Continuous Fractionation of Serum by Zone Electrophoresis

INGER BRATTSTEN

Institute of Biochemistry, University of Uppsala, Uppsala, Sweden

By electrophoresis in a supporting medium complete separation of components of a mixture can be obtained. By arranging a simultaneous transport of the liquid medium in a direction perpendicular to the electric field the fractionation can be performed as a continuous process, which allows the application of greater quantities of material than those conveniently used in a batch procedure. According to these principles an apparatus has been developed [1-3] and applied to the study of the proteins of blood serum [4]. Using a veronal buffer of the ionic strength 0.03 and pH 8.4 as the conducting medium, the dialyzed serum solution is injected as an 1 cm wide zone. In the velocity field this zone, which contains about 2 % of protein, is split into its component zones and finally covers a distance of about 20 cm horizontally. The fractions are collected at intervals of 0.5 cm. The protein contents of the fractions give a distribution curve, where the γ-, β-, α-globulins and serum albumin form the four main peaks (Fig. 1). By the fractionation of 20–30 ml of whole serum enough material is obtained for extensive analysis of the fractions. The rate of fractionation usually is about 0.8 ml of whole serum per hour.

The homogeneity of the fractions has been studied electrophoretically and in the ultracentrifuge. By paper electrophoresis it can be shown that material from the principal maxima of the distribution curve migrate as singular spots at rates characteristic of the γ-, β-, α-globulins and albumin, respectively. When material from successive fractions of the γ group are simultaneously submitted to electrophoresis on the paper, the line connecting the centres of the spots gradually becomes inclined, demonstrating that the differentiation achieved in the separation column is not an artefact. The two maxima in the γ peak can be shown to contain material of distinctly different mean mobility. In the ultracentrifuge the fractions containing the γ group and the maxima of the β-globulin and albumin zones are homogeneous, whereas the α peak always contains three main components and the dilute fractions between the γ and β groups two components each. On both sides of the α maximum the fractions are also heterogeneous in the ultracentrifuge, but those fractions which by paper electrophoresis can be split into albumin and α_1-globulin only contain one boundary, although the heterogeneity is apparent from the skewness in the optical pattern. By ultracentrifugations of solutions containing approximately 0.5 % of protein in a phosphate buffer of the ionic strength 0.05 and pH 6.83 the following sedimentation constants have been computed (*cf.* Fig. 1):

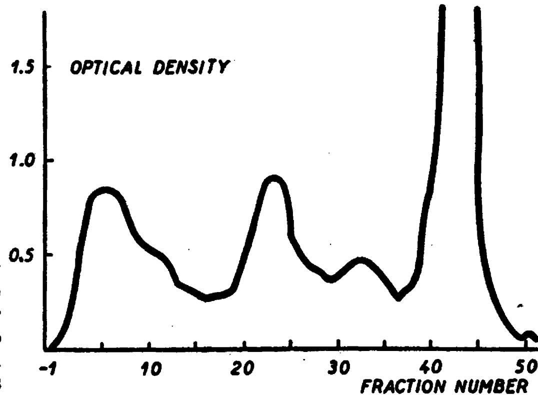

Fig. 1. Fractionation of normal human serum. The optical densities of the fractions at 284 mμ have been plotted against the fraction numbers.

Fraction No.	5	11	13	15, 16, 17	20	23	28	33	39	43
s_{20}	7.0	7.03	6.90 19.3	6.68 19.2	(5.23) 6.29	5.30	6.36 11.8	4.01 7.83 (11.6) 18.3	4.56	4.54

1. Svensson, H. and Brattsten, I. *Arkiv Kemi* **1** (1949) 401.
2. Brattsten, I. *Arkiv Kemi* **4** (1952) 503.
3. Brattsten, I. *Arkiv Kemi (In press).*
4. Brattsten, I. *Arkiv Kemi (In press).*

Received October 21, 1954.

*epi*Friedelinol and Derivatives, a Re-investigation

TORGER BRUUN and PHILLIP R. JEFFERIES

Institutt for Organisk Kjemi, Norges Tekniske Högskole, Trondheim, Norway, and the Johnson Chemical Laboratories, University of Adelaide, South Australia

The occurrence of *epi*friedelinol in *Ceratopetalum apetalum* D. Don (Cunoniaceae)[1] and the lichen *Cetraria nivalis* (L.) Ach.[2] has recently been reported simultaneously and independently by us. The rotations recorded in the two communications were, however, so widely differing (compare Table 1) that it could not be accounted for by experimental errors alone. The difference could conceivably be due to a small content of a contaminant in one of the samples. To settle this question a re-investigation was undertaken, the details of which are found in the experimental part. We believe that the rotations now found (see Table 1) should be regarded as the correct ones. The differences in the m.p.'s are not significant in view of their range and different measurement conditions.

As is seen from the experimental part, there is a strong indication that *epi*friedelinol from *Cetraria nivalis* did contain a small amount of some other material, but further investigations were prevented by lack of material.

Experimental. M.p.s are not corrected. Rotations were measured at room temperature (approximately 20°) in chloroform in a 1 dm tube, unless specified to the contrary.

The *epi*friedelinol used in these investigations, except in one case, was the original sample obtained from *Ceratopetalum apetalum* D. Don[1], $[\alpha]_D$ +19° (c. 0.91), +21° (c, 1.02), m. p. 272—275°, admixture with a sample of the lichen *epi*friedelinol (m. p. 273—276°) m. p. 272—276°, all three taken at the same time. Similarly, in evacuated tubes, the m.p.s were, respectively, 280—281°, (281—282°) and 281—282°.

epi*Friedelanyl acetate.* The acetate was prepared with acetic anhydride in pyridine with standing at room temperature for 4 days in the same way as previously used by Bruun[2]. Sufficient pyridine was used to keep the alcohol in solution. The product was filtered through alumina in benzene and crystallised from chloroform-methanol, m. p. 284—285°, admixture with the original acetate (prepared in the same way) of *epi*friedelinol from lichens (m. p. 273—274°)[2] m. p. 270—285°, all three taken at the same time, $[\alpha]_D$ +37° (c, 1.02). Recrystallised the acetate melted at 282—283°, $[\alpha]_D$ +38° (c, 0.96). After drying at 115° for 6 hours in a water-pump vacuum it melted at 282—285°, $[\alpha]_D$ +34° (c, 1.76).

A sample of the acetate was also prepared by refluxing for 1 hour 20 mg of *epi*friedelinol from *lichens* with acetic anhydride as used by Jefferies[1]. The crystals which deposited on cooling were filtered, m. p. 276—279°, admixture with the previously prepared acetate from lichens (m. p. 271—274°) m. p. 271—274°, all three taken at the same time, $[\alpha]_D$ +33° (c, 1.24). Recrystallised it melted at 281—283°, admixture with the sample above from *Ceratopetalum apetalum* (m. p. 282—285°) m. p. 282—285°, all three taken at the same time, $[\alpha]_D$ +33° (c, 1.11).

Table 1.

	Ceratopetalum apetalum D. Don		*Cetraria nivalis* (L.) Ach.		Present investigation	
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$
*epi*Friedelinol	279—283°	+24°	281—282° (vac.)	+15°	272—275° 280—281° (vac.)	 + 20°
Acetate	290—294°	+ 45°	275—276° (vac.)	+ 12°	282—285°	+ 35°
Benzoate	254—257°	+ 40°	232—233° (vac.)	+ 25°	246—248°	+ 34°