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Spectrophotometric Study of Complex Formation between Oxovanadium(IV) and Antiamebic Drugs

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Abstract □ Complex formation between oxovanadium(IV) and the antiamebic drugs 5,7-dibromo-8-quinolinol and 5,7-dichloro-8-quinolinol was studied in the pH 1.5–2.0 range, using ethanol, dioxane–water, and dimethylformamide as solvents. The composition of the formed complexes was determined by more than one procedure. In ethanol and dioxane–water, the 1:1 and 1:2 complexes were formed; in dimethylformamide, the 1:1, 1:2, and 1:3 complexes were formed. The stability constants were computed using two procedures: the molar ratio method and the extrapolation method. The reproducibility of results is satisfactory.

Keyphrases □ Complex formation—oxovanadium(IV) and substituted 8-quinolinols, spectrophotometric study in various solvents □ Oxovanadium(IV)—complex formation with substituted 8-quinolinols, spectrophotometric study in various solvents □ 8-Quinolinols, substituted—complex formation with oxovanadium(IV), spectrophotometric study in various solvents □ Spectrophotometry—determination of composition of complexes of oxovanadium(IV) and substituted 8-quinolinols in various solvents □ Antiamebic drugs—5,7-dibromo- and 5,7-dichloro-8-quinolinols, complex formation with oxovanadium(IV)

Quinoline derivatives, especially the iodinated ones, are active in amebiasis. Early work on these drugs was reviewed previously (1). Drugs such as 5,7-dibromo-8-quinolinol, 5,7-dichloro-8-quinolinol, and other 8-quinolinol derivatives were effective only in intestinal amebiasis (2). The chelating properties, ionization potential, and oil–water partition are predominant structure–activity factors (3).

The antibacterial action of the 8-quinolinol drugs, but not their efficiency as amebicides, is dependent on their chelating properties (4). The metal chelates of the studied drugs have tuberculostatic and fungitoxic activities (5, 6).

The chemistry of vanadium(IV) is almost entirely that of oxovanadium or vanadyl compounds. Selbin (7, 8) showed that VO^{+2} is probably the most stable diatomic ion known. The mixed ligand complexes of VO^{+2}

with 8-quinolinol and thiocyanate were studied (9). The formation constant and free energy of formation of $[VOL(NCS)_2]HL$, where HL is 8-quinolinol and L is its anion, have been computed.

The chemistry of some 8-quinolinol complexes of vanadium, iron, and nickel was studied (10, 11). The use of 5,7-dibromo-8-quinolinol in the detection of vanadium was recommended (12, 13). Vanadium complexes of 8-quinolinol and its derivatives were used as sensitive indicators for the colorimetric determination of phenols and alcohols (14).

In this work, the complex formation between VO^{+2} and some 8-quinolinols was investigated in several organic solvents. The composition and formation constants of the formed complexes were found to be solvent dependent. Vanadyl quinolinolates may have tuberculostatic and fungitoxic activities similar to those of the copper derivatives (5).

EXPERIMENTAL

Materials—5,7-Dibromo-8-quinolinol (I) and 6,7-dichloro-8-quinolinol (II) were prepared by direct halogenation of 8-quinolinol[1] in acetic acid (15, 16). Vanadyl sulfate[2] solution was standardized potentiometrically (17). Ethanol (96%), dioxane, and dimethylformamide were purified by conventional methods (18).

Apparatus and Method—Electronic absorption spectra were determined[3] using 1.0-cm fused silica cells. To determine the composition and stability constant of the complexes, solutions of the metal ion and ligands were mixed just before scanning the spectra.

The solution pH was measured on a precision pH meter[4] by using the millivolt scale, and the corresponding pH values were calculated.

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[1] British Drug Houses grade reagent.
[2] Prolabograde reagent.
[3] Unicam SP 8000 spectrophotometer.
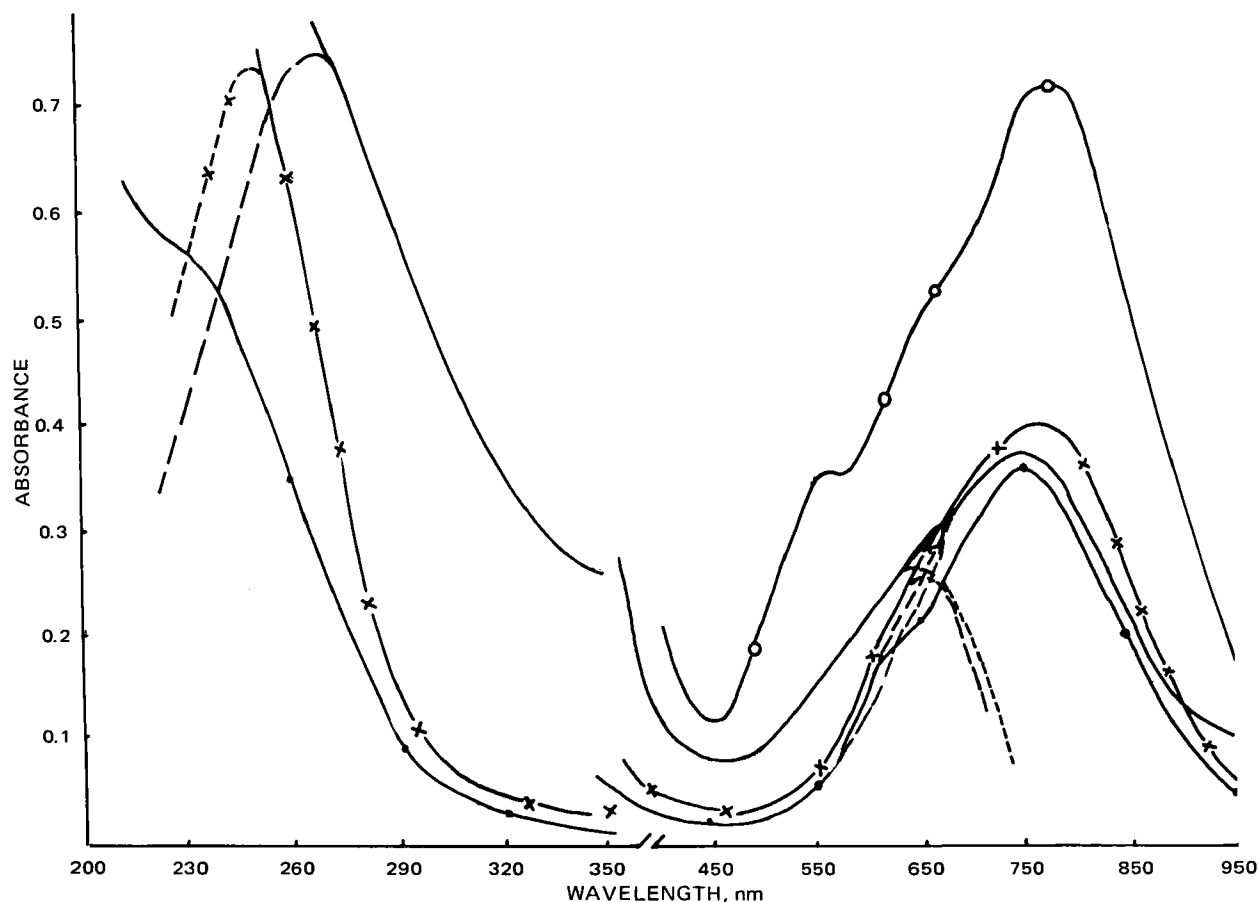[4] Radelkis type OP-205.

Figure 1—*Electronic absorption spectra of vanadyl sulfate. Key: —, in dioxane–water; O, in dimethylformamide; ×, in ethanol; and ●, in water.*

RESULTS AND DISCUSSION

Absorption Spectra of Oxovanadium(IV) Solutions—The electronic absorption spectra of oxovanadium(IV) complexes consist of three d–d transitions in the 8000–32,000-cm^{-1} range in addition to a strong charge transfer transition in the near UV region (42,000 cm^{-1}). The three transitions are designated band 1 ($b_2 \rightarrow e$, 11,000–14,700 cm^{-1}), band 2 ($b_2 \rightarrow b_1$, $10D_q$, 14,800–20,400 cm^{-1}), and band 3 ($b_2 \rightarrow a_1$, 21,000–31,250 cm^{-1}). In many cases, band 3 is obscured by the long wavelength tail of the more intense charge transfer band.

The spectra of the species $[VO(H_2O)_5]^{+2}$ have been extensively studied (19–21), and it was recommended (19) to treat the ion as a

Table I—Band Maxima, Molar Absorptivity, and f Values for Vanadyl Sulfate in Different Solvents

Solvent	Band	λ_{max}, nm	ϵ	$f^a \times 10^3$	Δ^b, cm^{-1}
Water	1	768	17.0	0.3	
	2	630	9.1	0.2	15,873
	3	—	—	—	
	4	240	275.0	0.1	
96% Ethanol	1	768	19.5	0.4	
	2	640	12.5	0.2	15,620
	3	—	—	—	
	4	245	—	—	
90% Dioxane—water	1	740	17.25	0.4	
	2	650	12.0	0.3	15,380
	3	—	—	—	
	4	266	—	—	
Dimethylformamide	1	775	35.75	0.8	
	2	650	24.00	0.5	15,150
	3	550	17.00	0.3	
	4	—	—	—	

^{a} Oscillator strength. ^{b} $10D_q$.

tetragonally distorted octahedron. Figure 1 shows the absorption spectra of vanadyl sulfate in water, ethanol (96%), 90% (v/v) dioxane–water, and dimethylformamide. All solutions were acidified with sulfuric acid to pH 1.5–2.0.

The absorbing species can be considered to be $[VO(A_xB_y)]^{+2}$, where A and B are solvent molecules. These molecules are of the same type when vanadyl sulfate is dissolved in water and may be different when it is dissolved in an organic solvent, $x + y = 5$. Band maxima are given in Table I.

The water molecules in the inner coordination sphere of VO^{+2} ions are replaced partially or completely by other solvent molecules when vanadyl sulfate pentahydrate is dissolved in an organic solvent. With water as the solvent, the $b_2 \rightarrow b_1$ transition occurs at 15,873 cm^{-1}, giving a value of 1587 cm^{-1} for D_q. The value of Δ is appreciably below that expected by extrapolation of the $10D_q$ values of $V(H_2O)_6^{+2}$ (11,800 cm^{-1}) and $V(H_2O)_6^{+3}$ (18,500 cm^{-1}). Consequently, the crystal field description of the VO^{+2} ion is not the best, and π-bonding is significant.

With ethanol as the solvent, the numerical value of D_q and the energy of the charge transfer transition are less than the corresponding values with water. This finding indicates that alcohol molecules replace some water molecules in the inner coordination sphere. The ligand field strength is in the order water > ethanol. Also, the ionization potential of ethanol (10.5 ev) is less than that of water (12.6 ev), which accounts for the decrease of the energy of the charge transfer transition. Similar results were observed with dioxane–water.

The spectrum of vanadyl sulfate pentahydrate differs significantly in dimethylformamide. All of the d–d transitions are red shifted, and band 3 appears clearly (not obscured). On the other hand, the charge transfer transition is not observed, since it occurs in the region where the solvent absorbs. The results indicate that the ligand field strength is in the order water > ethanol > dioxane > dimethylformamide.

**Complex Formation between Oxovanadium(IV) and I in Ethanol**—*Absorption Spectra*—When the alcoholic solution of I (pale yellow) is mixed with the alcoholic solution of vanadyl sulfate (blue), the mixture becomes red. The formed complex has an ab-
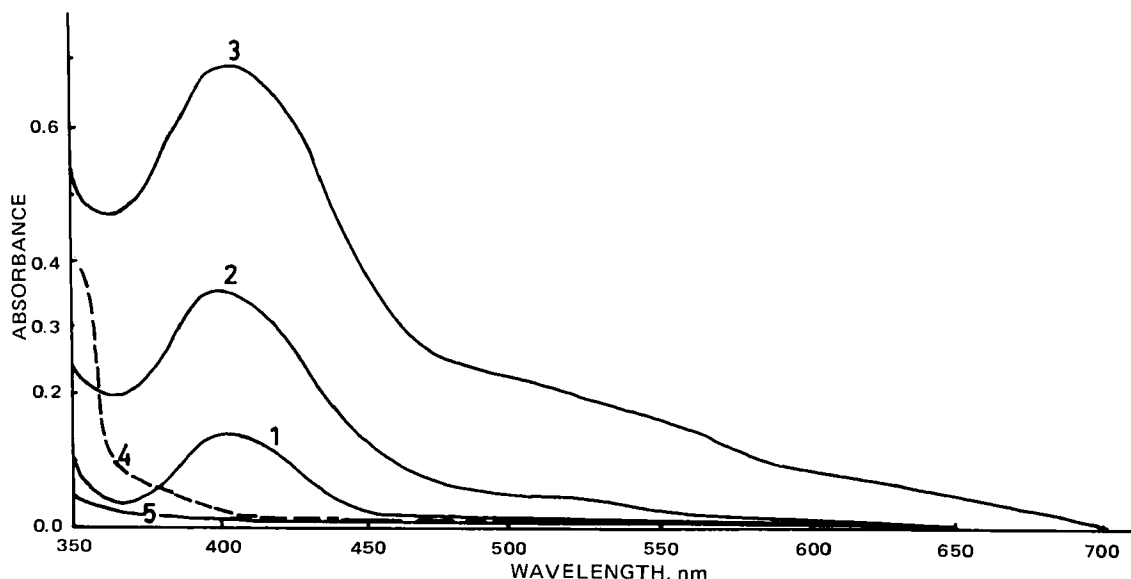
Figure 2—*Absorption spectra of vanadyl sulfate–I in ethanol. Total concentration was 5×10^{-4} M. The ratios of $[VO^{+2}]$–I were: 1, 5:1; 2, 1:9; and 3, 3:7. Curve 4 shows I at 4.5×10^{-4} M, and curve 5 is VO^{+2} at 4.5×10^{-4} M.*

sorption band of its own, with λ_{max} 396 nm (Fig. 2); this band is used to investigate its properties.

When applying Job's (22) method of continuous variations and the slope ratio and molar ratio methods (23, 24), the predominant complex proved to be the 1:2 vanadyl–I complex (Fig. 3).
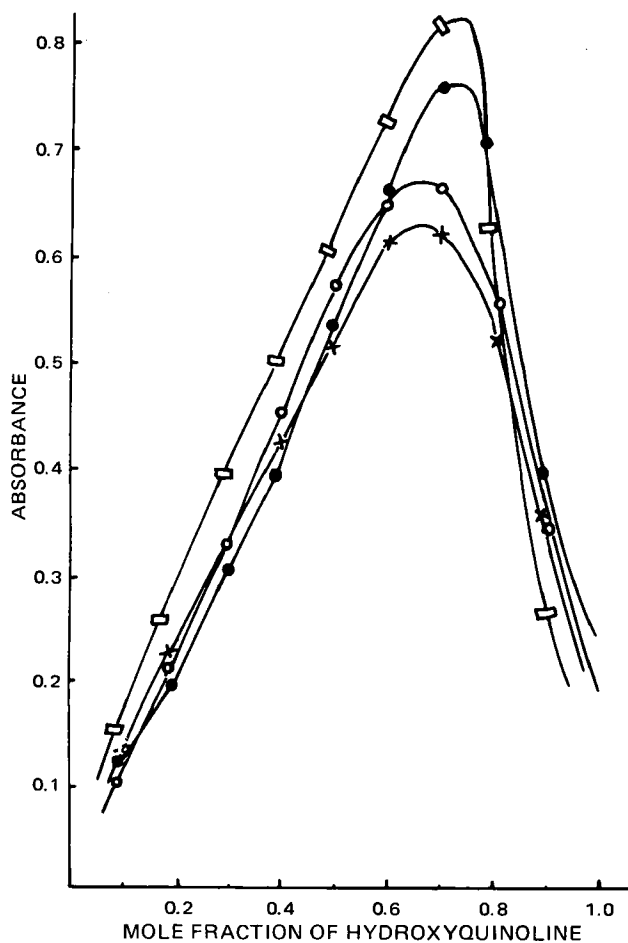
Figure 3—*Composition of vanadyl–8-quinolinol complexes using the continuous variation method. Key: O, I in ethanol; ×, II in ethanol; □, I in demethylformamide; and ●, II in dimethylformamide.*

The acid dissociation constant of I in 96% ethanol was determined potentiometrically (25). The quinolinol solution in ethanol (2.0×10^{-3} M) was titrated against a 2×10^{-2} M alcoholic solution of standardized potassium hydroxide; the average value of pKa was 9.79. Since complexation of VO^{+2} with I was studied in the pH 1.5–2.0 range, the concentration of the 8-quinolinol anion was negligible and the ligating species was considered to be 8-quinolinol itself.

Stability Constant of Formed Complexes—The stability constant of the 1:2 vanadyl–I complex was computed using the simple molar ratio method. The spectra of mixtures of VO^{+2} and I, in which the ratio of $[VO^{+2}]/[I]$ varied from 1:1 to 1:25, were determined. The relationship between the absorbance at 396 nm and the ratio of $[I]/[VO^{+2}]$ is shown in Fig. 4. By using the simple known relationship (25), a value of 9.11 was computed for log β_2.

Yatsimiriskii's method for computing $\beta_1, \beta_2, \ldots, \beta_n$ for the first, second, and nth complex is applicable to spectrophotometric data. Details of the procedure and mathematical formalism are found elsewhere (26). The equations needed for computation are:

$$f_1 = (E - \epsilon_0)/a \qquad \lim_{a \to 0} f_1 = \Delta\epsilon_1\beta_1 = X \qquad \text{(Eq. 1)}$$

$$f_t = (f_{t-1} - X)/a \qquad \lim_{a \to 0} f_t = \Delta\epsilon_t\beta_t - \Delta\epsilon_1\beta_1^t \qquad \text{(Eq. 2)}$$

$$f_1' = f_1 a \qquad \lim_{a \to \infty} f_1' = \Delta\epsilon_N = Y \qquad \text{(Eq. 3)}$$

$$f_t' = (f_{t-1}' - Y) \qquad \lim_{a \to \infty} f_2' = (\Delta\epsilon_1 - \Delta\epsilon_2)\beta_1/\beta_2 \qquad \text{(Eq. 4)}$$

Table II—Data Needed for Yatsimiriskii's Method to Compute β_1 and $\beta_2{}^a$

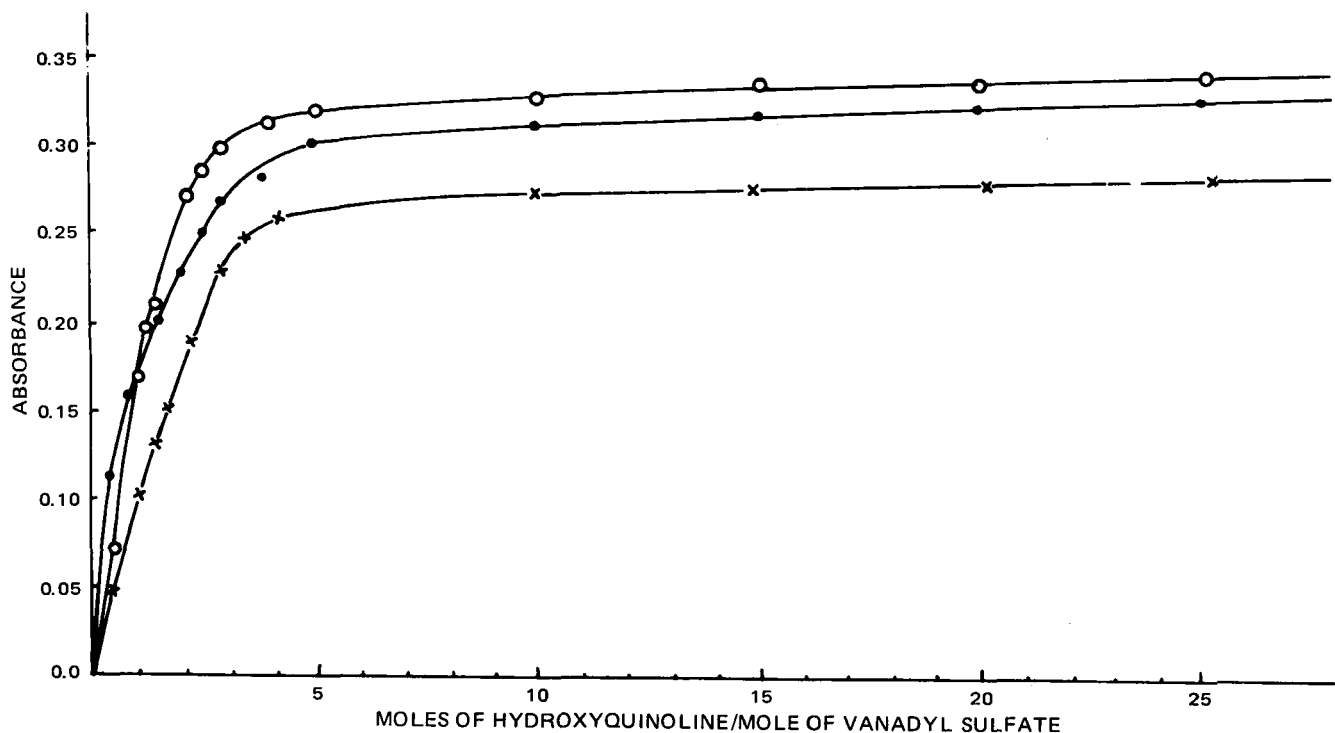| Ligand Concentration $[A] \times 10^4$	$[A]^{-1} \times 10^{-3}$	E	$f_1 \times 10^{-6}$	$-f_2 \times 10^{10}$
0.2	50.00	1350	67.5	17.5
0.4	25.00	2600	65.6	13.8
0.5	20.00	3200	64.0	14.0
0.6	16.00	3750	62.5	14.1
0.8	12.50	4800	60.0	13.75
1.0	10.00	5575	55.7	15.3
1.2	8.30	6075	50.6	17.0[b]
1.6	6.25	6525	40.8	18.8[b]
4.0	2.50	7325	18.3	13.2
6.0	1.66	7625	12.7	9.7
8.0	1.25	7750	9.7	7.6
10.0	1.00	7975	8.0	6.3
12.0	0.83	8225	6.8	5.3

^{a} $[VO^{+2}] = 4 \times 10^{-5} M$. ^{b} Neglected.

Figure 4—*Variation of absorbance of vanadyl sulfate–8-quinolinol solutions with 8-quinolinol concentration. Key: ×, I in ethanol, λ = 396 nm; ●, II in dimethylformamide, λ = 388 nm; and ○, I in dimethylformamide, λ = 386 nm.*

The spectra of solutions containing constant $[VO^{+2}]$ and increasing ligand concentrations were determined, and the value of E is given by:

$$E = (A_s - l\epsilon_A a)/Bl \qquad \text{(Eq. 5)}$$

where A_s = absorbance of the mixture at 396 nm, ϵ_A = molar absorptivity of the ligand at 396 nm, B = total metal concentration, and a = free ligand concentration.

The necessary data are given in Table II, and the values calculated for $\Delta\epsilon_1$, $\Delta\epsilon_2$, β_1, β_2 are given in Table III.

Complexes of Oxovanadium(IV) and II—To confirm these results, complex formation between VO^{+2} and II in 96% ethanol was investigated. The compositions of the formed complexes were similarly determined and proved to be 1:1 and 1:2. The acid dissociation constant of II was investigated, and a value of 9.83 was computed for the pKa. Consequently, the complexing species was thought to be 8-quinolinol at pH 1.5–2.0. In the kinetic study of complex formation of 8-quinolinol and 8-quinolinol 5-sulfonate (27) with nickel(II), the complexing species was considered to be 8-quinolinol.

The stability constants of the formed vanadyl–II complexes were computed as previously described. The molar ratio method gave 8.3 for log β_2, and results of Yatsimiriskii's procedures are given in Table III.

Comparison of the results in Table III indicates that vanadyl–I complexes are more stable than vanadyl–II complexes. This finding was expected on the basis of the stronger electron-donating properties of I compared to II.

Effect of Solvent on Complex Formation—The complex formation between VO^{+2} and I and II in dioxane–water or dimethylformamide was investigated spectrophotometrically. If the inner coordination sphere of the metal ion contains one or more solvent molecules, then the composition of the complex, band maxima of its absorption, molar absorptivity, and stability constant depend on the solvent. The compositions of the formed complexes were determined following the same procedures as before.

In dioxane–water, the main complexes between vanadyl ion and the studied 8-quinolinols were the 1:1 and 1:2 complexes. The 1:1, 1:2, and 1:3 complexes were formed when dimethylformamide was the solvent. The computed stability constants of the formed complexes using Yatsimiriskii's procedure are given in Table IV. With dimethylformamide, β_3 was calculated using the molar ratio method (Table IV).

CONCLUSIONS

Oxovanadium(IV) and 5,7-dibromo-8-quinolinol or 5,7-dichloro-8-quinolinol complex in the ratios of 1:1 and 1:2 when ethanol or dioxane–water is the solvent. The 1:1, 1:2, and 1:3 complexes are formed when dimethylformamide is the solvent. In all cases, complex formation was studied in the pH 1.5–2.0 range. If a distorted octahedron is assumed for the formed complex, the suggested composition for the 1:2 complex is $[VO(R)_2S]^{+2}$, where S stands for a solvent molecule; $[VO(R)_3]^{+2}$ stands for the 1:3 complex. In this case, two ligand molecules act as bidentate and the third acts as a unidentate ligand. It

Table III—Molar Absorptivities and Stability Constants of Vanadyl–I and Vanadyl–II Complexes

$[VO^{+2}]$ $\times 10^5$	I				II			
	$\Delta\epsilon_1$	$\Delta\epsilon_2$	Log β_1	Log β_2	$\Delta\epsilon_1$	$\Delta\epsilon_2$	Log β_1	Log β_2
4.0	6870	7650	4.01	7.76	8000	8900	3.80	7.01
5.0	6600	7500	3.86	7.28	—	—	—	—
6.0	7000	7200	3.75	6.4ᵃ	7800	8500	3.65	6.81
8.0	—	—	—	—	7500	8300	3.64	6.87
Average	6820	7420	3.67	7.52	7800	8600	3.69	6.93

ᵃ Neglected.

Table IV—Band Maxima, Molar Absorptivities and Stability Constants of Vanadyl-8-Quinolinol Complexes in Different Solvents

Solvent	Ligand	Band Maximum, nm	Log β_1	Log β_2	Log β_3	$\Delta\epsilon \times 10^{-3}$		
						$\Delta\epsilon_1$	$\Delta\epsilon_2$	$\Delta\epsilon_3$
Dioxane–water (90% v/v)	I	394	3.92	7.25	—	6.45	6.7	—
	II	392	3.88	7.25	—	4.6	6.5	—
Dimethylformamide	I	386	—	—	15.87	—	—	8.5
	II	388	—	—	15.36	—	—	8.2

is suggested that the complexing species is the 8-quinolinol molecule and not its anion. This conclusion is based on the fact that, in the pH 1.5–2.0 range, the anion concentration is negligible as computed from the acid dissociation constant of the 8-quinolinol in the specific solvent.

Variation of the solvent affected significantly the composition, stability, and d-orbital splitting of the metal ion in the formed complex. Due to steric factors, no dimethylformamide molecules appear to coordinate in the inner coordination sphere of metal ion; as a result, the 1:3 vanadyl–8-quinolinol complexes are predominant. In 96% ethanol and dioxane–water, one solvent molecule coordinates in the inner coordination sphere and 1:2 complexes are formed.

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Isolation and Characterization of the Cardiotonic Polypeptide Anthopleurin-A from the Sea Anemone *Anthopleura xanthogrammica*

TED R. NORTON ˣ, S. SHIBATA, M. KASHIWAGI, and JAMES BENTLEY

Abstract□ A highly potent cardiotonic polypeptide, anthopleurin-A, was isolated from the sea anemone, *Anthopleura xanthogrammica* (Brandt), using solvent partition, gel permeation chromatography, and cation-exchange chromatography. It is a pure basic polypeptide with a molecular weight of about 5500.

Keyphrases □ Anthopleurin-A—isolated from sea anemone *Anthopleura xanthogrammica* extract □ *Anthopleura xanthogrammica*—sea anemone extract, cardiotonic polypeptide anthopleurin-A isolated □ Cardiotonic polypeptide—anthopleurin-A isolated from sea anemone □ Sea anemone—extract, cardiotonic polypeptide anthopleurin-A isolated

Several extracts of coelenterates have been examined in this laboratory for antitumor activity (1–4) and, more recently, for a positive inotropic effect (4, 5). Interest was first stimulated in *Anthopleura xanthogrammica* when the crude extract was found to be highly toxic when administered intravenously to an anesthetized rat[1]. Later, the crude extract at much higher dilutions exhibited a strong positive inotropic effect on isolated rat atria; pretreatment with adrenergic receptor blocking agents, 6-hydroxydopamine or reserpine, did not affect the response, indicating that the extract acts by a nonadrenergic mechanism (5).

Many investigators have examined the chemical nature and pharmacology of toxins from coelenterates (6). An extract of the tentacles of *Calliactus polypus* (a sea anemone) caused a brief lowering of coronary outflow, heart rate, and amplitude of cardiac contractions in the isolated rabbit heart, and higher doses produced irregular cardiac contractions, usually resulting in cardiac

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[1] Dr. George W. Read and Ms. Charlotte Oda, Department of Pharmacology, University of Hawaii, personal communication.