preciably volatile at ordinary temperatures and readily hydrolyzable by water vapor.

PROVIDENCE, RHODE ISLAND

[CONTRIBUTION FROM THE LABORATORIES OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH]

COBALT COMPLEXES OF THIOGLYCOLIC ACID

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For reasons understandable from some preceding papers,[1] it seemed important to enhance our knowledge of metal complexes of sulfhydril compounds which also contain a carboxyl group. Though the behavior of various representatives of this group toward cobalt has some common aspects, there are individual differences. This paper is restricted to the simplest of these compounds, thioglycolic acid.

Compounds of cobalt and thioglycolic acid have been described by Rosenheim and Davidsohn.[2] Attempts to repeat their work did not yield readily crystallizable compounds and furthermore these authors were not aware of the fact that the formation of their brown compounds requires free oxygen and does not take place in the absence of air.

Another brief notice is given by Myers[3] but he evidently did not obtain a pure compound and was also unaware of the oxidation which takes place in its formation. Much more nearly related to the compounds described in this paper is Cremer's[4] observation of a complex established with cysteine and cobalt in the absence of air and in the presence of carbon monoxide. This complex, according to Cremer, contains two molecules of cysteine and one molecule of carbon monoxide for each atom of cobalt. The color of this complex is olive-green, more intense than the color of the cobaltous complex of cysteine without carbon monoxide. This author does not mention the fact that this cobalto complex in contact with air undergoes a considerable change and intensification of color, a fact which will be the starting point for our investigation. Cremer also describes an analogous complex of ferrous iron. One atom of iron combines with two molecules of cysteine and two molecules of carbon monoxide, so differing from the cobalt complex. This ferrous complex is highly optically active, is orange-yellow in color, and reversibly dissociates the carbon monoxide on being illuminated.

Closely related to the present work is the attempt of Cannan and Rich-

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[1] L. Michaelis and E. S. Guzman Barron, *J. Biol. Chem.*, **83**, 191 (1929); L. Michaelis, *ibid.*, **84**, 777 (1929).

[2] A. Rosenheim and J. Davidsohn, *Z. anorg. Chem.*, **41**, 231 (1904).

[3] C. N. Myers, *J. Lab. Clin. Med.*, **6**, 359 (1921).

[4] W. Cremer, *Biochem. Z.*, **206**, 228 (1929).

ardson[5] to establish by indirect physico-chemical analysis the composition of iron thiogylcolic acid complexes. Their conclusions, however, are quite different from what will be suggested here.

Yet among the innumerable papers on cobalt complexes by such authors as Werner, Pfeiffer, Jörgensen, Weinland, Tchugaeff, Ley and others, there is not a single one concerned with compounds such as cysteine or thioglycolic acid which has in addition to an SH group some other group capable either of direct salt or complex formation so that the whole molecule would give an inner complex in the sense of Ley.[6] This is the more remarkable as many other sulfur compounds have turned out to form quite stable complexes with cobalt; even in compounds in which the sulfur seems quite saturated, such as the thioethers and disulfides, there seems to be a strong affinity between sulfur and cobalt.

When a cobalt salt is mixed with a buffer around *P*H 6–10 (phosphate, carbonate, veronal) a pink precipitate arises which is perfectly stable in air and shows no trace of any oxygen consumption when observed in a microrespiration apparatus. In the same way, the soluble slightly pink cobalt complex of pyrophosphate is not oxidized by air, in contrast to the analogous ferro complex.

When the mixture of cobalt salt and phosphate buffer, containing the slightly pink precipitate, is mixed with thioglycolic acid under such conditions that the reaction never becomes acid, the precipitate is dissolved and the color rapidly becomes brown. Under favorable conditions one may see an intermediate state of deep blue-green color. This intermediate state, difficult to observe in air, is the permanent state in absence of air. On mixing the components under completely deaerated condition in a stream of nitrogen, the green color arises and is stable. The green color may be intense at high concentration but is by no means comparable in color intensity to the brown color established in air. Instead of oxygen, ferricyanide, or a dyestuff such as indophenol, methylene blue, or even indigo disulfonate can be used as oxidant with equal effect, the dye being reduced instantaneously. The green cobalt complex is so sensitive to air that we have not attempted as yet to prepare it in pure condition. The only statement one may make is that in absence of air approximately two molecules of thioglycolic acid are necessary to dissolve one molecule of cobalt present in the form of the phosphate precipitate.

When this mixture is exposed to the air it rapidly consumes oxygen, simultaneously turning brown. To get an idea of the oxidation level of this brown complex a study was made of the amount of oxygen taken up in its formation. This study has shown that there is more than one step to the oxidation process and that by adjusting the conditions these steps

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[5] R. K. Cannan and G. M. Richardson, *Biochem. J.*, **23**, 1242 (1929).

[6] H. Ley, *Z. Electrochem.*, **10**, 954 (1904).

can be quite sharply distinguished. It is particularly necessary to avoid too alkaline a reaction.

The oxygen uptake was measured in a 500-cc. Erlenmeyer flask with a one-holed rubber stopper leading to a calibrated air reservoir with a manometer attachment so that the whole system could be held at atmospheric pressure. In the flask two test-tubes were placed, one with thioglycolic acid and one with alkali, while in the bottom of the flask itself was placed the cobalt salt with some primary phosphate. The whole was sealed with paraffin and after standing for at least an hour in a constant temperature room at 30° and under slight pressure to make sure there were no leaks, the flask was tipped to allow the contents of the test-tubes to mix with those of the flask and then the decrease in volume of the gas in the system was read off at successive time intervals, the flask being shaken all the time.

Under these conditions and with the amounts of material noted in the table there was observed for the first thirty minutes a rapid oxygen uptake which then dropped off quite suddenly, falling to a rate which was about one-twentieth to one-hundredth of the average rate during the first thirty minutes. After an hour the oxygen uptake had dropped to a rate of the order of 1% or less of the rate during the first thirty minutes and continued for days without reaching a definite end. The amount of oxygen taken up during this first thirty-minute interval, which quite evidently corresponds to a distinct step of oxidation, is one-eighth mole of oxygen per mole of thioglycolic acid used, provided the molecular ratio of thioglycolic acid to cobalt is not greater than two to one and provided the mixture is not too alkaline. These are the conditions under which the crystalline complexes were made and frequently isolated within an hour with yields of over 70%. If the thioglycolic acid be in excess with respect to cobalt beyond the ratio noted above, then the oxygen uptake also increases somewhat, the rate depending on the alkalinity of the solution, for the excess thioglycolic acid is oxidized by oxygen to dithiodiglycolic acid. Under these conditions it is sometimes difficult to isolate the crystalline products which are our present concern, but even under these conditions there is an approximate step in the oxidation which calculated now to the cobalt amounts to one-fourth mole of oxygen per atom of cobalt. Also, if the mixture is made too alkaline

Table I

Oxygen Consumption in Mixtures of Cobalt Chloride and Thioglycolic Acid

The column headed "calcd. O_2 consump." is the theoretical oxygen consumption calculated as one-fourth mole of oxygen per atom of cobalt if thioglycolic is in excess to cobalt over 2 to 1. If the ratio of thioglycolic acid to cobalt is less, then one-eighth mole of oxygen per mole of thioglycolic acid is taken as the theoretical value.

Expts. 2 and 7, oxygen consumption under conditions for making complexes described; Expts. 1 and 9, oxygen consumption with excess cobalt; Expts. 3, 6 and 8 increased oxygen consumption with excess alkali; Expts. 4 and 5, increased oxygen consumption with excess thioglycolic acid.

Expt.	Composition of mixture, millimoles Volume of the whole 30–40 cc.				Calcd. O_2 consump., mmols.	Observed O_2 consump., mmols.			% Excess of O_2 consumed in first hour over calcd.
	Thiogly. acid	Co	KOH	KH_2PO_4		30 min.	3 hrs.	20 hrs.	
1	6.5	6.5	21	4.5	0.81	0.93	1.11	1.54	26
2	14.4	6.5	23	4.5	1.62	1.47	2.01	2.37	7
3	14.4	6.5	52	4.5	1.62	1.98	2.98	3.99	53
4	10.3	2.5	15	9	0.63	0.67	0.90	1.23	24
5	10.3	3.4	19	9	.85	.98	1.13	1.44	23
6	10.3	3.4	37	9	.85	1.42	1.59	2.11	76
7	10.3	5.0	22	9	1.25	1.21	1.44	1.62	6
8	10.3	5.0	45	9	1.25	1.65	1.80	3.32	38
9	10.3	10.0	37	9	1.29	1.26	1.52	1.80	8

the second slow step of oxidation seems to be speeded up so that the two steps are not as sharply separated and the total oxygen uptake is increased. This is probably due to a slow oxidation of the sulfur in the complex.

We were interested in the problem whether the consumed oxygen might be completely reduced or in part stay in the form of hydrogen peroxide. The only test for hydrogen peroxide in a solution of such a deep color is the test with chromic acid and extraction of the blue chrom-peroxide by ether (or better still ethyl acetate). When there is an excess of thioglycolic acid, with respect to cobalt (referred to the normal ratio 2 : 1), there is no hope of detecting hydrogen peroxide because thioglycolic acid, when not combined to form the metal complex, is rapidly oxidized by hydrogen peroxide; but on working with the ratio 2 : 1, traces of peroxide could be detected in some of the experiments. It is very likely that the formation of hydrogen peroxide depends on the partial pressure of free oxygen and on the rate of stirring, as Wieland and Franke[7] have shown for the oxidation of cobalto-hexacyanide by oxygen. At any rate, even in the best case we found only a minute trace of peroxide under such conditions where in the analogous case of cobalt cyanide a very conspicuous amount of peroxide could be found, as has been shown already by Manchot and Herzog[8] and also by Wieland.

So we may state with a fair degree of accuracy that in a mixture of one atom of cobalt and two molecules of thioglycolic acid, one-fourth of a molecule of oxygen is used up with no, or no appreciable, residue of hydrogen peroxide, during the first, rapid stage of the oxidation, with which this paper is concerned, and that the compounds to be described here represent such a state of oxidation that the cobalto-thioglycolic acid complex has consumed one-fourth of a molecule of oxygen for each atom of cobalt. This is no more oxygen than would be necessary to oxidize the cobaltous state to the cobaltic, without further oxidation of the sulfur groups. It should be emphasized that this stage of oxidation is not the highest obtainable but it is the first product of oxidation which is stable enough to isolate in pure condition. Only with this stage of oxidation is the present paper concerned.

The Preparation of the Complexes.—The simplest of these cobalt-thioglycolic acid complexes to prepare is the potassium salt. 10 cc. of thioglycolic acid (140 millimoles) is dissolved in 15 cc. of water and then 20 cc. of 7.5 *M* potassium hydroxide is added. This potassium thioglycolate solution is then poured into a mixture of 130 cc. of cobalt chloride (0.5 *M*), 50 cc. of potassium dihydrogen phosphate (0.9 *M*) and 19 cc. of potassium hydroxide (7.5 *M*) with rapid stirring. The mixture immediately turns a deep red-brown. On examination under a microscope the solution now appears practically clear. The mixture should be shaken or stirred in contact with the air or have air bubbled through it for an hour. If necessary it is filtered. The solution is

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[7] H. Wieland and W. Franke, *Ann.*, **473**, 289 (1929).

[8] W. Manchot and J. Herzog, *Ber.*, **33**, 1742 (1900).

evaporated either *in vacuo* or over steam to about one-third its volume. After cooling in the ice box for an hour or two, the crop of crystals is filtered off on a Büchner funnel and sucked as dry as possible. This product dissolves readily and completely in water, giving an intensely colored red-brown solution. It is dissolved in about 125 cc. of water and 95% alcohol is added slowly and with stirring until about 125 cc. has been added. An amorphous precipitate immediately appears which rapidly becomes crystalline. The crystals observed under low magnification are thin rectangular plates pointed at the ends. After standing for a few hours they can be filtered on a Büchner and washed first with 50% alcohol, then with 95% alcohol. The yield after drying for a day or two in the air is over 16.5 g. or over 75% calculated on the basis of the composition found by analysis. After drying by exposure to the air for a few days the following analytical results were obtained.

Anal. Calcd. for $[Co(SCH_2COO)_2KH]_2O{\cdot}5H_2O$: K, 11.76; S, 19.28; Co, 17.74; H_2O, 13.55. Found: K, 11.55; S, 19.31; Co, 17.83; H_2O, 13.07.

The potassium salt which has just been described is the best starting point for the preparation of the rest of the compounds studied here. To prepare the barium salt 3 g. of the recrystallized potassium salt is dissolved in 150 cc. of water and an aqueous barium chloride solution is added. An amorphous precipitate forms immediately, which begins to crystallize only after standing for about an hour. The crystallization is watched under a microscope and may take from one to four or five days for completion. Then the crystals are filtered off and washed several times with water and finally with 95% alcohol. The yield of the air-dried product is 3.2 g. This salt is quite insoluble in cold or hot water and dissolves only partially on addition of concentrated hydrochloric acid. The following analytical results are on the air-dried product.

Anal. Calcd. for $Ba[Co(SCH_2COO)_2H]_2O{\cdot}5H_2O$: Ba, 18.96; S, 17.72; Co, 16.30; H_2O, 12.45. Found: Ba, 18.87; S, 17.41; Co, 16.41; H_2O, 12.02.

Another crystalline product which appears to be an acid potassium salt may also be obtained from the potassium salt already described. About 15 g. of the potassium salt is dissolved in 75 cc. of water, then drop by drop and with constant stirring 3.5 cc. of concentrated hydrochloric acid is added. The mixture is set on ice for about twelve hours. The solution then turns almost solid with a light brown precipitate which under the microscope appears as exceedingly fine hair-like crystals. They are difficult to filter and are best separated by centrifuging. Then they are dissolved in about 250 cc. of water. In contrast to the former potassium salt these crystals dissolve only very slowly. After solution is complete alcohol is stirred in slowly and in small portions until about 150 to 200 cc. has been added. An amorphous precipitate appears which sometimes crystallizes in an hour or two and at other times does so only after standing for a week. The crystals are filtered off, washed with 50% alcohol, then with 95% alcohol and dried by exposure to air. This air-dried product amounts to 7 g.

Anal. Calcd. for $KH[Co(SCH_2COO)_2H]_2O{\cdot}4H_2O$: K, 6.42; S, 21.07; Co, 19.37; H_2O, 11.84. Found: K, 7.17; S, 20.39; Co, 19.72; H_2O, 12.66.

The free complex acid corresponding to the compounds which have been described can be prepared from the acid potassium salt by dissolving the latter in water and adding concentrated hydrochloric acid drop by drop with steady stirring until a permanent precipitate is formed. Under the microscope the precipitate appears as very short narrow bars. These are filtered off after several hours, washed with 30% alcohol and finally with 95% alcohol. After drying in the air, these crystals were analyzed.

Anal. Calcd. for $[Co(SCH_2COOH)_2]_2O{\cdot}3.5H_2O$: S, 22.87; Co, 21.01; C, 17.10; H, 3.42; H_2O, 11.24. Found: S, 23.04; Co, 21.45; C, 16.88; H, 3.21; H_2O, 11.26.

All of these complexes are quite stable and hydrogen sulfide, potassium hydroxide and potassium ferrocyanide all fail to precipitate the cobalt. The dry crystalline compounds are quite stable and can be heated to 140° without decomposition provided they are not heated for too long a time. The aqueous solutions of these compounds are intensely reddish-brown, with no band in the visible part of the spectrum but with increasing absorption toward the violet end.

The analytical results given are in all cases the mean results of duplicate analyses run on two or three entirely independent preparations.

For the C and H determinations we are indebted to Dr. Elek.

In the case of the potassium salt and the barium salt, water was determined by heating to constant weight at 110 to 120°. In the case of the potassium salt subsequent exposure to the air brought about, in a week, a recovery of most of the lost weight. The acid potassium salt and the free acid seem to begin to decompose slowly after heating for a few days at 120°, so in these cases water was determined in a vacuum desiccator over sulfuric acid.

Discussion of the Constitution.—In setting up formulas for the compounds which have been described, the oxygen consumption studies must be considered as well as the analytical figures. All the compounds show a sulfur to cobalt ratio of 2:1 and where carbon and hydrogen have also been determined the results show that the thioglycolic acid molecule behaves as a unit in coördinating with the cobalt. Furthermore, in none of these compounds has there ever been found any negative radical other than the thioglycolate although made in the presence of chloride, phosphate or pyrophosphate. The complex nucleus of the compounds considered here, therefore, consists of two molecules of thioglycolic acid distributed around the cobalt atom. This nucleus forms the negative ion of the compounds.

The oxidation level for a unit consisting of a cobalt atom and two thioglycolate radicals is such that one electron is lost for each such unit. This could mean either that the cobalt is oxidized from a cobaltous to a cobaltic condition or that half the thioglycolic acid is oxidized to a disulfide form.

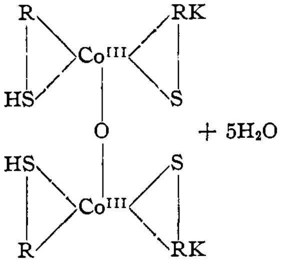
Finally, the existence of the acid potassium salt with one potassium atom to two cobalt atoms and four thioglycolate radicals suggests the possibility that these complexes may be dinuclear in structure.

To get some further evidence to show whether the compounds were mono- or dinuclear, the freezing point depressions of aqueous solutions of the potassium salt were determined. The results were: (a) 0.091° lowering of freezing point of water in a solution of 0.3884 g. of the potassium salt in 20.10 g. of water; (b) 0.147° lowering for 0.6389 g. of the potassium salt in 17.65 g. of water. This is, on the average, a freezing point depression of 0.0044° for each gram of substance dissolved in 1000 g. of water. The expected depression for a solution 1:1000 would be, assuming total dissociation of all potassium ions

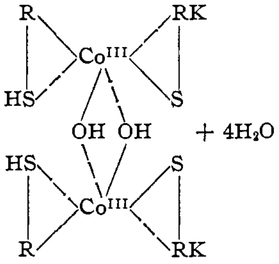
For the monomolecular formula	0.0112°
For the dimolecular formula	0.0084°

It can be seen that the monomolecular formula is not in accordance with the observed freezing point at all. The dimolecular formula is more compatible, taking into consideration that in electrolytes with polyvalent anions the assumption of 100% dissociation and the neglecting of osmotic coefficients (in the sense of Debye) are not justifiable and necessarily render the calculated depression greater than it can really be. Even a higher polymerization might be in agreement with the observed freezing point. Anyhow, this method is not very conclusive for polyvalent electrolytes and may be only utilized here to discredit a monomolecular formula.

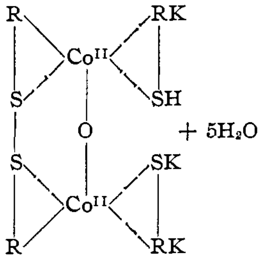
Putting all these facts together leads to the following tentative formulas in which Ia and Ib represent a cobaltic compound, IIa and IIb a cobaltodisulfide compound, both in the form of the potassium salt. The two b-formulas are analogous to what Werner designated as ol-complexes. They have the advantage that the six coördination places around the central cobalt are occupied and at the same time account for the fact that the crystals of both the free acid and the potassium salt, as well as the barium salt contain an odd number of water molecules (as reckoned for the bimolecular formula). The odd water molecule may be the one which the two b-formulas have in the inner spheres of the cobalt atoms, and which the a-formulas do not have.



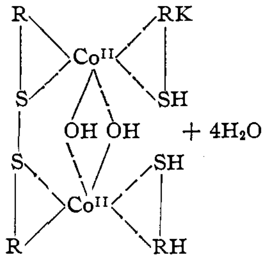
Ia



Ib



IIa



IIb

$R = SCH_2COO$

A similar formulation of some cobalt complexes which are somewhat analogous to the ones here described is given by Durrant,[9] who also at-

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[9] R. G. Durrant, *J. Chem. Soc.*, **87**, 1781 (1905).

tempted to determine the oxidation level as well as the molecular weight of the compounds he describes, particularly of the oxalate compounds.

So far no isomers have been found. It may be that the residual valences between pairs of sulfur atoms, even in the saturated form as SH groups, prevent them from taking a *trans*-position instead of *cis*, and so prevent the formation of isomers.

As we attempt to bring up various arguments for the preference of one or the other of the two possibilities I or II, the following ideas may be set forth.

Nickel sulfate (or chloride) reacts, under the same conditions as cobalt, with thioglycolic acid, giving a red color, but is the same in the absence or in the presence of oxygen. Oxygen consumption is, therefore, not involved in the establishment of this compound. This complex is, therefore, most likely analogous to the slightly green cobaltous complex which arises in the absence of oxygen. Ferrous salts, under similar conditions, and in the absence of oxygen, form a practically colorless soluble complex with thioglycolic acid, which combines avidly with oxygen and forms the red complex differing from the oxidized cobalt complex by its lability so that iron is a catalyst for the oxidation of thioglycolic acid to dithiodiglycolic acid. The behavior of the three complexes: ferrous, nickelous and cobaltous cysteine, toward oxygen is, therefore, what may be anticipated from the general properties of the metals, but scarcely from the general property of thioglycolic acid. Nickel compounds are under any conditions stable only in the nickelous state. Cobalt compounds are in certain compounds stable only in the cobaltous state (cobalt sulfate, chloride, etc.), in others they are, when produced in the cobaltous state, spontaneously and completely oxidized even by moleular oxygen to the cobaltic state and then cannot be reduced to the cobaltous state even by powerful reductants. Iron compounds can always be shifted easily from the ferric state to the ferrous and *vice versa*. From this point of view, the cobaltic formula No. 1 is preferable.

On the other hand, the high affinity of the two sulfur atoms, deprived of the H-atoms, in such a vicinity as indicated in the formula should be taken into account. This might involve a secondary change of formula I to II.

Many attempts have been made to decide between the two possible formulations, but with no decisive result as yet. One may think of a desmotropic relationship between the two formulas. This idea might account for the intense color of the complexes. Anyhow, the very fact that such a doubt is possible at all hints at the close relationship between a cobaltihydrosulfide form and a cobalto-disulfide form, and seems to be an approach toward an understanding of the metal catalysis in the oxidation of hydrosulfides to disulfides.

Summary

Some crystalline complex compounds of cobalt and thioglycolic acid have been prepared and on the basis of chemical analysis and oxygen consumption their possible constitutions have been discussed. The bearing of these constitutions on the heavy metal catalysis of the oxidation of thiol acids has been pointed out.

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[Contribution from the Chemical Laboratory of Brown University]

STUDIES RELATING TO METHYL TIN DERIVATIVES. VI. THE REACTION BETWEEN CHLOROFORM AND SODIUM TRIMETHYL STANNIDE IN LIQUID AMMONIA

By Charles A. Kraus and Arthur M. Neal

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As was shown in an earlier paper[1] sodium trimethyl stannide reacts with methylene chloride in liquid ammonia with the formation of di-trimethyl stannyl methane. It might, therefore, be expected that a corresponding reaction would take place between chloroform and sodium trimethyl stannide according to the equation

$$3(CH_3)_3SnNa + CHCl_3 = [(CH_3)Sn]_3CH + 3NaCl \qquad (I)$$

Indeed, when chloroform is added to a solution of sodium trimethyl stannide in liquid ammonia, reaction takes place immediately with the formation of a white precipitate which, in part at least, is solid. On evaporation of the ammonia, an oily liquid is left behind, which might be expected to be tri-trimethyl stannyl methane. A closer investigation of this liquid, however, showed that it consisted of at least two substances and it was found necessary to examine the product at length in order to establish the nature of the substances in question. The various experiments relating to this study are described below.

Preliminary Experiments.—A quantity of sodium trimethyl stannide was treated with chloroform in liquid ammonia. When the reaction was completed, the ammonia was allowed to evaporate and the oily liquid left behind was washed several times, first with ammonia water and, finally, with distilled water. The liquid was separated from the water layer and dried by filtering through anhydrous sodium sulfate in an atmosphere of nitrogen. Samples were sealed in weighed tubes from which the air had been displaced by nitrogen.

Samples of the product were analyzed for tin by the Carius method.

Anal. Subs., 0.3403, 0.3841: SnO_2, 0.3046, 0.3433. Calcd. for $[(CH_3)_3Sn]_3CH$: Sn, 70.64. Found: Sn, 70.73, 70.40.

The molecular weight of the compound was determined by the cryoscopic method with benzene as solvent.

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[1] Kraus and Neal, This Journal, **52,** 695 (1930).