TABLE I

Solvent	λ_a (nm)	λ_f (nm)	$\bar{\nu}_a$ (cm^{-1})	$\bar{\nu}_f$ (cm^{-1})	$\bar{\nu}_a - \bar{\nu}_f$ (cm^{-1})	φ (D, n)	Z
Pyridine	538	560	18587	17857	730	0·212	64·0
n-Butanol	528	548	18939	18248	691	0·216	77·7
Cyclohexanone	538	558	18587	17921	666	0·248	..
Amylalcohol	530	548	18867	18248	619	0·256	..
Glycerol	516	544	19379	18382	997	0·264	..
Iso-butanol	532	550	18796	18181	615	0·267	71·3
Acetic-anhydride	524	542	19083	18450	633	0·273	79·2
Formamide	516	544	19379	18382	997	0·283	83·3
Acetone	534	554	18726	18050	676	0·285	65·7
Ethanol	528	544	18939	18382	557	0·290	79·6
Methanol	526	546	19011	18387	624	0·308	83·6
Water	508	532	19685	18796	889	0·320	94·6

as well as in the excited state. The shifts in the wavelength depend upon the polarity of the solvent. It is obvious that a graph between D and $\bar{\nu}_a$ or $\bar{\nu}_f$ will not be quite linear, However, a plot of Z versus $\bar{\nu}_a$ or $\bar{\nu}_f$ as shown in Fig. 1, shows a good linear relationship. This indicates that the universal solvent polarity scale as suggested by Kosower[9] and given in terms of Z values is a better measure of the microscopic solvent polarity than D which gives the macroscopic solvent behaviour. It may be noted that the slope of lines $\bar{\nu}_a$ versus Z and $\bar{\nu}_f$ versus Z are almost equal indicating that the change in the dipole moment in the excited state from the ground state is not very large.

According to equation (1), when the Stoke's shift $\triangle\bar{\nu}$ is plotted against ϕ (D, n) of the solvent, it should be a straight line, the slope of which would give

$$2(\mu_e - \mu_g)^2/hca^3.$$

Fig. 2 shows such a plot between φ (D, n) and $\bar{\nu}_a - \bar{\nu}_f$. This straight line graph confirms the theoretical relationship stated in equation 1. Again the slope of the line here indicates that the change in the dipole moment is not very large.

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CHROMIUM (III) MYOGLOBIN

RECENT interest in the reaction of oxygen with chromium (II) porphyrin[1] and capability of inositol hexaphosphate in switching the quaternary structures of high-spin ferric hemoglobin from oxy- to deoxy- states[2,3] have prompted us to investigate the properties of chromium replaced hemoproteins. In this report we describe the preparation of chromium (III) myoglobin and its visible and electron paramagnetic resonance spectra. The chloro derivative of chromium(III) mesoporphyrin IX was prepared by known method[4]. The chromium(III) myoglobin was prepared by mixing the chloro derivative of chromium (III) and apomyoglobin in Tris-HCl buffer of pH 8. The reaction mixture was transferred immediately to a Sephadex G–25 gel permeable column equilibrated and eluted with 0·01 M potassium phosphate buffer of pH[5]. The first eluted fraction was chromium(III) myoglobin. The second fraction was excess metal porphyrin. The chromium (III) myoglobin was further purified by absorbing on to a CM–52 cellulose ion-exchange column and

then eluted with 0·1 M potassium phosphate buffer of pH 7. The most stable product was first eluted and stored at 0-5 C°.

The ultraviolet and visible spectrum of chromium (III) myoglobin in 0·1 M potassium phosphate buffer of pH 7 is given in Fig. 1. The spectral bands

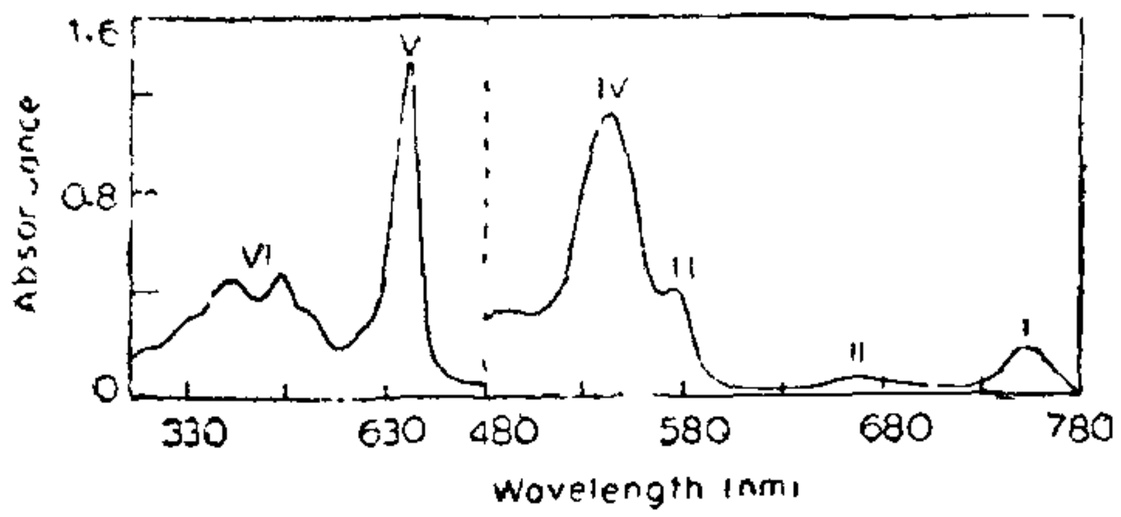

FIG. 1. Absorption spectrum of chromium (III) myoglobin in 0·1 M potassium phosphate buffer of pH 7. Concentrations of chromium(III) myoglobin were 11·5 μM in the Soret region and 115 μM in the visible region. The wevelength is in nm.

present in the protein complex have been assigned following the assignment of Gouterman and coworkers[6] on the chromium (III) octaethylporphyrin complexes. The protein complex shows two visible bands, an α band at 575·5 nm and a β band at 543 nm and a Soret band at 445 nm. The bands at 755 nm and 667 nm are assigned to a 'trip-quartet' excited states[6]. The intense near ultraviolet bands are assigned to charge transfer transitions. The effect of pH of the absorption spectrum of chromium(III) myoglobin in the Soret region has been studied in the pH range of 7 to 10. On plotting the changes in absorbance of the protein complex as a function pH at 441 nm, a S-shaped curve is obtained. The pK_a value obtained from this plot for 50% absorbance change is 8·8. This value is somewhat lower than the pK_a value of 9·2 for myoglobin derivative of reconstituted ferric mesoporphyrin IX[7]. This pK_a value of ferric myoglobin is assigned to an equilibrium involving dissociation of an aquo complex of ferric myoglobin into its hydroxo derivative and a proton[8]. Thus the above result suggests that the ferric myoglobin has a coordinated water molecule (or hydroxyl ion) at sixth coordination position of ferric ion. Similar coordination geometry around chromium (III) in chromium (III) myoglobin is suggested with fifth and sixth coordination positions around metal atom occupied by a proximal histidine and a water molecule (or an hydroxyl ion) respectively. The aquo and hydroxo derivatives of chromium (III) myoglobin must not show a thermal equilibrium between high- and low-spin forms, which exists in aquo and hydroxo derivatives of ferrimyoglobin[9]. Further studies on above complexes of the chromium(III) myoglobin will delineate the influence of equilibrium thermal mixture of high- and low-spin forms on the properties of ferrimyoglobin.

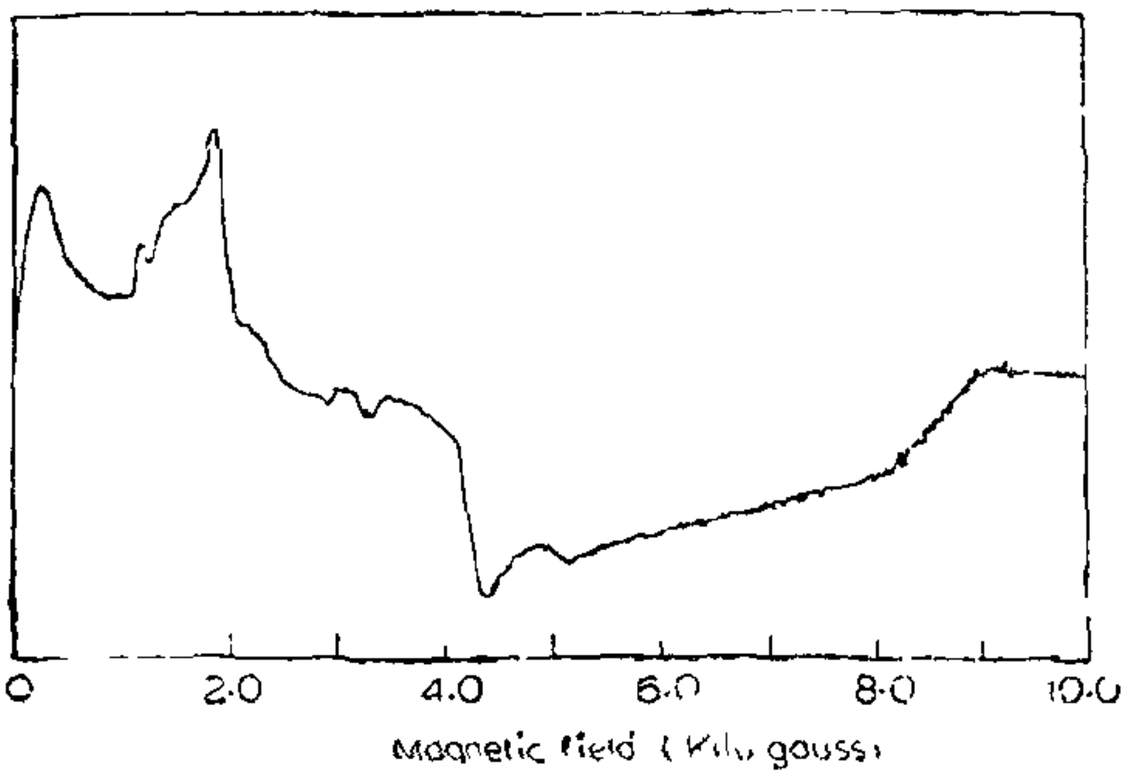

FIG. 2. Electron paramagnetic resonance spectrum of chromium (III) myoglobin in 0·1 M potassium phosphate buffer of pH 7 at 77° K. Concentration of chromium (III) myoglobin was 2·5 mM. The scanned magnetic field is from 1 to 10 kilogauss.

The electron paramagnetic resonance spectrum of chromium (III) myoglobin at liquid nitrogen shows five main signals at magnetic fields at 1225, 1925, 3325, 4400, and 5200 gauss, which are shown in Fig. 2. Two relatively weak additional signals at 1575 and 2225 gauss are also present. This spectrum is very similar to the spectrum of 1-methyl-imidazole adduct of chloromeso-tetraphenylporphinatochromium(III),[10] and suggests the similar coordination geometry around chromium(III) in the chromium (III) myoglobin and the above model compound. Thus the electron paramagnetic resonance result can be interpreted in terms of a strong and a weak coordinating ligands occuping the fifth and sixth coordination positions respectively of chromium(III) myoglobin, and, further, supports the above conclusion regarding the geometry of ligands around the metal atom in chromium(III) myoglobin.

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ACID-CATALYSED CONDENSATION OF FORMALDEHYDE WITH SOME PHENOLIC KETONES

IN connection with the synthesis of homocupressuflavone hexamethyl ether, the required intermediate, *viz.*, 3, 3′-methylenebis phloracetophenone-4, 6-O-dimethyl ether was prepared by the acid-catalysed condensation of formaldehyde (formalin) with phloracetophenone-4, 6-O-dimethyl ether[1,2]. We have studied similar condensations with other phenolic ketones and some of the results are reported in this communication.

Resacetophenone-4-O-methyl ether gave, on reaction with formalin and aqueous sulphuric acid (35%), four products A, B, C and D which were separated by fractional crystallisation from acetone: Compound A, m.p. 204–5° (diacetate, 188–9°), compound B, m.p. 255–6° (diacetate, 161–2°), Compound C, m.p. 161–2° and Compound D, m.p. 181–2° (acetate, 161–2°). Their PMR spectral data (60 MHz, δ, $CDCl_3$) are given below:

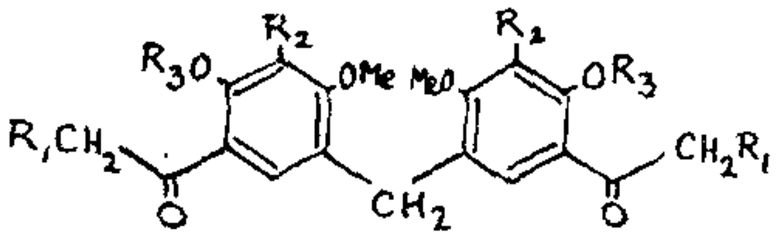
Compound A: 2·58 (s, 6H, two $CO-CH_3$), 3·96 (s, 2H, $-CH_2-$), 4·06 (s, 6H, two OCH_3), 6·75 (s, 2H two C_3–Ar H), 7·26 (s, 2H, two C_6–Ar H), 10·09 (s, 2H, two chelated OH).

Compound B diacetate: Since the compound B is not easily soluble in deuterochloroform, the PMR spectrum of its diacetate was recorded.

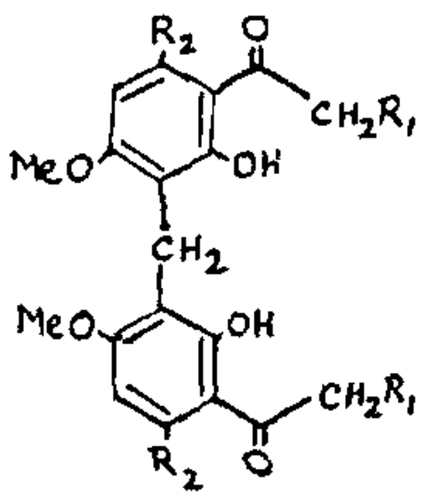
2·2 (s, 6H, two $CO-CH_3$), 2·45 (s, 6H, two $O-COCH_3$), 3·82 (s, 6H, two OCH_3), 3·9 (s, 2H, $-CH_2-$), 6·78 (d, 2H, J=9 Hz, two C_5–Ar H), 7·76 (d, 2H, J=9 Hz, two C_6–Ar H).

Compound C (90 MHz): 2·24 (s, 3H, $CO-CH_3$), 2·6 (s, 3H, $CO-CH_3$), 3·92 (s, 2H, $-CH_2-$), 3·98 (s, 6H, two $-OCH_3$), 6·55 (s, 1H, C_3–Ar–H), 6·7 (d, 1H, J=12 Hz, C_5–Ar H), 7·32 (s, 1H, C_6'–ArH), 7·95 (d, 1H, J=12 Hz, C_6–Ar H), 13·08 (s, 1H, chelated OH), 13·2 (s, 1H, chelated OH).

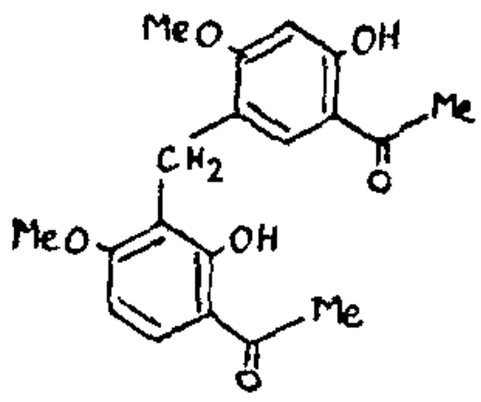
On the basis of the analytical and the PMR data the structures Ia, IIa and III were assigned to A, B and C respectively. The structure of the compound D is under investigation.



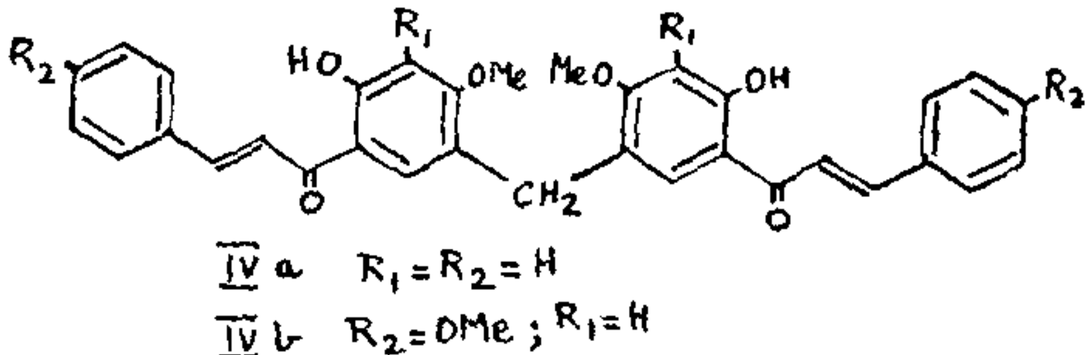
Ia $R_1=R_2=R_3=H$
Ib $R_2=OMe$; $R_1=R_2=H$
Ic $R_1=OMe$; $R_2=R_3=H$
Id $R_3=Bz$; $R_1=R_2=H$



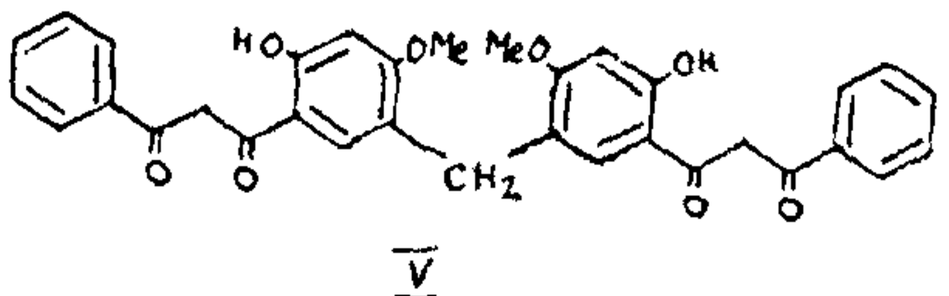
IIa $R_1=R_2=H$
IIb $R_1=R_2=OMe$



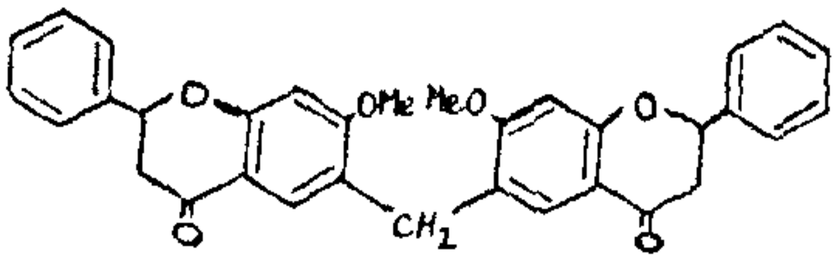
III



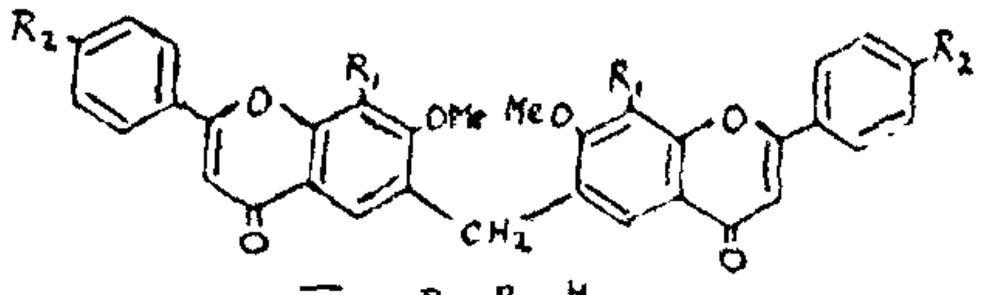
IVa $R_1=R_2=H$
IVb $R_2=OMe$; $R_1=H$
IVc $R_1=OMe$; $R_2=H$
IVd $R_1=R_2=OMe$



V



VII



VIa $R_1=R_2=H$
VIb $R_2=OMe$; $R_1=H$
VIc $R_1=OMe$; $R_2=H$