A Method for Quantitative Starch-gel Electrophoresis

EFFORTS to make use of starch-gel electrophoresis even in quantitative protein analysis has led to several techniques to make the gel transparent[1–5]. A further method, where the gel is stained in the same medium which helps it become transparent, is described here.

The gel is cut with a device giving a slice 1 mm. thick with even surfaces on both sides. The slice is immersed in a 95° C. bath of 47·5:47·5:5 (v/v/v) glycerol/water/acetic acid saturated with amido black 10 *B* for 10 min. and then washed at room temperature with slow, continuous shaking in a series of glycerol/water mixtures where the glycerol content is increasing, giving a final wash medium of pure glycerol. The washing procedure is somewhat slow, taking two days. The slice can then be analysed in a chromatograph for transmitted light using a supporting device. The data of two normal human sera and two myeloma sera analysed using an electrophoresis method enabling the separation of 14 fractions and a transmission chromatograph (Spinco 'Analytrol') are presented in Table 1. Paper electrophoresis data of the same normal sera are presented in Table 2. For comparison the data of some normal sera analysed using the same electrophoresis method as in Table 1, but the original methanol-staining method and a reflexion chromatograph (Joyce, Loewell and Co. 'Chromograph') are presented in Table 3. The striking difference in the amount of albumin fraction obtained by the two methods emphasizes the need for different normal values when using different methods.

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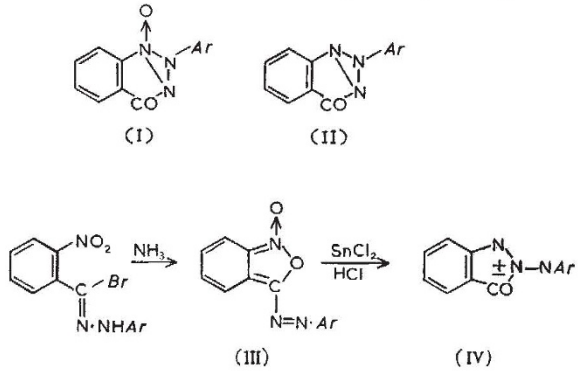
Structural Revisions in the ω-Halobenzaldehyde Arylhydrazone Series

SOME twenty compounds, described as 3-keto-1,2-endo-arylimino-1,2-dihydro-1,2-benzisodiazole 1-oxides (I), have been prepared by Chattaway *et al.*[1–8]; eleven of these have been reduced by stannous chloride in hydrochloric acid (or, in some cases, by prolonged boiling in ethanol) to deoxy compounds, formulated as (II)[2–6].

A recent study of the Chattaway–Adamson rearrangement[4,9] has directed attention to these interesting compounds. The 'oxides' result from mild basic treatment of the appropriate ω-halo-*o*-nitrobenzaldehyde arylhydrazones; from a mechanistic point of view[10], these compounds should be regarded as 3-arylazo-anthranil 1-oxides (III), and, as such, are seen to be analogous to the isoxazoline oxides of Kohler[11]. Such a conclusion is supported by the electronic and vibrational spectra of compound (III, *Ar* = 2,4-dibromophenyl), and is in harmony with the intense colour and with the chemical properties of these labile compounds.

Reduction to the near-colourless deoxy-compounds evidently involves scission of the heterocyclic ring, followed by recyclization to the mesoionic indazole derivatives (IV). This is implied by the general



(I) (II) (III) (IV)

Table 1. ELECTROPHORETIC DISTRIBUTION OF DIFFERENT PROTEIN FRACTIONS OF TWO NORMAL SERA AND TWO MYELOMA SERA MEASURED BY STARCH-GEL ELECTROPHORESIS AND TRANSMITTED LIGHT

Fraction	Normal serum 1 Run 1	Run 2	Run 3	Normal serum 2 Run 1	Run 2	Run 3	Mycloma 1	Myeloma 2
Pre-albumin 1	1·7	1·5	1·7	1·5	1·0	1·1	1·6	1·5
Pre-albumin 2	1·8	1·6	1·8	1·6	1·4	1·3	1·1	1·0
Albumin	57·0	56·5	56·9	64·8	65·0	61·2	43·4	45·6
Post-albumin 1	1·9	2·4	1·7	1·2	1·5	1·6	1·0	2·0
Post-albumin 2	4·2	4·8	1·7	1·9	1·0	1·0	1·5	
Cœruloplasmin			2·6		1·5	1·6	4·8	4·1
Transferrin	4·4	3·2	4·3	3·2	3·4	3·2		6·1
α_1-β-Globulins	1·9	2·4	2·6	0·8	1·5	1·0	4·2	6·1
Haptoglobins	5·2	4·0	3·5	1·6	2·9	1·6	2·6	3·1
						1·1		2·0
				0·8		1·1		1·0
'Slow' α_2-globulin	2·6	4·0	3·4	3·5	4·4	4·8	3·7	5·1
β-Lipoprotein (myelomaprotein)	6·1	6·8	5·2	2·9	1·9	3·2	29·2	5·6
γ-Globulin	13·2	12·8	14·6	16·2	14·5	16·2	6·9	8·2
Myelomaprotein	—	—	—	—	—	—	—	8·6

Table 2. ELECTROPHORETIC DISTRIBUTION OF DIFFERENT PROTEIN FRACTIONS OF THE SAME TWO NORMAL SERA AS IN TABLE 1 MEASURED BY PAPER ELECTROPHORESIS

Fraction	Normal serum 1	Normal serum 2
Albumin	58·3	64·5
α_1-Globulin	4·9	3·9
α_2-Globulin	8·7	5·2
β-Globulin	10·6	10·3
γ-Globulin	17·5	16·2

Table 3. ELECTROPHORETIC DISTRIBUTION OF DIFFERENT PROTEIN FRACTIONS OF FOUR NORMAL SERA MEASURED BY STARCH-GEL ELECTROPHORESIS AND REFLECTED LIGHT

Fraction	1	2	3	4
Pre-albumin 1	1·3	1·6	2·8	1·3
Pre-albumin 2	4·6	3·5	3·4	2·3
Albumin	33·1	36·0	37·3	39·3
Post-albumin 1	2·2	2·3	2·2	2·0
Post-albumin 2	2·9	2·7	3·5	2·1
Cœruloplasmin	8·7	4·9	12·2	5·2
Transferrin	4·3	7·7	5·2	6·8
α_1-β-Globulins	4·5	3·8	3·2	3·8
Haptoglobins	2·5	3·1	2·6	2·8
	4·5	3·5	3·2	3·6
	3·6			
'Slow' α_2-Globulin	11·2	7·7	6·4	6·9
β-Lipoprotein	3·4	8·5	4·5	7·0
γ-Globulin	13·2	14·7	13·5	16·9