Partition Chromatography of the Oxidised A and B Chains of Insulin

WILLIAM ANDERSEN

*Carlsberg Laboratory, Chemical Department,
Copenhagen, Denmark*

Partition columns were introduced by Martin and Porter^{1,2} for chromatographing proteins like ribonuclease and insulin. In order to obtain a sufficiently high partition coefficient in favour of the organic (stationary) phase these authors used rather high concentrations of salts in their solvent systems. When working with comparatively low molecular-weight, dialysable polypeptides, the removal of large amounts of salt from the peak fractions might conceivably cause difficulties or would at least be inconvenient. Salt-free solvent systems have been used in countercurrent distribution of naturally occurring polypeptides, particularly by Craig and his group. It must be borne in mind, however, that whereas in countercurrent distribution partition coefficients not too far from unity are preferable, values of at least 5 are required for high resolving power in partition chromatography.

In a recent paper on countercurrent distribution of insulin³ Craig and coworkers introduced solvent systems containing di- or trichloroacetic acid (TCA) in moderate concentration. The increase in partition ratio caused by these complexing agents was very remarkable. Accordingly we considered it worth while to investigate

whether solvent systems of this kind could also be used in partition chromatography of the oxidised A and B chains of insulin, which Sanger⁴ separated by conventional precipitation methods.

The solvent system 2-butanol-0.01 *N* TCA (1 : 2.5) proved useful for this purpose. The columns for preparative runs (25 × 4.5 cm) were made from silane-treated Hyflo Super-Cel (150 g), which carried 75 ml of the butanol phase. Prior to chromatography the oxidised insulin was separated into crude A and B fractions by a single fractionation in ammonium acetate buffer⁴. Each fraction (120 mg) was extracted with 60 ml of aqueous phase and the extracts were chromatographed, the chromatograms being developed with aqueous phase. The elution was followed by determining the optical density at 277 m μ of the fractions collected (Fig. 1).

The A fraction emerged as a single component whereas the B fraction gave two peaks, of which the later one was the B peptide. The peak fractions were combined in each case, freed of TCA by neutralising with Dowex-2 in the bicarbonate form and lyophilised. The chromatographed A and B peptides appeared as white, amorphous solids (about 45 mg of each). In paper electrophoresis experiments (barbital buffer at pH 8.6) each peptide travelled as a single spot, thus indicating that a complete separation had been achieved.

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Fig. 1. Elution chromatograms of A peptide (A) and B peptide (B). Abscissa: Number of fractions (20 ml). Ordinate: Optical density of fractions at 277 m μ .

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Dehydrogenation of Dihydro-Dehydro-Di-Isoeugenol and its Methyl Ether

ÅKE SPETZ

Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden

For various purposes, especially in relation to work on the U.V.-absorption spectra of lignin and lignin derivatives compound I and its methyl ether II were required.

These substances are easily obtained by sulphur dehydrogenation of the corresponding coumarane derivatives, dihydro-dehydro-di-isoeugenol and its methyl ether. For spectrochemical studies compare G. Aulin-Erdtman¹.

Experimental. 3-Methyl-5-propyl-7-methoxy-2-(4-hydroxy-5-methoxy-phenyl) coumarane (I): Dihydro-dehydro-di-isoeugenol (12.5 g) and sublimed sulphur (1.3 g) were mixed in a 25 ml Claisen distillation flask and heated in a salt bath. The temperature was slowly raised and at 215° evolution of hydrogen sulphide began. It ceased completely after 30 minutes, by which time the temperature had reached 250°. The reaction vessel was arranged for vacuum distillation and at 220–240° (1

mm) a yellow substance (9.0 g) was collected. The distillate was dissolved in benzene, filtered through aluminium oxide and evaporated to give an almost colourless substance (5.5 g) melting at 85–87°. Recrystallization from ligroin and from 50 % acetic acid raised the m.p. to 86.5–87.5° (needles). The substance exhibits a blueish violet fluorescence in U.V. light. (Found: C 73.6; H 6.78. Calc. for C₂₀H₂₂O₄ (326.4): C 73.6; H 6.80.)

Acetate: The phenol (I) was acetylated with acetic anhydride in pyridine. The reaction mixture was poured into water and the product crystallized from ethanol in needles, m.p. 124–125°. (Found: OCH₃ 16.9. Calc. for C₂₀H₁₈O₅ (OCH₃)₂ (368.4): OCH₃ 16.9.)

3-Methyl-5-propyl-7-methoxy-2-(4,5-dimethoxyphenyl) coumarane (II):

A. The phenol (I) was methylated with dimethyl sulphate and alkali in the usual way giving the methyl ether which crystallized from methanol in cubes, m.p. 91.5–92.0°. The substance exhibits a violet fluorescence in U.V. light. (Found: OCH₃ 27.5; Calc. for C₂₂H₂₄O (OCH₃)₂ (340.4): OCH₃ 27.4.)

B. Dihydro-dehydro-di-isoeugenol methyl ether² was dehydrogenated as described for the phenol above. Evolution of hydro-

gen sulphide began at 200°. Distillation at 220–225°, 1 mm, gave about the same yield of crude product as above. Purification by filtration through aluminium oxide was in this case without success but repeated crystallization from methanol gave the pure compound m.p. 91–92°, undepressed on admixture with the substance obtained according to method A.

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