Equilibria in Complexes of N-Heterocyclic Molecules. Part 34.† Iron Binding by Caerulomycins

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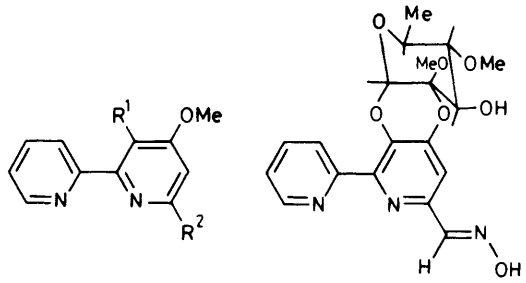
Spectroscopic properties of caerulomycin (Hcmn) complexes with iron(II) are discussed. The stoicheiometry of complexation (Job's method) is 2 cmn : 1 Fe; cmn acts as a terdentate ligand. The pK_a for the red $[Fe(Hcmna)(cmna)]^+$ (Hcmna = caerulomycin A) to form its purple conjugate base $[Fe(cmna)_2]$ is 3.5. Solid compounds were made containing both the red cation and the purple uncharged species. Visible c.d. spectra are reported for caerulomycin D and its iron(II) complex.

Siderophores, which are low-molecular-weight chelating agents manufactured by micro-organisms to facilitate the uptake of iron into the organisms in response to an iron deficiency, bind to iron(III) very strongly but have a much weaker affinity for iron(II).[1,2] Only a few powerful iron(II)-binding compounds are found in aerobic cells; these are apoferroverdin,[3] pyrimine,[4,5] caerulomycin,[6] streptonigrin,[7-9] and orelline.[10] Except for apoferroverdin and pyrimine, all these metabolites contain the 2,2′-bipyridyl (bipy) system.‡

Streptomyces caeruleus produces a series of caerulomycins [13,14] (Hcmn) which have antibiotic properties. The principal component is caerulomycin A (Hcmna), with smaller amounts of caerulomycins B (Hcmnb), C (Hcmnc), and D (Hcmnd). The structures are outlined in Figure 1.

The caerulomycins are active against some filamentous fungi and yeasts, and have weak activity against certain bacteria. Divekar and co-workers [15] have suggested that their phytotoxic (plant-wilting) properties may be associated with their ability to chelate heavy metals. This is in agreement with Vining's [6] conclusions concerning the mode of action of caerulomycins on micro-organisms.

We report here our investigations of the chelating properties of Hcmna with iron(II), and the circular dichroism (c.d.) spectra of the iron(II)–cmnd complex.



(1)-(3) (4) (Hcmnd)

	R^1	R^2
(1) Hcmna	H	CH=NOH
(1a)	H	C≡N
(1b)	H	CO_2H
(1c)	H	$CONH_2$
(2) Hcmnb	OH	CH=NOH
(3) Hcmnc	OMe	CH=NOH

Figure 1. Structures of the caerulomycins

Results and Discussion

Isolation of the Caerulomycins.—Cultures of *S. caeruleus* were incubated as described by Funk and Divekar.[16] Extraction of the culture filtrate with diethyl ether gave the crude caerulomycins. T.l.c. indicated that only Hcmna and Hcmnd were present. These were purified by chromatography on Sephadex LH-20,[13] the major product being Hcmna, with Hcmnd being obtained only in low yield as an oil. Attempts to crystallize the latter compound were unsuccessful.

Caerulomycin A.—Caerulomycin A, in ethanol, forms a violet-blue complex when mixed with Fe^{2+} ions in aqueous solution. By the method of continuous variation (Figure 2) using ammonium iron(II) sulphate, the composition of the complex was found to be 2 mol of ligand per mol of iron(II). The formulation $[Fe(Hcmna)_2(H_2O)_2]^{2+}$ is unlikely since bis complexes of the type $[FeL_2X_2]$ [L = bipy or 1,10-phenanthroline (phen); X = halide or thiocyanate] can be prepared only in non-aqueous solvents and dismute readily in aqueous solutions to give the tris complex, $[FeL_3]^{2+}$.[17-20] This suggests that the oxime group must also co-ordinate to the iron(II) ion.

The criterion for the oxime group of substituted pyridine-2-carbaldoximes to participate in metal binding is that it must have a *syn* configuration (see Figure 3); the *anti* isomers shown do not give the rapid colouration on mixing with iron(II), cobalt(II), and copper(II) [21-23] salts which would indicate complexation (although cases are known of metal ions promoting *anti–syn* isomerization, this is slow).

Vining and co-workers [6] have assigned the *E* (*syn*) configuration to the oxime group of Hcmna. Hence Hcmna and/or $cmna^-$ would act as a terdentate ligand through the three nitrogen atoms. Other examples of a substituent in the α position of bipy or phen producing a terdentate ligand are 2,2′ : 6′,2″-terpyridyl, 1,10-phenanthroline-2-carboxylic acid,[24] 1,10-phenanthroline-2-carboxamide,[25] and 1,10-phenanthroline-2-carboxamide oxime.[26] A molecular model of the iron(II) bis-cmna complex indicates that it can have only the *mer* configuration.

The iron(II) complex of cmna is violet-blue in neutral and alkaline solutions, with an absorption maximum at 530 nm, and red in the range pH 2.5—5, with a maximum at 520 nm and a shoulder at 460 nm (see Figure 4). Under more acidic solutions the complex dissociates. These reversible colour changes are due to the ionizations of the oxime protons, as shown in the Scheme. Similar equilibria have been observed

† Part 33 is ref. 4(*c*),
‡ Orelline, which has been assigned the structure 3,3′,4,4′-tetrahydroxy-2,2′-bipyridyl, gives a red colouration with Fe^{2+} ions.[10] However, when there are substituents in the 3,3′ positions of bipy, the N heterocycles are known not to give a red colour with Fe^{2+} ions.[11,12]

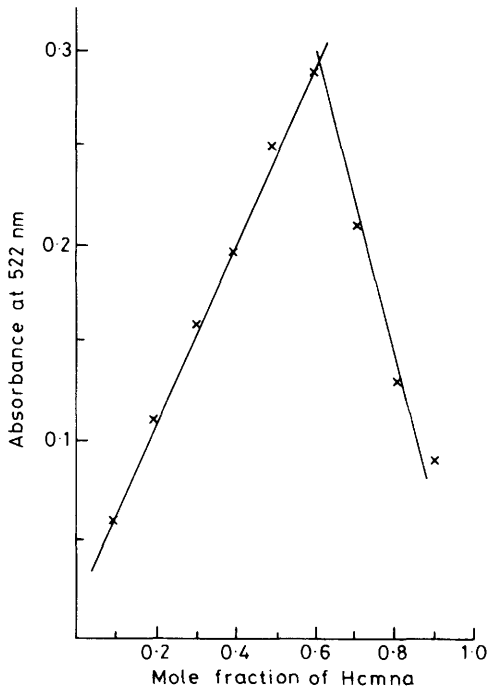


Figure 2. Job's method of continuous variation for the iron(II)-caerulomycin A complex

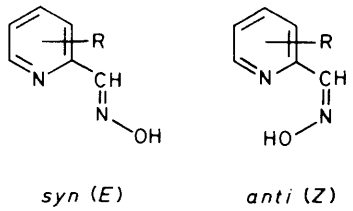


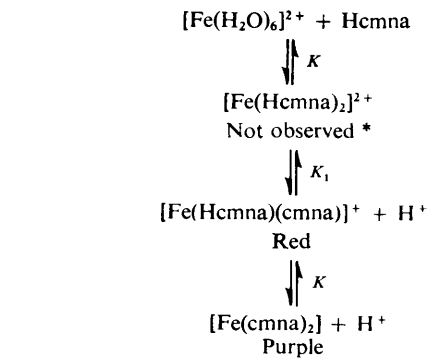
Figure 3. Configurations of pyridine-2-carbaldoxime (R = Me, Et, or Ph)

for tris(pyridine-2-carbaldoxime)iron and bis(phenanthroline-2-carboxamide oxime)iron complexes,[26] although for the latter the changes in spectra were not reported.

From the spectral changes with pH, the pK_a for the second ionization (K_2) was calculated as 3.5 (pK_a of Hcmna = 9.87 [6,27]); *i.e.*, on complexation, the dissociation constant of the oxime proton increases in value. This type of 'linked effect' between complex formation and proton dissociation of the ionizable side group is common in haem proteins and enzymes, and known elsewhere.

The red form of the complex was isolated both as the perchlorate and hexafluorophosphate salts, and found to be [Fe(Hcmna)(cmna)]X (X = ClO_4^- or PF_6^-). The purple complex was obtained as a powder which analysed as [Fe(cmna)$_2$]. The diffuse-reflectance spectrum of [Fe(Hcmna)(cmna)]ClO_4 shows a broad band in the visible region, with a maximum at 480 nm, while the spectrum of [Fe(cmna)$_2$] has a broad band centred at 525 nm with a shoulder at 725 nm. This is similar to the solution spectra at pH 2.76 and 6.67 respectively (Figure 4).

The oxime groups in bis(cmn) complexes are disposed *cis* as a consequence of the *mer* configuration; it may be that the remaining proton in [Fe(Hcmna)(cmna)]$^+$ forms a hydrogen bond between the two *cis*-disposed oxime oxygen atoms.† The electronic spectra suggest that the chromophore present is low-spin FeN_6 {akin to that in [Fe(bipy)$_3$]$^{2+}$}, indicating that the ligand-field strength of Hcmna is large enough to induce



Scheme. Ionization in the iron(II) complex of caerulomycin A. * Or, less likely, with electronic spectrum identical to its conjugate base

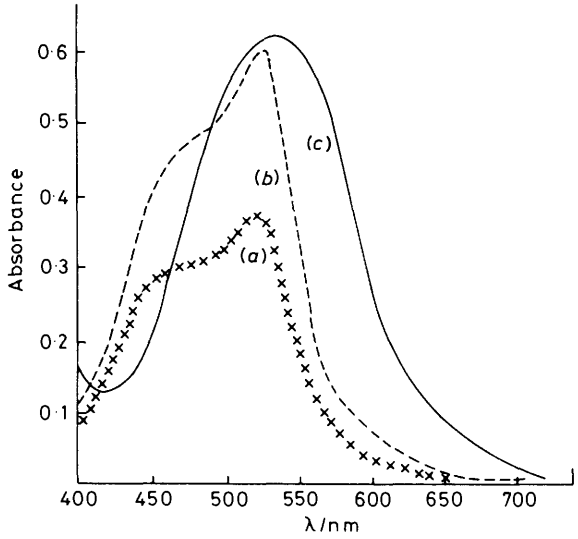


Figure 4. Variation of the electronic spectrum of the iron(II)-caerulomycin A complex (6×10^{-5} mol dm^{-3}, 1 cm cell) with pH: (*a*) 1.76; (*b*) 2.76; (*c*) 6.67

spin pairing. Certainly, for what it is worth, no e.s.r. signal was observed for a frozen aqueous solution at 88 K.

Caerulomycin D and its Iron(II) *Complex*.—The glycoside moiety of Hcmnd has been said,[14] from the sign of the optical rotation, to have the L configuration at C^2. This portion is thought, on the basis of values of the specific optical rotatory power (α_m), to be a derivative of the rare monosaccharide 6-deoxy-L-*arabino*-hexos-2-ulose. The c.d. and electronic spectra of Hcmnd were run immediately after eluting the compound from the chromatography column, in the solvent system used for the elution (benzene–ethyl acetate–acetic acid). The spectra could not be recorded below 250 nm because of solvent interferences. The c.d. spectrum ‡ of the compound has a positive band at 305 nm, and broad negative bands at 250 and 265 nm. The solution was acidic (the spectrum would be expected to change in an alkaline solution).

The metal complexes of cmnd should show optical activity

† Such bonds are observed in other bis(oximate) species, such as bis(dimethylglyoximato)nickel(II) and the 'cobaloximes,' although there the two oxime groups are *trans*-planar.

‡ This spectrum differs sufficiently from that of a typical hexose monosaccharide as to reinforce our suggestion [9] that the unusual monochromatic rotations for Hcmnd may stem from preferential atropisomer distribution between the skewed pyridine rings rather than the presence of the rare or unique monosaccharide proposed.[14]

Table 1. Isotropic absorption (a.b.) and c.d. spectra of the iron(II)–caerulomycin D complex in acidic and basic media [a]

Acidic				Basic			
A.b.		C.d.		A.b.		C.d.	
λ/nm	$10^{-4}\ \varepsilon$ [b]	λ/nm	$10^{-1}\ \Delta\varepsilon$	λ/nm	$10^{-4}\ \varepsilon$ [c]	λ/nm	$10^{-1}\ \Delta\varepsilon$
		410	−0.32			430	−0.56
445	7.45	470	+0.86	465	8.46	530	+2.21
530	10.0	535	+0.22	530	10.34	640	−0.35
		600	−0.32				

[a] ε and Δε in $dm^3\ mol^{-1}$. [b] Based on the absorption coefficient of the $[Fe(Hcmna)(cmna)]^+$ complex ($\varepsilon_{530} = 1.0 \times 10^5\ dm^3\ mol^{-1}$). [c] Based on the absorption coefficient of the $[Fe(cmna)_2]$ complex ($\varepsilon_{530} = 1.03 \times 10^5\ dm^3\ mol^{-1}$).

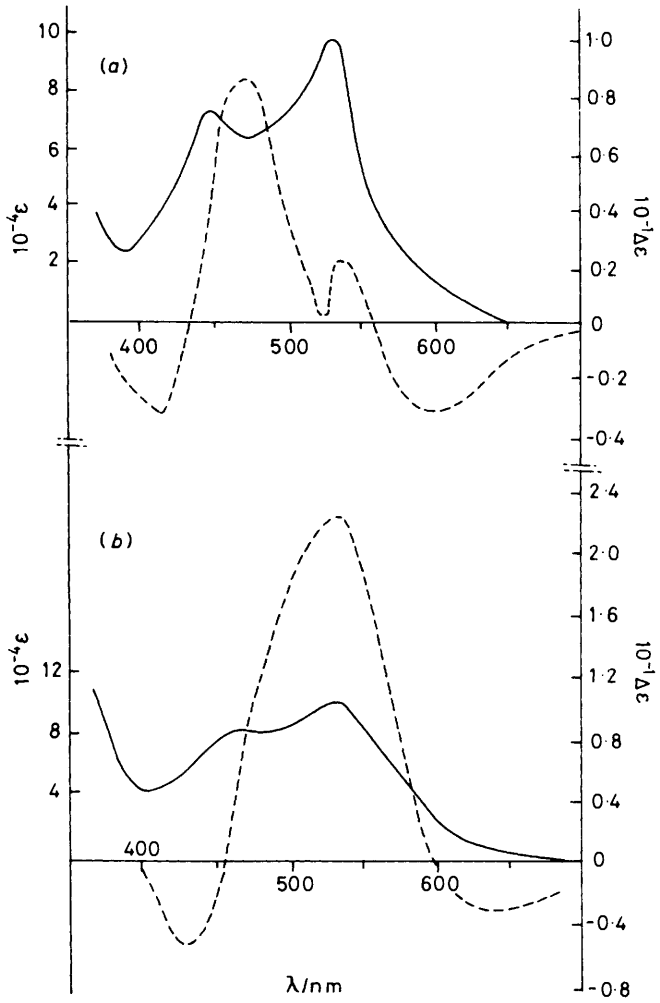


Figure 5. Absorption (——) and c.d. (– – – –) spectra of the iron(II)–caerulomycin D complex in (*a*) acidic solution, (*b*) basic solution; ε and Δε values in $dm^3\ mol^{-1}$

due to the metal ion, either because the asymmetry of the sugar moiety is transmitted to the electronic transitions localized on the metal orbitals, or the ligand binds stereoselectively to the metal, producing an excess of one diastereoisomer.

The iron(II)–cmnd complex was obtained by adding an aqueous solution of Mohr's salt to Hcmnd dissolved in the eluting solvent mixture. The resulting red complex was formed in the aqueous phase. At least one of the ligands must be protonated since the solution is acidic (owing to the acetic acid present). The transitions in the c.d. and electronic spectra of this solution correlated well and are given in Figure 5(*a*). The electronic spectrum in the visible region is typical [28] of the $[Fe(NNN)_2]^{2+}$ chromophore. The absorption at 445 nm in the electronic spectrum may in fact be a combination of the two bands at 470 and 410 nm observed in the c.d. spectrum (see Table 1 and Figure 5).

The addition of a dilute solution of sodium hydroxide to the acidic solution of the iron(II)–cmnd complex caused a slight darkening. The electronic spectrum [Figure 5(*b*)] still shows a band at 530 nm but the band at 445 nm for the red complex in acidic solution shifts to 465 nm. The c.d. spectrum of this species is illustrated in Figure 5(*b*).

Conclusions

Both the bipy and the aldoxime groups of Hcmn appear to be necessary for the antibiotic and phytotoxic properties of the molecule. Divekar and co-workers [15] have shown that modification of the 2 substituent from an oxime to a nitrile group [compound (1a)] renders the compound less toxic and the minimum concentration required for plant wilting is then the same as for bipy. However, converting the aldoxime group of Hcmna to either a carboxylic group [compound (1b)] or a carboxamide group [compound (1c)] does not alter the wilting properties of the compounds with respect to Hcmna itself. It is possible that the biological properties of compounds (1b) and (1c) are due to their ability to form 2 : 1 iron(II) complexes,* whereas the nitrile group cannot co-ordinate to metals and this makes compound (1a) bidentate, forming a 3 : 1 complex with iron(II). It may not be the free ligand, but rather its metal complex, which contributes to the overall wilting activity observed. The minimum concentration required for wilting (and thus probably the antibiotic activity) would then be related to the number of potential co-ordination sites on the molecule.

Experimental

Isolation of the Caerulomycins.—The cultures of *S.caeruleus* were grown in the Microbiology Department of University College, Cardiff by Dr. Barbara Evans as described by Funk and Divekar.[16] The culture filtrate was extracted with diethyl ether and evaporation of the ether phase gave the crude caerulomycins as a buff-coloured powder. Qualitative separation of the crude caerulomycins from various cultures by t.l.c. on silica gel HF_{254}, using benzene–acetic acid–water (42 : 24 : 1), showed that there were either one or two

* 1,10-Phenanthroline-2-carboxylic acid and 1,10-phenanthroline-2-carboxamide have been shown [24,25] to behave as terdentate ligands (although these compounds have not been tested for biological activity).

Table 2. Analytical data

Complex	Formula	Analyses (%) [a] C	H	N [b]	Cl
[Fe(Hcmna)(cmna)]$ClO_4 \cdot H_2O$	$C_{24}H_{23}ClFeN_6O_9$	46.2 (45.7)	4.35 (3.3)	11.1 (13.35)	5.1 (5.6)
[Fe(Hcmna)(cmna)]$PF_6 \cdot 2H_2O$	$C_{24}H_{25}F_6FeN_6O_6P$	40.7 (41.6)	3.6 (3.6)	8.8 (12.1)	
[Fe(cmna)$_2$]$\cdot 3H_2O$	$C_{24}H_{20}FeN_6O_7$	49.25 (50.9)	4.5 (4.6)	11.7 (14.8)	

[a] Calculated values in parentheses. [b] These combustion results for nitrogen are very low, a common experience with N heterocycles.

metabolites present; comparison of the R_f values with the reported values [13] suggested that these were Hcmna and Hcmnd. The crude caerulomycins were separated and purified on a Sephadex LH-20 column [13] (90 × 2.6 cm) equilibrated with the solvent system benzene–ethyl acetate–acetic acid–water (1 : 12 : 4 : 12). The eluant was collected in 10-cm^3 fractions which were monitored by adding aqueous ammonium iron(II) sulphate to a small sample of the fraction: those which showed no colouration were discarded; those which showed a strong colouration in sequence were pooled and their purity checked by t.l.c.

Job's Method of Continuous Variation.—Since the ligand Hcmna is colourless and the absorbance due to the Fe^{2+} ions at the concentrations used is negligible, any absorbance observed in the visible region of the electronic spectrum is due to the formation of the metal complex. The reagent solution (x cm^3; 1×10^{-4} mol dm^{-3}) was mixed with the metal-ion solution ($10 - x$ cm^3) of the same concentration, and absorbance was plotted as a function of the mole fraction of the ligand. For the Fe^{2+} ion sharp maxima were obtained at a mole fraction close to 0.66 for Hcmna.

Determination of Charges on the Iron(II)–cmna Complex at Various pH *Values.*—Sephadex C-25 and Sephadex A-25 resins were each made up in pH 9 and pH 3 buffers and packed in columns (7 × 0.4 cm). The iron(II)–cmna complex was made up in these two buffer solutions and applied to the appropriate C-25 and A-25 columns of the same pH. The red complex, in pH 3 buffer, remained at the top of the C-25 column, but passed through the A-25 column. The mauve complex, in pH 9 buffer, passed through the C-25 and A-25 columns.

Preparation of [Fe(Hcmna)(cmna)]ClO_4 *and* [Fe(cmna)$_2$].—To a solution of Hcmna (0.18 g, 0.81 mmol) in a minimum amount of hot ethanol was added a warm solution of ammonium iron(II) sulphate (0.14 g, 0.35 mmol). Solid sodium perchlorate was added to the purple solution, which was then cooled to room temperature. The brick-red precipitate of [Fe(Hcmna)(cmna)]ClO_4 was collected, washed with ice-cold water, and dried *in vacuo* over P_2O_5.

To the red filtrate a few drops of sodium hydroxide solution (2 mol dm^{-3}) were added whereupon the solution became purple. The solution was evaporated to dryness and the purple compound extracted into absolute ethanol (3×). The combined ethanolic extracts were evaporated to a small volume and diethyl ether was added to precipitate [Fe(cmna)$_2$] which was recrystallized twice from ethanol–diethyl ether.

The C, H, and N analyses (Table 2) were determined by the Microanalytical Service, University College, using a Technicon C,H,N Auto Analyser, and the halogen analyses were determined using the oxygen flask combustion method.[29] Electronic spectra were recorded using a Beckman model DK-2A ratio-recording spectrophotometer and a Beckman model DK2 reflectance spectrophotometer. C.d. measurements utilized the Jobin-Yvon (C.N.R.S.-Rousell-Jouan) Dichrographe III.

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References

1 'Microbial Iron Metabolism,' ed. J. B. Neilands, Academic Press, New York, 1974.
2 J. B. Neilands, in 'Inorganic Biochemistry,' ed. G. Eichorn, Elsevier, New York, 1973, p. 167.
3 A. S. Ballio, A. H. Bertholdt, A. Carieli, E. B. Chain, V. D. Vittoria, A. Tonolo, and L. Vero-Barcellona, *Proc. R. Soc., London, Ser. B*, 1963, **158**, 43; A. S. Ballio, E. B. Barcellona, A. Tonolo, E. B. Chain, and L. Vero-Barcellona, *ibid.*, 1964, **161**, 384.
4 (*a*) R. Shiman and J. B. Neilands, *Biochemistry*, 1965, **4**, 2233; (*b*) J. G. D. M. Atton, M.Sc. Thesis, University of Wales, Cardiff, 1979; (*c*) *cf.* J. G. D. M. Atton and R. D. Gillard, *Transition Met. Chem.*, 1981, **6**, 351.
5 M. Pouteau-Thouvenot, M. Choussy, A. Gaudemer, and A. Barbier, *Bull. Soc. Chim. Biol.*, 1970, **52**, 51.
6 P. V. Divekar, G. Read, and L. C. Vining, *Can. J. Chem.*, 1967, **45**, 1215.
7 K. Rao, K. Biemann, and R. B. Woodward, *J. Am. Chem. Soc.* 1963, **85**, 2532.
8 B.P., 1 012 684/1965.
9 S. Dholakia and R. D. Gillard, *Tetrahedron*, 1981, **16**, 2929.
10 W. Z. Antkowiak and W. P. Gessner, *Tetrahedron Lett.*, 1979, 1931.
11 G. E. Inglett and G. F. Smith, *J. Am. Chem. Soc.*, 1950, **72**, 842.
12 B. R. James, M. Parris, and R. J. P. Williams, *J. Chem. Soc.*, 1961, 4630.
13 A. G. McInnes, D. G. Smith, J. L. C. Wright, and L. C. Vining, *Can. J. Chem.*, 1977, **55**, 4159.
14 A. G. McInnes, D. G. Smith, J. A. Walter, J. L. C. Wright, L. C. Vining, and G. P. Arsenault, *Can. J. Chem.*, 1978, **56**, 1836.
15 R. R. Chandran, R. Sankaran, and P. V. Divekar, *J. Antibiot.*, 1967, **21**, 243.
16 A. Funk and P. V. Divekar, *Can. J. Microbiol.*, 1959, **5**, 317.
17 K. Madeja and E. Konig, *J. Inorg. Nucl. Chem.*, 1959, **25**, 377.
18 W. A. Baker and H. M. Bobonich, *Inorg. Chem.*, 1964, **3**, 1184.
19 E. Konig and K. Madeja, *Proc. Chem. Soc.*, 1960, 61.
20 T. Fujiwara, K. Matsuda, and Y. Yamamoto, *Inorg. Nucl. Chem. Lett.*, 1980, **16**, 301.
21 A. A. Schilt and P. J. Taylor, *Talanta*, 1969, **16**, 448.
22 F. H. Case and P. J. McMenamin, *J. Heterocycl. Chem.*, 1968, **5**, 161.
23 F. H. Case and A. A. Schilt, *J. Heterocycl. Chem.*, 1977, **14**, 1.
24 H. A. Goodwin and R. N. Sylva, *Aust. J. Chem.*, 1967, **20**, 217.
25 H. A. Goodwin and F. E. Smith, *Aust. J. Chem.*, 1972, **25**, 37.
26 H. A. Goodwin and F. E. Smith, *Aust. J. Chem.*, 1970, **23**, 1545.
27 S. Dholakia, Ph.D. Thesis, University of Wales, Cardiff, 1981.
28 P. Krumholz, *Inorg. Chem.*, 1965, **4**, 612.
29 G. Ingram, 'Methods of Organic Microanalysis,' Chapman and Hall, London, 1962, p. 161.

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