

RESEARCH PAPERS

THE CHEMISTRY OF ANTI-PERNICIOUS ANÆMIA FACTORS

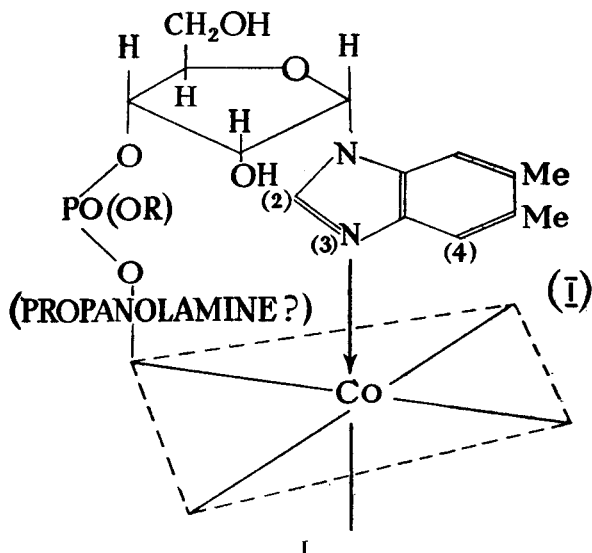
PART IX. THE PREPARATION AND SPECTROGRAPHIC PROPERTIES OF SOME BENZIMINAZOLE-COBALT CO-ORDINATION COMPOUNDS

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THE evidence upon which Beaven, Holiday, Johnson, Ellis, and Petrow (Part VI)¹ based their conclusion that the phosphoryl 1 α -D-ribofuranosyl-5:6-dimethylbenziminazolyl residue present in the B₁₂ molecule was coordinately linked to cobalt, as shown in (I), was largely spectroscopic in character. In the main it rested on the recognition of two anomalies



in the contribution of the benziminazole chromophore to the spectrum of the parent vitamin, and the association of these anomalies with co-ordination of the type specified.

Two spectroscopic features were held to distinguish the co-ordinated glycosylbenziminazole structure from an unattached phosphoryl-1 α -D-ribofuranosyl-5:6-dimethylbenziminazole: (i) the absence of a short wave shift in passing from pH 12 to pH 2, and (ii) the absence of a well-resolved fine structure band at $\lambda = 2885\text{\AA}$, characteristic of glycosyl-5:6-dimethylbenziminazoles, but only evident in the cyanocobalamin spectrum as a slight inflection.

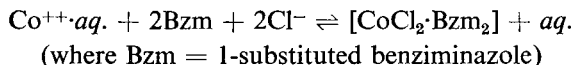
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Their validity as criteria for co-ordination, however, was open to criticism, as the results were not based upon data derived from authentic benziminazolocobalt co-ordination compounds, *per se*, the preparation of which had not at the time been described in the literature. Additional studies were therefore initiated in order to provide the remaining links in the chain of evidence leading to partial structure (I).

The π -electron density at N^3 of a 1-substituted benziminazole must clearly play an important part in determining the stability of the resulting cobalt co-ordination complex.² At the same time the electron density must also bear some relation to the pK_a of the base. The first part of our study was therefore concerned with the relationship between chemical constitution and basicity in the benziminazole and glycosylbenziminazole series (Davies, Mamalis, Petrow and Sturgeon, Part VIII)³. The present communication takes the investigation a stage further by describing the preparation of some authentic benziminazolocobalt co-ordination compounds, and by showing that such materials fulfil the spectral criteria (i) and (ii) laid down in Part VI as evidence for co-ordination.

Our first experiments were directed to the preparation of quadrico-ordinate cobaltous complexes of the type $[\text{CoCl}_2 \cdot \text{py}_2]$ ^{4,5,6} (where py = pyridine), which are generally prepared by simple admixture of the components in solvent media. Extension of this preparative method to 1-alkylbenziminazoles proved unsuccessful in those cases in which the pK_a of the base was less than 3.22 in ethanol (50 per cent.) (1-methyl-5:6-dichlorobenziminazole, Part VIII³). Glycosylbenziminazoles, though relatively basic compounds (pK_a 3.92 to 4.70 in water), likewise failed to react, a result probably due to the operation of unknown factors associated with the sugar group. 1-Alkylbenziminazoles of $pK_a \leq 3.88$ in ethanol (50 per cent.), in contrast, passed readily into the required dibenziminazolocobaltous chloride complexes $[\text{CoCl}_2 \cdot \text{Bzm}_2]$ (II) (see Table IV), which were obtained as crystalline blue solids of striking appearance.

Attempts to dissolve these compounds in water led to complete hydrolysis as shown by (a) the colour change from deep blue to pink, (b) the identification of free benziminazole in the solution by spectroscopic methods (*vide infra*), (c) conductimetric measurements, the molar conductance at infinite dilution being that of cobaltous chloride itself, and (d) solvent extraction, when over 90 per cent. of the ligand could be recovered. Concentration of such solutions led to reversal of the hydrolytic reaction through a mass-action effect



with separation of the complex in its crystalline form. In this state the benziminazole molecules were very firmly combined, not being removed by heating *in vacuo* at 150° to 160° C. We had thus to seek for organic solvents in which the compounds could be dissolved without dissociation in order to obtain solutions suitable for spectroscopic study.

Success was ultimately achieved by employing dry acetone and dry *n*-butanol, wherein the compounds dissolved to give solutions retaining

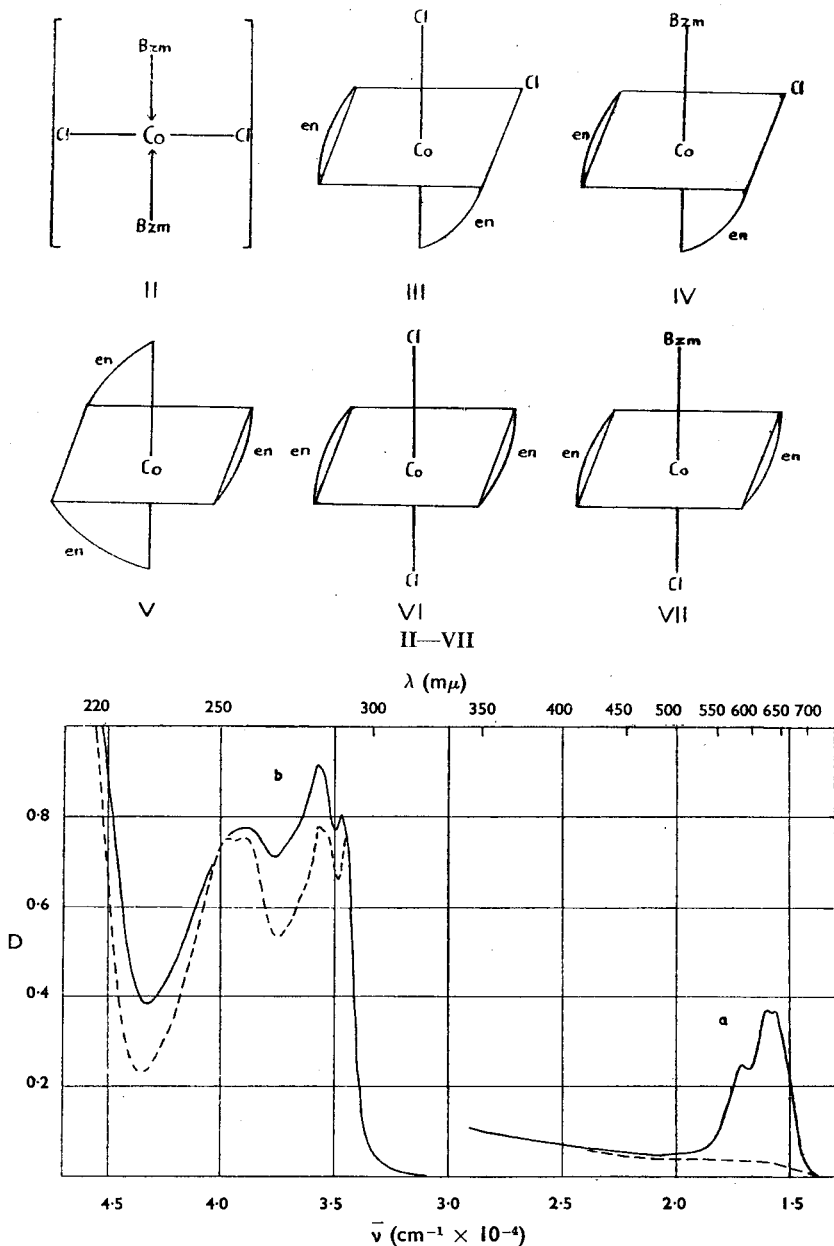


FIG. 1. Di-[1 : 5 : 6-trimethylbenziminazolo]-cobaltous chloride

Solvent .. *n*-Butanol
 Concentration .. Saturated at 20 C° (ca. 1 g./litre, 0.002 M)
 Cell length .. (a) 4.75 mm., (b) 0.475 mm.
 ——— .. Dry *n*-butanol
 - - - .. After adding 5 per cent. vol. of water.

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unchanged the blue colours characteristic of the undissociated salts. Spectroscopic study of such solutions showed unmistakably that the authentic dibenziminazolocobaltous chloride co-ordination compounds (II) satisfied the spectral criterion (ii) (above) laid down by Beaven *et al*¹ as evidence for co-ordination, and thus provided the first direct evidence for its validity. The results obtained are illustrated in Figure 1 by reference to di-(1:5:6-trimethylbenziminazolo)-cobaltous chloride in dry *n*-butanol solution. Inspection of the curve shows that the absorption of the benziminazole chromophore is anomalous with reduced resolution of the fine structure and with both the general form and location of the absorption bands intermediate between those of a free benziminazole in acid (cation) and alkaline (free base) solution. Addition of 5 per cent. volume of water increases the fine-structure resolution, and the spectrum alters towards that of the free base, though the full resolution of the fine structure is not regained. Solution in water, in contrast, leads to complete destruction of the complex, as shown in Figure 2, with reversion of the spectrum to that of the free benziminazole (*cf.* (b), p. 449).

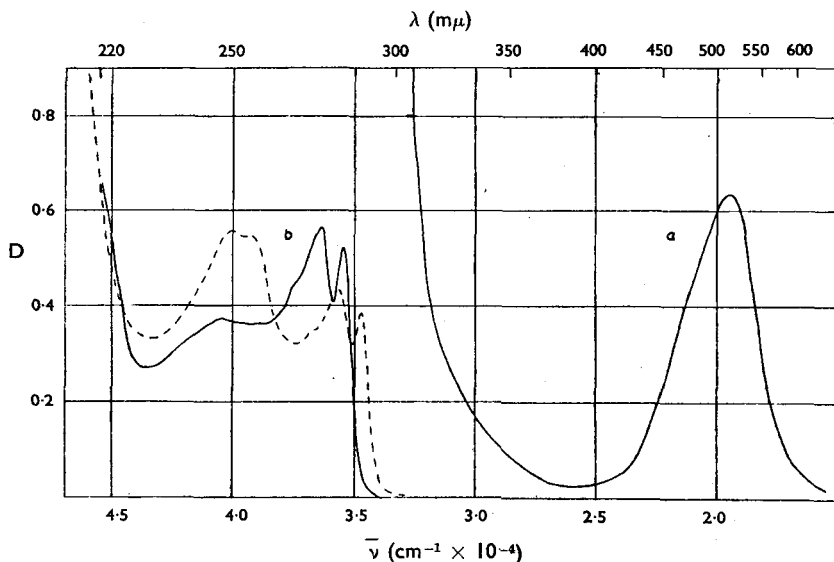


FIG. 2. Di-[1:5-dimethylbenziminazolo]-cobaltous chloride

Solvent .. Water
 Concentration (a) Saturated at 20° C. ca. 0.1 M
 (b) ca. 0.0001 M
 Cell length .. (a) 2 cm. (b) 1 cm.
 — .. pH 2
 - - - .. pH 10.

The absorption spectra in the visible region of the blue dibenziminazolocobaltous chloride complexes (II) differ markedly from that of the pink hydrated cobaltous ion, but resemble that of the blue $[\text{CoCl}_4]^{--}$ ion, which

has been studied by Macwaller and Barratt,⁷ Grøh and Schmidt,⁸ and others (see Macwaller and Barratt for references).

Compound	$\lambda_{\max.}(\text{m}\mu)$				Solvent
$[\text{CoCl}_2 \cdot (1\text{-MeBzm})_2]$	(518)	(592)	617	637	acetone
$[\text{CoCl}_2 \cdot (1:5:6\text{-TriMeBzm})_2]$	(526)	(592)	625	637	acetone
Co^{++}	(ca. 530)	585	621	637	<i>n</i> -butanol
$[\text{CoCl}_4]^{--}$		510			water*
			625	680	ethanol/HCl†
			585	678	acetone†

* See *Tables Annuelles de Constantes et Donnes Numeriques*, No. 39, vol. 13, section 33, p. 12 (Hermann, Paris, 1943).

† This work.

The molar extinction coefficient ($\epsilon_{\max.}$) of the 637 $\text{m}\mu$. band (Fig. 1) of (II) is *ca.* 200, compared with *ca.* 5 for the 510 $\text{m}\mu$. band of hydrated Co^{++} , and with *ca.* 300 for the 678 $\text{m}\mu$. band of $[\text{CoCl}_4]^{--}$ (in acetone). The latter figure is in good agreement with the values (630, 609) found by Macwaller and Barratt⁷ and by Grøh and Schmidt,⁸ respectively, as in neutral acetone only one-half of the cobalt is in the form of the complex ion:—



The intense 678 $\text{m}\mu$. band present in the spectrum of the $[\text{CoCl}_4]^{--}$ ion is not evident in the spectra of the dibenziminazolocobaltous chloride complexes (II). This observation provides further proof that the latter exist in acetone and *n*-butanol solutions in the undissociated forms.

It was, of course, not possible to confirm spectral criterion (i) (above) with the dibenziminazolocobaltous chloride complexes (II), owing to their marked instability to traces of moisture (see also Experimental part). We therefore turned our attention to the preparation of co-ordinated complexes derived from cobaltic cobalt, as it was thought that such compounds might show greater stability in aqueous solution (*cf.* Pauling⁹). Meisenheimer and Kiderlen,¹⁰ Ablov,^{11,12} and Bailar and Clapp¹³ had previously shown that *trans*-bisethylenediamino-dichlorocobaltic chloride $[\text{Co.en}_2\text{Cl}_2]\text{Cl}$ (VI; en = ethylenediamine) reacts readily with arylamines and with heterocyclic bases to give compounds of the type $[\text{Co.en}_2\text{Base.Cl}]\text{Cl}_2$. We now find that by heating (VI) with 1-methylbenziminazole in aqueous ethanolic solution at 100° C. for 3 to 4 hours, or preferably by allowing the reaction to occur at room temperature for 1 to 2 days, a rose-red compound of empirical constitution $[\text{CoCl}_3.en_2.1\text{-MeBzm}]$, is readily obtained in excellent yield.

Conductance measurements show that the molar conductance of this complex at infinite dilution corresponds to a salt of the type $[\text{Co complex}]^{++}\text{Cl}_2$. Conductance titrations with silver nitrate likewise show that two-thirds of the chlorine content is ionic. There is little doubt, on this evidence alone, that the compound has the desired constitution of a [bis-ethylenediamino-1-methylbenziminazolochlorocobaltic] dichloride (IV, *cis*-form; (VII), *trans*-form). It thus represents the first example of an authentic benziminazolocobaltic complex to be prepared in the laboratory. In addition, its physico-chemical characteristics

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make it a suitable model compound for spectroscopic study. At the same time it is impossible to define rigidly its stereochemical configuration. The balance of evidence derived from chemical (p. 454) and spectroscopic studies (p. 457), however, appears to indicate that the *cis*-structure (IV) and not the *trans*-structure (VII) is the preferred form. (IV) has, therefore, been adopted for usage in the present communication.

Extension of the reaction to other 1-substituted benzimidazoles has given the results listed in Table I, alongside the basicity of the ligands.

Three types of behaviour are evident:—

(a) 1:5:6-Trimethyl-, 1:5-dimethyl-, 1-ethyl-, and 1:2-hydroxyethyl-benzimidazole react readily to give the co-ordination compound (IV) in yields greater than 60 per cent.

(b) 1-*iso*Propyl-, 1-*n*-propyl-, 1-allyl-, and 1:6-dimethyl-5-chloro-benzimidazole give mixtures consisting largely of *cis*[bis-ethylenediaminodichlorocobaltic] chloride (III), admixed with small quantities (< 5 per cent.) of the co-ordination compound, which is separated by fractional crystallisation from aqueous acetone. 1-*n*-Butyl-, and 1-methyl-5-chloro-benzimidazole also appear to react in this way, as distinctly red fractions are obtained. Purification of the co-ordination compounds, however, could not be effected.

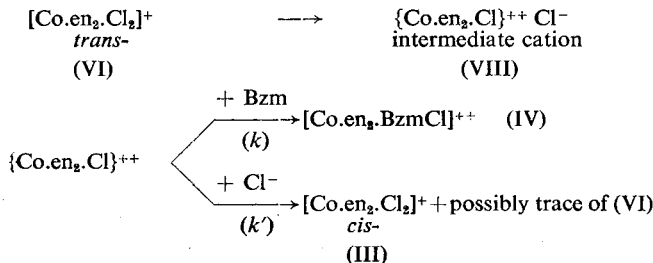
(c) 1:2:5:-Trimethyl-, 1 α -D-arabopyranosyl-5:6-dimethyl-, and 1 β -D-glucopyranosylbenzimidazole afford only the *cis*-compound (III), no evidence for complex formation being obtained.

TABLE I

Benzimidazole Bzm	pKa Ethanol (50 per cent.)	Reaction product(s)
1:5:6-Trimethyl-	5.45	} [Co.en ₂ .BzmCl]Cl ₂ yield \gtrsim 60 per cent.
1:5-Dimethyl-	5.22	
1-Ethyl-	4.88	
1-Methyl-	4.88	
1:2-Hydroxyethyl-	4.82	
1- <i>iso</i> Propyl-	4.97	} (III) plus [Co.en ₂ .BzmCl]Cl ₂ in less than 5 per cent. yield
1- <i>n</i> -Propyl-	4.83	
1-Allyl-	4.58	
1:6-Dimethyl-5-chloro-	4.1†	} (III) plus possibly some [Co.en ₂ .BzmCl]Cl ₂
1- <i>n</i> -Butyl-	4.75	
1-Methyl-5-chloro-	3.88	
1:2:5-Trimethyl	6.07	} (III)
1 α -D-Arabo-5:6-dimethyl-	4.2*	
1 β -D-Glucopyranosyl-	3.69	

* Estimated from value measured in aqueous solution.

† Calculated from figures given in Table V, Part VIII².



The explanation for this multiplicity of behaviour lies in the dual nature of the changes which can occur in mixtures of (VI) and a benziminazole in aqueous ethanolic solution. In addition to formation of (IV), we now find that (VI) undergoes spontaneous conversion into (III), a change previously thought to occur through participation of the co-present ligand.^{10,13}

Both these competing reactions may be visualised as proceeding through initial formation of the intermediate cation (VIII) from the *trans*-salt (VI) by an S_N1 type of mechanism. Addition of benziminazole or chloride ion then takes place, the relative proportions of the two products formed being governed by the specific reaction rates k and k' . Thus benziminazoles in category (a) react quickly with formation of complex (IV) in excellent yield. Category (b) benziminazoles, in contrast, show slow reaction with concomitant production, in major amount, of the auto-transformation product (III), whilst category (c) compounds form either a special instance of such behaviour, or do not react at all.

Inspection of Table I shows that, apart from the exceptions mentioned below, the yields of $[\text{Co.en}_2\text{.Bzm}\cdot\text{Cl}]\text{Cl}_2$ run parallel to the basicity of the ligands. The relationship is not quantitatively proportional, however, as there is a definite break in the yield at about pK 4.8. The benziminazoles thus resemble the arylamines, in which compounds stronger than aniline (pK_a 4.62 in 30 per cent. ethanol) give co-ordination compounds in high yield, whereas weaker bases appear to give solely the *cis*-compound (III) (Ablov¹¹). At the same time the marked influence of basicity on complex formation may sometimes be overshadowed by the effect of other factors which cannot be strictly defined at present. Thus the basic *l*-isopropylbenziminazole gives only a minute yield of complex with (IV), whilst the strongly basic 1:2:5-trimethylbenziminazole fails to give evidence of reaction. The reason for this anomalous behaviour is not clear, but in the case of the latter compound may well be related to steric factors associated with the 2-substituent.

In spite of the general similarity between the reactions of benziminazoles and arylamines with (VI), a point of difference is nevertheless observed in the behaviour of the former series with the *cis*-salt (III). Bailar and Clapp¹³ had previously reported that arylamines react with both (III) and (VI) to give similar products, but that "it cannot be stated with certainty that the compounds from the two series are identical." Benziminazoles, in contrast, fail to react with (III) under the conditions studied. This observation, however, does not provide data for a decision on the stereochemistry of the [bis-ethylenediamino-1-methylbenziminazolo-chlorocobaltic]dichloride complexes (p. 453), as their formation is thought to occur through the intermediate cation (VIII), from which both *cis*-(IV) and *trans*-(VII) types can be formed. It is known, moreover, that aqueous ammonia converts both (III) and (VI) into the *cis*-chloroamine $[\text{Co.en}_2\cdot(\text{NH}_3)\text{Cl}_2]$ whilst methyl- and ethylamine react with *dextro*-(III) to give derivatives which are optically active (though readily racemised) and thus unambiguously formulated as *cis*-complexes analogous to (IV).¹³ It seems reasonable to assume, on this evidence, that the *cis*-

TABLE II
ABSORPTION SPECTRA OF (BIS-ETHYLENEDIAMINO-BENZIMINAZOLOCHLOROCOBALTIC)DICHLORIDE COMPLEXES
IN WATER (pH ca. 6)

Benzimidazole substituents	λ_{max} .		ϵ_{max} .		λ_{min} .		ϵ_{min} .		λ_{max} .		ϵ_{max} .		λ_{max} .	ϵ_{max} .	λ_{max} .	ϵ_{max} .
	513	70	(370)	(110)	32	429	(370)	(110)	32	429	(370)	(110)				
1-Methyl	510	88	—	—	29	430	—	—	—	—	—	—	—	—	233	19,400
1:5-Dimethyl	521	84	(385)	(120)	31	433	(385)	(120)	—	—	—	—	—	—	—	—
1-Ethyl	513	85	(370)	(120)	30	427	(370)	(120)	—	—	—	—	—	—	—	—
1:2'-Hydroxyethyl	505	50*	—	—	24*	425	—	—	—	—	—	—	—	—	—	—
1-iso-Propyl	515	97	(370)	(130)	38	425	(370)	(130)	—	—	—	—	—	—	235	19,700
1-Allyl	513	85	(375)	(120)	32	428	(375)	(120)	—	—	—	—	—	—	234	19,500
Mean values	535	60	380	70	24	405	380	70	(303)	(815)	—	—	—	—	244	15,500
<i>cis</i> -[Co(en) ₂ Cl ₂]Cl	621	34	(445)	(28)	8	524	(445)	(28)	(400)	(36)	—	—	304	1100	241	15,500
<i>trans</i> -[Co(en) ₂ Cl ₂]Cl	464	83	337	70	12	383	337	70	—	—	—	—	—	—	—	—

* Not corrected for unknown water of crystallisation: excluded from mean. † Also λ inf. 603 m μ . ϵ inf. 44. Wavelengths in m μ : values in parentheses denote unresolved inflections.

TABLE IV
DIBENZIMINAZOLO-COBALTOUS CHLORIDE COMPLEXES [CoCl₂·Bzm₂]

Substituted Benzimidazole	pKa Ethanol (50 per cent.)	Product	Analysis														
			Found per cent.						Required per cent.								
			C	H	N	Cl	C	H	N	Cl	C	H	N	Cl			
1-Methyl-	4.88	[CoCl ₂ (C ₆ H ₄ N ₂) ₂]	48.6	4.0	—	18.6	48.8	4.1	—	18.0	—	—	—	—	—	—	—
1:5-Dimethyl-	5.22	[CoCl ₂ (C ₆ H ₃ N ₂) ₂]	51.6	4.6	13.1	—	51.2	4.8	13.3	—	—	—	—	—	—	—	—
1:5:6-Trimethyl-	5.45	[CoCl ₂ (C ₆ H ₂ N ₂) ₂]	53.4	4.8	12.3	16.6	53.4	5.4	12.4	15.8	—	—	—	—	—	—	—
1:2:5-Trimethyl-	6.07	[CoCl ₂ (C ₆ H ₃ N ₂) ₂]	53.0	5.3	11.7	15.3	53.4	5.4	12.4	15.8	—	—	—	—	—	—	—
1- <i>n</i> -Butyl-	4.75	[CoCl ₂ (C ₁₁ H ₁₄ N ₂) ₂]	—	—	—	14.9	—	—	—	—	—	—	—	—	—	—	—
1-Methyl-5-chloro-	3.88	[CoCl ₂ (C ₆ H ₃ N ₂ Cl) ₂]	—	—	12.2	30.2	—	—	—	—	—	—	—	—	—	—	—
1-Methyl-6-chloro-	3.88	[CoCl ₂ (C ₆ H ₃ N ₂ Cl) ₂]	—	—	12.0	30.4	—	—	—	—	—	—	—	—	—	—	—

structure (IV) may well be the preferred form in the present instance. Spectroscopic results, detailed below, point likewise in this direction.

In contrast to the cobaltous complexes (II), the cobaltic salts (IV) proved readily soluble in water to give solutions stable at room temperature over the *pH* range 2 to 10. We were thus able to obtain further experimental evidence in support of criterion (ii) page 448), and, in addition, to establish beyond reasonable doubt the validity of criterion (i). For this purpose 6 of the complexes listed in Table I were examined spectroscopically in aqueous solution. The results obtained are presented in such a way as to separate the absorption due to the "cobalt complex" chromophore (Table II) from that of the co-ordinated benziminazole moiety (Table III):

TABLE III
FINE STRUCTURE BANDS OF
[bis-ETHYLENEDIAMINO-1-METHYLBENZIMINAZOLOCHLOROCOBALTIC] DICHLORIDE
AND OF 1-METHYLBENZIMINAZOLE

Complex (IV)	<i>pH</i> 2 <i>pH</i> 10	λ_{\max}	ϵ_{\max}	λ_{\max}	ϵ_{\max}	λ_{\max}	ϵ_{\max}
			278.2 278.5	7600 7000	270.6 271.7	9400 9100	(c. 262.5) 263.9
1-McBz*	<i>pH</i> 2	274.4	6600	267.8	6950	261.0	5400
	<i>pH</i> 10	279.8	4600	272.9	5000	265.2	4500

Values in parentheses refer to unresolved inflections.
Wavelengths in $m\mu$.

* See Beaven, Holiday and Johnson.¹⁴

(a) The absorption of the "cobalt complex" chromophore is characterised by a band in the visible region at *ca.* 515 $m\mu$., an inflection in the near ultra-violet at *ca.* 375 $m\mu$., and an intense band at *ca.* 234 $m\mu$.. The absorption contribution of the benziminazole moiety is superimposed on the long-wave side of this complex band, which thus makes an appreciable contribution to the apparent densities of the benziminazole bands (*cf.* Table III).

(b) The absorption of the benziminazole moiety is characterised by:

- (1) The absence of a long-wave shift when the *pH* of the solution is changed from 2 to 10, corresponding for a free benziminazole to complete conversion of the "acid" form (benziminazolinium cation) to the free base.
- (2) The low resolution of the fine structure in the long-wave band at 270 to 290 $m\mu$.
- (3) The positions of the fine structure bands, which lie between the positions occupied by the corresponding bands of the free benziminazoles in acid and alkaline solution, respectively.

These features, which are illustrated in Figure 3 by reference to the absorption of [bis-ethylenediamino-1-methylbenziminazolochlorocobaltic] dichloride (IV), are clearly in marked agreement with those postulated in criteria (i) and (ii) (p. 448) as evidence for co-ordination. In addition, it may be pointed out that donation of an unshared electron pair from $N^{(3)}$ of the benziminazole to a d^2sp^3 orbital of hexavalent Co^{+++} is formally analogous to donation to a proton with formation of the

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benziminazolium cation. The observation that the absorption spectrum of a co-ordinated benziminazole tends towards the cationic form may therefore be regarded as further evidence for the concepts formulated in the present work.

Comparison of the absorption spectrum (Fig. 3) of [bis-ethylene-diamino-1-methylbenziminazolochlorocobaltic]dichloride (IV) with those

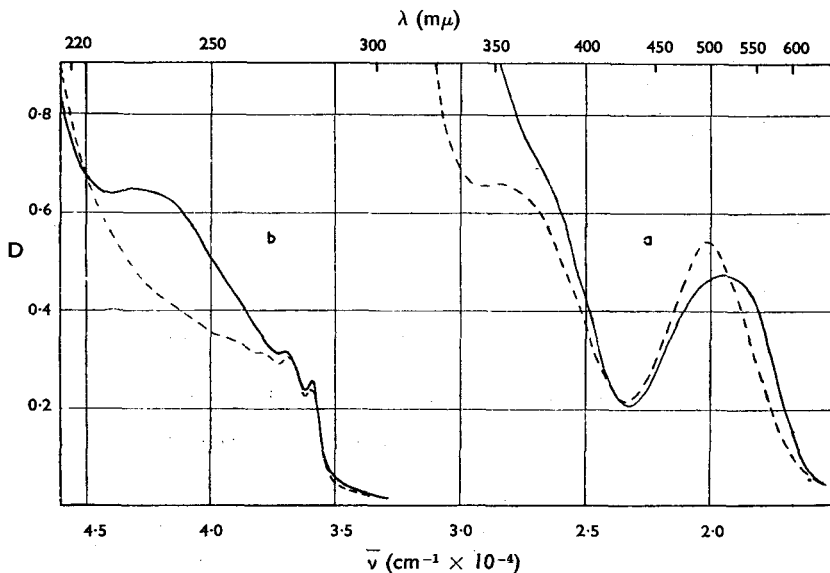


FIG. 3. [Bis-ethylenediamino-1-methylbenziminazolochloro-cobaltic] dichloride

Solvent	..	Water
Concentration	(a) 0.00632 M ; (b) 0.0000335 M	
Cell length	..	1 cm.
—	..	pH 2.
— —	..	pH 10.

obtained from *cis*-[Co.en₂.Cl₂]Cl (III) (see Fig. 4) and *trans*-[Co.en₂.Cl₂]Cl (VI) (see Fig. 5), gives some information regarding the configuration of the benziminazolochlorocobaltic complexes. The spectra of all three compounds show general similarity, although both (III) and (VI) show features at *ca.* 300mμ. which are not present in the benziminazolochlorocobaltic complexes. In (VI) however, the visible band occurs at longer wavelengths (621 mμ.) and is lower in intensity than in (III), and the inflection is similarly altered. It is, therefore, the *cis*-salt (III) which resembles the benziminazole complexes more closely with respect to absorption spectrum. More data on similar pairs of complexes of known configuration would be required, however, before this similarity could be considered as evidence of *cis*-configuration for the benziminazolochlorocobaltic complexes themselves. At the same time it seems reasonable to infer that the spectroscopic results support (IV) rather than the *trans*-structure (VII).

Inter alia, we have examined the absorption spectrum of [Co.en₃]Cl₃ (V) (see Fig. 6), which is noteworthy for the absence of an intense shortwave

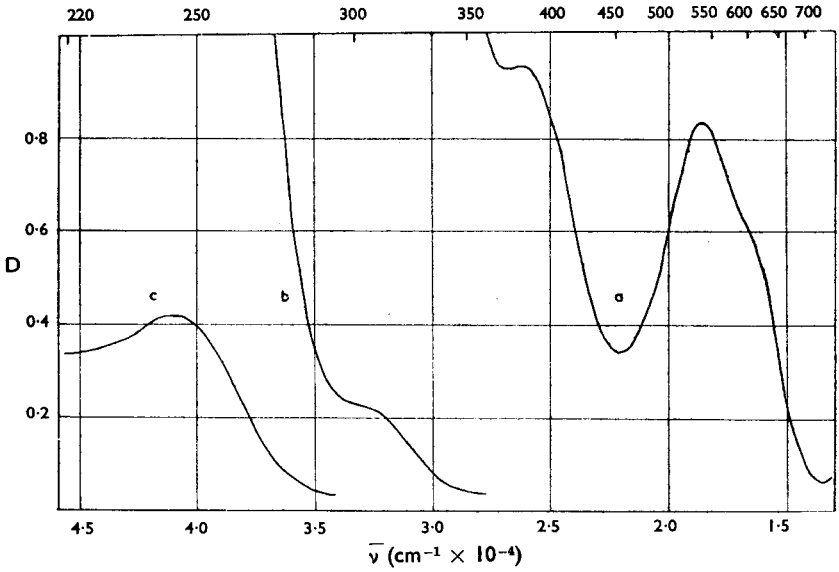


FIG. 4. *Cis*-[bis-ethylenediaminodichlorocobaltic] chloride
 Solvent .. Water
 Concentration (a) 0.0135 M; (b) 0.00027 M; (c) 0.000027 M
 Cell length .. 1 cm.

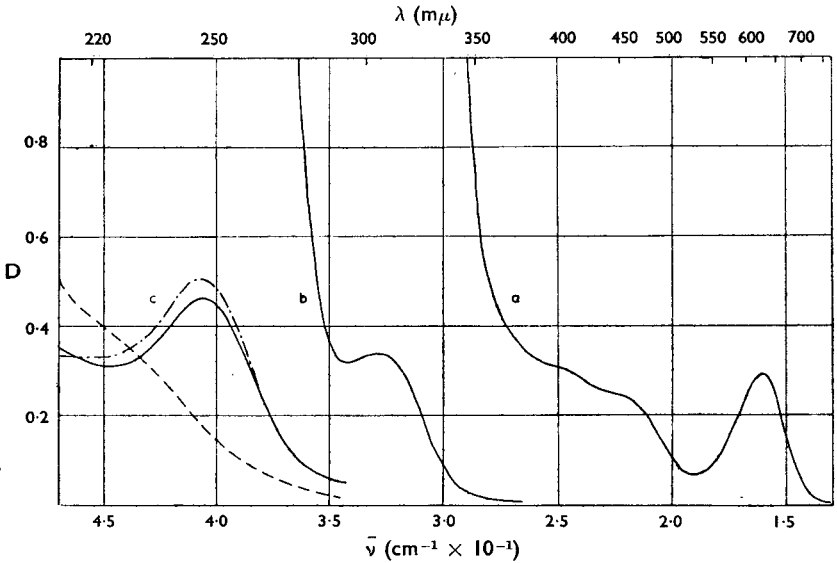


FIG. 5. *Trans*-[bis-ethylenediaminodichlorocobaltic] chloride
 Solvent .. Water
 Concentration (a) 0.0085 M; (b) 0.00034 M; (c) 0.0000272 M
 Cell length .. 1 cm.
 · - · - · .. pH 2
 ——— .. pH 6
 - - - .. pH 10.

[CoBr₂·(C₈H₈N₂)₂] requires N, 11·6, and *thiocyanate*, Found: N, 18·6; S, 14·8 [Co(SCN)₂·(C₈H₈N₂)₂] requires N, 19·1; S, 14·6 per cent., were obtained. Both complexes crystallised in dark blue prisms of indefinite melting point.

Di[1:5-dimethylbenziminazolo] *cobaltous nitrate trihydrate* was prepared by adding an aqueous solution of cobalt nitrate (880 mg.) to a warm solution of 1:5-dimethylbenziminazole (800 mg.) in acetone (5 ml.). It formed magenta needles. Found: C, 40·8; H, 4·8; N, 16·3. Co(NO₃)₂·(C₉H₁₀N₂)₂·3H₂O requires C, 40·8; H, 5·0; N, 15·9 per cent.

[*Bis*-ethylenediamino-1-methylbenziminazolochlorocobaltic] *dichloride* (IV):— (a) A mixture of *trans*-(bis-ethylenediaminodichlorocobaltic) chloride (1 g.), 1-methylbenziminazole (1 g.), water (8 ml.), and a few drops of ethanol, was heated at 100° C. in an evaporating basin for 3 hours. The residue was washed with ethanol and then recrystallised twice from water and acetone. The *complex* separated in rose-red prismatic needles, m.pt. 255° C. (decomp.). Found: N, 20·2; Cl, 25·4. C₁₂H₂₄N₆Cl₃Co requires N, 20·1; Cl 25·5 per cent.

(b) (VI) (2·9 g.), 1-methylbenziminazole (1·3 g.), water (5 ml.), and ethanol (3 ml.) were shaken together, when the chloride rapidly dissolved and the solution began to assume a red colour. Red prisms began to separate after 3 to 4 hours when ethanol (10 ml.) was added. The complex was collected after standing at 0° C. overnight. Found: N, 19·8 per cent.

[*Bis*-ethylenediamino-1-ethylbenziminazolochlorocobaltic] *dichloride* was obtained (90 per cent.) by reacting 1-ethylbenziminazole (1·5 g.) with (VI) (3 g.) by method (b). It separated from aqueous acetone in deep red prisms, m.pt. 255° to 260° C. (decomp.) after turning green at 240° C. Found: N, 19·4; Cl, 24·1. C₁₃H₂₆N₆Cl₃Co requires N, 19·5; Cl, 24·7 per cent.

[*Bis*-ethylenediamino-1(2-hydroxyethyl)-benziminazolo chlorocobaltic] *dichloride*, prepared (65 per cent.) by method (b), formed dark red prisms from aqueous acetone, m.pt. 235° C. (decomp.). Found: N, 19·2; Cl, 23·7. C₁₃H₂₆ON₆Cl₃Co requires N, 18·4; Cl, 23·3 per cent.

[*Bis*-ethylenediamino-1:5-dimethylbenziminazolochlorocobaltic] *dichloride* was obtained (72 per cent.) from aqueous acetone in small pink platelets, m.pt. 202° to 204° C. (decomp.). Found: N, 17·4; Cl, 22·2. C₁₃H₂₆N₆Cl₃Co·3H₂O requires N, 17·4; Cl, 21·9 per cent.

[*Bis*-ethylenediamino-1:5:6-trimethylbenziminazolochlorocobaltic] *dichloride* was isolated (80 per cent.) from aqueous acetone in felted pink needles, m.pt. 255° C. (decomp.), after turning green at 230° C. Found: N, 19·0; Cl, 23·6. C₁₄H₂₈N₆Cl₃Co requires N, 18·9; Cl, 23·8 per cent.

[*Bis*-ethylenediamino-1-isopropylbenziminazolochlorocobaltic] *dichloride*:—The benziminazole (1·7 g.) was allowed to react with the *trans*-dichloride (VI) (2·9 g.) in cold aqueous ethanol for 1 week. Acetone was then added and the purple crystalline product collected the next day and identified as (III). A second crop, obtained by evaporation of the mother liquor, was red in colour and proved to be a mixture of the desired complex and (III). The latter was removed by repeated crystallisation from aqueous acetone

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when [*bis-ethylenediamino-1-isopropylbenziminazolochlorocobaltic*] dichloride (50 mg.) was obtained as dark red prisms, m.pt. 210° to 212° C. (decomp.). Found: N, 17.0. $C_{14}H_{28}N_6Cl_3Co \cdot 2H_2O$ requires N, 17.4 per cent.

The following complexes were prepared and isolated in essentially the same way:—

[*Bis-ethylenediamino-1-n-propylbenziminazolochlorocobaltic*] dichloride, rose-red needles, m.pt. 215° C. (decomp.). Found: N, 18.9; Cl 23.4. $C_{14}H_{28}N_6Cl_3Co$ requires N, 18.9; Cl, 23.8 per cent.

[*Bis-ethylenediamino-1-allylbenziminazolochlorocobaltic*] dichloride, purple red crystals, m.pt. 208° to 209° C. (decomp.). Found: N, 17.5; Cl, 22.7. $C_{14}H_{26}N_6Cl_3Co \cdot 2H_2O$ requires N, 17.5; Cl, 23.3 per cent.

[*Bis-ethylenediamino-5-chloro-1:6-dimethylbenziminazolochlorocobaltic*] dichloride, fine pink needles, m.pt. 255° to 256° C., after turning green at 235° C. Found: N, 17.0; Cl, 29.3. $C_{13}H_{26}N_6Cl_4Co \cdot H_2O$ requires N, 17.4; Cl, 29.3 per cent.

Molar Conductivities. Specific conductivities of aqueous solutions were determined employing the bridge and enclosed type cells of 3 ml. capacity manufactured by Mullard Electronic Products, Ltd. Temperature control was effected by immersion of the cells in a thermostatically controlled water-bath maintained at $25 \pm 0.05^\circ C.$, bridge readings being taken after an equilibration period of 20 minutes. The electrodes were replatinised prior to each set of determinations. Potassium chloride, A.R., was employed as a standard in cell-constant determinations, the equivalent conductivity value of the salt being taken from the results of Shedlovsky, Brown and MacInnes.¹⁵ The overall accuracy of the determinations was within ± 3 per cent.

(1) *Complexes of the type [CoCl₂:2 Base] (II).*

The specific conductivities of solutions of (a) dipyridinocobaltous chloride, (b) di[1:5-dimethylbenziminazolo]cobaltous chloride, and (c) cobaltous chloride, A.R., determined with respect to the bridge standard internal impedance, are given in Table V. On plotting the corresponding values for Λ_M against $100 M^{\frac{1}{2}}$, linear plots are obtained (Fig. 7) for the

TABLE V
MOLAR CONDUCTIVITIES OF COBALTOUS CHLORIDE COMPLEXES (II) IN
AQUEOUS SOLUTION AT 25° C.

Compound	10 ⁴ M	100M ^½	10 ⁴ k	Λ _M
CoCl ₂ ·6H ₂ O	44.00	6.63	10.55	240
	22.00	4.69	5.38	244
	4.40	2.10	1.12	213
[CoCl ₂ :py ₂]	100.00	10.00	21.74	217
	50.0	7.07	11.49	230
	25.0	5.00	5.96	238
[CoCl ₂ :(1:5-DiMeBzm) ₂] ..	65.40	8.09	13.97	222
	29.94	5.47	7.06	236
	13.08	3.62	3.17	242
	2.99	1.73	0.76	254

Concentration (M) expressed in moles/l., specific conductivity (k) expressed in mhos.cm⁻¹, molar conductivity (Λ_M) expressed in mhos.cm².

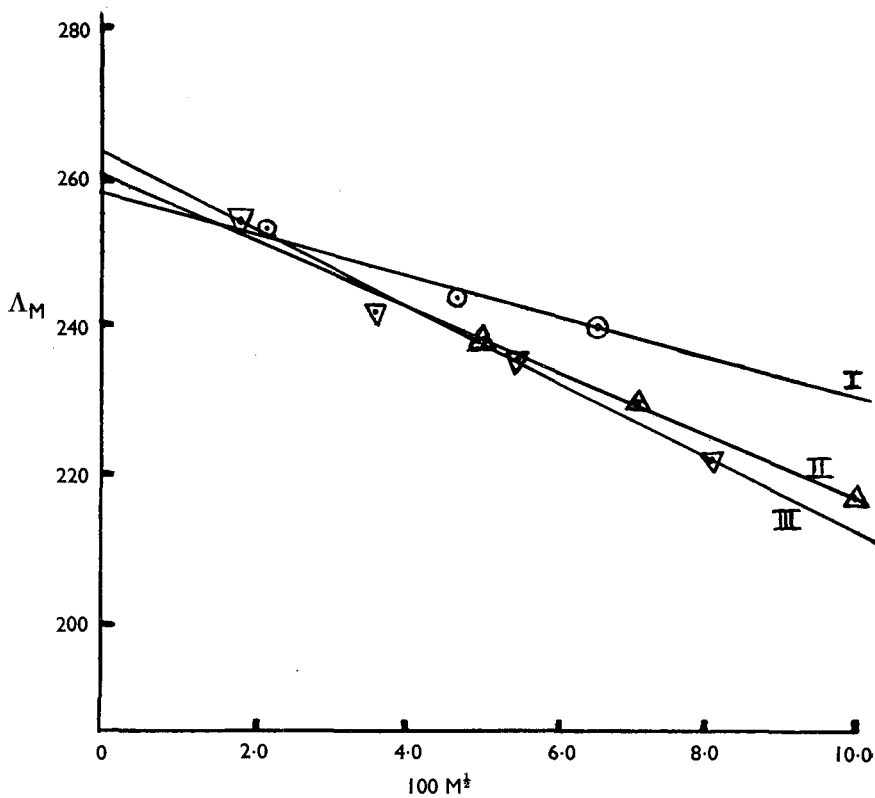


FIG. 7.

- | | | |
|-------|---|--|
| (I) | ○ | CoCl ₂ .6H ₂ O |
| (II) | △ | [CoCl ₂ .Py ₂] |
| (III) | ▽ | [CoCl ₂ .(1-meBzm) ₂] |

three substances which co-average on to the same limiting value for Λ_M (where $c = 0$). Complete dissociation of (a) and (b) therefore occurs in dilute solution. Deviations of the molar conductivity values of (a) and (b) from those of cobaltous chloride at the higher concentrations are possibly due to the presence of undissociated complex.

(2) *Complexes of the type* [Co.en₂.Bzm.Cl]Cl₂ (IV).

The results obtained (Table VI) shown graphically in Figure 8, reveal that the complexes are of the type $M^{II} Cl_2$.

Estimation of Ionic Chlorine. Ionic chlorine was estimated by titration of the aqueous solutions against 10^{-3} N silver nitrate solution. Equivalence points were determined conductimetrically, the bridge being employed in conjunction with a robust dip-type cell of standard design. Smooth bright platinum foil electrodes were employed.

The results obtained are given in Table VII.

Absorption measurements. Absorption spectra were measured with a twin-beam automatic recording spectrophotometer designed and built

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 TABLE VI
 MOLAR CONDUCTIVITIES OF COMPLEXES $[\text{Co.en}_2\text{Bzm.Cl}]\text{Cl}_2$ (IV) IN
 AQUEOUS SOLUTION AT 25° C.

Bzm	10 ⁴ M	100 M [‡]	10 ⁴ k	Λ_m
(Pyridine)	123.1 24.62 12.31	11.09 4.96 3.51	23.98 5.46 2.75	194 222 224
1-Methylbenzimidazole— (a) run No. 1	20.49 10.25 5.12	4.53 3.20 2.26	4.69 2.47 1.27	229 241 247
(b) run No. 2	20.11 10.06 5.03	4.48 3.17 2.24	4.61 2.42 1.25	229 241 250
1-Ethylbenzimidazole— (a) run No. 1	50.24 12.56 10.05 5.02	7.09 3.54 3.17 2.24	10.37 2.80 2.28 1.18	206 223 227 236
(b) run No. 2	50.10 12.52 10.02 5.01	7.08 3.54 3.17 2.24	10.40 2.81 2.33 1.20	208 225 232 240
1:2'-Hydroxyethylbenzimidazole— (a) run No. 1	20.45 10.23 5.11	4.52 3.20 2.27	4.44 2.35 1.21	217 229 237
(b) run No. 2	12.02 6.01 3.01	3.47 2.45 1.74	2.68 1.40 0.72	222 233 238
1:5-Dimethylbenzimidazole (·3H ₂ O)— (a) run No. 1	18.08 9.04 4.52	4.25 3.01 2.13	4.22 2.16 1.08	233 239 240
(b) run No. 2	17.83 8.92 4.46	4.22 2.99 2.11	4.17 2.13 1.08	234 239 243

 TABLE VII
 ESTIMATION OF IONIC CHLORINE IN COMPLEXES OF TYPE $[\text{Co.en}_2\text{BzmCl}]\text{Cl}_2$ (IV)
 IN AQUEOUS SOLUTION, AT LABORATORY TEMPERATURE

Bzm	10 ⁴ M	10 ⁴ M'	Ionic chlorine per cent.	Number of chlorine ions per molecule
1-Methylbenzimidazole	5.16	4.3	19.1	2.25
1-Ethylbenzimidazole	10.61	8.2	17.9	2.18
1:5-Dimethylbenzimidazole(·3H ₂ O)	9.10	7.0	17.4	2.38
1:2'-Hydroxyethylbenzimidazole	0.84	0.58	19.7	2.50

M = concentration of complex solution, expressed in moles/l.

M' = concentration of complex in the titration vessel, near equivalence point, expressed in moles/l.

by Holiday and Sutton.¹⁶ The spectrograms obtained from it are linear in wave number ($\bar{\nu}$) and optical density (D), as shown in Figures 1 to 6, which are direct tracings of the records after correction for instrumental zero errors. Important features of the spectra listed in Table III were checked manually on a Unicam SP.500 single-beam spectrophotometer.

Observations on (II) in the ultra-violet were made difficult by the sensitivity of the compounds to traces of water in the solvent. Satisfactory results were obtained, however, by using a variable-length cell. It was

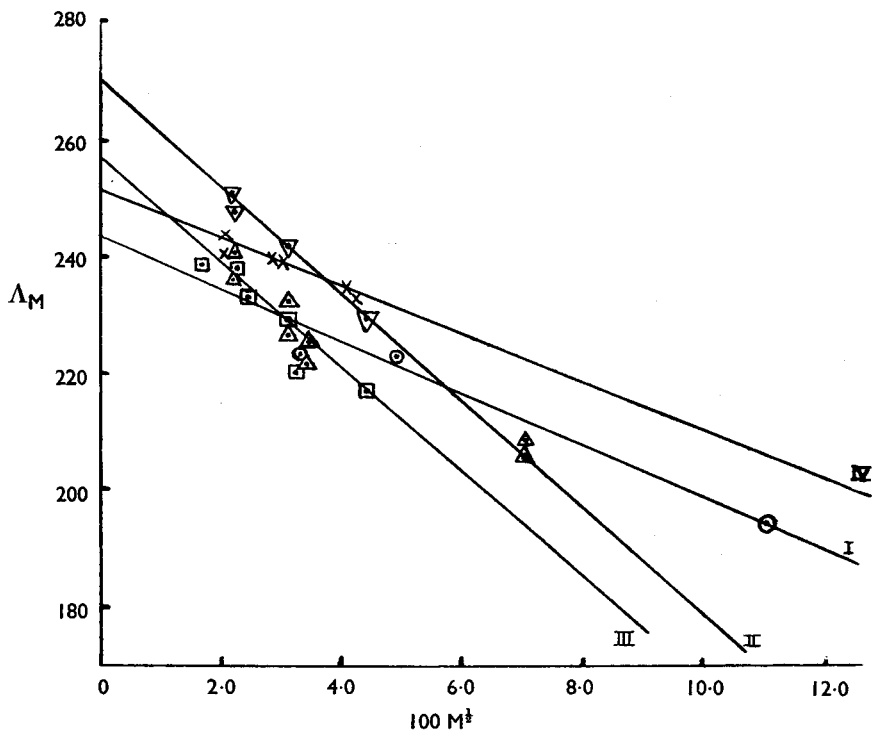


FIG. 8.

- Pyridine (I)
- ▽ 1-methylbenziminazole (II)
- △ 1-ethylbenziminazole (not graphed)
- 1:2'-hydroxyethylbenziminazole (III)
- × 1:5-dimethylbenziminazole (IV)

thus possible to determine the absorption spectra of the blue solutions in the visible at long path lengths, and then to reduce the path length for ultra-violet determinations without further dilution of the solutions. By working in this way it was possible to confirm that the blue solutions of (II) had not decomposed before absorption in the ultra-violet had been determined.

Effect of cyanide on the cobaltic complexes (IV). Addition of cyanide to the cobaltic complexes (IV) led to rupture of the benziminazole-cobalt co-ordination linkage with formation of $K_3Co(CN)_6$ (*cf.* Pt VI¹).

SUMMARY AND CONCLUSIONS

1. The preparation of some authentic benziminazolo-cobaltous and -cobaltic co-ordination compounds has been effected.

2. Their spectroscopic study has proved the validity of the criteria [(i) and (ii), p. 448] employed in Part VI¹ to establish the existence of the benziminazole-cobalt co-ordination linkage in vitamin B₁₂.

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