Schiff Bases of Salicylaldehyde and Their Cobalt(II) Derivatives as Antitumor Agents

ERNEST M. HODNETT and WINFRED WILLIE[1]

Department of Chemistry, Oklahoma State University, Stillwater

The purpose of this investigation was to prepare derivatives of cobalt(II) which could be tested for antitumor activity. Some cobalt compounds, such as *trans*-dichlorotetrapyridinocobalt(II) and fluoropentamminecobalt(III) nitrate have shown significant inhibition of tumor growth in mice. It was hoped that cobalt compounds of new and different organic ligands would show even greater antitumor activity.

Complexes formed from Schiff bases and metals such as cobalt, nickel, and copper have been studied as "oxygen carriers" (Calvin et al., 1946; Bailes et al., 1947). Although no metal compounds of this series had been tested for antitumor activity it seemed possible that some of them would show this property since their ability to act as "oxygen carriers" might give them a role in the metabolic processes of the body. The methods employed in the synthesis of Schiff bases and their cobalt complexes allow the preparation of a large number of compounds with the same basic structure, but with different solubilities in water and other solvents which is an important factor in determining the usefulness of drugs.

Many aldehydes react with a variety of primary amines to produce imines, which are often referred to as Schiff bases, as shown in Fig. 1. The reaction sometimes occurs spontaneously when the aldehyde and amine are mixed; heat is evolved, and the products may separate as solids. The Schiff bases (I in Fig. 1) described in this paper have all been derived

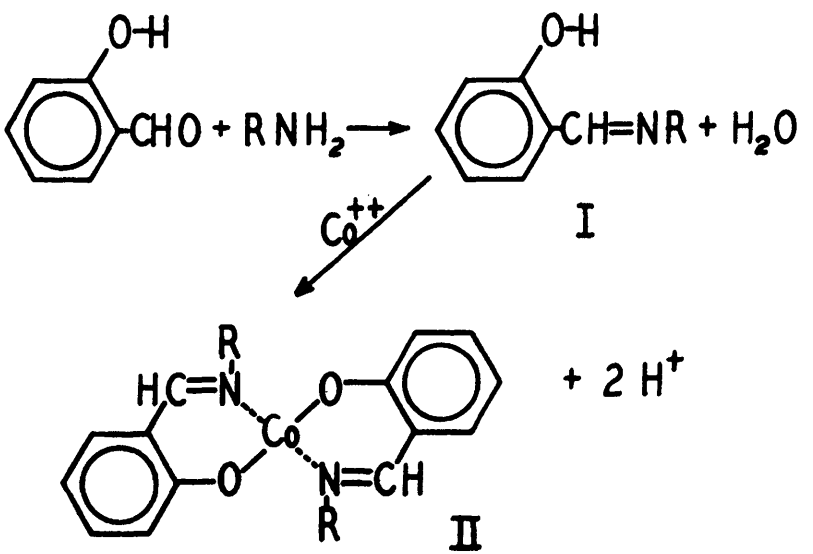

Fig. 1. Syntheses of Schiff bases and their cobalt(II) derivatives.

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from salicylaldehyde, which has a hydroxyl group ortho to the aldehyde group. This hydroxyl group makes possible the formation of metal derivatives of the resulting Schiff bases. Since the cobalt complexes usually have one atom of cobalt for two molecules of Schiff base it is assumed that their structure is that shown as II in Fig. 1.

EXPERIMENTAL

Preparation of Schiff Bases—To 0.25 mole of freshly distilled salicylaldehyde 0.25 mole of primary amine was added with stirring and cooling if necessary. The mixture was stirred for 20 minutes to insure complete reaction. The mixture was allowed to stand an additional 15 minutes and 50 ml of a 1:1 water-alcohol mixture was added with rapid stirring. The resulting mixture was then cooled in an ice bath in order to crystallize the product. The product obtained was washed with dilute alcohol solution, separated, and dried in a vacuum desiccator. The solid was recrystallized from toluene or toluene-heptane solutions until pure.

All these Schiff bases are yellow, presumably because of the carbon-nitrogen double bond which is conjugated with the benzene ring. Identities were established by infrared spectroscopy. New Schiff bases thus prepared, together with their melting points, are: 2-salicylideneamino-2-methyl-1,3-propanediol, 128 - 129°; 2-salicylideneamino-2-methyl-1-propanol, 65. 5 - 66.0°.

Preparation of Complexes of Schiff bases and Cobalt (II)—The method was similar to one which had been previously used for other metal derivatives (Diehl et al., 1948). To 0.05 mole of the Schiff base dissolved in 25 ml of 95% ethanol in a three-neck round-bottom flask equipped with a mechanical stirrer and nitrogen inlet and outlet tubes was added with constant stirring in a nitrogen atmosphere, a pulverized mixture of 0.025 moles of cobalt(II) acetate and 0.1 mole of sodium bicarbonate. The stirring was continued until the evolution of carbon dioxide ceased. The flask was then heated for 15 minutes to just below the boiling point of alcohol. Distilled water was then added to the solution until precipitation was complete. The precipitate was filtered, washed with dilute alcohol, and dried. The new cobalt complexes are listed in Table I with some of their properties.

Determination of Cobalt in the Complexes—The cobalt content of each complex was determined by a method described by Laitinen and Burdett (1951). A sample of the complex was weighed into a porcelain crucible and decomposed by heating carefully with a gas burner. The resulting oxide was fused with potassium pyrosulfate and the contents of the crucible were transferred with water to a 250-ml Erlenmeyer flask. Five g of sodium bicarbonate and 5 ml of 30% hydrogen peroxide were added to the solution, which was heated gently until effervescence ceased. The solution was cooled in an ice bath, diluted to 100 ml, and 5 g of potassium iodide was added. The solution was made acidic with hydrochloric acid (1:1) and the liberated iodine was titrated with standard thiosulfate solution. The results of the analyses are listed in Table I.

ANTITUMOR TESTS

All compounds prepared in this study were submitted to the Cancer Chemotherapy National Service Center for determination of their antitumor activities. Of twelve compounds submitted only one had been previously tested for this purpose. Each compound was tested in a tissue culture of human epidermoid carcinoma of the nasopharynx (KB) and in three mouse cancers: sarcoma 180 (SA), L-1210 lymphoid leukemia (LE), and Lewis lung carcinoma (LL). The results are reported in Table II where each tumor system is identified by the letters in parentheses above. It should be noted that compounds with significant activities have T/C

TABLE I. NEW COBALT DERIVATIVES OF SCHIFF BASES

Compound	Color	Melting Point, °C	Yield, %	Cobalt, % Found	Cobalt, % Calc.
Bis[o-(*N*-phenylformimidoyl)-phenolato]cobalt	Reddish yellow	188-92	52	13.19	13.08
Bis[2-methyl-2-(salicylideneamino)-1,3-propanediolato]-cobalt	Brown	above 300	75	12.86	12.42
Bis[2-ethyl-2-(salicylideneamino)-1,3-propanediolato]cobalt	Dark brown	above 300	62	10.99	11.73
Bis[o-[*N*-(3-morpholinopropyl)-formimidoyl]phenolato]-cobalt	Orange	61-64	29	11.15	10.67
Bis[3-(salicylideneamino)-1-propanolato]cobalt	Light green	171-78	38	15.10	14.21
2-[[3-(Salicylideneamino)propyl]-amino]ethanol, cobalt deriv.	Greenish yellow	130-32	30	10.53	11.78
Bis[2-methyl-2-(salicylideneamino)-1-propanolato]cobalt	Pink	above 300	80	12.46	13.31
Bis[o-[*N*-(2-ethoxyethyl)formimidoyl]-phenolato]cobalt	Orange red	127-30	91	12.01	13.33

TABLE II. RESULTS OF ANTITUMOR TESTS

NSC No.	Compound	SA Dose, mg/kg	SA T/C	LE Dose, mg/kg	LE T/C	LL Dose, mg/kg	LL T/C	KB
92396	*o*-Formimidoylphenol	500	0.74	400	0.91	400	0.85	9.6
92397	2-Methyl-2-(salicylideneamino)-1-propanol	125	1.03	100	0.98	100	0.59	*
92398	2-Methyl-2-(salicylideneamino)-1,3-propanediol	500	1.16	400	0.90	400	1.17	M19
92400	Bis[3-(salicylideneamino)-1-propanolato]cobalt	12.5	0.93	10	0.98	10	0.91	M19
92401	Bis[2-methyl-2-(salicylideneamino)-1-propanolato]cobalt	50	0.96	20	1.04	40	0.58	14
92402	Bis[2-methyl-2-(salicylideneamino)-1,3-propanediolato]cobalt	100	0.74	80	1.16	80	0.57	27
92403	Bis[2-ethyl-2-(salicylideneamino)-1,3-propanediolato]cobalt		*	100	1.21	25	0.79	17
92404	Bis[*o*-(*N*-phenylformimidoyl)-phenolato]cobalt		*	100	1.13		*	M19
92405	Bis[*o*-[*N*-(2-ethoxyethyl)-formimidoyl]phenolato]cobalt	125	0.64	100	0.95		*	M10
92406	Bis[*o*-[*N*-(3-morpholinopropyl)-formimidoyl]phenolato]cobalt	25	0.72	20	1.08	40	0.92	19
92407	2-[[3-(Salicylideneaminopropyl)]-amino]ethanol, cobalt deriv.	100	0.69	80	1.13	40	0.67	18

* Compounds are active, but tests are incomplete

values as follows: for SA, 0.53 or less; for LE, 1.25 or greater; and for LL, 0.53 or less. The value for KB should be 6 or less. Each compound was administered at its maximum tolerated dose, so the dose level is indicative of the toxicity of the compound to mice. Testing still continues for some compounds which have shown significant activity.

Examination of the data in Table II shows that the cobalt compounds have much more toxicity than the original Schiff bases, and are effective in reducing the growth of mouse cancers at much lower doses. Only one Schiff base shows significant activity; NSC-92397 is definitely active in the KB tissue culture and has some activity in Lewis lung carcinoma. One cobalt compound, NSC-92404, shows significant inhibitory activity in two tumor systems. All tumor systems except L-1210 lymphoid leukemia responded to at least one compound in this series. No general relationship was found between any physical or chemical property of these compounds and their antitumor activity; the search for such a relationship continues.

CONCLUSION

Eleven Schiff bases and cobalt derivatives have been prepared and tested for their antitumor activities. Several of these, particularly the cobalt derivatives, have shown significant inhibitory action against mouse cancers. These compounds are being studied further in order to discover a relationship between the antitumor activities of the compound and some physical or chemical property of the compounds.

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