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PREPARATION AND PROPERTIES OF STABLE ORGANOLEAD DIAZONIUM SALTS FOR USE AS ELECTRON HISTOCHEMICAL REAGENTS

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SUMMARY

The preparation, properties and reactions of the diazonium chlorides derived from triphenyl(*p*-aminophenethyl)lead and triphenyl(*p*-aminophenyl)lead are described. The susceptibility of the latter compound to acid degradation is discussed.

INTRODUCTION

For some time work in this laboratory has been directed towards the preparation and application of heavy-atom containing compounds as marker molecules for electron histochemistry. One particular problem being studied required stable diazonium compounds containing heavy atoms capable of being employed in aqueous buffer solution in the pH range of 3–7.

The preparation and diazotisation of triphenyl(*p*-aminophenyl)lead (II) has been reported previously[1,6]. However, neither of the described methods gave satisfactory yields of product. A new route, through a *p*-lithio-*N*,*N*-bis(trimethylsilyl) intermediate, is described.

Protection of the amino group in the synthesis of triphenyl(*p*-aminophenethyl)-lead (III) was achieved by conversion to the lithium salt.

$$p\text{-}(Me_3Si)_2N\text{-}C_6H_4\text{-}Li + Ph_3PbCl \rightarrow p\text{-}(Me_3Si)_2N\text{-}C_6H_4\text{-}PbPh_3 \text{ (I)} \rightarrow$$

$$\rightarrow p\text{-}H_2N\text{-}C_6H_4\text{-}PbPh_3 \text{ (II)}$$

$$3\ Ph_3PbLi + Cl\text{-}CH_2\text{-}CH_2\text{-}C_6H_4\text{-}NH_2\text{-}p \rightarrow Ph_3Pb\text{-}CH_2\text{-}CH_2\text{-}C_6H_4\text{-}NH_2\text{-}p \text{ (III)}$$

DISCUSSION

Triphenyl(p-aminophenyl)lead

The methods previously described[1,6] for the preparation of triphenyl(*p*-aminophenyl)lead involve the intermediate *p*,*N*,*N*-trilithioaniline, prepared by treatment of *p*-bromoaniline with n-butyllithium. It was found that, under the conditions described, this material was extremely insoluble in anhydrous ether and reacted slowly with triphenyllead chloride to give poor yields of product. Consequently an improved

synthesis was devised.

p-Bromoaniline was converted to *p*-bromo-*N*,*N*-bis(trimethylsilyl)aniline[2,3]. Lithiation of this material with n-butyllithium and subsequent treatment with triphenyllead chloride afforded *p*-(triphenylplumbyl)-*N*,*N*-bis(trimethylsilyl)aniline. The N–Si bond in this compound exhibited a high resistance to alkaline hydrolysis compared with those in other compounds of this type[2]. The compound was recovered unchanged after being refluxed in 2% sodium hydroxide in 95% aqueous methanol for periods up to eight hours. Desilylation was achieved readily in acid (0.5 *N* aqueous acetic acid for $1\frac{1}{2}$ h at room temperature). However it was noted that extended exposure of the compound to acid led to degradation with formation of triphenyllead acetate.

This susceptibility to acid degradation must be considered when diazotisation of the amine is being carried out. The method described previously[1] suggested cold glacial acetic acid as a solvent. It was found that a stirred suspension of the triphenyl(*p*-aminophenyl)lead in glacial acetic acid under the described conditions (without addition of sodium nitrite) led to formation of quantities of triphenyllead acetate which was characterised by melting point and by the chromatographic methods of Krebs and Henry[4]. This observation suggested extensive cleavage of the Pb–C bond *para* to the amino group under these acidic conditions. The sensitivity of this bond to acid conditions has been confirmed by our inability to desilylate the dodecasilyl derivative of hexaanilinodilead under acidic conditions without bringing about complete degradation of the molecule[5].

When diazotisation of the triphenyl(*p*-aminophenyl)lead was carried out in glacial acetic acid and the diazonium chloride coupled with 2-naphthol in alkaline solution, the azo dye produced appeared to be identical in every respect with an authentic sample of 1-(phenylazo)-2-naphthol.

Conditions were therefore sought by which triphenyl(*p*-aminophenyl)lead could be diazotised with minimal Pb–C bond cleavage. The procedure selected was that of aprotic diazotisation in absolute ethanol employing two equivalents of hydrogen chloride. The solid diazonium chloride was isolated by ether precipitation. Fifteen separate diazotisations gave good yields of product (*q.v.*). The diazonium salt prepared in this manner was an off-white hygroscopic solid having the expected lead content. The material retained its coupling properties after storage under anhydrous conditions at $-20°$ for periods in excess of three weeks. It was found that successful diazotisations were critically dependent upon the exclusion of moisture.

Triphenyl(p-aminophenethyl)lead

Lithium has been employed previously[1,6,7] as a method of protection of amino groups in synthesis. In the present work (triphenylplumbyl)lithium was prepared by cleavage of hexaphenyldilead with lithium metal[12]. This reagent was employed to treat *p*-aminophenethyl chloride, a sufficient excess being present to protect the amino group as the lithium salt. The *p*-aminophenethyl chloride was generated from its hydrochloride immediately prior to use.

The aprotic diazotisation procedure devised for use with triphenyl(*p*-aminophenyl)lead was also found to be applicable to the phenethyl compound. The solid diazonium chloride was readily isolable in yields similar to those obtained with the diazonium chloride of the phenyl compound. Its stability and coupling properties

were also similar to those of the *p*-(triphenylplumbyl)benzenediazonium chloride.

Coupling reactions

For histochemical reasons the prime interest was in the coupling reactions between the diazonium chlorides and 7-bromo-3-hydroxy-2-naphth-*o*-anisidide (Naphthol AS-BI). This was found to occur instantaneously with both compounds at pH 5.5 in 0.1 *M* acetate buffer containing 1% (v/v) dimethylformamide. The azo dyes produced were virtually insoluble in the solvent medium and could be readily isolated. Both azo dyes contained lead in the expected amounts and had Pb/Br atomic ratios approaching unity. Similar results[5] were obtained in coupling experiments with 8-amino-1-naphthol-3,6-disulphonic acid (H acid).

A successful series of histochemical investigations have been carried out with these lead-containing diazonium compounds[10] in the electron microscope.

EXPERIMENTAL

Elemental analyses (of elements other than silicon) were carried out by the Organic Micro-analytical Laboratory of the Imperial Cancer Research Fund. Silicon analyses were carried out by Southern Consultant Analytical Laboratory, Surrey, England. Hexaphenyldilead and triphenyllead chloride were prepared by standard methods[8]. A gift of these materials was also received from Schering A.G., Bergkamen, Germany, through the kind offices of Dr. M. Buschoff. Ether, tetrahydrofuran and n-hexane were dried over sodium–lead alloy (B.D.H.) and absolute alcohol over molecular sieve (Union Carbide, Type 3A).

p-Aminophenethyl chloride was prepared by catalytic hydrogenation of the nitro compound[9] in ethanol containing one equivalent of hydrogen chloride, for 30 min at 10 psi over a 5% palladium on charcoal catalyst.

p-Bromo-*N*,*N*-bis(trimethylsilyl)aniline was prepared by the methods of Walton[2] and Broser and Harrer[3].

All reactions involving lithium or organolithium intermediates were carried out under an atmosphere of dry, oxygen-free nitrogen. n-Butyllithium was prepared according to Vogel[11]. Melting points are uncorrected.

p-(Triphenylplumbyl)-N,N-bis(trimethylsilyl)aniline (I)

n-Butyllithium (60 mmole) in ether (10 ml) was added dropwise to a stirred solution of *p*-bromo-*N*,*N*-bis(trimethylsilyl)aniline (18.2 g, 60 mmole) in n-hexane (45 ml) at 0°. The mixture was refluxed for 15 min and cooled to 10°. Solid triphenyllead chloride (28.4 g, 60 mmole) was added portionwise over a period of 10 min and the mixture refluxed for 1½ h. After cooling, lithium salts were filtered off and the ether/hexane removed by rotary evaporation. Methanol (250 ml) was added to the residual oil and the mixture stirred for 10 min at room temperature, whereupon crystallisation took place. The solid was filtered off, washed with cold methanol and dried *in vacuo* at room temperature. The crude solid (22.4 g) was recrystallised twice from boiling methanol containing a small quantity of activated charcoal to yield pure *p*-(triphenylplumbyl)-*N*,*N*-bis(trimethylsilyl)aniline; m.p. 120–121°; 17.1 g, 42.2%. (Found: C, 53.61; H, 5.26; N, 1.95; Pb, 30.42; Si, 8.4. $C_{30}H_{37}NPbSi_2$ calcd.: C, 53.38; H, 5.52; N, 2.07; Pb, 30.70; Si, 8.3%.)

Triphenyl(p-aminophenyl)lead (II)

To finely ground *p*-(triphenylplumbyl)-*N*,*N*-bis(trimethylsilyl)aniline (10.0 g, 14.8 mmole) was added aqueous acetic acid (250 ml, 0.5 *N*). The mixture was stirred rapidly at room temperature for 1½ h. During this period the suspension became pink in colour. The solid was filtered off, washed well with water and dried *in vacuo* over phosphoric oxide at room temperature. The crude material (7.1 g) was recrystallised twice from methanol containing a small quantity of activated charcoal to yield pure triphenyl(*p*-aminophenyl)lead; 4.4 g, 56.1%; m.p. 169–170° (lit.[6] 166–167°). (Found: C, 54.26; H, 3.75; N, 2.41; Pb, 39.40. $C_{24}H_{21}NPb$ calcd.: C, 54.32; H, 3.99; N, 2.64; Pb, 39.05%.)

Triphenyl(p-aminophenethyl)lead (III)

Hexaphenyldilead (21.9 g, 25 mmole) was dissolved in dry tetrahydrofuran (90 ml); finely cut lithium metal (1.2 g, 200 mmole) was added and the mixture stirred rapidly at room temperature[12]. After a period of about 20 minutes the reaction solution became black and the temperature rose rapidly. Stirring was continued for 3.5 h at room temperature. The black suspension was filtered, under nitrogen, through a glass-wool pad.

Immediately prior to the filtration of the (triphenylplumbyl)lithium solution, *p*-aminophenethyl chloride hydrochloride (2.74 g, 14.3 mmole) was dissolved in water (10 ml) containing sodium hydroxide (30 mmole). This solution was extracted with ether (2 × 20 ml) and the ethereal extract containing the free base dried over anhydrous magnesium sulphate for 10 min. The magnesium sulphate was removed by rapid filtration under nitrogen and the solution of (triphenylplumbyl)lithium added dropwise with stirring, at such a rate as to maintain refluxing. After the addition was complete the mixture was refluxed for 2 h. The mixture was cooled and hydrolysed with ice/ice water. The aqueous suspension was extracted with ether (2 × 100 ml) and the combined extracts dried (anhydrous magnesium sulphate). Ether was removed by rotary evaporation and residue dried *in vacuo* to give crude material (5.1 g).

Recrystallisation from methanol containing a small quantity of activated charcoal yielded triphenyl(*p*-aminophenethyl)lead; 3.1 g, 38.8%; m.p. 95–96°. (Found: C, 55.92; H, 4.48; N, 2.24; Pb, 37.08. $C_{26}H_{25}NPb$ calcd.: C, 55.89; H, 4.51; N, 2.51; Pb, 37.09%.)

Diazotisation of the organolead amines

The amine (0.55 mmole, ca. 300 mg) was suspended in dry absolute ethanol (3.0 ml) containing hydrogen chloride (1.2 mmole) at 0–5°. Dry (magnesium sulphate) isopentyl nitrite (3 × 0.03 ml) was added portionwise at 60 sec intervals, the temperature being kept at 0–5°. One minute after the addition of the final aliquot of the isopentyl nitrite dry ether (100 ml) was added to precipitate the diazonium salt. The salt was filtered off and washed with dry ether (3 × 50 ml). The solid was kept under ether until the washing was complete. The ether was removed and the solid diazonium salt dried *in vacuo* over phosphoric oxide for 1 h at room temperature. The yields of dry solids obtained in 30 separate diazotisations (fifteen samples of each amine) varied from 58–78% of the theoretical values. The material, stored at −20° under anhydrous conditions, retained its coupling activity for periods in excess of three weeks. The diazonium salts were extremely hygroscopic off-white solids, and proved difficult

to analyse. Immediately after isolation, however, samples of the diazonium chlorides prepared for histochemical experiments were rapidly assayed (by atomic absorption spectroscopy) to ensure that expulsion of lead had not taken place. It was generally found that the lead content varied by no more than 0.8% from that of the theoretical value. About half the samples examined showed agreement of better than 0.4%.

Coupling reactions of the diazonium chlorides

(*a*). *With 7-bromo-3-hydroxy-2-naphth-o-anisidide.* The diazonium chloride (50 mg) was dissolved in acetate buffer (0.1 *M*, pH 5.5, 100 ml) containing dimethylformamide (1% v/v). A solution of 7-bromo-3-hydroxy-2-naphth-*o*-anisidide (400 mg/100 ml) in acetone was added dropwise until precipitation of the azo dye appeared to be complete. The coupling reaction (with both diazonium chlorides) took place rapidly, the azo dye being completely insoluble in the solvent medium. The red azo dye was filtered off, washed with a small volume of acetone and dried at room temperature *in vacuo*. The material was crystallised from ethanol containing a small quantity of benzene. The results are shown in Table 1.

TABLE 1

RESULTS OF COUPLING REACTIONS OF ORGANOLEAD DIAZONIUM CHLORIDES

Diazonium chloride derived from	Coupled to	Yield (%)	M.p. of azo dye (°C)	Found (calcd.) (%)		Pb/Br
				Pb	Br	
Triphenyl-(*p*-aminophenyl)lead	Naphthol AS-BI	43.1	194–195	22.50 (22.64)	9.01 (8.74)	0.96
Triphenyl-(*p*-aminophenethyl)lead	Naphthol AS-BI	58.7	177–178	22.31 (21.97)	8.78 (8.48)	0.95
Triphenyl-(*p*-aminophenyl)lead	2-Naphthol	52.2	190–191	30.41 (30.12)		

(*b*). *With 2-naphthol.* *p*-(Triphenylplumbyl)benzenediazonium chloride (100 mg) was dissolved in dimethylformamide (40 ml) and this solution added dropwise to a stirred solution of 2-naphthol (250 mg) in aqueous sodium hydroxide solution (1 *N*, 10 ml) at room temperature. Coupling took place instantaneously with formation of the red azo dye. The dye was filtered, washed with sodium hydroxide solution (1 *N*) followed by water and dried *in vacuo*. The material was recrystallised from ethanol containing a small quantity of benzene. The results are shown in Table 1.

Diazotisation of triphenyl(p-aminophenyl)lead in glacial acetic acid[1]

Triphenyl(*p*-aminophenyl)lead (530 mg, 1 mmole) was suspended in glacial acetic acid cooled to the freezing point. Cold, aqueous sodium nitrite solution (100 mg/2 ml) was added dropwise with stirring. The temperature of the mixture was allowed to rise to 20° and held at this value for 15 min. The mixture was poured into a solution of 2-naphthol (500 mg) in sodium hydroxide solution (1 *N*, 20 ml). The azo dye was filtered off, washed with sodium hydroxide solution, followed by water and dried *in vacuo*. The yield of dye was 173 mg melting at 130–131°. Mixed melting point

with an authentic sample of 1-(phenylazo)-2-naphthol[13] showed no depression. The product and the latter compound exhibited identical properties when subjected to chromatographic analysis (Kieselgel G, Merck) in two different solvent systems [(*i*). 3% ethanol in toluene; (*ii*). 1% ethyl acetate in dichloromethane]. The azo dye did not contain lead.

Action of acid on triphenyl(p-aminophenyl)lead

Triphenyl(*p*-aminophenyl)lead (530 mg, 1 mmole) was suspended in glacial acetic acid (10 ml) at 20° and the suspension stirred for 15 min. Some of the solid dissolved as the reaction proceeded. The reaction mixture was diluted with water (10 ml) and the white solid filtered off, washed well with water and dried *in vacuo* over phosphoric oxide at room temperature. The material (380 mg) was recrystallised from ethanol to give 310 mg of a white crystalline solid, m.p. 206–207°. Mixed melting point with an authentic sample of triphenyllead acetate showed no depression. The product and the latter compound exhibited identical properties when subjected to thin-layer chromatographic analysis (Kieselgel G) in three different solvent systems [(*i*). benzene; (*ii*). hexane; (*iii*). 3% ethanol in benzene]. Both compounds gave the same (yellow) reaction with dithizone in chloroform[4].

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