

Metal Chelates of Oxazolidinones as Central Nervous System Stimulants

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In an attempt to determine the effect of chelation upon central nervous system activity, a series of metal chelates have been prepared of 2-imino-5-phenyl-4-oxazolidinone, 2-imino-5,5-diphenyl-4-oxazolidinone, and 2-imino-5-*p*-biphenyl-4-oxazolidinone, using Cu^{++} , Ni^{++} , Mg^{++} , and Fe^{+++} ions. Spectral and analytical evidence has indicated a 1:1 ratio of metal to oxazolidinone in the respective chelates of all four metals. The magnesium chelate of 2-imino-5-phenyl-4-oxazolidinone exhibited the selective central nervous system stimulating characteristics of the parent compound and it also provided two therapeutic advantages, an earlier onset of action and a relatively shorter span of activity.

IN 1913 Traube (1) reported the synthesis of a series of oxazolidinones, but it was not until 1956 that Schmidt (2) called attention to the central stimulant activity of one of the compounds, 2-imino-5-phenyl-4-oxazolidinone. In 1957 Lienert and Janke (3) in a further study of the same compound showed an activity similar to one parameter of caffeine activity, namely central nervous system stimulation, without the objectionable side-effects of the alkaloid.

The presence of a center for chelation in the oxazolidinone molecule suggested that chelates

could be prepared. The possibility then exists that metal chelates of an oxazolidinone might provide a water-insoluble compound with more desirable pharmacological activity. This then would be a start in a study of the effects of chelation on drugs which must cross the blood-brain barrier. Accordingly, various oxazolidinones were reacted with a number of metal salts capable of forming chelates, and several metal chelates have been prepared.

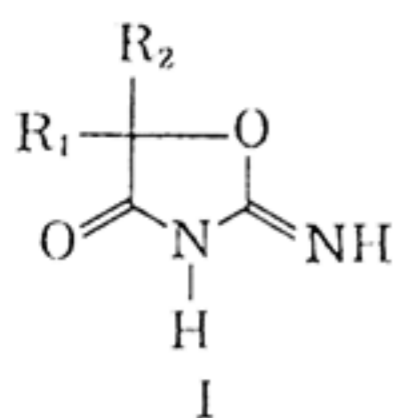
DISCUSSION

In order to determine the influence of substitution on the central nervous system stimulant activity of the oxazolidinone molecule, the following oxazolidinones were prepared.

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- Ia. $R_1 = C_6H_5$, $R_2 = H$ 2-imino-5-phenyl-4-oxazolidinone
 Ib. $R_1 = C_6H_5$, $R_2 = C_6H_5$ 2-imino-5,5-diphenyl-4-oxazolidinone
 Ic. $R_1 = C_6H_5-C_6H_5$, $R_2 = H$ 2-imino-5-*p*-biphenyl-4-oxazolidinone

Chelates (1:1) with cupric, nickel, ferric, and magnesium ions were isolated and the analytical results are recorded in Table I. A stable 1:1 chelate with neither zinc nor ferrous iron could be obtained.

Structural Considerations.—The results of elemental analyses of the chelates, as shown in Table I, indicate a structure containing one metal atom per molecule of oxazolidinone. The presence of water of chelation was shown by a loss of weight corresponding to approximately the calculated number of molecules of water without a change in color when the chelates were dehydrated at 225° . In the case of the magnesium chelate, the additional molecule of water of hydration was determined by Karl Fischer titration without drying at 110° as was done with the other chelates. Saturated aqueous solutions of the nickel and copper chelates gave negative tests for the metal ions. A positive test was given by magnesium and ferric iron indicating the ionic character of these complexes.

The infrared spectral characteristics of the oxazolidinones and their chelates were compared in an attempt to show the site of chelation. Owing to the complexity of the spectra of amide chelates in the 3μ region, it is not suitable for detailed study. However, in the 6μ region the intense amide I band can easily be identified. This band is usually considered to be a stretching wavelength of the amide carbonyl in resonance with the C—N band. In the oxazolidinone compounds the amide II band thought to be due to C—N stretching or N—H deformation is also present. Table II lists the wavelengths of the absorption bands for the various oxazolidinones and their chelates.

TABLE I.—ANALYSES OF OXAZOLIDINONE METAL CHELATES^a

Chelate ^b	Formula	C, %	H, %	N, %	M, ^c %	Water ^d	Color
Ia-Mg ⁺ OH ⁻ ·2H ₂ O	C ₉ H ₁₀ MgN ₂ O ₄ ·H ₂ O	Calcd. 42.81 Found 42.0	3.99 4.3	11.09 9.9	9.64 10.4 ^e	21.4 20.5 ^f	white
Ia-Mg ⁺ OH ⁻ ·H ₂ O	C ₉ H ₉ MgN ₂ O ₄	Calcd. 46.10 Found 45.85	4.21 4.0	11.01 12.2	10.42 10.45	15.7 15.8	white
Ia-Cu·2H ₂ O	C ₉ H ₁₀ CuN ₂ O ₄	Calcd. 39.49 Found 40.9	3.68 3.6	10.23 9.9	23.21 22.3	13.2 12.7	blue
Ia-Ni·4H ₂ O	C ₉ H ₁₄ N ₂ NiO ₆	Calcd. 35.44 Found 35.0	4.63 4.9	9.18 8.7	19.24 18.7	23.6 24.4	green
Ia-Fe ⁺⁺ 2OH ⁻ ·2H ₂ O	C ₉ H ₁₂ FeN ₂ O ₆	Calcd. 35.91 Found 35.5	4.35 3.9	9.31 8.9	18.55 19.2	23.9 25.0	brown
Ib-Cu·2H ₂ O	C ₁₅ H ₁₄ CuN ₂ O ₄	Calcd. 51.47 Found 51.0	4.03 3.8	8.0 7.7	18.17 17.3	10.3 9.4	purple
Ic-Cu·2H ₂ O	C ₁₅ H ₁₄ CuN ₂ O ₄	Calcd. 51.47 Found 51.6	4.03 3.8	8.0 7.8	18.17 17.5	10.3 10.9	blue

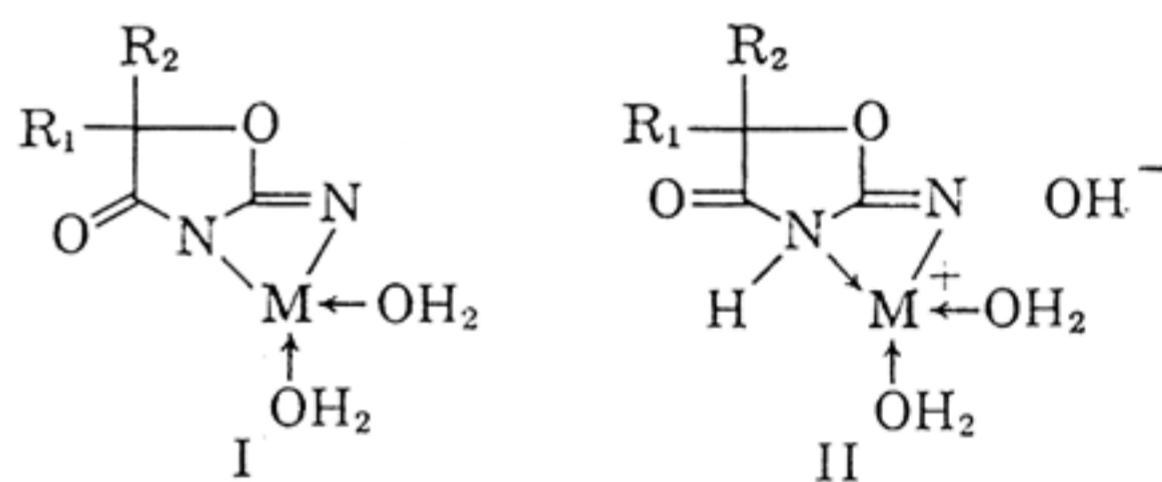
^a The carbon-hydrogen analyses were determined by Weiler and Strauss, Oxford, England, and by Dr. C. K. Fitz, Needham, Mass. ^b Ia = 2-imino-5-phenyl-4-oxazolidinone; Ib = 2-imino-5,5-diphenyl-4-oxazolidinone; Ic = 2-imino-5-*p*-biphenyl-4-oxazolidinone. ^c The metal content of the chelates was determined by ashing to constant weight at 1000° . ^d This quantity is the loss of weight on dehydration at 225° after initial drying at 110° . ^e Determined gravimetrically as the pyrophosphate. ^f This sample was determined by the Karl Fischer method without drying at 110° .

TABLE II.—INFRARED ABSORPTION BANDS, μ , OF OXAZOLIDINONE CHELATES^a

Compound	Bands		
	Amide I	Amide II	NH or OH
Ia	5.95	6.35 ^b	7.73
Ia-Copper chelate	6.25	...	7.67
Ia-Nickel chelate	6.18	...	7.69
Ia-Magnesium chelate	6.00	6.35, 6.62	7.80
Ia-Ferric chelate	6.00	6.35, 6.42	7.75, 7.82
Ib	6.00	6.30	7.65
Ib-Copper chelate	6.10	...	7.70
Ic	6.00	6.38	7.80
Ic-Copper chelate	6.20	...	7.72

^a The compounds were examined as solids in Nujol mulls using a Perkin-Elmer model 137 recording spectrophotometer. ^b A broad band extending to 6.65μ .

It will be noted that in the case of the copper and nickel chelates the band corresponding to the amide I has a higher wavelength than the parent compound and the amide II band disappears. This could be due to a definite shift of the resonance equilibrium of the amide group and the formation of covalent bonds. The fact that there is no change in the intensity of the carbonyl or amide I band could indicate structure I as the most likely for the copper and nickel chelates.



In the case of the ferric and magnesium chelates, the amide I band remains unchanged while the amide II band shows two maxima. This could be due to coordination with the imino group giving rise to definite bands rather than the broad amide II band of the parent compound. These compounds then could have a structure corresponding to II which might indicate either a cyclic complex or a chelate. In general, chelate bonding of the alkaline earth ions is primarily ionic and the bond

strength is related to the ratio of charge of the ion to its ionic radius. The charge/radius ratio for magnesium is 2.44 which is at the lower limit for chelate formation. Thus, the relatively weak bonding of magnesium as compared with the strong bonding of copper accounts for the difference in the infrared spectra of the two chelates.

The band at 7.73 μ for 2-imino-5-phenyl-4-oxazolidinone, as may be seen in Table II, was found to undergo a regular shift from one chelate to another. It is interesting to note that the relative order of stability of the various divalent chelates was found to parallel the wavelength shifts of this band (Cu > Ni > Mg). The results agree with those obtained by Harkins and co-workers (4) with five membered chelate ring imidazole derivatives except for the position of the magnesium.

Examination of another physical property, the oil-water partition coefficient, was made not only to discover differences between the chelates and cyclic complexes or ionic chelates, but also to obtain information regarding the capabilities of the metal derivatives to penetrate the bloodbrain barrier. Oleyl alcohol was selected for this determination in accordance with the advantages ascribed by Albert (5). The coefficients were obtained by measuring spectrophotometrically the percentage of compound extracted by oleyl alcohol in the manner described by Foye and Duvall (6). Reference to Table III shows that the copper chelate is considerably more fat-soluble than any of the others. It will be noted in respect to biological applications that the increased liposolubility modifies the onset of action of these compounds. However, this effect does not appear to be the only determinant of central nervous system stimulant activity.

TABLE III.—OIL-WATER PARTITION COEFFICIENTS AT 25°

Compound	Concn., Aq. Phase \times $10^4 M$	pH Aq. Phase	Partition Coeff. Oleyl Alc./H ₂ O
Ia ^a	2.8	7.9	3.2
Ia-Magnesium chelate	2.1	8.9	4.3
Ia-Copper chelate	0.7	6.0	18.0
Ia-Nickel chelate	1.3	6.6	11.0

^a Ia = 2-imino-5-phenyl-4-oxazolidinone.

Biological Results.—Initial tests on 2-imino-5-phenyl-4-oxazolidinone, known generically as pemoline, confirmed the findings of Schmidt (2). This compound induces central nervous system stimulation in various laboratory animals while the homologs described by Traube (1) were inactive. The results of preliminary pharmacological screening of related compounds and their chelates which we had prepared are given in Table IV. The compounds were screened in male albino mice (18–22 Gm.) for pharmacological activity. Attention was especially directed toward central nervous system stimulant action determined by visual observation. The compounds were tested at dose levels of 250 and 500 mg./Kg., p.o. It is apparent from the results shown in Table IV that substitution at position 5 is critical while chelation does not effect activity *per se*.

TABLE IV.—CNS STIMULANT ACTIVITY OF VARIOUS OXAZOLIDINONES AND THEIR CHELATES^a

Compound ^b	No. of Mice	Dosage ^c	Activity ^d
Phenyl	20	250	A
	20	500	A
Phenyl, Cu	20	250	A
	20	500	A
Phenyl, Fe	20	250	A
	10	500	A
Phenyl, Mg	20	250	A
	20	500	A
Phenyl, Ni	20	500	A
Diphenyl	10	250	I
	20	500	I
Diphenyl, Cu	20	500	I
<i>p</i> -Biphenyl	20	250	I
	20	500	I
<i>p</i> -Biphenyl, Cu	20	500	I
Methyl	20	500	I
Diphenylene	10	250	I
	20	500	I
<i>p</i> -Bromophenyl	20	500	I
<i>p</i> -Hydroxy phenyl	20	500	I
<i>p</i> -Methoxy phenyl	20	500	I

^a The results were obtained by visual observation. ^b The 5-position substituents of the 2-imino-4-oxazolidinone molecule is given and the metal when chelates are referred to. ^c The dosage is in mg./Kg., p.o. ^d A, active; I, inactive.

The relative lack of toxicity of magnesium in comparison with the other metals led to the selection of the magnesium chelate for further study. The compound induced central nervous system stimulation in monkeys, dogs, cats, rabbits, rats, and mice. Due to the insoluble nature of the compound, the oral route of administration was used throughout these studies except in rare instances. The duration and degree of central nervous system stimulatory activity in laboratory animals appears to be directly related to dosage. Although the acute oral LD₅₀ value in mice was approximately 1500 mg./Kg. it was possible to note increased locomotor activity in doses as low as 15 mg./Kg. The profile of central nervous system stimulation noted does not resemble that induced by sympathomimetic amines, i.e., pilomotor erection salivation, and widening of the palpebral fissures. Antihistaminic, cholinolytic, and adrenolytic effects were not observed in the pharmacological evaluation of this compound.

Unlike the monamine oxidase inhibitors, the serotonin and norepinephrine levels of the brains of the mice pretreated with magnesium pemoline did not differ from those of untreated control animals. Female albino mice weighing 18–24 Gm. were pretreated with magnesium pemoline (100 mg./Kg., p.o.) and sacrificed at scheduled times (1 hour, 3 hours, and 24 hours). Whole brains were removed and pooled. Serotonin and norepinephrine measurements were carried out by spectrophotofluorimetric methods (7, 8).

To measure the onset and duration of effect of the magnesium chelate compared to the base compound, screening studies were undertaken in mice, measuring the response to supramaxial electroshock by the methods of Toman and co-workers (9, 10).

Mice received supramaximal electroshock through cranial electrodes. The latency period was measured for convenience from onset of shock to onset of tonic extensor phase. It normally includes a

phase of increasing limb flexion. The threshold for tonic extensor convulsions is 7–10 ma. The latency period between shock and the extensor component of the convulsion was recorded to the nearest 0.2 sec. following 20 ma. (approximately twice the threshold shock).

After establishing that pemoline and magnesium pemoline significantly prolonged the supramaximal electroshock latency period following oral administration, each drug was administered orally to mice, generally in groups of 20. The dose of each compound was 100 mg./Kg., p.o. Treated groups and control groups were challenged at specified intervals and onset and duration of action were determined. Analysis of data presented in Table V reveals that the onset of activity of magnesium pemoline was more rapid. Statistically significant responses were obtained at 15 minutes while the parent compound was inactive at 15 minutes and 30 minutes, but was active at the 1-hour test period.

TABLE V.—ELECTROSHOCK SEIZURE LATENCY EXPERIMENTS

Expt. No.	Time of Electroshock Challenge after Drug, hr. ^a	No. of Mice/Group	Increase in Seizure Latency Time Over Control Group	
			Mg-Pemoline	Pemoline
1	1/4	10	S ^b	N.S
2	1/4	10	S	N.S
3	1/2	10	S	N.S
4	1/2	10	S	N.S
5	1	20	S	S
6	4	20	S	S
7	6	10	N.S	S
8	6	10	S	S
9	8	10	N.S	S
10	10	10	N.S	N.S
11	10	10	N.S	N.S

^a The dosage used was 100 mg./Kg. orally. ^b S, Statistically significant increase in latency seizure time; N.S, not significant statistically (*t* test).

EXPERIMENTAL

2-Imino-5-phenyl-4-oxazolidinone.—This product was obtained by the procedure of Traube (1). A 83% yield of colorless needles was obtained which melted at 254–256° (decompn.) which agrees with the reported value.

2-Imino-5,5-diphenyl-4-oxazolidinone.—This compound was prepared by the procedure of Iwaya and workers (11). A white solid was obtained which melted at 250–252° (decompn.) which agrees with the reported value.

Ethyl-*p*-xenyglyoxalate.—This intermediate was prepared by the procedure of Blicke and Grier (12). Recrystallization of the product from an ether-petroleum ether mixture gave a white solid which melted at 38–39° which agrees with the reported value.

Ethyl-*p*-xenyhydroxyacetate.—In a pressure

bottle was placed 25.4 Gm. (0.1 mole) of ethyl-*p*-xenyglyoxalate in 175 ml. of anhydrous ethanol, 0.85 Gm. platinized charcoal, and 6 ml. of water containing 0.35 Gm. of palladium chloride. The mixture was hydrogenated for 8 hours at three atmospheres pressure. The catalyst was removed by filtration and the solvent evaporated under reduced pressure. The residue was recrystallized from aqueous alcohol to give 23.0 Gm. (90%) of ethyl-*p*-xenyhydroxyacetate; m.p. 160–162°.

Anal.—Calcd. for C₁₆H₁₆O₃: C, 74.97; H, 6.29. Found: C, 74.81; H, 6.50.

2-Imino-5-*p*-biphenyl-4-oxazolidinone.—A solution of 12.8 Gm. (0.05 mole) of ethyl-*p*-xenyhydroxyacetate and 9.2 Gm. (0.05 mole) of guanidine nitrate in 50 ml. of ethanol and sodium ethoxide (1.5 Gm. sodium) was heated 30 minutes on a water bath. The ethanol was removed *in vacuo* and the residue dissolved in sodium bicarbonate. The aqueous solution was washed with ether and acidified with dilute hydrochloric acid to give 9.0 Gm. (70%) of 2-imino-5-*p*-biphenyl-4-oxazolidinone as a crystalline solid melting at 283–286° with decomposition.

Anal.—Calcd. for C₁₅H₁₂N₂O₄: C, 71.43; H, 4.79. Found: C, 71.11; H, 4.92.

Preparation of the Metal Chelates.—The chelates of the various oxazolidinones were all prepared by the same procedure. The metal salts which were employed were: cupric chloride, magnesium sulfate, ferric sulfate, nickel nitrate.

To a mixture of the oxazolidinone (0.025 mole) in 50 ml. of ethanol was added sufficient 20% sodium hydroxide solution to solubilize the oxazolidinone. The solution was diluted with 100 ml. of water and the metal salt (0.025 mole) in 20 ml. of water was slowly added with constant stirring. A sufficient quantity of 20% sodium hydroxide solution was added from time to time to maintain the pH at 10.5. The mixture was stirred for 1 hour after the final addition of the metal salt solution and the product collected by filtration. The yields of the various chelates ranged from 83 to 95% of the theoretical. Analyses and colors of the various chelates are shown in Table I.

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