48 hours, a 50-fold excess of the reagent is used. Beer's law is obeyed from 2 to 20 mg of vanadium (IV) in 100 ml of solution. It is interesting to note that the extraction with the solvent mixture is greater than the sum of the extractions with either of the solvents individually. The first extraction will collect 95% of the total amount of vanadium (IV).

Reagents.—A 4% solution of sodium thiocyanate (B.D.H. AnalaR) is prepared.

Vanadium(IV) solution is prepared by reducing 50 ml of approximately 0·1 N sodium vanadate solution, mixed with 20 ml of 5 N sulphuric acid, with sulphur dioxide and standardised by the method of Dikshitulu and Gopala Rao[1].

All other reagents employed are of analytical reagent quality.

Procedure.—The sample solution containing 2 to 20 mg of vanadium (IV) is taken into a 150 ml separatory funnel, 2·5 to 25·0 ml of the reagent added and the pH adjusted to 0·5 to 2·5 with either dilute acid or alkali using a pH meter. The solution is shaken with 45 ml of the solvent mixture of *n*-butanol and ethyl acetate (8 : 3 v/v). The aqueous layer is collected after the two layers separate and the non-aqueous layer is run off into a 150 ml beaker. The aqueous layer is washed again with the solvent mixture. The non-aqueous extracts, collected in the beaker, are dried over anhydrous sodium sulphate and transferred to a 100 ml volumetric flask and the solution is made up to the mark. The optical density is then measured against the solvent blank at 730 nm using Hilger UVISPEK spectrophotometer using 1 cm glass cells.

Interferences.—100-fold excess of chloride, sulphate, nitrate, and perchlorate, and equal amount of phosphate do not interfere. Chromium (III) interferes, but the interference can be overcome by increasing the reagent concentration by 1½ times. Ce(III) precipitates out under the conditions of the experiment. Fe(III), Mo(VI), Ni(II), and Cu(II) interfere.

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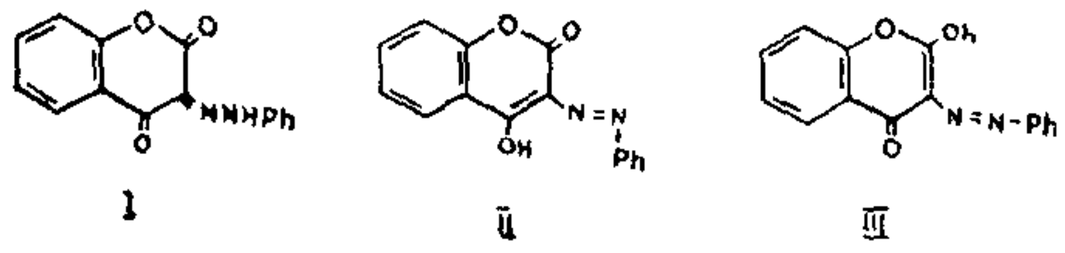
COMPLEX FORMATION WITH 3-PHENYLAZO-4-HYDROXY COUMARIN

THE report[1,2] on the complex formation of 3-phenyl-4 : 5 : 7-trihydroxy coumarin with various metal ions turned our attention to a study on the chelating capacity of 3-phenylazo-4-hydroxy coumarin. Although this compound was first made in 1945 by Huebner and Link[3], and its derivatives were later on used as important intermediates in the synthesis of the antibiotic Novobiocin and its analogues[4-7], very little is known regarding the binding capacity of this interesting ligand with metal ions. Moreover, since three tautomeric structures (I, II & III) are possible with this ligand theoretically[8], it has been considered that a study of the spectral data of these metal complexes may provide an explanation as to which tautomeric form predominates under a particular set of experimental conditions.

CHART-I



I II III

The reaction of this ligand, prepared by earlier reported procedures, with Ag(I), Tl(I), Cu(II), Mg(II), Mn(II), Al(III) and Th(IV) yielded the corresponding complexes. The general method of preparation consisted of mixing the equimolar quantities of the respective metal ions (in aqueous solution) and the ligand in methanol at room temperature. The products, obtained in good yield, were crystallised from dioxane and dried over calcium chloride *in vacuo*. The compounds were stable, non-volatile, soluble in non-polar solvents and non-conductors in dioxane and methanol solutions.

Gravimetric determinations on these complexes based on standard procedures indicated that the metal to ligand ratio is 1 : 1 in the case of silver and thallium, 1 : 2 in the case of copper, manganese and magnesium, 1 : 3 with aluminium and 1 : 4 with thorium. These results are also well in agreement with the analytical data presented in Table 1.

Spectral Data.—The ultraviolet spectrum of the ligand consists of two bands at 246 nm and 420 nm. The former is attributable to the α, β-unsaturated

TABLE I

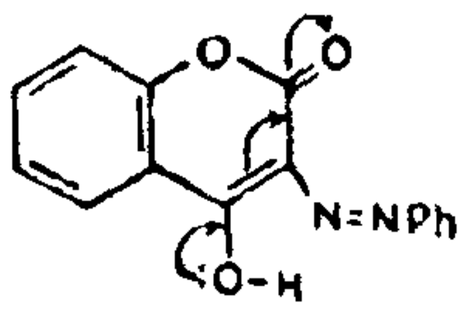
Analytical and spectroscopic data of complexes

Name of Complex	M.P.* (°C)	Yield (%)	% N**		% Metal		λ_{max} (nm)	C=O (cm^{-1})
			Found	Calcd.	Found	Calcd.		
Silver 3-phenylazo-4-hydroxy coumarinate	Above 300	50	6·8	6·4	47·8	48·0	244 420	1660
Thallium 3-phenylazo-4-hydroxy coumarinate	260	60	5·8	5·5	29·0	28·8	253 418	1650
Copper 3-phenylazo-4-hydroxy coumarinate	Above 300	90	10·0	9·5	10·9	10·7	252 422	1700
Magnesium 3-phenylazo-4-hydroxy coumarinate	185	50	4·5	4·3	10·5	10·1	253 425	1650
Manganese 3-phenylazo-4-hydroxy coumarinate	170	50	10·0	9·4	9·8	9·5	252 424	1660
Aluminium 3-phenylazo-4-hydroxy coumarinate	210	20	3·6	3·2	10·8	10·3	256 421	1650
Thorium 3-phenylazo-4-hydroxy coumarinate	182	60	8·5	8·7	18·1	18·0	256 425	1650

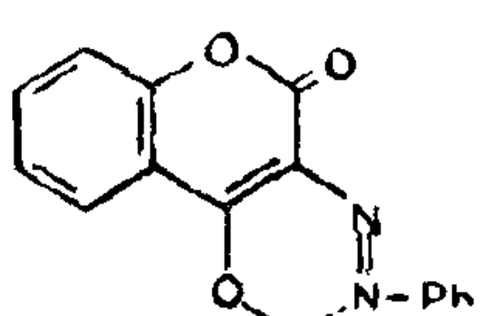
* All the melting points are uncorrected.

** All the complexes satisfactorily analysed for C and H also.

ketonic transition (*a*) and the latter to the hydrogen bonding (*b*).



(a)



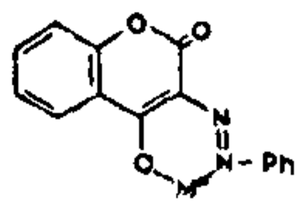
(b)

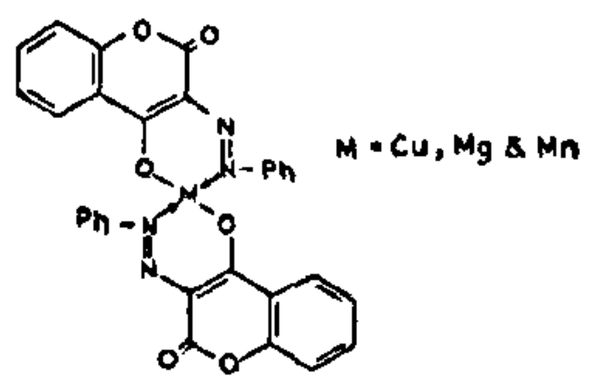
All the complexes exhibit peaks around the same region, *viz.*, 250 ± 6 nm and 420 ± 5 nm but with higher intensities.

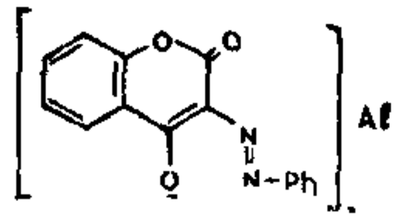
In the infrared, the ligand exhibits the carbonyl frequency at 1740 cm^{-1} and a band at 3300 cm^{-1} for the chelated hydroxyl group. The complexes exhibit no band in the hydroxyl region; however, there have been enormous shifts in the carbonyl frequencies. While the copper complex absorbs around 1700 cm^{-1}, all the others showed this band around 1670–1650 cm^{-1}. In addition, all the complexes exhibited a band at 1550 cm^{-1} for the N=N group.

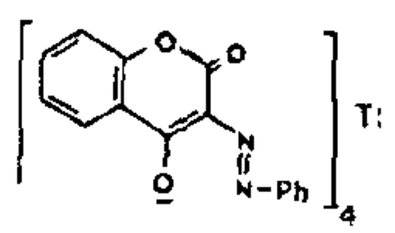
On the basis of the above spectral data, the diketo (I) and the chromone (III) tautomeric structures have been found to be untenable and it has been concluded that the ligand exists in solution only in the 4-hydroxy coumarin tautomeric form (II). Hence, the following structures have been proposed for the complexes:

CHART - III









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A POSSIBLE INVOLVEMENT OF CYCLIC 3′ : 5′ ADENOSINE MONOPHOSPHATE IN THE MATURATION OF MAMMALIAN RETICULOCYTES*

THE mammalian reticulocyte is formed in the bone marrow by differentiation of the normoblasts. After shedding the nucleus, the reticulocyte begins to mature in the bone marrow but attains full maturity in peripheral circulation[1,2]. Maturation is accompanied by depletion of reticulum and the loss of ability to synthesize haemoglobin. The mammalian reticulocyte is thus an interesting system for study of molecular regulatory mechanisms. Results suggestive of a role of cyclic 3′ : 5′ adenosine monophosphate in this process are presented in this communication.

Reticulocytosis was induced in adult male rabbits by administration of phenylhydrazine[3]. A sample of blood drawn from an anaemic patient containing 28% reticulocytes was used as the human sample. Adenyl cyclase activity was demonstrated by measuring the increase in intracellular content of cyclic AMP-^{14}C formed from *in situ* synthesized ATP-^{14}C by incubation of cells under appropriate conditions with glucose and theophylline[4-6]. Uptake of epinephrine by cells was followed by increase in radioactivity in the membrane of cells exposed to epinephrine-^{14}C.

The results obtained by us indicate that rabbit reticulocytes are four times more active than rabbit erythrocytes in their ability to incorporate adenine-8-^{14}C into cyclic AMP-^{14}C. Anaemic human RBC containing 28% reticulocytes also showed three to four fold more activity in this respect than normal human RBC. Epinephrine at 10^{-4} M stimulated adenyl cyclase activity of rabbit reticulocytes by 40% over the controls but had no effect on mature RBC. Studies with epinephrine-^{14}C revealed that reticulocyte membranes bind nearly thirteen times more of epinephrine than mature RBC (Table I) although the km values of binding of epinephrine to membrane were similar in mature RBC or reticulocytes.

Since maturation of reticulocytes is known to result in the depletion of glycogen[2], we studied the glycogen phosphorylase activity of mature RBC and reticulocytes. The activity of this enzyme was hardly detectable in mature RBC but was present in significant amounts in reticulocytes and prior exposure of reticulocytes to epinephrine led to 30–40% stimulation of its activity. We have also obtained evidence for the presence of a phosphodiesterase that acts as cyclic AMP in both reticulo-

TABLE I

Effect of epinephrine on the synthesis of c-AMP and binding of epinephrine by rabbit RBC

RBC type	*p*-moles 8–^{14}C adenine incorporated per 5×10^9 cells per hour		Epinephrine binding molecules × 10^7 bound per RBC		
	None	Epinephrine (0·1 mM)	0·5 mM*	5·0 mM*	10·0 mM*
Reticulocyte	670	953	12·0	141·1	306·7
Erythrocyte	170	164	3·0	11·0	36·7

* Concentration of epinephrine present in incubation medium for binding studies. For *c*-AMP synthesis the incubation medium contained NaCl 140 mM, KCl 6 mM, $MgSO_4$ 2 mM, Tris HCl buffer (pH 7·2) 15 mM (basal medium) adenine-8-^{14}C (5 μCi) glucose, 10 mg and $0{\cdot}7 \times 10^9$ cell. After incubation at 37°C in a Dubnoff metabolic shaker 4 mg theophylline and where required epinephrine (0·1 mM) were added and flask incubated for varying time. After required period *c*-AMP isolated and measured as described earlier (*4*).

For epinephrine-^{14}C binding the incubation medium contained NaCl 140 mM, KCl 5 mM, $MgSO_4$ 2 mM, Tris HCl buffer (pH 7·5) 15 mH, 0·5, 5·0 or 10·0 mM. epinephrine containing 25×10^3; 250×10^3 or 500×10^3 cpm respectively of epinephrine-^{14}C and 1×10^9 cells in a final volume of 2·5 ml. Incubation time was 20 min. after which membranes isolated and counted in Packard Liquid Scintillation spectrometer.