[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WISCONSIN]

The Antitubercular Action of 1,1,1-Trichloro-2,2-bis-(*p*-aminophenyl)-ethane[1]

BY S. KIRKWOOD AND P. H. PHILLIPS

Studies in our laboratory on the Läuger hypothesis[2] for the mechanism of action of D.D.T. using fluorine analogs[3] suggested that the compound 1,1,1-trichloro-2,2-bis-(*p*-aminophenyl)-ethane (I) might possess antitubercular activity. Kirkwood and Phillips[4] hav eshown that 1,1-dichloro-2,2-bis-(*p*-chlorophenyl)-ethylene when fed to rats was accumulated in the depot fat to approximately the same extent as was D.D.T. fed at the same level. From this evidence it seemed possible that 1,1-dichloro-2,2-bis-(*p*-aminophenyl)-ethylene (II) might likewise show lipid affinity and that both I and II might possess antitubercular properties.

We have succeeded in preparing and establishing the structure of both of these compounds by the steps shown in the accompanying flow-sheet. The diamine I was expected to be an unstable substance since similar trichloroethanes are known to lose hydrogen chloride readily under the influence of alkali[5] and the molecule contains two amino groups. The route of synthesis shown was worked out because of the relatively mild conditions under which it was hoped the reduction of the nitro to the amino group would be accomplished. Burger, Graef and Bailey,[6] in their synthesis of mono- and dibenzoyl derivatives of I, found them to be so unstable as to lose hydrogen chloride under the relatively mild conditions of acid hydrolysis which they employed. Further, an attempt to prepare a water soluble compound by partial hydrolysis of the *p*-phthalimidophenyl derivative[7] resulted in an alkali soluble product which resinified during isolation.

Synthesis by the route chosen by us seemed possible since Lange and Zufall[8] showed that 1,1-dichloro-2,2-bis-phenyl-ethylene (VI) gave the compound 1,1-dichloro-2,2-bis-(*p*-nitrophenyl)-ethylene (VII) as the main product of nitration. They proved the structure of VII by oxidative hydrolysis to the known bis-(*p*-nitrophenyl) ketone (IX). From these reactions we thought it possible that 1,1,1-trichloro-2,2-bis-phenyl-ethane (III) might give 1,1,1-trichloro-2,2-bis-(*p*-nitrophenyl)-ethane (IV)[9] as the main product of nitration. This possibility was realized as we found that the compound resulting from the nitration of III could be quantitatively transformed to VII by ethanolic potassium hydroxide. Lange and Zufall[8] mention the reduction of the nitro compound VII by stannous chloride and the isolation of an amine and the preparation of its acetyl derivative, but they give no physical constants, analyses or structures. The reduction of both nitro compounds was found by us to proceed smoothly at room temperature using Raney nickel catalyst with hydrogen at four atmospheres pressure. The diamine I proved to be so unstable that it lost hydrogen chloride even when aqueous solutions of its hydrochloride were carefully neutralized with sodium bicarbonate. However, when the product was worked up rapidly and the free amine prepared by the careful addition of a saturated aqueous solution of sodium acetate to an aqueous solution of the hydrochloride, a crystalline substance was obtained. The crude product was unstable on standing at room temperature but when recrystallized several times proved to be stable at room temperature. The technique of using sodium acetate to prepare the free amines from aqueous solutions of their hydrochlorides was used in the preparation of both diamines I and II.

The tendency for compounds of this type to rearrange, especially under the influence of reducing agents, is well known.[10,11,12] This made it desirable to present proof that no rearrangement had occurred during our reduction. It was found possible to convert the diamine I to the diamine II by the use of methanolic potassium hydroxide. Further it was found possible to convert 1,1,1-trichloro-2,2-bis-(*p*-acetylaminophenyl)-ethane (V) to 1,1-dichloro-2,2-bis-(*p*-acetylaminophenyl)-ethylene (VIII) in very good yield by the use of ethanolic potassium hydroxide. The structure of VIII was proven by oxidative hydrolysis to bis-(*p*-acetylaminophenyl) ketone (XI).

The diamines I and II have been tested *in vitro* with the coöperation of Dr. Elizabeth McCoy of the Department of Agricultural Bacteriology. They completely inhibit the growth of a human-virulent strain of *M. tuberculosis* at concentrations of 1/100,000. Preliminary *in vivo* testing indicates that the diamine I gives marked control of experimentally induced tuberculosis in guinea-pigs. Further *in vivo* work on the diamines I and II is in progress.

Experimental

1,1,1-Trichloro-2,2-bis-(*p*-nitrophenyl)-ethane (IV).—Fifty grams of 1,1,1-trichloro-2,2-bis-phenyl-ethane pre-

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These researches were supported in part by the Wisconsin Alumni Research Foundation.

(2) Läuger, Martin and Müller, *Helv. Chim. Acta*, **27**, 892 (1944).

(3) (a) Kirkwood and Dacey, *Can. J. Research*, **24B**, 69 (1946); (b) Kirkwood and Phillips, *J. Pharmacol. Exptl. Therapy*, **87**, 375 (1946).

(4) Kirkwood and Phillips, unpublished data, University of Wisconsin, 1946.

(5) Müller, *Helv. Chim. Acta*, **29**, 1560 (1946).

(6) Burger, Graef and Bailey, THIS JOURNAL, **68**, 1725 (1946).

(7) Graef and Burger, *ibid.*, **68**, 2400 (1946).

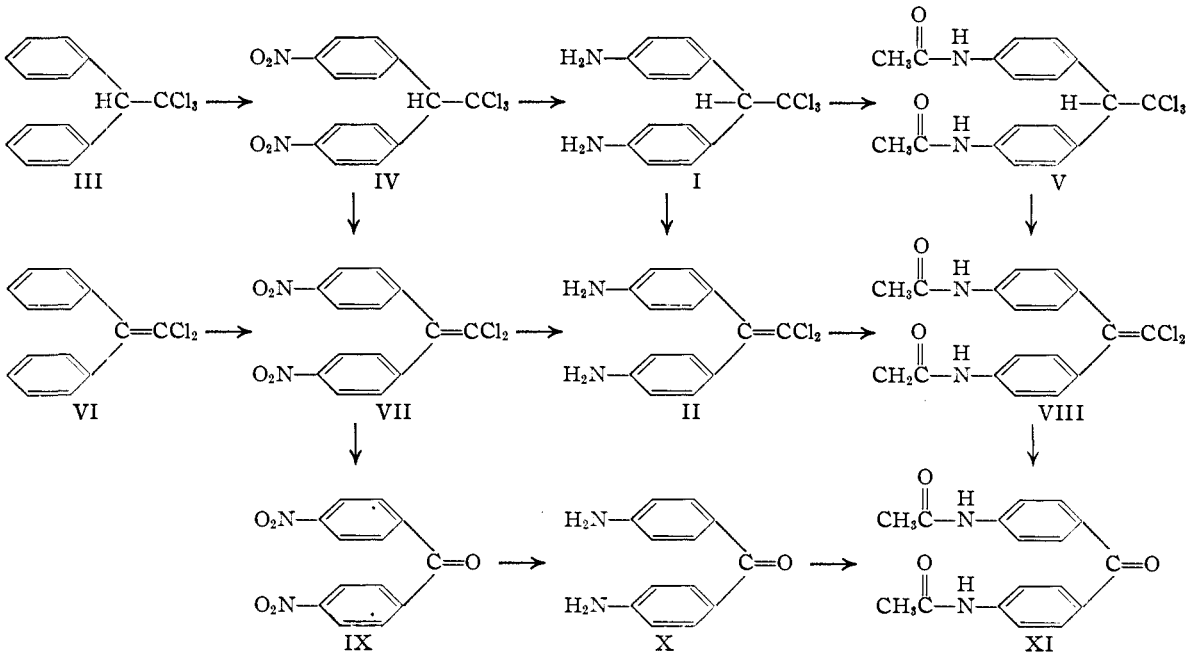
(8) Lange and Zufall, *Ann.*, **271**, 1 (1892).

(9) This compound may have action against murine typhus; Kikuth, Office of the Publication Board, Department of Commerce, Report No. 248, p. 63.

(10) Elbs, *J. prakt. Chem.*, [II] **47**, 44 (1893).

(11) Brand and Krücke-Amelung, *Ber.*, **72**, 1029 (1939).

(12) Brand and Krücke-Amelung, *ibid.*, **72**, 1036 (1939).



pared by the method of Baeyer[13] was added slowly to 500 g. of white fuming (1.5) nitric acid under vigorous stirring. The temperature was controlled at 30° with ice cooling and the mixture was left for four hours at room temperature after the addition of the diphenyl compound. It was then poured onto crushed ice, the precipitated material filtered off and crystallized from glacial acetic acid (yield 41.5 g., 63%). It crystallized in the form of colorless needles melting at 166–167°.[14]

Anal. Calcd. for $C_{14}H_9O_4N_2Cl_3$: N, 7.46; Cl, 28.34; hydrolyzable Cl, 9.44. Found: N, 7.12; Cl, 28.18; hydrolyzable Cl, 9.36.

1,1-Dichloro-2,2-bis-(*p*-nitrophenyl)-ethylene (VII).—A total of 0.6 g. of potassium hydroxide (0.2 g. at a time) was added with shaking to a suspension of 2.0 g. of 1,1,1-trichloro-2,2-bis-(*p*-nitrophenyl)-ethane (IV) in 50 cc. of ethanol. The mixture was left for four hours at room temperature and then poured into water. The crystalline product in the form of colorless prisms melted at 172–173° and weighed 1.8 g. (quantitative yield). This melting point could not be raised by recrystallization from glacial acetic acid. A mixture melting point with an authentic sample of 1,1-dichloro-2,2-bis-(*p*-nitrophenyl)-ethylene prepared by the method of Lange and Zufall[8] showed no depression.

Oxidative Hydrolysis of 1,1-Dichloro-2,2-bis-(*p*-nitrophenyl)-ethylene (VII).—Two grams of 1,1-dichloro-2,2-bis-(*p*-nitrophenyl)-ethylene was added to a solution of 3.0 g. of chromium trioxide in 75 cc. of glacial acetic acid. The mixture was heated for one hour on the steam-bath, poured into water and the ketone filtered off. It crystallized in the form of platelets from glacial acetic acid which melted at 187–188° (yield 1.52 g., 95%). Lange and Zufall[8] report 187–188°.

1,1,1-Trichloro-2,2-bis-(*p*-aminophenyl)-ethane (I).—Fifteen grams of 1,1,1-trichloro-2,2-bis-(*p*-nitrophenyl)-ethane was hydrogenated in 100 cc. of glacial acetic acid over 4 cc. of Raney nickel catalyst at *ca.* 60 lb. pressure. Hydrogen uptake ceased, after the theoretical amount was absorbed, in *ca.* one hour. The solvent was removed *in vacuo* at 40–50°, the residual acetate was taken up in 200 cc. of water containing 5 g. of sodium acetate and the amine was extracted with 100 cc. of ether. The ether layer was washed once with 200 cc. of a 2.5% aqueous sodium acetate solution and then extracted twice with 200 cc. of dilute hydrochloric acid (1–20 dilution of concentrated hydrochloric acid). The aqueous extracts were combined and decolorized with norit. A saturated aqueous solution of sodium acetate was added with stirring to the decolorized solution until no more amine was precipitated. The crude crystalline amine was filtered off (7.2 g., 57%) and recrystallized from methanol or ethanol in the form of colorless needles melting at 154–155° with decomposition.[15]

Anal. Calcd. for $C_{14}H_{13}N_2Cl_3$: C, 53.31; H, 4.15; N, 8.88; Cl, 33.72. Found: C, 53.26; H, 4.31; N, 8.84; Cl, 33.71.

1,1-Dichloro-2,2-bis-(*p*-aminophenyl)-ethylene (II).—Fifteen grams of 1,1-dichloro-2,2-bis-(*p*-nitrophenyl)-ethylene was hydrogenated in 100 cc. of glacial acetic acid over 4 cc. of Raney nickel catalyst. Hydrogen uptake ceased, after the theoretical amount was absorbed, in *ca.* one hour. The product was worked up in a similar fashion to the diamine I and the result was 11.1 g. (90%) of crystalline diamine melting at 142–144°. The substance crystallized from methanol in the form of colorless platelets melting at 144–145°.[16]

Anal. Calcd. for $C_{14}H_{12}N_2Cl_2$: C, 60.25; H, 4.30; N,

(13) Baeyer, *Ber.*, **5**, 1094 (1872).

(14) All melting points reported in this paper are uncorrected.

(15) When originally prepared in these laboratories the diamine I occurred in the form of platelets melting at 92–95° with decomposition. The nitrogen analysis was 8.80 (calcd. 8.88) [Kirkwood, Phillips and McCoy, THIS JOURNAL, **68**, 2405 (1946).] It has since been obtained in the form of needles melting at 154–155° with decomposition and we have been unable to isolate the low-melting form again. Since the material melting at 92–95° gives the same analysis and affords the same diacetyl derivative V as that melting at 154–155°, we conclude that it is a low-melting crystalline form of the diamine I. Three recrystallizations of the material melting at 154–155° from methanol, followed by three from ethanol and a further recrystallization from methanol failed to raise this melting point.

(16) Burger, *et al.*, prepared this compound by the acid hydrolysis of 1,1,1-trichloro-2,2-bis-(*p*-benzamidophenyl)-ethane and reported a melting point of 174–175°.[6] Our preparation of this compound from the corresponding nitro compound has been repeated many times and always affords us material melting at 144–145°. This melting point cannot be raised by further repeated recrystallization. It is possible that this diamine II shows dimorphism as does the diamine I and that we have the low-melting form.

10.05; Cl, 25.42. Found: C, 60.34; H, 4.93; N, 9.89; Cl, 25.48.

Dehydrochlorination of 1,1,1-Trichloro-2,2-bis-(*p*-aminophenyl)-ethane (I).—To a solution of 1.0 g. of potassium hydroxide in 30 cc. of methanol was added 2.8 g. of 1,1,1-trichloro-2,2-bis-(*p*-aminophenyl)-ethane. The solution was refluxed on the steam-bath for one hour and then poured into dilute hydrochloric acid and worked up in a similar fashion to the diamine I. The material crystallized from methanol (yield 1.2 g., 48%) in the form of colorless platelets and melted from 143–145°. A mixture melting point with a sample of 1,1-dichloro-2,2-bis-(*p*-aminophenyl)-ethylene prepared by the reduction of the nitro compound VII showed no depression.

1,1,1-Trichloro-2,2-bis-(*p*-acetylaminophenyl)-ethane (V).—One gram of 1,1,1-trichloro-2,2-bis-(*p*-aminophenyl)-ethane was added to a mixture of 15 cc. of glacial acetic acid, 2 cc. of pyridine and 5 cc. of acetic anhydride. The mixture was allowed to stand four hours at room temperature during which time the acetyl compound crystallized out in the form of platelets. The mixture was poured into water, the acetyl compound filtered off and recrystallized from glacial acetic acid (yield 1.2 g., 95%). It did not melt but decomposed at about 275°.

Anal. Calcd. for $C_{18}H_{17}O_2N_2Cl_3$: N, 7.01; Cl, 26.64. Found: N, 6.97; Cl, 26.61.

1,1-Dichloro-2,2-bis-(*p*-acetylaminophenyl)-ethylene (VIII).—Four grams of 1,1-dichloro-2,2-bis-(*p*-aminophenyl)-ethylene was added to a mixture of 20 cc. of glacial acetic acid, 3 cc. of pyridine and 10 cc. of acetic anhydride. The mixture was allowed to stand at room temperature for four hours and the acetyl compound precipitated out in the form of colorless needles. The mixture was poured into water, the diacetyl compound filtered off and recrystallized from glacial acetic acid (yield 5.0 g., 96%). It melted at 296–297° without decomposition.

Anal.[17] Calcd. for $C_{18}H_{16}O_2N_2Cl_2$: N, 7.74; Cl, 19.53. Found: N, 7.50; Cl, 19.37.

Dehydrochlorination of 1,1,1-Trichloro-2,2-bis-(*p*-acetylaminophenyl)-ethane (V).—One-half gram of 1,1,1-trichloro-2,2-bis-(*p*-acetylaminophenyl)-ethane was suspended in a solution of 0.2 g. of potassium hydroxide in 20 cc. of ethanol. The mixture was refluxed for twenty minutes on the steam-bath and then poured into water. The material was filtered off and recrystallized from glacial acetic acid. The result was 0.41 g. (91%) of material melting at 296–297°. A mixture melting point with a sample of VIII prepared by the acetylation of the diamine II showed no depression. This material as well as that from the acetylation of II gave the same diacetylaminoketone XI on oxidative hydrolysis.

Oxidative Hydrolysis of 1,1-Dichloro-2,2-bis-(*p*-acetylaminophenyl)-ethylene (VIII).—A suspension of 0.40 g. of 1,1-dichloro-2,2-bis-(*p*-acetylaminophenyl)-ethylene in 20 cc. of glacial acetic acid was heated on the steam-bath and 0.60 g. of chromium trioxide was added in small portions over a period of ten minutes. The mixture was heated for twenty minutes more and then poured into water. Two grams of sodium bicarbonate was added and the product filtered off; yield 0.16 g., 50%. It crystallized from ethanol in the form of needles melting at 238–239°. Rivier and Farine report 237°.[18] A mixture melting point with a sample prepared by the acetylation of X showed no depression.

bis-(*p*-Aminophenyl) Ketone (X).—1.88 grams of bis-(*p*-nitrophenyl) ketone was hydrogenated in 50 cc. of glacial acetic acid over 2 cc. of Raney nickel at *ca.* 60 lb. pressure. Hydrogen uptake ceased, after the theoretical amount was absorbed, in *ca.* one hour. The material was worked up in the same fashion as the diamine I. It crystallized from ethanol in the form of needles melting at 242–244°. Rivier and Farine report 244–245°[18]; yield 1.3 g., 89%.

Acetylation of Bis-(*p*-aminophenyl) Ketone (X).—0.34 grams of bis-(*p*-aminophenyl) ketone was added to a mixture of 15 cc. of glacial acetic acid, 1 cc. of pyridine and 4 cc. of acetic anhydride. The solution was heated on the steam-bath for one and one-half hours, poured into water and the diacetyl compound filtered off; yield 0.33 g., 70%. It crystallized from ethanol in the form of needles melting at 238–239°. Rivier and Farine report 237°.[18]

We wish to thank Mr. R. G. Hansen for the nitrogen analyses and Mr. Phil Wilcox and Mrs. Eva Mason for the carbon and hydrogen analyses reported in this paper.

Summary

1. The preparation of 1,1,1-trichloro-2,2-bis-(*p*-aminophenyl)-ethane (I) and 1,1-dichloro-2,2-bis-(*p*-aminophenyl)-ethylene (II) from the corresponding nitro compounds is described. These compounds exhibit marked antitubercular activity *in vitro* and preliminary experiments indicate that compound I may have activity *in vivo*.

2. The structure of compounds I and II is proved by degradation to known substances.

MADISON, WISCONSIN RECEIVED DECEMBER 26, 1946

(17) Both compounds V and VIII showed a remarkable tendency to retain methanol. Crystals of both compounds when washed on the Buchner funnel with methanol gave carbon, hydrogen and chlorine analyses indicating 0.5 mole of methanol per mole of substance. This methanol could not be removed by drying overnight in the Abderhalden apparatus at 100° and 0.02 mm. pressure. Recrystallization from glacial acetic acid removed the methanol and gave material with the correct analysis.

(18) Rivier and Farine, *Helv. Chim. Acta*, **12**, 865 (1929).