A_{260}} = 0.57$ for Pal-CoA was taken as a criterion of purity as well as a method for the identification of eluted material. The actual ratios obtained varied from 0.52 to 0.62 for the second peak from the silicic acid column. The material was freed from silicic acid by repeated flash evaporation at 37° to remove solvent followed by dissolving the dry material in methanol. This procedure leaves the silicic acid behind as a residue in the flask used for drying the sample.

Pal-CoA prepared in this manner was enzymatically active when tested for ability to acylate α-glycerol phosphate using a rat brain microsomal enzyme system for the synthesis of phosphatidic acid.[6]

This procedure has also been used to purify synthetic stearyl-Coenzyme A.

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8. Commercial AR grade absolute methanol was used.

* From a thesis submitted by S. S. Kuwahara in partial fulfillment of the requirements for the degree of Master of Science in Biochemistry to the University of Wisconsin.

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