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AZO LEAD DYES

by

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INTRODUCTION

Since its introduction in the chemotherapeutic treatment of cancer by Blair Bell[1] in 1922, lead has attracted more attention than any other metal in this particular field of therapeutics.

An ideal drug for the successful treatment of cancer should be relatively non-toxic toward benign tissue; should have a selective affinity for malignant tissue; and should preferably be water-soluble.

That lead compounds are, by and large, quite toxic is a matter of common knowledge. To circumvent this objectionable property chemists have resorted to the preparation of compounds which contain the lead in a highly masked form in complex molecules.[2,3]

The problem of endowing lead compounds with a highly specific affinity for carcinogenic tissue is a more involved task, and success is yet to be attained in this direction.

Compounds can generally be made more water-soluble by the introduction of polar groups such as $-SO_3H$, $-OH$, $-COOH$, $-NH_2$,

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1. Bell, Lancet, 203, 1005 (1922); 206, 267 (1924); 209, 1003 (1925).
2. Datnow, Am. J. Cancer, 24, 531 (1935) [C.A., 29, 7490 (1935)].
3. Schmidt, Med. u. Chem. Abhandl. Med-chem. Forschungstätten I. G. Farbenind., 3, 418 (1936) [C.A., 31, 5866 (1939)].

and so forth. In the past chemists have encountered great difficulty in finding a general method for introducing such groups into organolead compounds. Schmidt[3] was able to reduce dinitrodiphenyllead oxide with titanium trichloride and obtained diaminodiphenyllead dichloride. The amino groups could be detected by diazotization and coupling and also by the formation of yellow Schiff's bases.

The first recorded aminoaryllead compound having all four valences of the lead atom satisfied by lead-carbon linkages is triphenyl-*p*-aminophenyllead.[4] This compound was diazotized and coupled with β-naphthol. Isolation and analysis of the coupling product showed that it was an azo lead dye.

Since cancerous tissue can be stained *in vitro* by certain dyes[5] the possibility of preparing organolead dyes having a preferential staining capacity for neoplasms seems inviting. Also, the formation of large molecules, masking the lead, and the introduction of water-solubilizing groups should be possible by means of the coupling reaction. Two problems are involved: (1) The synthesis of organolead compounds capable of undergoing diazo coupling, and (2) a study of the applications and limitations of diazo coupling in organolead compounds in view of the preparation of lead dyes as possible anticarcinogenics.

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[4] Gilman and Stuckwisch, *J. Am. Chem. Soc.*, *64*, 1007 (1942).
[5] Geschickter, *Stain Tech.*, *5*, *49 (1930)*; *5*, 81 (1930).

PART I

ORGANOLEAD COMPOUNDS HAVING FUNCTIONAL GROUPS THAT FACILITATE DIAZO COUPLING

HISTORICAL

The coupling reaction was discovered by Peter Griess in 1866[6] and has since played an important role in organic chemistry, particularly in the preparation of azo dyes, many of which have commercial value.

In general primary aromatic amines can be diazotized and coupled with phenols, some phenolic ethers and with primary, mono- and dialkyl aromatic amines.

The number of lead compounds containing such functional groups is very limited. They will be discussed in the following section.

Alkoxy Groups

Lead can be introduced into aromatic and aliphatic compounds by various methods, the simplest being that of Pfeiffer using the Grignard reagent.[7]

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[6] Griess, Ann., 137, 67 (1866).
[7] Pfeiffer and Truskier, Ber., 37, 1125 (1904).

$$4RMgX + 2PbCl_2 \longrightarrow R_4Pb + 4MgX_2 + Pb$$
$$2R'MgX + R_2PbX_2 \longrightarrow R_2PbR'_2 + 2MgX_2$$
$$R'MgX + R_3PbX \longrightarrow R_3PbR' + MgX_2$$

This method is of general applicability whenever the desired Grignard reagent can be prepared. The observations of Krause and Schmitz,[8] later verified by Robinson,[9] indicate, however, that the higher homologs of tetraphenyllead cannot be prepared from lead chloride and the Grignard reagent in satisfactory yields.

Bailie[10] prepared tetra-o-anisyllead, tetra-p-anisyllead and the corresponding tetraphenetyl compounds from the Grignard reagent and lead chloride. The ortho and para trianisyl and triphenetyllead compounds have been obtained by the same method.[11]

Cleavage of lead compounds with a reactive metal such as sodium in liquid ammonia yields a lead-sodium derivative which can be used as an intermediate for the synthesis of other lead compounds. When tri-o-anisyllead is dissolved in liquid ammonia and then treated with sodium, tri-o-anisylleadsodium is formed. This reacts with benzyl chloride to give tri-o-anisylbenzyllead.[11] The corresponding p-phenetyllead compound has been prepared in an analogous manner.

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[8] Krause and Schmitz, Ber., 52, 2165 (1919).
[9] Robinson, Doctoral Dissertation, Iowa State College (1929).
[10] Bailie, Doctoral Dissertation, Iowa State College (1938).
[11] Gilman and Bailie, J. Am. Chem. Soc., 61, 731 (1939).

In some instances the Grignard reagent can be replaced by an organolithium compound. Thus Towne[12] obtained *p*-anisyltriphenyllead from triphenyllead chloride and *p*-anisyllithium, the latter being made from *p*-bromoanisole and lithium.

The trialkoxyaryllead halides are made by conventional procedures.[10]

Hydroxyl Groups

Hitherto no hydroxyaryllead compounds have been recorded. Kocheskov[13] introduced hydroxyaryl groups into tin compounds by using hydroxyarylmercury compounds as precursors.

$$R_3SnSnR_3 - R'_2Hg \;\text{———}\; 2R_3SnR' - Hg$$

In this manner he prepared triethyl-*o*-hydroxyphenyltin from hexaethylditin and di-*o*-hydroxyphenylmercury. Analogous reactions between lead and mercury compounds failed; but this is not surprising in view of results obtained earlier by Austin.[14]

It is of interest to note here that β,γ-dihydroxypropyltriphenyllead has been prepared by Austin[15] through the cautious oxidation of triphenylpropenyllead with potassium permanganate.

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[12] Towne, *Iowa State Coll. J. Sci.*, *8*, 229 (1933) [*C.A.*, *28* 232 (1933)].

[13] Kocheskov, *Ber.*, *69*, 1639 (1936).

[14] Austin, *J. Am. Chem. Soc.*, *54*, 3287 (1932).

[15] Austin, *ibid.*, *53*, 3514 (1931).

Primary Amino Groups

Primary amino groups can generally be obtained by the reduction of nitro groups. An attempt to extend the reaction

$$R_3PbNa + R'X \longrightarrow R_3PbR' + NaX$$

to aryl halides containing nitro groups proved abortive.[16]

Vorländer nitrated tetraphenyllead with fuming nitric acid and obtained m-dinitrodiphenyllead dinitrate.[17] He does not record an attempted reduction of the nitro compound. Kocheskov tried to reduce m-dinitrodiphenyllead dibromide by various methods. Stannous chloride gave aniline and lead bromide as did iron and hydrochloric acid. Ammonium sulfide yielded aniline and lead sulfide, while titanium trichloride had no reaction. Since m-dinitrodiphenyllead dibromide is not cleaved by hydrogen bromide, Kocheskov concluded that the amino group has a labilizing effect, placing it above the nitro group in the Kharasch series.[18]

By the gradual addition of an excess of 15 per cent titanium trichloride solution to dinitrodiphenyllead oxide, dissolved in concentrated alcoholic hydrochloric acid, Schmidt[3] obtained m-diaminodiphenyllead dichloride.

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16. Bindschadler, Doctoral Dissertation, Iowa State College (1941).
17. Vorländer, Ber., 58, 1893 (1925).
18. Kocheskov, Bull. Acad. Sci. (U.R.S.S.) Ser. Chim., 569 (1937), /Chem. Zentr., 110 I, 4934 (1939)/.

Dialkylamino Groups

It has been mentioned that in some instances organolithium compounds may be substituted for the Grignard reagent in the preparation of organolead compounds.[12] Tetra-p-dimethylaminophenyllead, di-p-dimethylaminophenyldiphenyllead and p-dimethylaminophenyltriphenyllead have been obtained by the interaction of p-dimethylaminophenyllithium and the required lead halide, i.e. $PbCl_2$, diphenyllead dichloride and triphenyllead chloride.[19]

The halogen-metal interconversion[20] reaction is a method of choice for the preparation of organolithium compounds inaccessible through other, more direct methods. By this method aryllithium compounds containing nuclear hydroxyl,[21] primary amino[22,4] and carboxyl[21] substituents have been obtained. When the lithium compounds obtained by halogen-metal interconversion were used as intermediates in the preparation of lead compounds complex mixtures were obtained.[4,23] This may be due in part to the metal-metal interconversion.[24]

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19. Austin, J. Am. Chem. Soc., 54, 3726 (1932).
20. Gilman, Langham and Jacoby, Ibid., 61, 109 (1939); Wittig and Pockels, Ber., 72, 89 (1939).
21. C. E. Arntzen, Doctoral Dissertation, Iowa State College (1942).
22. Gilman and Stuckwisch, J. Am. Chem. Soc., 63, 2844 (1941).
23. W. Gregory, Master's Thesis, Iowa State College (1942).
24. Gilman and Moore, J. Am. Chem. Soc., 62, 3206 (1940).

The difficulty was overcome by converting the lithium compound to the Grignard reagent by means of magnesium bromide.[4,25] This may prove to be a general method for the synthesis of a variety of organolead compounds hitherto inaccessible.

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[25] Gilman and Kirby, *ibid.*, *63*, 2046 (1941).

EXPERIMENTAL

In all of the reactions involving active organometallic compounds, the conventional three-necked flask provided with a mechanical stirrer and Hopkins condenser was used. An anhydrous, inert atmosphere was maintained by keeping a slight pressure of dry, oxygen-free nitrogen over the reaction.

The procedures for the preparation of starting materials, unless otherwise stated, were the best available in the literature. Modifications of procedures are given in detail.

Analyses for lead were carried out on approximately 0.3 g. samples. The samples were decomposed in sulfuric acid with nitric acid and finally hydrogen peroxide. The lead was determined as lead sulfate.

Nitrogen was determined by the micro-Dumas method.

Halogen-Metal Interconversion Studies

Preparation of *n*-Butyllithium.

n-Butyllithium was used for all the halogen-metal interconversions employed in this investigation. Heretofore *n*-butyllithium was generally prepared at room temperature in the conventional three-necked flask. This procedure is relatively time consuming and is limited to small runs because ether cleavage of *n*-butyllithium is rapid at the reflux temperature of ether.[26] In interconversions involving active hydrogen atoms an additional equivalent of organometallic compound is required for each active hydrogen present in the compound. Since such reactions require large amounts of n-butyllithium, a more rapid method of preparation for this reagent was desirable.

n-Butyl bromide in ether was added to lithium suspended in ether until a rapid rate of reflux was obtained. The reaction flask was then immersed in an ice-water bath and the n-butyl bromide added at such a rate that gentle refluxing was maintained. After all the n-butyl bromide had been added, the ice-water bath was removed and stirring was continued until refluxing ceased. In runs using 0.30 mole of n-butyl bromide the time of preparation was reduced from 2-1/2 hours to 40 minutes and the average yield increased by 10%. By this method as high as 2 moles of n-butyllithium were prepared in 58% yields.

The method has one chief disadvantage. A large amount of colloidal lithium is formed and does not settle out on standing, nor can it be removed by filtration. In reactions where a clear solution of n-butyllithium is desirable the old procedure is recommended.

o-Bromoaniline and n-Butyllithium.

To 0.128 mole of n-butyllithium in 125 ml. of ether was

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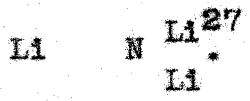
[26] Haubein, Doctoral Dissertation, Iowa State College (1942).

added dropwise 7.4 g. (0.042 mole) of o-bromoaniline in 25 ml. of ether. After 1/3 of the o-bromoaniline solution was added, rapid refluxing ceased and the remaining solution was added more rapidly. Evidently the replacement of the first active hydrogen is more rapid than the second. After 15, 30, and 45 minutes, respectively, 50 ml. aliquot portions were withdrawn by means of a pipet and carbonated in the usual manner. After working up the reaction by conventional procedures, the alkaline extract was treated with benzenesulfonyl chloride. The benzenesulfonamide of anthranilic acid was precipitated with concentrated hydrochloric acid. This method of isolation was used because of the difficulty encountered in quantitatively isolating a water-soluble acid. Preliminary experiments with anthranilic acid and benzenesulfonyl chloride showed that the reaction between these two compounds is practically quantitative. The sulfonamide was recrystallized from dilute ethanol. The yield of sulfonamide for the 15, 30, and 45-minute runs was 10, 25, and 40 per cent, respectively. Increasing the reaction time beyond 45 minutes did not increase the percentage of interconversion.

m-Bromoaniline and n-Butyllithium.

To 0.30 mole of n-butyllithium in 250 ml. of ether was added dropwise 17.2 g. (0.10 mole) of m-bromoaniline dissolved in 50 ml. of ether. After 10, 15, 30, 45, 60, and 90 minutes respectively, 50 ml. aliquot portions were carbonated in the usual manner. The various carbonated mixtures were treated as described for the *o*-bromoaniline reaction. No product of interconversion was isolated and 14 g. of *m*-bromoaniline was recovered.

p-Iodoaniline and *n*-Butyllithium.

Six and seven-tenths grams (0.035 mole) of *p*-iodoaniline in 50 ml. of ether was added dropwise to 0.1 mole of *n*-butyllithium in 100 ml. of ether. Fifty-ml. aliquot portions were withdrawn and carbonated at 15-, 30-, and 45-minute intervals. The reaction mixture was treated as previously described, but no interconversion product was isolated. This was rather surprising in view of the fact that *p*-bromoaniline undergoes interconversion to the extent of 70 per cent. A precipitate was formed immediately when the *p*-iodoaniline was added to the organometallic solution. The insolubility of the N-Li compound may inhibit further reaction. In the reaction between *p*-bromoaniline and *n*-butyllithium a precipitate is formed after about fifteen minutes of reaction. This precipitate is the compound  $\mathrm{Li}\qquad\mathrm{N}\begin{smallmatrix}\mathrm{Li}\\ \\ \mathrm{Li}\end{smallmatrix}$[27]. The apparent course of the reactions is

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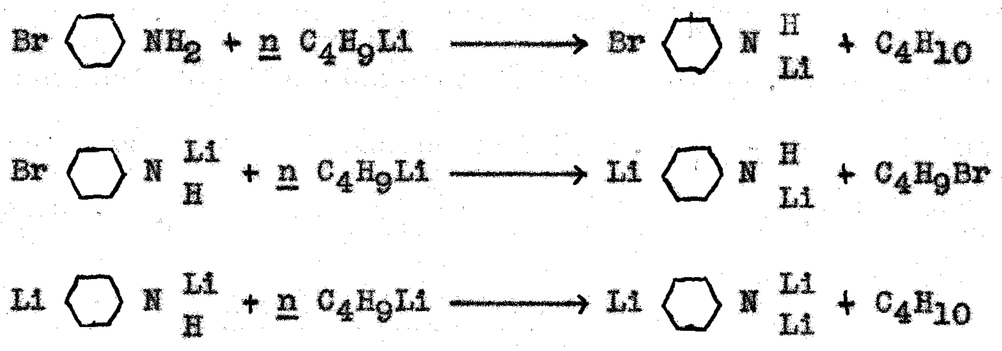
[27] Abbott, Doctoral Dissertation, Iowa State College (1942).



Preparation of 4-Iodoresorcinol[28]

4-Iodoresorcinol was prepared in essential accordance with the procedure of Stenhouse. Fifty grams of resorcinol and 120 grams of iodine were dissolved in 300 ml. of ether and the mixture was gradually decomposed with 550 grams of litharge. When the iodine color had disappeared, the ether was removed and the residue was extracted with benzene. The benzene solution was evaporated to dryness and the residue crystallized from hot water. The yield of 4-iodoresorcinol was 40% (m.p., 67°).

4-Iodoresorcinol and n-Butyllithium.

When 3 equivalents of *n*-butyllithium were added dropwise to 1 equivalent of 4-iodoresorcinol dissolved in ether, a precipitate was formed immediately. The reaction was allowed to continue for 30 minutes and then carbonated. No 4-carboxyresorcinol was isolated. Several repetitions of the reaction employing both shorter and longer times failed to give 4-carboxyresorcinol subsequent to carbonation and hydrolysis.

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[28] Stenhouse, *Ann.*, *171*, 311 (1874).

Preparation of p-Bromo-N-methylaniline.

Several methods for the preparation of p-bromo-N-methylaniline are given in the literature. Fischer [29] prepared the compound by methylating p-bromoaniline. Another preparation described by Fries[30] involves the bromination of N-methylaniline. The former method is not very satisfactory because it gives some tertiary amine in addition to unreacted primary amine. The bromination of N-methylaniline always gives some of the dibromination product. When either of these two methods of preparation was used, the acid obtained from the interconversion with n-butyllithium followed by carbonation and hydrolysis could not be isolated in a pure condition.

Bromination of N-methylacetanilide followed by hydrolysis of p-bromo-N-methylacetanilide[31] gave a product sufficiently pure for interconversion purposes. A mixture of 250 g. (2.34 moles) of methylaniline, free from primary or tertiary amines, and 250 ml. of acetic anhydride was refluxed on a hot plate for 25 hours. The mixture was then concentrated to one-half its original volume. On cooling the N-methylacetanilide crystallized from the solution. The yield was 228 g. or 66%. The compound melted at 102°.

Two hundred and twenty-eight grams (1.53 moles) of N-methylacetanilide in 820 ml. of glacial acetic acid was

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29. Fischer, Ber. 45, 1100(1912).
30. Fries, Ann., 346, 174 (1906).
31. Huntress, J. Ind. Chem. Soc., 10, 637 (1933).

treated with 41 ml. of bromine in 41 ml. of glacial acetic acid in the presence of ultra-violet light. In the course of 2 or 3 hours, shining platelets appeared. The reaction was continued for 5 hours longer, and the mixture was diluted with 4 volumes of water and kept over night. The solid was then filtered off and crystallized from dilute ethanol. The yield of *p*-bromoacetanilide melting at 98-99° was 140 g. or 50%.

Eighty grams of *p*-bromo-N-methylacetanilide was hydrolyzed by boiling with 150 ml. of concentrated hydrochloric acid for 5 hours. The free amine was precipitated with sodium hydroxide and was extracted with ether. The ether layer gave on distillation 44.8 g. or 60% of the free amine boiling at 109-111° at 0.5 mm. $D^{20}_{20} = 1.4701$. The picrate prepared from *p*-bromo-N-methylaniline melted at 176-177° and was analyzed for nitrogen.

Anal. Calcd. for $C_{13}H_{11}O_7N_4Br$: N, 13.49. Found: N, 13.12.

p-Bromo-*N*-methylaniline and *n*-Butyllithium.

To a solution of 11.9 g. (0.065 mole) of *p*-bromo-N-methylaniline in 25 ml. of ether was added 0.13 moles of *n*-butyllithium in 150 ml. of ether. The mixture was stirred at room temperature for one hour and was then carbonated by pouring on crushed, solid carbon dioxide. Subsequent to hydrolysis, acidification of the alkaline extract yielded 2.5 g. or 27% of an acid melting at 145-152°. When the acid was recrystallized from hot water the melting point was

156-157°. This is the same as the melting point recorded for p-N-methylaminobenzoic acid.[32] A mixed melting point with an authentic specimen[32] showed no depression.

Preparation of N-(p-Iodophenyl)-phthalimide.[33]

Phthalic anhydride [37 g. (0.25 mole)] and 54.5 g. (0.25 mole) of p-iodoaniline were heated together in a metal bath at 250-260° for 2 hours. The melt was then poured into cold glacial acetic acid. The mixture was heated to boiling and filtered. On cooling, the filtrate deposited crystals of N-(p-iodophenyl)-phthalimide. The yield was 64 g. or 72% (m.p., 226-228°).

N-(p-Iodophenyl)-phthalimide and n-Butyllithium.

Run 1. To a solution of 0.03 mole of n-butyllithium in 250 ml. of ether, cooled to -5° was added 10.5 g. (0.03 mole) of N-(p-iodophenyl)-phthalimide. The mixture was stirred for 10 minutes and then carbonated by pouring on crushed, solid carbon dioxide. After working up the reaction by conventional procedures, only a trace of solid acid material was obtained. No odor of valeric acid was present.

Run 2. In a second run the same quantities of reagents were used as in the first. The n-butyllithium solution was cooled to -50° in a dry-ice-acetone bath before the N-(p-iodophenyl)-phthalimide was added. After 70 minutes the

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[32] Houben and Shottmueller, Ber., 42, 3739 (1909).
[33] Gabriel, Ber., 11, 2260 (1878).

reaction mixture was carbonated and worked up by conventional procedures. The alkaline extract on acidification yielded 3.5 g. of solid acidic material. After crystallization from dilute ethanol the melting point was 284-286°.

Anal. Calcd. for $C_{15}H_9O_4N$: neut. equiv., 267; N, 5.24. Found: neut. equiv., 266; N, 5.15.

The yield of N-(p-carboxyphenyl)-phthalimide was 37%. The ethyl ester prepared from the acid melted at 150°.[34]

Increasing the length of reaction time at -50° did not increase the amount of interconversion.

Preparation of Organolead Compounds

Triphenyl-p-aminophenyllead.

Three variations were used for the preparation of triphenyl-p-aminophenyllead. The first method which consists of adding anhydrous magnesium bromide to the p-aminophenyllithium solution is described in the literature.[4]

Method No. 2. To 5.67 g. (0.033 mole) of p-bromoaniline in 25 ml. of ether was added dropwise 0.1 mole of n-butyllithium in 180 ml. of ether. After 60 ml. of the organometallic solution had been added, refluxing ceased and the remaining 120 ml. was added more rapidly. The mixture was stirred for an hour. A yellow crystalline precipitate of the

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[34] Crippa and Galimberti, Gazz. chim. ital., 63, 81 (1933); Coniglio, Rend. acad. sci., 36, 56 (1930) /Chem. Zentr., 102, 1276 (1931)/.

p-N,N-dilithioaminophenyllithium separated from solution. The reaction flask was fitted with a stopcock at the bottom so that the solid could be filtered from the liquid. To the solid residue remaining in the flask was added 250 ml. of ether and then 10.3 g. (0.022 mole) of triphenyllead chloride. The mixture was stirred for one hour at room temperature and then hydrolyzed with iced ammonium chloride solution. The organic layer was dried over sodium sulfate. Hydrogen chloride was cautiously passed into the dried ether solution cooled in an ice-bath to precipitate the hydrochloride of triphenyl-p-aminophenyllead. The hydrochloride was converted to the free amine by suspending it in water and adding dilute ammonium chloride. The yield of triphenyl-p-aminophenyllead thus obtained was 6 g. or 59%. After crystallization from a mixture of benzene and petroleum ether, the compound melted at 172°. A mixed melting point with the compound obtained by the first method showed no depression.

Method No. 3. To 5.3 g. (0.031 mole) of p-bromoaniline in 30 ml. of ether was added dropwise 0.093 mole of n-butyllithium in 200 ml. of ether. The mixture was stirred at room temperature for one hour and was then poured into a suspension of 14.2 g. (0.03 mole) of triphenyllead chloride in ether. After three minutes the mixture was poured into ice water. The ether layer was separated and extracted with dilute HCl to remove unreacted p-bromoaniline. (The hydrochloride of triphenyl-p-aminophenyllead is insoluble in water.) Dry hydrogen chloride was passed into the dried ether solution to precipitate the hydrochloride of triphenyl-p-aminothenyllead. The free amine was obtained as described in method No. 2. The yield was 9.5 g. or 60%.

In the interconversion of p-bromoaniline the n-butyllithium solution must be clear, or a gummy precipitate which is difficult to work with will be obtained.

Triphenyl-o-aminophenyllead.

Run 1. To a solution of 8.6 g. (0.05 mole) of o-bromoaniline in 25 ml. of ether was added dropwise 0.15 mole of n-butyllithium in 175 ml. of ether. After 45 minutes 18.9 g. (0.04 mole) of triphenyllead chloride was added to the reaction mixture. The mixture was stirred vigorously for three minutes and was then poured into an aqueous ammonium chloride solution. The ether layer was separated, extracted with dilute hydrochloric acid and then dried over sodium sulfate. When the ether was removed under reduced pressure, a solid residue was left behind. Fractional crystallization of the residue from ethanol gave 5.3 g. or a 50% yield (based on 40% interconversion of o-bromoaniline) of a solid melting at 164-165°.

Anal. Calcd. for $C_{24}H_{21}NPb$: Pb, 39.01. Found: Pb, 38.73.

In addition to the triphenyl-o-aminophenyllead there was isolated 6 g. of triphenyl-n-butyllead.

Run 2. To a solution of 8.6 g. (0.05 mole) of o-bromoaniline in 25 ml. of ether was added dropwise 0.15 mole of n-butyllithium in 175 ml. of ether. After 45 minutes a slight excess of anhydrous magnesium iodide in ether (prepared from magnesium and iodine in ether) was added. To this mixture was added 18.9 g. (0.04 mole) of triphenyllead chloride. The mixture was stirred for two hours and was hydrolyzed with an iced ammonium chloride solution. The ether layer was separated, extracted with dilute hydrochloric acid and then dried over sodium sulfate. After the ether was evaporated, the solid residue was extracted with boiling petroleum ether (b.p. 60-68°). The petroleum ether-soluble material after being crystallized from ethanol melted at 142-143°. A qualitative analysis showed that the compound contained no nitrogen but gave a positive test for iodine. A mixed melting point with triphenyllead iodide showed no depression. The total amount of triphenyllead iodide isolated was 11.2 g. or 50%. No triphenyl-o-aminophenyllead was obtained.

The formation of triphenyllead iodide is difficult to explain. The slight excess of magnesium iodide added to the reaction could react with triphenyllead chloride to yield magnesium chloride and triphenyllead iodide, but the excess beyond that required to convert the lithium compound to the Grignard reagent was insufficient to account for the total amount of the latter product. Furthermore, the failure to obtain triphenyl-*o*-aminophenyllead is also surprising. A repeat run gave the same results as the experiment just described.

Triphenyl-*p*-*N*-Methylaminophenyllead.

To a solution of 9.7 g. (0.05 mole) of *p*-bromo-N-methylaniline in 25 ml. of ether was added dropwise 0.1 mole of *n*-butyllithium in 150 ml. of ether. After one hour 18 g. (0.037 mole) of triphenyllead chloride was added to the solution. The reaction mixture was stirred at room temperature for three hours and then was hydrolyzed with iced ammonium chloride. The ether layer was separated; extracted with 10% hydrochloric acid; and dried; the ether was removed at the water pump. The solid residue was extracted with hot methanol. The methanol-insoluble fraction, after being crystallized from benzene, melted at 224°. A mixed melting point with tetraphenyllead showed no depression. The yield of tetraphenyllead was 15%.

On cooling, the methanol extract deposited white crystals which melted at 90 to 96°. Two crystallizations from methanol gave a trace of material melting at 97-98°.

When the original methanol extract was concentrated, more material was obtained. This proved to be triphenyl-*n*-butyllead (mixed m.p.).

Run No. 2. A second run was made in which the mixture was hydrolyzed three minutes after the triphenyllead chloride had been added. The reaction was worked up as in the first run. No tetraphenyllead was found in the reaction. After crystallization from methanol, the yield of triphenyl-p-N-methylaminophenyllead melting at 97-98° was 10%.

Anal. Calcd. for $C_{25}H_{23}NPb$: Pb, 38.07; N, 2.5. Found: Pb, 37.79; N, 2.6.

Besides some recovered triphenyllead chloride there was isolated 50% of triphenyl-n-butyllead.

Triphenyl-o-dimethylaminophenyllead.

To 0.5 g. of lithium suspended in 25 ml. of ether was added 5.6 g. (0.03 mole) of o-bromodimethylaniline in 50 ml. of ether. The mixture was heated gently to start the reaction and then the o-bromodimethylaniline was added at such a rate that gentle refluxing was maintained. After all of the amine had been added, stirring was continued for thirty minutes. The solution was filtered through glass wool and 5 ml. of the filtrate was hydrolyzed and titrated with standard base. The yield of organometallic compound was 66%. The solution of organometallic compound thus prepared was added to 9.5 g. (0.02 mole) of triphenyllead chloride suspended in 50 ml. of ether. After 15 minutes the mixture was hydrolyzed with iced ammonium chloride solution. The ether layer was separated and dried and the ether removed at reduced pressure. Crystallization of the residue from 95% ethanol gave 5.4 g. or 50% of triphenyl-o-dimethylaminophenyllead melting at 101-102°.

Anal. Calcd. for $C_{26}H_{25}NPb$: Pb, 37.1. Found: Pb, 36.9.

Triphenyl-o-hydroxyphenyllead.

To 7.8 g. (0.045 mole) of o-bromophenol in 50 ml. of ether was added dropwise 0.09 mole of n-butyllithium in 175 ml. of ether. After 30 minutes a slight excess of anhydrous magnesium bromide was added. To the mixture thus obtained was added 14 g. (0.03 mole) of triphenyllead chloride. The mixture was stirred at room temperature for two hours and then hydrolyzed with iced ammonium chloride solution. The ether layer was dried and the ether removed at reduced pressure. The solid residue was extracted with 95% ethanol.

Seven grams of the material was insoluble in alcohol, contained no halogen, and melted at 231°. A quantitative lead analysis gave 43.7% lead. This agrees with the calculated value for triphenyllead carbonate. When boiled with concentrated hydrochloric acid, the compound evolved carbon dioxide and was converted to triphenyllead chloride.

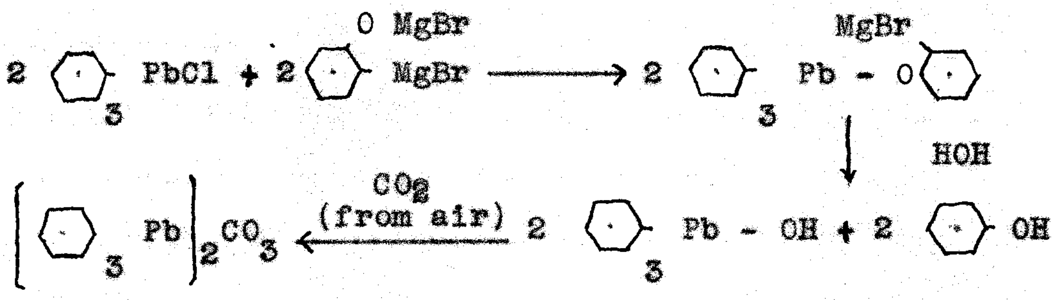
The alcohol-soluble portion after several crystallizations from an alcohol-petroleum ether mixture melted at 217-218° with decomposition. The yield of triphenyl-o-hydroxyphenyllead was 3 g. or 20%.

Anal. Calcd. for $C_{24}H_{20}OPb$: Pb, 38.98. Found: Pb, 38.81.

Attempted Preparation of Triphenylphenoxylead.

A suspension of 0.39 g. (0.010 mole) of potassium was heated in 20 ml. of anhydrous toluene with vigorous stirring. When the potassium was in the form of a sand, 0.94 g. (0.01 mole) of phenol in 10 ml. of toluene was added dropwise. The mixture was stirred and heated for five hours and then was allowed to cool. To the cold mixture was added 4.7 g. (0.01 mole) of triphenyllead chloride. After two hours of refluxing the mixture was filtered. On cooling, white crystals deposited (m.p., 160-185°). Repeated crystallization from ethanol decreased the solubility of the product and brought the melting point to 224-230°. No pure product could be isolated.

Triphenyllead carbonate might be formed in the reaction between the Grignard reagent of *o*-bromophenol and triphenyllead chloride by the following steps:



The failure to obtain pure triphenylphenoxylead but rather a product that approached the properties of triphenyllead carbonate substantiates this mechanism.

If the above reaction actually takes place, then, if two equivalents of triphenyllead chloride were added to the reaction for each equivalent of organometallic compound present, the yield should be increased. Accordingly, an experiment was carried out under conditions identical with those above, with the exception that twice as much triphenyllead chloride was used. The results, however, did not differ appreciably from those of the first run.

Triphenyl-*o*-hydroxyphenyllead.

In a third preparation of triphenyl-*o*-hydroxyphenyllead, the magnesium bromide was omitted and the reaction was hydrolyzed three minutes after 0.05 mole of *o*-lithiolithiumphenoxide had been added to 0.05 mole of triphenyllead chloride. From this reaction the yield of triphenyl-*o*-hydroxyphenyllead was 70% and no high-melting material corresponding to that which was previously obtained was present.

Triphenyl-*p*-hydroxyphenyllead.

To a solution of 17.3 g. (0.1 mole) of *p*-bromophenol in 30 ml. of ether was added from a dropping funnel 0.2 mole of *n*-butyllithium in 200 ml. of ether. After one and one-half hours 47.0 g. (0.1 mole) of triphenyllead chloride was added to the solution. The mixture was stirred vigorously for four minutes and was poured into an aqueous ammonium chloride solution. The ether layer was separated and dried over sodium sulfate. After the ether had been removed, the residue was subjected to crystallization from various organic solvents. All the fractions obtained melted between 190 and 230°.

Vacuum sublimation of a part of the material gave a crystalline solid melting at 204°. The compound analyzed for triphenyllead oxide.

In a second attempt to prepare triphenyl-p-hydroxyphenyllead, 5.3 g. (0.01 mole) of triphenyl-p-aminophenyllead was suspended in glacial acetic acid cooled to 15°. Two grams of sodium nitrite dissolved in 10 ml. of water was added to the suspension. After 30 minutes the mixture was made neutral with sodium hydroxide and was warmed to 60°. The solid material was filtered off and crystallized from ethanol. A small amount (0.3 g.) of material melting at 230° was obtained.

Anal. Calcd. for $C_{24}H_{20}OPb$: Pb, 38.98. Found: Pb, 40.1.

Further crystallization of the material did not improve the analysis. Whether triphenyl-p-hydroxyphenyllead was present in an impure state is not certain.

Triethyl-p-dimethylaminophenyllead.

A solution of p-dimethylaminophenyllithium, prepared from 32 g. (0.16 mole) of p-bromodimethylaniline and 2 g. of lithium, in 100 ml. of ether was added to 50 g. (0.154 mole) of triethyllead chloride in 200 ml. of ether. The mixture was stirred for two hours at room temperature and then hydrolyzed with iced ammonium chloride solution. The ether layer was dried, the ether evaporated at the water pump, and the residue distilled at 1 mm. Two fractions were collected.

The first distilled at 60° and the second at 125°.

The first fraction, weighing 5 g., had an index of refraction and a density corresponding to that of tetraethyllead. A qualitative analysis showed that no nitrogen was present.

The second fraction gave a qualitative test for nitrogen and had the following physical constants:

$$n_D^{25} = 1.5442; \qquad D_4^{25} = 1.4982.$$

Anal. Calcd. for $C_{14}H_{25}PbN$: Pb, 50.7. Found: Pb, 50.4.

The yield of triethyl-p-dimethylaminophenyllead was 15 g. or 20%.

Preparation of Trimethyllead Chloride.

Grüttner and Krause prepared trimethyllead chloride by the low temperature chlorination of tetramethyllead[35]. This compound can be more readily prepared by cleaving tetramethyllead with hydrogen chloride.

Hydrogen chloride was passed into a solution of 22 g. (0.083 mole) of tetramethyllead[36] in 400 ml. of petroleum ether (b.p. 28-30°). After fifteen minutes a white solid began to separate. The flow of hydrogen chloride was continued until gas evolution ceased. This required about two

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[35] Grüttner and Krause, Ber., 49, 1415 (1916).

[36] The author is grateful to Dr. Calingaert of the Ethyl Gasoline Corporation for the tetramethyllead.

hours. The trimethyllead chloride was filtered off and dried in a desiccator over potassium hydroxide. The yield was 20 g. or 85%.

Attempted Preparation of Various Triethyl- and Trimethylaryllead Compounds.

Since the halogen-metal interconversion reaction proved successful in the preparation of amino and hydroxyl substituted aryllead compounds, attempts were made to extend the reaction to alkyllead compounds. Triethyllead chloride and trimethyllead chloride were used as starting materials. Triethyllead chloride was allowed to react with the interconversion products of n-butyllithium and o-bromophenol, p-bromoaniline, o-bromoaniline, and p-bromo-N-methylaniline. Likewise, the interconversion products of o-bromophenol and o-bromoaniline were tried with trimethyllead chloride. Attempts to isolate the desired pure products from any of these reactions failed.

Theoretically there should be no difference in the course of reaction between these compounds and triphenyllead chloride under comparable conditions, and it is entirely possible that the desired compounds are actually formed. The difficulty resides in the separation of the reaction products from the unreacted materials. The alkyllead compounds are liquids and hence must be purified by distillation. At atmospheric pressure the alkyllead compounds are unstable at their boiling points. At reduced pressure the boiling points of the desired organolead compounds and the unreacted amine or phenol are too close together to permit separation.

Numerous experiments under varying conditions were tried and all gave the same result. The final products were low in lead content and high in nitrogen where amines were involved. A few typical runs are described in the following section.

Run 1. To 15.5 g. (0.09 mole) of *o*-bromophenol in 25 ml. of ether was added 0.18 mole of *n*-butyllithium in 185 ml. of ether. After 30 minutes the reaction mixture was added to 29.7 g. (0.09 mole) of triethyllead chloride suspended in anhydrous ether. After one minute hydrolysis was effected by pouring the mixture into an iced ammonium chloride solution. The ether layer was separated and dried, the ether was evaporated and the residue was distilled at reduced pressure (1 mm). Thirteen grams of a colorless liquid distilled between 45 and 55°. The solid residue in the distilling flask was inorganic lead. The distillate had the following constants:

$$D_4^{25} = 1.5344; \qquad n_D^{25} = 1.5219.$$

The material contained 46.6% lead. The calculated value for triethyl-*o*-hydroxyphenyllead is 54.33.

The material was redissolved in ether and the ether solution was extracted with dilute sodium hydroxide, dried and again distilled. The boiling point of the distillate was unchanged, and the lead analysis was 46.7%.

Varying the reaction time or the addition of magnesium bromide to the interconversion product did not alter the results. The lead analyses were always from four to eight per cent low.

Run 2. To 10 g. (0.05 mole) of p-bromo-N-methylaniline in 25 ml. of ether was added 0.10 mole of n-butyllithium in 175 ml. of ether. The solution was stirred for one hour after addition was complete and was then added to a suspension of 16.5 g. (0.05 mole) of triethyllead chloride in ether. After two minutes the mixture was poured into an iced ammonium chloride solution. The ether layer was separated and dried, and the ether was removed at the water pump. The residue was distilled at 0.001 mm. Seven and one-half grams of a colorless liquid distilled at 46-48°. The liquid gave a positive qualitative test for nitrogen and contained 38.8% lead. The calculated value for triethyl-p-N-methylaminophenyllead was 50.31%. Redistillation at 5 mm. did not improve the purity of the compound.

Triethyl-p-(N-phthalimido)-phenyllead.

To a solution of 0.05 mole of n-butyllithium in 250 ml. of ether, cooled to -60° in a dry-ice-acetone bath was added 17.5 g. (0.05 mole) of N-(p-iodophenyl)-phthalimide. After ten minutes 16.4 g. (0.05 mole) of triethyllead chloride was added. The cooling bath was removed, the mixture was allowed to come to room temperature, and was then hydrolyzed by pouring into an aqueous ammonium chloride solution. The ether layer was separated and dried and the ether was removed under vacuum. Repeated attempts to crystallize the gummy residue from various organic solvents failed. Distillation at 0.001 mm. gave 4 g. of a light yellow glass-like material. Attempts to crystallize this glass were unsuccessful; hence it was analyzed as such, and the analysis agreed for triethyl-p-(N-phthalimido)-phenyllead.

Anal. Calcd. for $C_{20}H_{23}O$ NPb: Pb, 40.1. Found: Pb, 39.9.

Hydrolysis of Triethyl-p-(N-phthalimido)-phenyllead.

A suspension of 5.16 g. (0.01 mole) of N-(p-triethylplumbylphenyl)-phthalimide in 50 ml. of 10% sodium hydroxide solution was warmed on a steam plate for three hours. The mixture was allowed to cool and was then extracted with benzene. The aqueous layer was acidified to precipitate the phthalic acid formed during the hydrolysis. The yield of phthalic acid was 1.2 g. or 75%. The benzene solution was dried over sodium sulfate and the benzene was removed under reduced pressure (12 mm.). The gummy residue left behind could not be purified either by distillation or by crystallization.

Cleavage Studies with Hydrogen Chloride

Triphenyl-p-aminophenyllead.

A stream of dry hydrogen chloride was passed through a boiling solution of 5.31 g. (0.01 mole) of triphenyl-p-aminophenyllead until a precipitate began to appear. The solution was then filtered and the filtrate was extracted with dilute hydrochloric acid. The hydrochloric acid extract was made basic with ammonium hydroxide. The oil which separated was treated with acetyl chloride. The acetanilide was crystallized from dilute ethanol. The yield was 0.8 g. or 60%. A mixed melting point (140°) with an authentic specimen of acetanilide gave no depression.

The chloroform solution was evaporated to dryness and the residue crystallized from 95% ethanol. A mixed melting point showed that this was triphenyllead chloride. The yield was 80%.

Triphenyl-o-aminophenyllead.

When triphenyl-o-aminophenyllead was treated in a manner similar to that described for triphenyl-p-aminophenyllead, the yield of acetanilide was 65%, and the yield of triphenyllead chloride was 78%.

Triphenyl-o-dimethylaminophenyllead.[19]

A solution of 5.58 g. (0.01 mole) of triphenyl-o-dimethylaminophenyllead in 75 ml. of chloroform was treated with dry hydrogen chloride. When the solution became cloudy, it was filtered and the filtrate was extracted with dilute hydrochloric acid. The hydrochloric acid extract was made alkaline with ammonium hydroxide solution. The oil which separated was dissolved in 95% ethanol and treated with an excess of picric acid (saturated solution in 95% ethanol). The yield of picrate, melting at 162°, was 2.1 g. or 66%.

The yield of triphenyllead chloride was 75%.

Triphenyl-o-hydroxyphenyllead.

Dry hydrogen chloride was passed through a solution of 5.32 g. (0.01 mole) of triphenyl-o-hydroxyphenyllead in 75 ml. of boiling chloroform. When the solution became cloudy, the flow of hydrogen chloride was stopped and the solution was filtered. The filtrate was extracted with 10% potassium hydroxide solution. The alkaline extract was acidified with dilute hydrochloric acid and the oil which separated was extracted with ether. The ether solution was dried and then distilled. The yield of phenol was 0.5 g. or 59%. The phenol was characterized as phenoxyacetic acid, melting at 98°.

The yield of triphenyllead chloride in this experiment was 65%.

Triethyl-p-dimethylaminophenyllead.

A solution of 8 g. (0.02 mole) of triethyl-p-dimethyaminophenyllead in 50 ml. of ether was treated with a stream of dry hydrogen chloride for 30 minutes. The precipitate of triethyllead chloride was filtered off and the filtrate extracted with dilute hydrochloric acid. Dimethylaniline was precipitated from the acid extract with dilute ammonium hydroxide. The dimethylaniline was weighed in the form of its picrate (m.p. 162°). The yield was 5.7 g. or 90%. The yield of triethyllead chloride was 95%.

DISCUSSION OF RESULTS

Halogen-Metal Interconversion Reactions

The halogen-metal interconversion reaction has been studied extensively in recent years.[37] It is clear that the extent of halogen-metal interconversion depends upon the nature of the reactants, the solvent, time, and temperature.

In general, the chlorine atom is not very amenable to X-M interconversion. The only cases are those recently noted with 2,4,5-triphenylfuran and phenylethynyl chloride.[38] The iodo compounds usually react more rapidly than the bromo compounds. The solubility of the reactants, however, has a pronounced effect. This effect may be marked enough to cause the interconversion to be greater with a bromo compound than with the corresponding iodo compound. In the course of this investigation it was found that p-iodoaniline does not interconvert with n-butyllithium at room temperature in diethyl ether; whereas p-bromoaniline can be interconverted to the extent of 70%. This is probably due to the fact that the initially formed p-iodo-N-lithioaniline is insoluble in ether and thus retards reaction.

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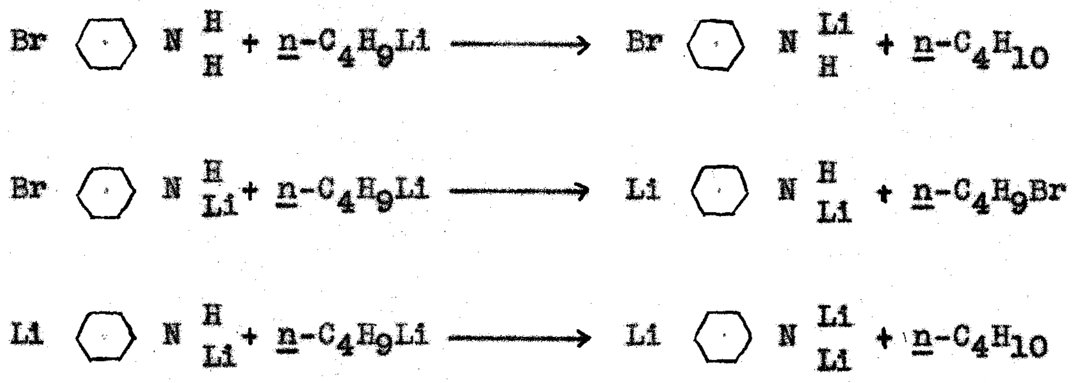
[37] Gilman, Langham and Moore, J. Am. Chem. Soc., 62, 2327 (1940).

[38] Studies by Dr. Haubein and Mr. Melstrom. See, also, Wittig and Witt, Ber., 74, 1474 (1941).

In the interconversion of p-bromoaniline with n-butyllithium it has been found that the apparent course of the reaction is



The replacement of the bromine atom occurs before that of the second so-called active hydrogen of the amino group. In that event, it might be expected that two equivalents of n-butyllithium would be sufficient for the interconversion. It has been shown, however, that with two equivalents of n-butyllithium the reaction did not proceed to as great an extent as when three equivalents were used. A plausible explanation lies in the demonstrated reversibility of the halogen-metal interconversion reaction.[39] p-Dilithioaminophenyllithium is insoluble in ether and is precipitated during the course of the reaction. This forces the equilibrium toward the right in the above equation.

No generalization can be made concerning the relative effect of the position of the halogen atom in disubstituted benzene derivatives. In the isomeric bromoanilines the order

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[39] Gilman and Jones, ibid., 63, 1441 (1941).

of decreasing activity is p > o > m; o-bromophenol gives better interconversion yields than the para isomer; in the bromodimethylanilines the order is o > p > m[40]; p-iodobenzoic acid gives better yields than the ortho isomer; and of the chlorobromobenzenes, o-chlorobromobenzene gives the poorest yields.

The interconversion of o- and p-iodobenzoic acids and N-(p-iodophenyl)-phthalimide are examples of the interconversion of compounds having a functional group that is highly reactive toward organolithium compounds. Here the X-M interconversion reaction is in competition with the addition to the carbonyl group. The final product is determined by the rapidity of the individual reactions. At a low temperature and short time the X-M interconversion predominates.

At -50° the carbonyl groups in N-(p-iodophenyl)-phthalimide are not attacked at all, even during a period of time as long as one hour. The interconversion, however, goes to the extent of but thirty-seven per cent. This is probably due to the extreme insolubility of N-(p-iodophenyl)-phthalimide in ether at -50°.

In halogen-metal interconversions involving an active hydrogen atom the order of addition of reagents is important. Better results were obtained when n-butyllithium was added to the compound to be interconverted rather than vice versa. The reason for this may be illustrated with o-bromophenol as

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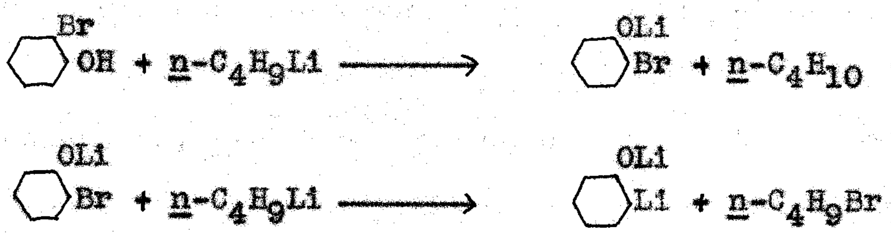
[40] Gilman and Banner, ibid., 62, 344 (1940).

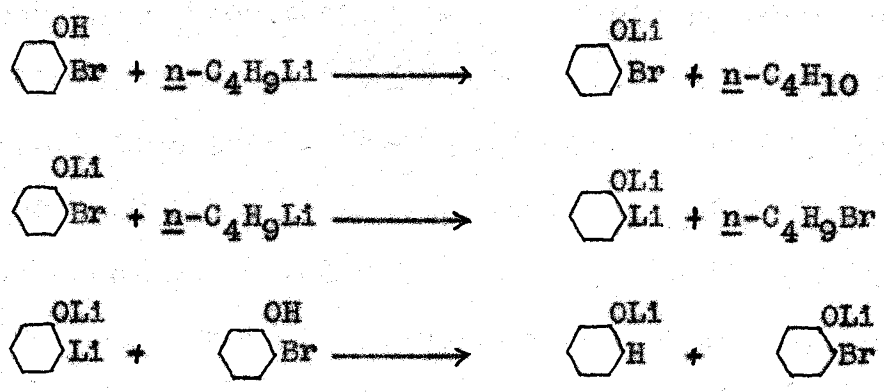
an example. When n-butyllithium is added to o-bromophenol the order of reaction is as follows:



I

II

When the addition is reversed and an excess of butyllithium is present, reactions III and IV may occur simultaneously and then may give rise to reaction V.



III

IV

V

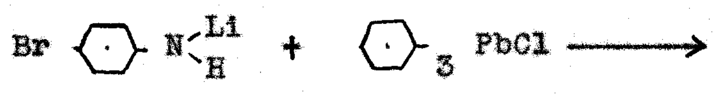
The latter is substantiated by the fact that more phenol was isolated from the reaction when o-bromophenol was added to n-butyllithium.

Preparation of Organolead Compounds

The most general method for the preparation of unsymmetrical organolead compounds is that employing the Grignard reagent. Heretofore organolithium compounds have had but limited use in the synthesis of organolead compounds.[12,19] Since the inception of the work on X-M interconversions, organolithium compounds containing "reactive" functional groups have been prepared.

When the organolithium compounds obtained by the halogen-metal interconversion reaction were first used for the preparation of organolead compounds complex reaction mixtures were obtained.[4]

In this investigation it has been shown that, in the reaction of the lithium compound with triphenyllead chloride, some tetraphenyllead was always formed. The amount of tetraphenyllead increased as the length of the reaction time was increased. At present the most plausible explanation for the formation of tetraphenyllead is the occurrence of disproportionation reactions. Towne[12] reported the isolation of a small amount of tetraphenyllead from the preparation of triphenyl-*p*-dimethylaminophenyllead using *p*-dimethylaminophenyllithium and triphenyllead chloride. He prepared the organolithium compound directly from lithium and *p*-bromodimethylaniline. It is possible that a disproportionation reaction is catalyzed by organolithium compounds and is accentuated when the organolithium compound contains an -OLi or -NLi linkage. It was possible to isolate a small amount of tetraphenyllead from the reaction



That this disproportionation is due to the organolithium compounds and also possibly to an -OLi or -NLi linkage is substantiated by the fact that the difficulty could be overcome by converting the lithium compound to the Grignard reagent.

The reaction between organolithium compounds and triphenyllead chloride is almost instantaneous. A negative color test I was obtained within three minutes. The short time of reaction practically eliminates the disproportionation reaction and is more convenient for preparative purposes than conversion of the lithium compound to the Grignard reagent.

An interesting observation was that noted in the preparation of triphenyl-o-hydroxyphenyllead. Large amounts of triphenyllead carbonate were isolated from this preparation when the reaction was permitted to go for an hour or longer. A possible mechanism for the formation of triphenyllead carbonate was given in the experimental section. This mechanism becomes rather doubtful when we consider the fact that reducing the reaction time reduces the amount of triphenyllead carbonate formed and increases the yield of triphenyl-o-hydroxyphenyllead. Since triphenyl-o-hydroxyphenyllead is formed in seventy per cent yields in three minutes and this yield decreases when the reaction time is lengthened, then the only possible conclusion is that in some manner the o-hydroxyphenyl group is gradually cleaved from the lead atom during the progress of the reaction. Here again it is possible that disproportionation results but takes a different course from that in the preparation of aminoaryllead compounds, where tetraphenyllead results.

Both tetraphenyllead and triphenyllead carbonate could result if we assume that the initial product of disproportionation is the triphenyllead radical. When an hydroxyl compound is involved, it could react with triphenyllead to give triphenylphenoxylead which might be stable before hydrolysis of the reaction; whereas in the case of an amino compound the nitrogen-lead linkage might be unstable before hydrolysis and the triphenyllead radical would disproportionate to tetraphenyllead as one of the products.

When the halogen-metal interconversion reaction was extended to the preparation of triethylaryllead compounds the results were less gratifying. Theoretically there should be no difference in the course of the reactions. The difficulty apparently resides in the separation of the desired products from unreacted materials. In the case of triethyl-*p*-(N-phthalimido)-phenyllead the unreacted material could be separated from the desired compound and the triethylaryllead compound was obtained.

In general, it can be said that the halogen-metal interconversion reaction is a general method for the preparation of organolead compounds hitherto inaccessible.

Cleavage Studies with Hydrogen Chloride

When mixed aryllead compounds are subjected to cleavage with hydrogen chloride the group with the highest molecular weight cleaves first.[41] In the light of present knowledge, this statement needs modification. It was, no doubt, a matter of chance that, in the mixed aryllead compounds on which this conclusion was based, these compounds all possessed more negative groups which at the same time were heavier than the phenyl or other group to which they were compared. The furyl and thienyl radicals, though lighter than the phenyl radical, are more readily cleaved than the phenyl radical. Furthermore, the furyl radical cleaves in preference to the thienyl radical.[42] In this investigation it has been shown that the substituted aryl groups cleave in preference to the phenyl group.

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[41] Krause and von Grosse, "Die Chemie der metall-organischen Verbindungen," Gebruder Bomtraeger, Berlin, 1937, pp. 419-420.

[42] Gilman and Towne, Rec. trav. chim., 51, 1054 (1932). For pertinent citations on previous cleavage studies see reference 1 of this paper. See, also, Kharasch and Flenner, J. Am. Chem. Soc., 54, 674 (1932).

SUMMARY

1. Some halogen-metal interconversion studies have been reported.
2. A general procedure for the preparation of hitherto inaccessible organolead compounds has been devised.
3. A theory for the formation of certain by-products has been presented.
4. Some cleavage studies with hydrogen chloride have been reported.

PART II.

AZO LEAD DYES AS POSSIBLE ANTICARCINOGENICS

HISTORICAL

The history of the application of lead and its compounds to the treatment of cancer is both interesting and informative. From his observations,[43] the first on the use of lead in cancer therapy, Bell concluded that metallic lead has a selective action upon sex cells, blood cells, nerve and embryonic cells, and tissue containing an abundance of phosphatides, especially lecithin. The action is general in young, vigorously growing tissue and local in adult cells. More lead accumulated in the cancerous tissue of men and dogs than in any part of the body except the gonads. Bell suggested a direct chemical combination between lead and the phosphatides. Ensuing investigations[43] gave varying degrees of success, and in addition, the toxic side action of the required therapeutic doses militated against an extended use of this method of treatment. Bell's publications stimulated

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[43] Bell, Brit. Med. J., 1926 I., 687 [C.A., 20, 2700 (1926)];
Wood, J. Am. Med. Assoc., 87, 717 (1926) [C.A., 21, 136 (1927)];
Shreiner and Wende, Surgery Gynecol. Obstet., 48, 115 (1929) [C.A., 24, 425 (1930)];
Newman, J. Cancer Res. Comm. Univ. Sidney, I, 101 (1929) [C.A., 24, 895 (1930)];
Roffo, Neoplasms, 8, 270 (1929) [C.A., 24, 3277 (1930)].

clinical trials as well as attempts to cure experimental carcinoma with lead. From the chemist's point of view, the most interesting feature is the form or type of compound in which the lead is administered; hence, in this discussion the compounds which have hitherto been investigated will be grouped according to structural relationships. These include salts of inorganic acids, salts of organic acids, complex compounds, and organolead compounds.

Salts of Inorganic Acids

Bischoff and Blatherwick[44] made a preparation of colloidal lead phosphate in gelatin solution containing 0.4 per cent lead. The preparation, injected intravenously into rabbits, caused no decided fall in hemoglobin count and was less toxic than lead acetate, colloidal lead or lead oxide. Ionic lead mixed with serum or red blood cells containing enough phosphate to react with the lead also was non-toxic.

According to Ulman[45] insoluble lead phosphate has essentially the same effect on tumors as colloidal-metallic lead, is much less toxic, has no effect on the kidneys and, in most instances, little effect on the blood. The preparation is stable and does not become more toxic with age.

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44. Bischoff and Blatherwick, J. Pharmacol., 31, 361 (1927) /C.A., 22, 3233 (1928)/.
45. Ulman, Southwestern Med., 13, 73 (1929) /C.A., 23, 3742 (1929)/.

The double phosphate of lead and calcium was found even less toxic and apparently as effective therapeutically.[46]

Since ionic lead could react with inorganic constituents in the blood, the toxicity of lead combinations that might result as intermediate reaction products was determined.[47] These included basic lead carbonate, dilead phosphate, lead oxychloride, lead sulfide, lead glycerophosphate and lead carbonate. All were toxic and hence of no therapeutic value.

Bargen and co-workers of the Mayo Clinic had less favorable results with lead phosphate. Of the eighty cases treated, sixty patients died and fourteen were entirely cured. Of the fourteen that were cured, seven had been given radiation treatment in addition to the lead.[48]

Potassium trichloroplumbate is reported as favorably successful by Collier and Kraus.[49] The lead salts of silicic, boric, and molybdic acids have only a slight inhibitory action.[50]

Schürch treated thirty-two carcinomatus patients with a 0.5% solution of lead iodide dissolved in 10% calcium gluconate and was able to produce only temporary improvement.[51]

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[46] Ulman, *Radiology*, *13*, 403 (1929) [*C.A.*, *24*, 1428 (1930)].

[47] Bischoff, *J. Pharmacol.*, *34*, 85 (1928).

[48] Bargen, *Am. J. Cancer*, *23*, 762 (1935).

[49] Collier and Kraus, *Z. Krebsforsch.*, *34*, 526 (1931).

[50] Collier, *Z. Hyg. Infectionskrankh.*, *110*, 236 (1929) [*C.A.*, *34*, *2183* (1930)].

[51] Schürch, *Deut. Med. Wochschr.*, *39*, 1494 (1933) [*C.A.*, *29*, 1773 (1934)].

Twenty to thirty mg. per kilogram of body weight caused the patients to lose weight and hemoglobin, but normalcy was again attained in four to five weeks.

More recently Woodhouse reported no success with lead carbonate and lead pyrophosphate.[9]

On the whole, lead salts, particularly the phosphates, are less toxic than metallic lead, but the therapeutic results leave much to be desired.

Salts of Organic Acids

One of the earliest extensive studies on lead salts of organic acids was made by Collier.[50] Lead formate was found most effective, curing 35.3% of the cases tested and having a retarding action on 64.7%. The following table lists the compounds investigated and the results obtained.

Table I. Effect of Lead Salts of Simple Organic Acids.

Acid	No. of animals	Per cent unaffected	Per cent affected	Per cent cured
tannic	19	63.2	36.8	26.3
formic	17	35.3	64.7	35.3
oxalic	16	75.0	25.0	18.7
oleic	18	77.8	22.2	16.6
maleic	18	61.2	38.8	33.3
phthalic	21	80.9	19.1	14.3
citric	16	68.8	31.2	18.7
salicylic	14	78.6	21.4	7.1
tartaric	15	80.0	20.0	13.3
naphthalene-sulfonic	17	76.5	23.5	7.1
fumaric	17	76.5	23.5	5.9
stearic	20	90	10.0	5.0

Rothman[52] converted various mercaptopyridine carboxylic acids into heavy metal salts containing the metal in the mercapto groups by reaction with metal oxides, hydroxides, or salts. The lead compounds were found effective in arresting metastases in transplanted rabbit carcinoma when applied early and repeatedly.[53] The same compounds protected against Row-sarcoma[54] and have a relatively low toxicity.

Dalimier and Schwartz found that triamyllead phenylacetate had a favorable influence in five isolated cases.[55]

Since selective intoxication of neoplasms seemed desirable, Eggers turned to carbohydrate-containing lead compounds. He prepared lead salts of carbohydrates derived from hexose and bearing a carboxyl group.[56]

Table II. Effect of Carbohydrate-containing Lead Salts.

Salt	Dose (mg)	No. of rats	No. cured
Gluconate	5	4	1
Gluconate	8	1	0
Gluconate	5	4	2
Gluconate	5	5	1
Gluconate	5	4	1
Glycuronate	5	4	4
Arabonate	4.3	3	1
Galactonate	4.1	4	4
Glucoheptonate	5	4	3
d-Mannonate	5	4	0
Rhamnohexonate	5	4	0

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52. Rothman, Brit. patent 380,083, Sept. 5, 1932 [*C.A.*, 27, 403 (1933)].
53. Collier, *Klin. Wochshr.*, 1932, 235.
54. Zadick, *ibid.*, 1933, 1145.
55. Dalimier and Schwartz, *Presse med.*, 42, 922 (1934) [*C.A.*, 28, 6849 (1934)].
56. Eggers, *Arch. Path.*, 18, 507 (1934) [*C.A.*, 29, 843 (1935)].

The tabulated data show the results on FRC carcinoma. The compounds were less effective against R39 rat-sarcoma.

Datnow prepared a series of lead salts, mostly derivatives of glycine.[2] The salts were dissolved shortly before use with the addition of gum arabic and sodium thiosulfate. The results are tabulated below.

Table III. Effect of Lead-containing Glycine Derivatives.

Salt	Clinical reactions
Lead benzenesulfonylglycinate	favorable
Lead p-toluenesulfonylglycinate	no value
Lead p-iodobenzenesulfonylglycinate	favorable
Lead toluene-2,4-disulfonylglycinate	very favorable
Lead m-carboxybenzenesulfonylglycinate	fair
Lead 4-acetylaminophenetole-2-sulfonylglycinate	no value
Lead fumarate	no value
Lead 2,6-dithiolisonicotinate	produces thrombosis
Sodium-lead complex of o-thiolbenzoic acid	fair
Lead phenylthioglycollate	fair

More recently Schmidt[3] made a study of the following lead salts:

1. Sodium salt of lead catecholdisulfonic acid
2. Sodium-calcium salt of lead catecholdisulfonic acid
3. Calcium lead gluconate
4. Lead salt of yeast nucleic acid
5. Lead salt of thymus nucleic acid
6. Lead salt of nucleic acid from mouse carcinoma
7. Lead salt of nucleic acid from rabbit carcinoma

8. A lead arsenostibio compound
9. Compound formed from lead and 3-amino-4-hydroxyphenylarsine
10. Selenourea lead chloride.

These compounds were tested on mouse cancer and to some extent on Brown-Pearse tumor in rabbits. Only compound "6" gave a retarding reaction and that very feeble.

A desire to decrease the toxic effect of lead by administering it in masked form led to the preparation of more complex compounds.

Complex Compounds

Complex compounds of lead have been subjected to fewer investigations than any of the types mentioned. Woodhouse[57] studied the therapeutic value of lead trypan blue, lead trypan red, and lead pyrrole blue. He also prepared lead fluorescein, lead nucleotide, and lead neosalvarsan. These compounds were tolerated in fairly large doses but had little or no inhibiting effect on cancerous growths.

When lead salts, lead oxide or lead hydroxide are treated with hydroxycarboxylic acids and aliphatic or hydrogenated heterocylic bases in aqueous medium, complex organic lead compounds are formed. Compounds of this type have some value as anticarcinogenics.[58]

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57. Woodhouse, Am. J. Cancer, 27, 285 (1936) /C.A., 30, 7217 (1936)/.
58. Streitwolf, I. G. Farbenind. A. G., Ger. patent 522,789, July 22, 1928 /C.A., 25, P 3776 (1931)/.

Organolead Compounds

Recently organolead compounds have attracted considerable attention as possible therapeutic agents for neoplasms.

Collier[59] studied eleven of the organolead compounds prepared by Krause.[60] The results are given in the following table.

Table IV. Effect of Some Organolead Compounds.

Compound	No. of animals	Distinct effect (%)	Complete healing (%)
Tri-_n_-propyllead fluoride	14	71.4	50.0
Tri-_i_sobutyllead bromide	28	60.7	21.4
Tri-isoamyllead bromide	13	61.5	7.7
Tetraphenyllead	22	59.1	40.9
Triphenyllead	18	16.6	5.5
Triphenyllead bromide	13	7.7	0.0
Triphenyllead fluoride	20	55.0	25.0
Diphenyllead dibromide	15	46.6	6.6
Tricyclohexyllead	13	53.8	30.7
Tricyclohexyllead iodide	16	43.8	25.0
Triphenylcyclohexyllead	20	20.0	15.0

The toxicity of the compounds was dependent upon their structure rather than solubility in water. Tri-_n_-propyllead fluoride was most effective.

Buck and Kumro[61] conducted a series of toxicity studies on various types of organolead compounds. They found that tetramethyllead has a relatively low toxicity and placed some

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[59] Collier, _Z_. _Hygiene_, _110_, 169 (1928).
[60] Krause, _Ber_., _62_, 135 (1929).
[61] Buck and Kumro, _J_. _Pharm_. _Exp_. _Therapeut_., _40_, 85 (1930).

hope on organolead compounds containing two or three methyl groups and having the remaining valencies of lead satisfied by an aromatic nucleus with amino or hydroxyl substituents.

Another series of toxicity studies is reported by Gilman and co-workers.[62] The following table gives the results obtained.

Table V. Toxicity of Some Organolead Salts.

Compound	Solubility (%)	M.T.D. (mgs.)	M.L.D. (mgs.)
Triethyllead *p*-toluenesulfonate	0.5-1.0	12-15	15
Triethyllead salicylate	0.25	15-18	18-20
Triethyllead phenylacetate	0.3	12-15	15
Triethyllead furoate	0.3-0.4	12-15	15
Triethyllead *p*-aminobenzoate	0.5-1.0	12-15	15
Triethyllead furylacrylate	0.3-0.5	12-15	15-18

One of the later and more extensive studies of organolead preparations for cancer therapy is that reported by Schmidt.[3] In addition to the ten salts mentioned earlier, seven organolead compounds were tested on experimental mouse cancer and to some extent on Brown-Pearse tumor in rabbits.

1. Trimethyllead chloride
2. Tetraphenyllead
3. Dimethyllead oxide
4. Trixylenyllead
5. Sodium salt of diphenyllead catecholdisulfonic acid

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[62] Gilman and Gruhzit, *ibid.*, *41*, 1 (1931); Gilman, Gruhzit, Robinson and Towne, *Proc. Iowa Acad. Sci.*, *37*, 248 (1930).

6. Sodium calcium salt of diphenyllead catecholdisulfonic acid
7. Sodium salt of diaminodiphenyllead catecholdisulfonic acid.

A distinct action could be obtained only with compounds 5 and 6 and to a lesser extent with 7. Using a radioactive isotope of lead in these compounds failed to increase their efficacy. The calcium was added from the pharmacological point of view as an antagonist to possible acute blood pressure symptoms during intravenous injections.

Schmidt concludes,

> Whether success will be obtained in endowing lead with a more specific action on cancer by progress in synthesis, only the future can show. The results obtained hitherto justify a certain doubt, and the more so in view of experience gained in the chemotherapy of infectious diseases that the more pronounced the metallic character of the element the less can fundamental changes in and increases of activity be obtained by chemical variations.

The latter statement, though substantiated to a certain extent, is not entirely justified. A striking case in point is mercury and its derivatives. Theoretically mercury is more metallic in character than lead and metallic mercury is extremely toxic; nevertheless, numerous mercurials are used as internal medicinals.

One approach toward increasing the specific action of lead on cancer is the synthesis of lead-containing dyes which selectively stain cancerous tissue.

The following section is an attempted correlation of the structure of dyes with tissue-staining properties.

Correlation of the Structure of Dyes with Staining Properties

Carmine, a naturally occurring dye, was the first to be used in microscopic work. Hill,[63] in 1770, employed this dye in accentuating the visual structure of timber under the microscope. There is no reference to the use of this or any other dye in microscopic staining techniques until 1836 when Ehrenberg used powdered indigo and carmine in the study of living microscopic organisms. Their use became common around 1850. The aniline dyes were produced commercially about 1856.

The hundreds of dyes which have since been marketed as a result of extensive synthetic research have resulted in a vast amount of study on staining technique and applications. Examination of this material shows that the ability of a dye to stain tissue is often more dependent upon the properties of the tissue than upon the dye itself, and because of this the following pages will treat the variations in tissue and staining procedures as well as the staining properties which are attributed to the dye alone.

The following table contains a list of the most important dyes and some of their physical properties which are important in dye classification.

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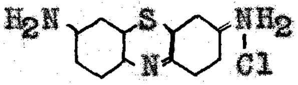
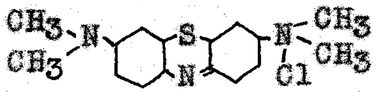
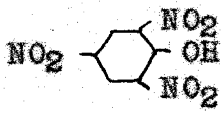
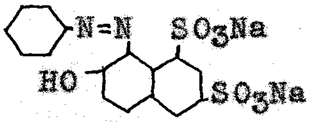
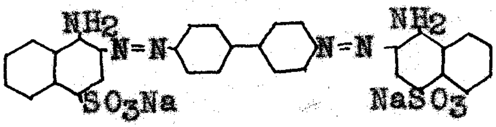
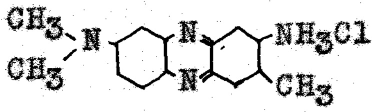
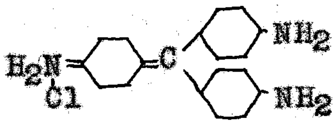
63. Hill, Dissertation, London (1770).

Table VI. Structure and Physical Properties of Dyes

Name	Formula	Absorption maxima	Absorption ratio	Solubility at 26° in alcohol (%)	Solubility at 26° in water (%)
Thionin		602	0.51	0.25	0.25
Methylene blue		667	0.59	1.48	3.55
Picric acid		360		8.96	1.18
Orange G		485	5.24	0.22	10.86
Congo red		497		0.19	
Neutral red		526	0.75	2.45	5.64
Basic fuchsin		540	0.755	5.93 to 8.16	0.26 to 0.39

(Continued)

Table VI (continued).

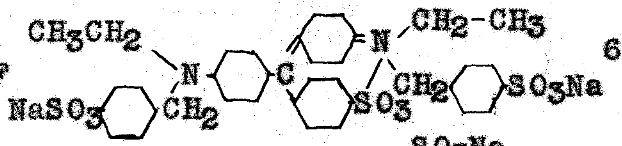
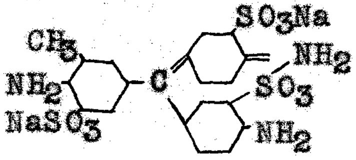
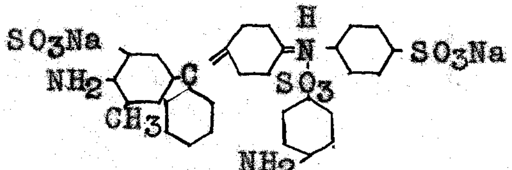
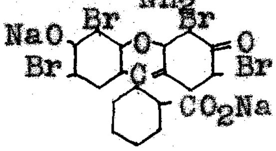
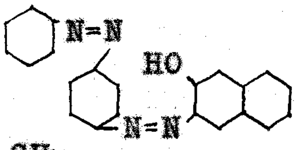
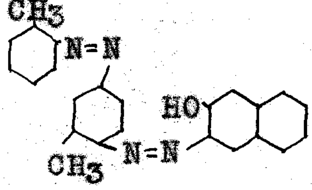
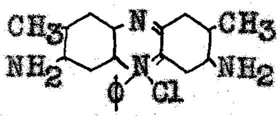
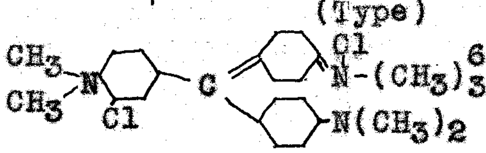
Name	Formula	Absorption maxima	Absorption ratio	Solubility at 26° in alcohol (%)	Solubility at 26° in water (%)
Light green SF yellowish		633.5	0.98	0.82	20.35
Acid fuchsin		545	0.81		
Aniline blue W.S.		600	1.08		Soluble
Eosin y		516	0.92	2.18	44.2
Sudan III		641	1.11	0.15	0.00
Sudan IV		657.4 with SO_4	0.97	0.09	0.05
Safrinin O		515	1.02	3.41	5.45
Methyl green	 (Type)	633.8	0.90		

Table VI (continued).

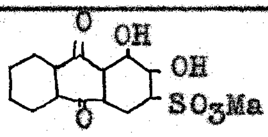
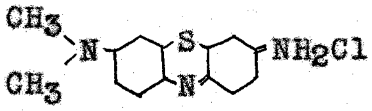
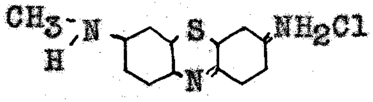
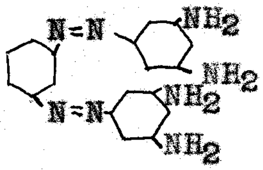
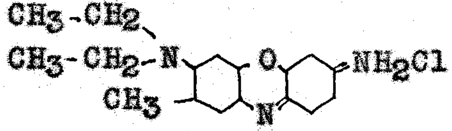
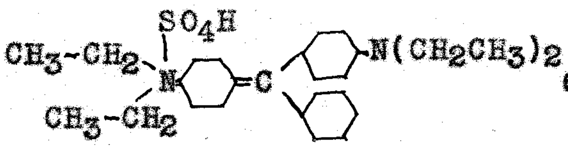
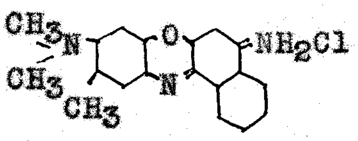
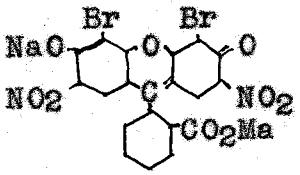
Name	Formula	Absorption maxima	Absorption ratio	Solubility at 26° in alcohol (%)	Solubility at 26° in water (%)
Alizarin red S		566-5	1.21	6.15	7.69
Azure A		638			
Azure C		611			
Bismarck brown y			1.88	1.08	1.36
Brilliant cresyl blue		631.8	0.90		
Brilliant green		623	1.51		
Cresyl violet		585	1-16	0.25	0.38
Eosin blue		521.5	0.92	0.75	39.11

Table VI (continued).

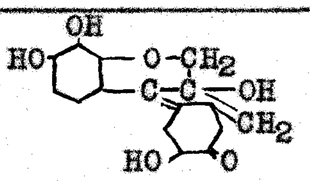
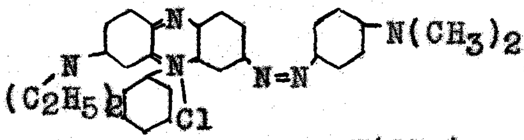
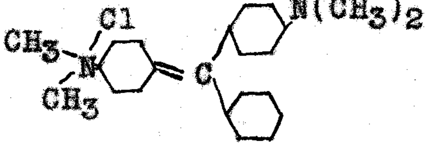
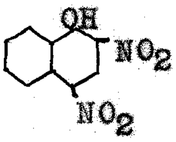
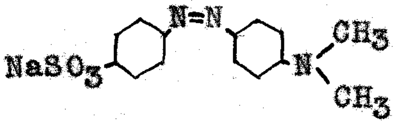
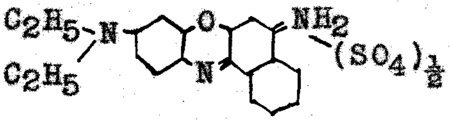
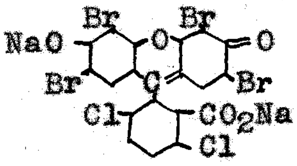
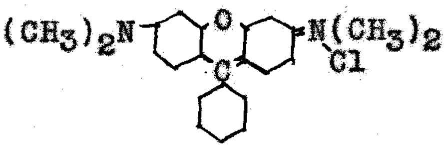
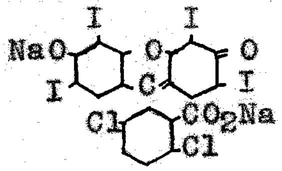
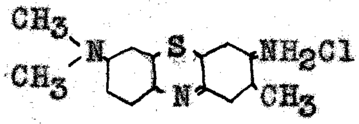
Name	Formula	Absorption maxima	Absorption ratio	Solubility at 26° in alcohol (%)	Solubility at 26° in water (%)
Hematein					
Janus green B		592.7	6.97	1.12	5.18
Malachite green		616	0.83		
Martius yellow		445	14.8	0.16	4.5
Methyl orange		506		0.08	0.52
Nile blue A		644.5	0.90		
Phloxine		535.7		9.02	50.9
Pyronine		545	7.75	0.60	8.96

Table VI (concluded).

Name	Formula	Absorption maxima	Absorption ratio	Solubility at 26° in alcohol (%)	Solubility at 26° in water (%)
Rose bengal		548	0.92	7.53	36.25
Toluidine blue		635	1.04	0.57	3.82

In the above table each absorption maximum is expressed in wave length (mu) and is the wave length of light which corresponds to the highest value of the extinction coefficient in the plot, wave length vs. extinction coefficient. The absorption ratio, R, is defined as the ratio of the extinction coefficients of the solution of the colored substance at two specific wave lengths. These two values are important in the determination of the purity of dyes and in defining the spectral position of the absorption band. The nature of the acidity or basicity, as indicated by the formulas, will later be shown to be important in relation to tissue-staining properties. Solubilities are also extremely important. This list of dyes is on the certification basis, Jan. 1, 1936.[64]

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[64] Holmes and Peterson, Stain Tech., 5, 65 (1930).

There are three physical factors which together or separately can explain many staining phenomena. The first of these is the ability of all more or less porous substances to be penetrated by virtue of their porosity or through osmosis. Regarding these two properties of tissues it is obvious that the particle size of the dye in solution is very important. Solubility (table VI) must also be a very important factor since a dye of limited solubility must be considered inferior from the point of view of limited applicability. Secondly, the action of adsorption can account for many staining phenomena. In the third place, a dye may penetrate tissue by straight-forward absorption and then be coagulated in a solid state.

From the physical point of view the theory of adsorption seems to be the most plausible explanation for selective staining. It is a well-known principle that certain substances show selective adsorption tendencies for certain ions.

It is equally well known that ions have a marked effect on adsorption properties. In the field of dyes, Wertheimer[65] has demonstrated this effect. Neutral salts modify the solubility of basic dyestuffs according to the anion series $SCN > I > Br > NO_3 > Cl > SO_4$, acetate and citrate; SCN has the strongest precipitating action. For the precipitation of colloidal

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[65] Wertheimer, _Arch_. _ges_. _Physiol_. (Pflugers), _202_, 383-94 (1924).

acid dyestuffs the cation series is $Ca > Mg > Na > K > NH_4$. The precipitation of basic dyes by neutral salts varies with the concentration of the dye and with the temperature. High temperatures inhibit precipitation while high concentrations of dyes favor precipitation. With frog tissue the capacity to stain is best explained by polar adsorption according to the following principles: The stainability of basic dyes is favored by anions in proportion to their valence and is diminished by cations. The reverse is true with acid dyes. Hydrogen ions act like multivalent cations, while hydroxyl ions act like anions.

It is difficult to determine whether certain staining mechanisms should be classified as chemical or physical in nature, and hence it is unwise to attempt such a distinction in all cases. Drawert[66] has studied the relation of pH to the adsorption and distribution of acid and basic dyes in plant tissue. A difference in behavior between the cell wall and plasma was explained by the fact that the cell wall is much more weakly buffered than the plasma of its cell. The cell wall is ordinarily not stained by acid dyes. Basic dyes stain only in the pH range which is limited by the discharging point of the cell wall and the transition point of the dye. The cytoplasm, plastids and nucleus take up basic dyes independently of the transition point of the dye from their

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[66] Drawert, Flora, 32, 91 (1937).

isoelectric points to well into strongly alkaline reaction. Various effects of salts on staining are explained by the effects of the salts on the pH of the medium. Pulcher[67] contributed some information where pH of staining solution vs. isoelectric point of tissue is concerned. The results of his work showed that the critical point of staining was displaced only -0.7 pH unit from the known isoelectric point. His conclusion was that the protein must be at the isoelectric point in order to take the dye most effectively.

Since hydrogen-ion control has been found essential to consistent results with most stains, some information on buffer mixtures is necessary. French[68] has worked out a series of buffer solutions containing ingredients, as shown in table VII, which cover a range of pH from 5.3 to 9.2.

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67. Pulcher, Boll. soc. ital. biol. spec., 2, 223-4 (1927).
68. French, Stain Tech., 5, 87 (1930).

Table VII. Composition of Buffer Solutions.

pH	KH_2PO_4	Na_2HPO_4-H_2O
5.3	0.227 grams*	11.583 grams*
5.6	0.454 "	11.286 "
5.9	0.908 "	10.692 "
6.2	1.816 "	9.504 "
6.5	2.723 "	8.316 "
6.8	4.539 "	5.940 "
7.0	5.447 "	4.752 "
7.2	6.355 "	3.564 "
7.4	7.262 "	2.376 "
7.7	8.170 "	1.188 "
8.0	8.624 "	0.594 "

Table VII (continued).

pH	$Na_2B_4O_7$-$10H_2O$	H_3BO_3	NaCl
8.2	6.69 grams	8.00 grams	1.89 grams
8.4	8.59 "	6.82 "	1.59 "
8.6	10.51 "	5.58 "	1.31 "
8.8	13.37 "	3.72 "	0.87 "
9.0	15.28 "	2.48 "	0.58 "
9.2	19.10 "		

*These amounts were dissolved in 100 ml. of water.

Further insight into the nature of staining was accomplished by Craig and Wilson[69] of the University of California. Their work is in accord with the theory of chemical combination of dyes on the acid side of their isoelectric points. If the pH of the medium is basic to the isoelectric point of the protein, the latter will combine with a dye cation or basic dye; whereas, if the pH is acid to the isoelectric point of the protein, it will combine with a dye anion or an acid dye. If the pH of the solution is fixed at four, then all proteins whose isoelectric points are less than four will stain with a basic dye. In general, there will be many proteins of different isoelectric points in any tissue, and its acidic or basic character is determined by the average value of the isoelectric points. The isoelectric point of a tissue may be very nearly determined by the use of dyes.

Investigation reveals the complexity in developing staining techniques. It is not possible to stain the cell nucleus, which is acid in character, with an acid dye without staining the cytoplasm. The entire tissue may be stained and then treated with the buffered dye which will remove stain from all proteins whose isoelectric points are less than the pH of the buffer; thus the nucleus may be destained. In order to stain the nucleus alone, it is necessary to use a basic dye which will combine with more and more proteins the

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69. Craig and Wilson, Stain Tech., 12, 99 (1937).

more basic the solution, until the nucleus is completely stained. The usual basic dyes are the amines. The amino group, however, has a strong tendency to add hydrogen ions and act as an anion, usually combining with proteins acting as cations. This further complicates any simplified correlation.

The fact that the acid dyes as a group show variation in staining ability in a solution of constant pH would indicate that the acid dyes might be classified with respect to one another, and the basic dyes in a similar manner. However, there seem to be too many variables involved, and available information is so intermingled that an accurate classification cannot be made.

Consideration of the heretofore presented material brings one to conclude that the selective staining of each tissue by the choice of an adequate dye and staining technique is a distinct and involved research problem in itself. The approach to each problem can be guided in part by the material referred to.

The following table includes a list of important dyes, together with their biological application. The reference numbers refer to the bibliography at the end of this section.

Table VIII. Dye Applications and References.

Name of stain	Application	Bibliographic reference to technique
Picric acid	For cytoplasm. Contrast stain	71
Martius yellow	For cancer tissue; with acid fuchsin and malachite green	70
Orange G	Counterstain to thionin; in plant pathology	79
Sudan R	Employed in a modification of the Kahn test for syphilis	72
Janus green	For sections of embryos; with neutral red	73
Methyl orange	For determining reaction of cell sap in plants	76
Sudan III	For fat in tissues	74
Sudan IV	For fat in tissues	75
Bismarck brown y	Chromosomes in smear prep. of eggs	76
Congo red	For sections of embryos	77
Trypan red	For vital staining	74
Alizarin	To distinguish cow's milk from goat's milk	78
Thionin	For staining bacteria and fungi in plant tissue	79
Azure A	As nuclear stain; with eosin and orange II	80
Azure B	For negri bodies	81
Methylene blue	Nuclear stain	74

(Continued)

Table VIII (continued)

Name of stain	Application	Ref. No.
Methylene green	For wood and fixed chromatin	
Toluidine blue O	Diagnosis of diphtheria	82
Brilliant cresyl blue	As a vital stain	83
Nile blue sulfate	Supravital stain for embryos	84
Neutral red	Stain for parasites in tissue	85
Cresyl violet	For fresh tumor tissue	86
Safranin O	For Chromatin; with light green	87
Malachite green	With Martius yellow; for cancer tissue	88
Light green SF yellowish	For general histological tissue	88
Basic fuchsin	Powerful nuclear stain	71
Aniline blue	For connective tissue	89
Acid fuchsin	Plasma stain and for connective tissue	89
Pyronin Y	Stain for gonorrhoeal pus	90
Eosin Y	Cytoplasm stain	91
Eosin ethyl	Stain for Negri bodies in smears of nervous tissue	92
Phloxine	For staining algae	93
Victoria blue	For granules in virus disease	94

(Continued)

Table VIII (continued)

Name of stain	Application	Ref. No.
Victoria blue B	For nervous tissue	95
Auramin	For plant sections	96
Indulin	For negative staining of bacteria	97

The involved nature of each application can be shown by a study of the application of dyes in the cancer problem for the purpose of early diagnosis.[5,70] The three fundamentals of the problem are the discovery of some unique property of the cancer cell, a proper dye to serve as indicator, and a procedure for tissue examination. Engel has shown that many inoculated tumors take as vital stains several acid dyes of the triphenylmethane group (fuchsin S, red violet, light green, and isamine blue). The coloring occurs in the living tumor cell and not in the necrotic cells.

The result of this survey shows that few certain predictions can be made beyond the principle of basic-tissues-acid-dye-affinity and _vice versa_. Each problem involves sufficient variables to merit separate detailed consideration.

The problem becomes still more complicated when the preferential staining of cancerous tissue, _in vivo_, is considered.

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70. Engel, _Z. Krebsforsch._, _22_, 365-72 (1925).

When inoperable cancers are involved, the dye cannot be applied directly. If the dye is administered into the body remote from neoplasm, the intermediate metabolic fate of the administered compound complicates the picture. Only by continued, untiring efforts in the research laboratories can we hope to find a solution to the problem of cancer cure.

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92. List, *Z. Wiss. Mikr.*, *2*, 145 (1885).

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94. Chamberlain, *Stain Tech.*, *2*, 91 (1927).

95. Herzberg, Abs. in *Stain Tech.*, *10*, 67 (1934).

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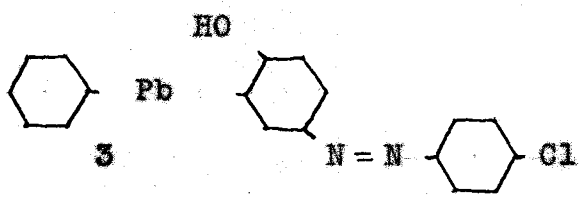
EXPERIMENTAL

Two avenues of approach were open for the preparation of organolead compounds containing an azo linkage joi[illegible] to the lead atom through carbon atoms. First, aminoaryllead compounds could be diazotized and coupled with various hydroxyl, methoxyl, amino, and alkylamino compounds. Second, primary amines could be diazotized and coupled with organolead compounds having functional groups capable of making the compounds amenable to diazo coupling. The experimental results obtained, when these possibilities were tried, are described in this section.

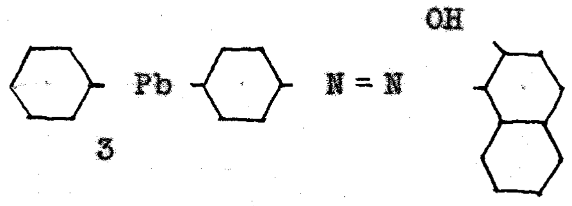
In naming the compounds that were prepared, the following system was used for each of the three chief types.

Type 1.



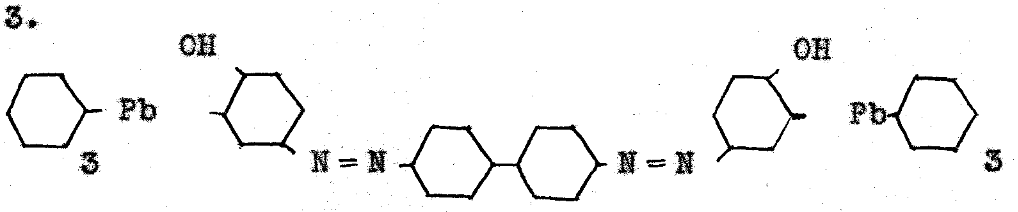
Triphenyl-1-[2-hydroxy-5-(*p*-chlorophenylazo)-phenyl]-lead

Type 2.



Triphenyl-1-(2-hydroxynaphthyl)-azophenyl-4-lead

Type 3.



p,*p*'Biphenylenedi-(5-azo-2-hydroxyphenyltriphenyllead)

Triphenyl-1-(2-hydroxynaphthyl)-azophenyl-4-lead

The reported[4] diazotization of triphenyl-*p*-aminophenyllead and subsequent coupling with β-naphthol were carried out in dilute hydrochloric acid. Since the yield of dye was very small, a better means of diazotization was sought.

When the aminoaryllead compound was dissolved in ethanol saturated with dry hydrogen chloride, treated with isoamyl nitrite at 0°, and then poured into a sodium hydroxide solution of β-naphthol, the yield of dye was no better. The same held true when 50% sulfuric acid was used as the reaction medium.

The following procedure gave the best results. Five-tenths of a gram of triphenyl-*p*-aminophenyllead was dissolved in 10 ml. of glacial acetic acid cooled to the freezing point. Sodium nitrite was then slowly added with stirring. The temperature was held between 15° and 20° for 15 minutes and the mixture was then poured into a sodium hydroxide solution containing 0.5 g. of β-naphthol. The solid which separated was filtered off and crystallized from dilute ethanol. The yield of dye melting at 135° (dec.)[4] was 0.2 g. or 35%.

Attempted Preparation of Triphenyl-1-(2-hydroxy-6,8-disulfononaphthyl)-azophenyl-4-lead

When 0.8 g. of triphenyl-*p*-aminophenyllead was diazotized as described in the previous section and the diazonium solution was poured into a basic solution of 0.7 g. of 2-naphthol-6,8-disulfonic acid, a red solution was obtained. The solid material was filtered off and the colored filtrate saturated with sodium chloride. Only traces of a red dye could be salted out in this manner. The residue from the reaction mixture contained a small amount of triphenyllead acetate and also some tetraphenyllead.

The same result was obtained with 2-naphthol-3,6-disulfonic acid.

Attempted Preparation of Triphenyl-2-(1-amino-3,6,8-trisulfononaphthyl)-azophenyl-4-lead

To a solution of 2.0 g. of triphenyl-*p*-aminophenyllead in 20 ml. of glacial acetic acid cooled to 15° was gradually added 1.0 g. of sodium nitrite. After fifteen minutes the mixture was poured into an aqueous solution containing 2.0 g. of 1-naphthylamine-3,6,8-trisulfonic acid and enough sodium hydroxide to almost neutralize the glacial acetic acid. The reaction mixture was allowed to come to room temperature and was then filtered. The colored filtrate was saturated with sodium chloride. The red dye which separated was collected on a filter and subjected to a qualitative test for lead. No lead was present; hence the dye probably resulted from the reaction of the diazonium salt of 1-naphthylamine-3,6,8-trisulfonic acid or the coupling of benzenediazonium chloride, resulting from the cleavage of triphenyl-p-aminophenyllead, with 1-nathphylamino-3,6,8-trisulfonic acid.

Triphenyl-1-(2-hydroxynaphthyl)-azophenyl-2-lead

To a solution of 2.0 g. of triphenyl-o-aminophenyllead in glacial acetic acid cooled to 15° was added 1.0 g. of sodium nitrite. The mixture was allowed to stand for 15 minutes and was then poured into an alkaline solution containing 1.0 g. of β-naphthol. The yellow solution was filtered and the residue crystallized from ethanol. No appreciable quantity of dye was isolated. The alcohol-insoluble fraction was crystallized from chloroform. A mixed melting point with tetraphenyllead showed no depression. The alcohol-soluble portion yielded triphenyllead acetate, and on dilution with petroleum ether, triphenyl-o-hydroxyphenyllead.

Triphenyl-1-/2-hydroxy-3,5-di-(p-nitrophenylazo)-phenyl7-lead

p-Nitroaniline (5.0 g.) was diazotized in the usual manner. The diazotized solution was then added to a mixture of 5.3 g. (0.01 mole) of triphenyl-o-hydroxyphenyllead, 20 ml. of ethyl acetate, 20 ml. of 95% ethanol, and 40 ml. of 10% sodium hydroxide solution. A deep, blood-red color was immediately obtained. The mixture was stirred rapidly until all the ethyl acetate had evaporated. This took about two hours. The solid residue was then collected on a filter and washed thoroughly with water and cold 50% ethanol. The dark red powder which remained on the filter was crystallized from ethanol containing a small amount of acetic acid. The compound decomposed above 180°. The yield was 7.1 g. or 86%.

Anal. Calcd. for $C_{36}H_{26}O_5N_6Pb$: Pb, 24.9; N, 10.1. Found: Pb, 24.6; N, 9.9.

The analysis of the compound showed that two molecules of p-nitrobenzenediazonium chloride had coupled with the lead compound.

Three grams of the dye was suspended in 10 ml. of hot water, and 8 g. of stannous chloride in 20 ml. of hot concentrated hydrochloric acid was added. The mixture was warmed until decolorization had taken place and was then filtered. The filtrate was made alkaline with sodium hydroxide and then extracted with ether. The water layer was next saturated with carbon dioxide and again extracted with ether. The ether extract was dried and the ether was evaporated. The residue was crystallized from dilute ethanol. The melting point was 79-80°. The picrate melted at 120°. This corresponds to the melting point of the picrate of 2,4-diaminophenol.[98] A mixed melting point with an authentic specimen[98] showed no depression.

The following equations show the structure determination:

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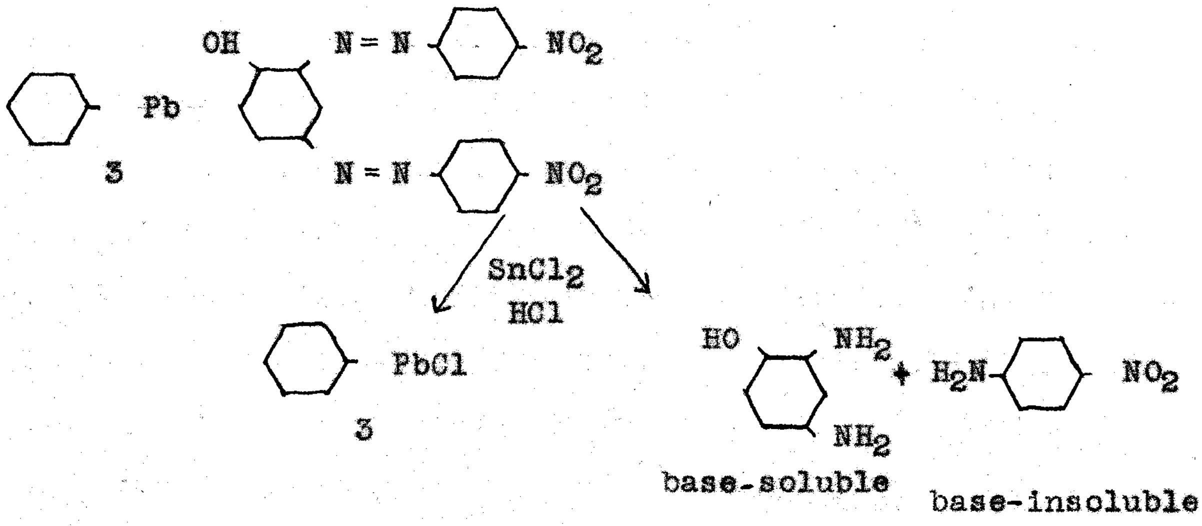
[98] Lumiere, Compt. rend., 116, 1204.



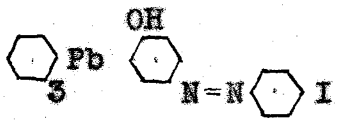
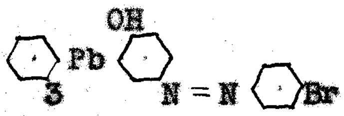
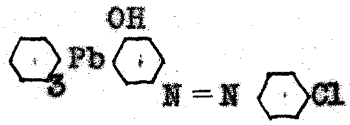
That this structure is correct is substantiated by the analysis, both for lead and nitrogen, and by the 2,4-diaminophenol isolated from the cleavage of the dye. Since *p*-nitroaniline is not directly involved in the structure determination, it was not isolated.

Triphenyl-1-[2-hydroxy-5-(*p*-iodophenylazo)-phenyl]-lead

To a suspension of 5.3 g. (0.01 mole) of triphenyl-*o*-hydroxyphenyllead in a solution containing 25 ml. of water, 3 g. of sodium hydroxide, 25 ml. of ethanol and 10 ml. of ethyl acetate was added 2 g. of *p*-iodoaniline diazotized in the usual manner. A red solid separated immediately. The mixture was stirred rapidly until all the ethyl acetate had evaporated. The solid was filtered; thoroughly washed with water, dried, and extracted with hot benzene. The benzene extract was evaporated to dryness and the residue was crystallized from a benzene-petroleum ether mixture. The yield of triphenyl-1-[2-hydroxy-5-(*p*-iodophenylazo)-phenyl]-lead was 5.4 g. or 71%.

Identical reactions were carried out using *p*-bromoaniline and *p*-chloroaniline. The results are summarized in Table IX. The dyes do not have a definite melting point but decompose slowly when heated above 150° or when left in the light at room temperature for several weeks.

Table IX. Results of the Coupling of Triphenyl-*o*-hydroxyphenyllead with Various Arylamines.

Compound	Yield		Pb analyses		Color	Solubility
	gm.	%	Calcd.	Found		
	5.4	71	25.9	25.5	Reddish brown	Insoluble in H_2O. Soluble in most organic solvents
	5.2	74	28.9	28.6	do.	do.
	5.1	75	30.9	30.8	Dark brown	do.

The structure of each of the dyes was proved by reduction with stannous chloride and subsequent isolation of *p*-aminophenol (m.p. 184°). Identity of the *p*-aminophenol was established by a mixed melting point with an authentic specimen.

The procedure was essentially the same as that described for the reduction of the dye resulting from the coupling of p-nitrobenzenediazonium chloride with triphenyl-o-hydroxyphenyllead.

Triphenyl-1-[2-hydroxy-5-(p-carboxyphenylazo)-phenyl]-lead

To a suspension of 5.3 g. (0.01 mole) of triphenyl-o-hydroxyphenyllead in a solution containing 25 ml. of water, 3 g. of sodium hydroxide, 25 ml. of ethanol and 15 ml. of ethyl acetate was added 4 g. of p-aminobenzoic acid diazotized in the usual manner. The mixture immediately became dark red and was stirred for two hours and then filtered. The filtrate was acidified with acetic acid. The solid which separated on acidification was collected on a filter and was washed with water. Crystallization from dilute ethanol gave a deep red dye decomposing above 190° and soluble in dilute alkali. The yield was 1 g. or 15%.

Anal. Calcd. for $C_{31}H_{24}O_3N_2Pb$: N, 4.1. Found: N, 3.9.

Several attempts were made to increase the yield of final product, but without success.

p,p'-Biphenylenedi-(5-azo-2-hydroxyphenyltriphenyllead)

To a suspension of 5.3 g. (0.01 mole) of triphenyl-o-hydroxyphenyllead, in a solution similar to that described in the preceding experiment, was added 1.8 g. of benzidine, tetrazotized in the usual manner. The mixture turned dark brown. After stirring for two hours, the mixture was filtered. The residue was washed with water, 95% ethanol, and chloroform, respectively. The brown solid which remained was insoluble in all the ordinary organic solvents and did not melt at 340°. The yield was 1.8 g. or 17%.

Anal. Calcd. for $C_{60}H_{46}O_2N_4Pb_2$: N, 4.88. Found: N, 4.95.

Attempted Preparation of Triphenyl-1-[2-hydroxy-5-(*p*-sulfonophenylazo)-phenyl]-lead

Several attempts were made to couple diazotized *p*-aminobenzenesulfonic acid with triphenyl *o*-hydroxyphenyllead. When the diazonium solution and a suspension of triphenyl-*o*-hydroxyphenyllead were mixed, no immediate coloration took place. After several hours the mixture turned slightly orange, but no lead-containing dye could be isolated. Triphenyl-*o*-hydroxyphenyllead was recovered almost quantitatively.

Triphenyl-1-[2-dimethylamino-5-(*p*-nitrophenylazo)-phenyl]-lead

To a vigorously stirred suspension of 5.58 g. (0.01 mole) of triphenyl-*o*-dimethylaminophenyllead in a solution containing 75 ml. of water, 75 ml. of ethanol, 50 ml. of ethyl acetate, and 3 g. of sodium acetate was added 2 g. of *p*-nitroaniline diazotized in the usual manner. A deep red color immediately appeared. After fifteen minutes air was bubbled through the mixture to expel the ethyl acetate. The gummy solid was filtered from the aqueous mixture and was dried on a porous plate. The dried material was boiled with ethanol to remove any of the starting lead compound, was then filtered and the residue was washed with water and dried. The yield of dark red dye was 4.2 g. or 70%.

Anal. Calcd. for $C_{32}H_{28}O_2N_4Pb$: N, 7.9. Found: N, 8.2.

Three grams of the dye was suspended in hot water (10 ml.) and 8 g. of stannous chloride in 20 ml. of hot concentrated hydrochloric acid was added. The mixture was warmed until decolorization was complete. The solid residue was collected on a filter. The filtrate was made basic with sodium hydroxide and then was extracted with ether. The ether layer was dried over sodium sulfate and the ether was evaporated. The crude bases were warmed on a water bath with 1 g. of acetic anhydride. This mixture was diluted with water and filtered. The filtrate was neutralized with sodium carbonate to precipitate p-dimethylaminoacetanilide. This compound, when crystallized from hot water, melted at 130°. A mixed melting point with an authentic specimen[99] showed no depression.

The following diagram shows the structure proof.

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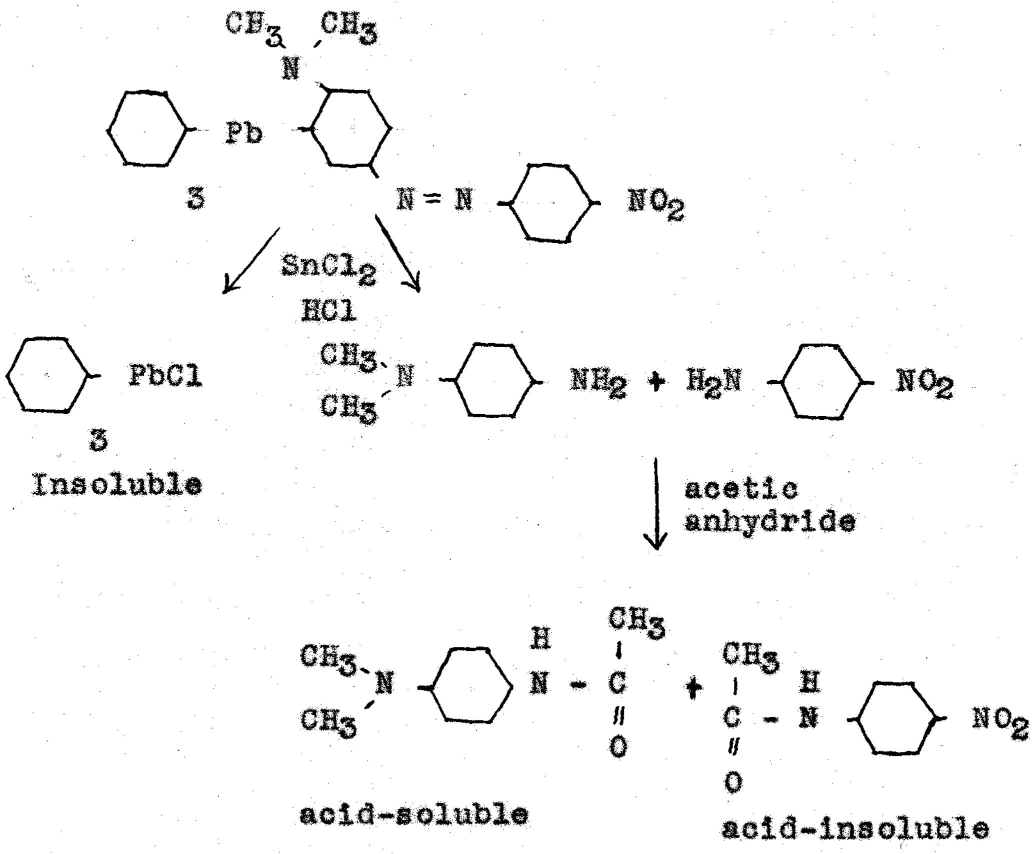
[99] Gattermann and Wieland, "Laboratory Methods in Organic Chemistry." The Macmillan Company, New York (1937), p. 302.



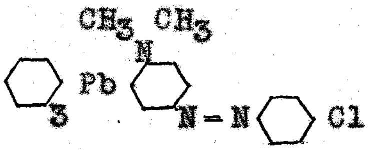
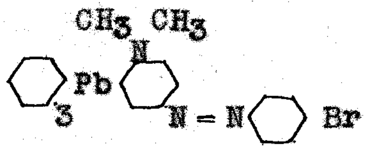
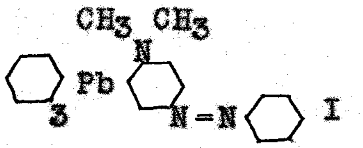
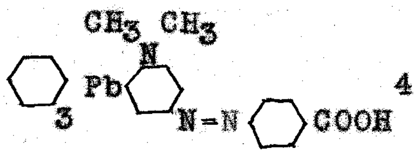
In this and the related dyes which follow, only the p-dimethylaminoacetanilide was isolated and identified by a mixed melting point with an authentic specimen. It is obvious that the other products obtained from the reductive cleavage of the dye are not important as far as structure determination is concerned.

Triphenyl-1-/2-dimethylamino-5-(p-chlorophenylazo)-phenyl7-lead

To a vigorously stirred suspension of 5.58 g. (0.01 mole) of triphenyl-o-dimethylaminophenyllead in a solution containing 75 ml. of water, 75 ml. of ethanol, 50 ml. of ethyl acetate, and 3 g. of sodium acetate was added 2 g. of p-chloroaniline subsequent to diazotization in the usual manner. A dark red color immediately appeared. After fifteen minutes air was bubbled through the mixture to expel the ethyl acetate. The solid material was then collected on a filter and washed thoroughly with water and ethanol. Purification was effected by crystallizing from hot alcohol.

Identical reactions were carried out using the diazonium salts of p-bromoaniline, p-iodoaniline and p-aminobenzoic acid. The results of all the experiments are tabulated as shown. The dyes decompose above 140°, but the decomposition point is not characteristic.

Table X. Results of the Coupling of Triphenyl-o-dimethylaminophenyllead with Various Arylamines.

Compound	Yield		Pb analyses		Color	Solubility
	gm.	%	Calcd.	Found		
	3.7	51	29.9	29.6	brownish red	Insoluble in H_2O. Soluble in most organic solvents.
	4.2	57	28.0	27.7	dark red	do.
	5.32	60	27.2	27.0	brown	do.
	4.06	57	29.8	29.6	bright red	Slightly soluble in dilute NaOH. Soluble in ordinary organic solvents.

The structures of the four dyes just described were proved by reductive cleavage with stannous chloride and concentrated hydrochloric acid. The procedure was identical with that used for the cleavage of triphenyl-1-[2-dimethylamino-5-(p-nitrophenylazo)-phenyl]-lead.

The critical product isolated in each cleavage was p-dimethylaminoacetanilide. After crystallization from hot water this compound melted at 130°.

Triphenyl-1-[4-methoxy-5-(p-nitrophenylazo)-phenyl]-lead

To a suspension of 5.5 g. (0.01 mole) of triphenyl-p-anisyllead in 75 ml. of 10% sodium hydroxide and 20 ml. of ethyl acetate was added 2 g. of diazotized p-nitroaniline. The brownish-red solid which formed was collected on a filter. Crystallization from 95% ethanol gave 3 g. or 33% of light brown dye melting at 180°.

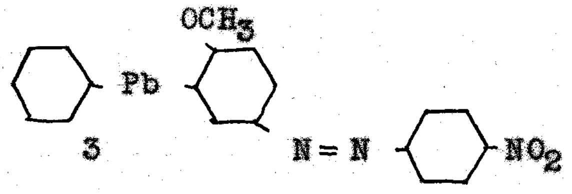
Anal. Calcd. for $C_{31}H_{25}O_3N_3Pb$: N, 6.7. Found: N, 6.8.

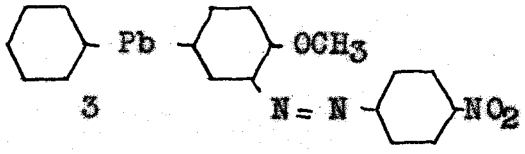
Triphenyl-1-[2-methoxy-5-(p-nitrophenylazo)-phenyl]-lead

When triphenyl-o-anisyllead was treated in the same manner as described in the preceding experiment for triphenyl-p-anisyllead, a light brown dye was obtained in 45% yield. The melting point of the dye was 178°.

Anal. Calcd. for $C_{31}H_{25}O_3N_3Pb$: N, 6.7. Found: N, 6.5.

The structures of the coupling products of p-nitrobenzenediazonium chloride with triphenyl-o-anisyllead and with triphenyl-p-anisyllead were established by reductive cleavage with stannous chloride and hydrochloric acid. The critical products that were isolated were o-anisidine as the p-toluenesulfonamide (m.p. 127°) and p-anisidine (m.p. 57°), respectively. The acetyl derivative prepared from p-anisidine melted at 125-126°. The structures are shown below.





Triethyl-p-dimethylaminophenyllead and p-Nitrobenzenediazonium Chloride

To a suspension of 4.1 g. (0.01 mole) in 75 ml. of water containing 5 g. of ethyl acetate was added 2 g. of p-nitroaniline diazotized in the usual manner. A bright red dye separated immediately. The dye was collected on a filter, dried, and crystallized from benzene. The yield was 3 g. Qualitative analysis for the elements showed that no lead was present. The melting point (198°) corresponds to that of the dye obtained by coupling dimethylaniline with p-nitrobenzenediazonium chloride. A mixed melting point showed no depression. Evidently the diazonium salt cleaved the triethyl-p-dimethylaminophenyllead.

DISCUSSION OF RESULTS

Two methods are available for the preparation of azo lead dyes: (1) diazotization of aminoaryllead compounds followed by coupling with a hydroxyl-, amino-, or methoxylaryl compound; or (2) coupling of diazonium salts with hydroxyl-, amino-, or methoxylaryllead compounds.

The diazotization of aminoaryllead compounds is not a good preparative procedure because of the difficulty encountered in obtaining the diazonium salt in good yields. Mild conditions must be used in the diazotizations to avoid cleavage of the organolead compounds. Acetic acid was the best solvent for this purpose.

The failure of diazotized aminoaryllead compounds to couple with sulfonic acid-substituted naphthalene compounds is probably due to steric hindrance. This same reasoning may be applied to the negative results that were obtained when triphenyl-*o*-aminophenyllead was diazotized and added to a basic solution of β-naphthol.

Azo lead compounds were obtained in good yields when diazonium salts were coupled with organolead compounds containing functional groups that facilitated diazo coupling. When hydroxyl and methoxyl substituted organolead compounds were used, the coupling was carried out in an alkaline solution. Ethyl acetate was added to the solution to increase the solubility of the reagents. When ethyl acetate was omitted the yields were markedly reduced. Ethyl alcohol increases the miscibility of water and ethyl acetate.

Aminoaryllead compounds were coupled in a medium weakly acid with acetic acid. Sodium acetate was used as a buffer.

A rather surprising observation was that p-sulfonobenzenediazonium chloride would not undergo coupling under conditions which gave good yields with other diazonium salts. A plausible explanation for this has not yet been found.

Apparently the lead atom, orienting to the meta position, increases the amount of coupling, since p-nitrobenzenediazonium chloride does not couple with anisole,[100] but does couple with triphenyl-o-anisyllead and triphenyl-p-anisyllead.

When the para position was open, coupling took place para to the functional group involved and meta to the lead atom.

Triethyl-o-dimethylaminophenyllead was cleaved by p-nitrobenzenediaxonium chloride under conditions favorable for coupling.

Generally, the dyes prepared did not have characteristic melting points. The presence of the lead atom gives the dyes a dull appearance. The shades varied between brown and red, dependent upon the type of compound.

The results of pharmacological tests have not yet been obtained.

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[100] Meyer, Ann., 398, 66 (1913); Ber., 47, 1741 (1914).

SUMMARY

1. A survey of lead compounds in the treatment of cancer has been presented.
2. An attempt has been made to correlate chemical structure and staining properties.
3. Some azo lead dyes have been prepared.
4. The positions of coupling have been determined.
5. Pharmacological results are awaited.