80° in contrast to acylchloride hydrochlorides of amino acids dealt with by us hitherto.

Anal. Calc'd for $C_5H_9Cl_2NO$: Cl, 41.6. Found: Cl, 40.8.

Polymerization of L-*prolyl chloride hydrochloride.* L-Prolyl chloride hydrochloride (2 g.) was heated in a high vacuum at 115° for 10 hours. The polymer thus obtained was soluble in water, slightly soluble in ethanol, and insoluble in benzene, dioxane, etc. Purification was effected by dissolving in the minimum amount of water and precipitation with absolute ethanol. Ninhydrin and biuret reactions were negative. According to the chlorine content the polymer was composed of 6 units on the average. It decomposed at 165°.

Anal. Calc'd for $C_{30}H_{46}ClN_6O_7$: C, 56.5; H, 7.1; N, 13.2; Cl, 5.6. Found: C, 53.6; H, 7.5; N, 13.0; Cl, 5.5.

DL-*Leucylglycylglycyl chloride hydrochloride.*[9] The tripeptide (0.9 g.) was suspended in 20 ml. of freshly distilled acetyl chloride, and after cooling in an ice-bath, 1 g. of phosphorus pentachloride was added in two portions and the mixture was shaken at room temperature for four hours. Then 0.5 g. of phosphorus pentachloride was added and shaking was resumed for another 13 hours. Finally, after addition of 0.3 g. of the chlorinating agent, shaking was continued for 7 more hours. The substance was treated and isolated in the usual way. Yield 0.85 g. (77.5%).

Anal. Calc'd for $C_{10}H_{19}Cl_2N_3O_3$: Cl, 23.7. Found: Cl, 21.4.

Polymerization of DL-*leucylglycylglycyl chloride hydrochloride.* The tripeptide chloride hydrochloride (0.8 g.) was heated in a high vacuum at 135° for seven hours. The polymer thus obtained was soluble in water and ethanol. It gave positive biuret and ninhydrin reactions. It was purified by dissolving in ethanol and reprecipitation with dry ether. According to the chlorine content and free amino end groups (Van Slyke's manometric method), it was composed of 5 tripeptide residues.

Anal. Calc'd for $C_{50}H_{88}ClN_{15}O_{16}$: C, 50.1; H, 7.4; N, 17.6; Cl, 3.0; Van Slyke nitrogen, 1.2. Found: C, 46.7; H, 7.5; N, 16.8; Cl, 3.1; Van Slyke nitrogen, 1.4. Amino nitrogen after hydrolysis 16.3.

The two constituent amino acids were identified on paper chromatograms made from the hydrolyzate.

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[9] E. Fischer, *Ber.*, **38**, 2914 (1905).

Partial Degradation of the Benzene Ring of Estradiol-17β

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The biosynthesis of carbon-14 labeled estrone and estradiol-17β from acetate-C^{14} has been reported recently under both *in vitro* and *in vivo* conditions.[1–4] Insight into the mechanisms involved in the biosynthesis may be forthcoming when methods become available for the total degradation of the aromatic and alicyclic parts of the estrogen molecules enabling a determination of the total distribution of the radioactive carbon atoms in the labeled estrogens. Recently, the partial degradation of the aromatic ring of estrone was reported.[5] This was accomplished by applying the bromopicrin split to several nitroestrones.[6]

This note describes the synthesis of 2,4-dinitroestradiol-17β and its partial degradation to tribromonitromethane (representing both carbon atoms 2 and 4 of the benzene ring) by a similar application of the bromopicrin split. The 2,4-dinitroestradiol-17β was characterized by its ultraviolet and infrared absorption spectra. The steroid had absorption bands at 741 cm.$^{-1}$, 759 cm.$^{-1}$, and 904 cm.$^{-1}$ which had been found previously to be characteristic of dinitrophenolic pentasubstituted compounds.[5] The ultraviolet spectrum of the dinitrosteroid showed absorption maxima in absolute ethanol at 277 mμ (ε 6655), 352–354 mμ (ε 3340), and a plateau of 424–426 mμ (ε 910). These values are in excellent agreement with those recorded for similar pentasubstituted aromatic compounds.[5] The 2,4-dinitroestradiol-17β was also characterized by conversion to a colorless 3,17-diacetate which was purified by filtration in ether over charcoal. The diacetate was hydrolyzed readily to a yellow 17-monoacetate by elution from a column of aluminum oxide. The bromopicrin resulting from the cleavage of the benzene ring of the dinitrosteroid was identified by infrared analysis.

EXPERIMENTAL[7]

2,4-Dinitroestradiol-17β. Estradiol-17β (100 mg.) was dissolved in 3 ml. of glacial acetic acid, and 60 μl of concentrated nitric acid was added slowly at room temperature with good mixing. After standing for 24 hours, the solution was poured into 50 ml. of water and was extracted several times with ether. The ether solution was concentrated to dryness *in vacuo.* The yellow residue was treated with 5 ml. of 5% methanolic potassium hydroxide for 2 hours to hydrolyze any ester that may have formed, after which the solution was acidified and centrifuged. The precipitate was washed several times with water and was crystallized from aqueous ethanol, m.p. 240–250° (dec.).

Anal. Calc'd for $C_{18}H_{22}N_2O_6$: C, 59.66; H, 6.12; N, 7.73. Found: C, 60.12; H, 6.50; N, 7.35.

2,4-Dinitroestradiol-3,17β-diacetate. 2,4-Dinitroestradiol-17β (20 mg.) was mixed with 100 μl. of acetic anhydride and 50 μl. of dry pyridine. After standing at room temperature overnight, the mixture was evaporated to dryness with a stream of nitrogen. The residue was taken up in ether, 70 mg. of activated charcoal was added, and the solution was filtered. Evaporation of the ether left a colorless solid that was crystallized from aqueous methanol, m.p. 161.5–164.5°.

Anal. Calc'd for $C_{22}H_{26}N_2O_8$: N, 6.28. Found: N, 6.30.

2,4-Dinitroestradiol-17β-acetate. The diacetate was taken up in benzene and was placed on a column of 5 g. of aluminum oxide (Merck acid-washed). Benzene containing 0.5%

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[1] Heard and O'Donnell, *Endocrinology*, **54**, 209 (1954).

[2] Rabinowitz and Dowben, *Biochim. Biophys. Acta*, **16**, 96, (1955).

[3] Levitz, Condon, and Dancis, *Fed. Proc.*, **14**, 245 (1955).

[4] Wotiz, Davis, and Lemon, *J. Biol. Chem.*, **216**, 677 (1955).

[5] Werbin and Holoway, *J. Biol. Chem.*, in press.

[6] Since estriol can be converted to estrone, the partial degradation of the benzene ring of the three most abundant estrogens found in human urine now is possible.

[7] Melting points were taken on a Fisher-Johns melting block and are uncorrected.

methanol caused elution of a yellow solid that was crystallized from aqueous methanol, m.p. 197.5–198.5°.

Anal. Calc'd for $C_{20}H_{24}N_2O_7$: N, 6.93. Found: N, 6.97.

Bromopicrin split. The procedures for the bromopicrin split and the isolation of tribromonitromethane have been described.[5] Method B was employed. From 48 mg. of 2,4-dinitroestradiol-17β there was obtained 30.5 mg. of tribromonitromethane. The infrared spectrum of this material was compared with the spectrum of a sample of tribromonitromethane from Eastman Kodak that was vacuum-distilled. The spectra were identical except for a few additional weak bands in the bromopicrin from the cleavage of the dinitrosteroid, indicating the presence of a minor component.[8] After evaporation of the bromopicrin on a watchglass at room temperature, a few micrograms of colorless crystals remained, subliming at 123–127°, $\nu_{\text{Max}}^{\text{KBr}}$ 1475, 1442, 1323, 1118, 1058, 881, and 642 cm.$^{-1}$. These bands accounted for the weak bands in the spectrum of the bromopicrin from the degradation of the dinitrosteroid. The small quantity precluded identification.

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[8] Scholl and Brenneisen, *Ber.*, **31**, 654 (1898), isolated dibromodinitromethane, m.p. 10°, as a minor component of tribromonitromethane resulting from the bromopicrin split of picric acid.

The Molecular Weights of Phosphonic and Phosphinic Acids

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There is considerable evidence that phosphonic and phosphinic[1] acids are associated in the solid state and in certain organic solvents by hydrogen bond formation. This concept is supported by infrared absorption studies[2] and by molecular weight determinations.[3] On the other hand, freezing point depressions obtained with several phosphonic acids in aqueous solution are *larger* than the calculated values based on monomolecular formulas.[3c] Although these results are difficult to interpret unambiguously, since there must be considerable acidic dissociation in aqueous solutions of these acids, it has been suggested[3b,c,4] that hydrogen bonded aggregates of phosphonic acid molecules are present even in aqueous solution and are in fact responsible for the formation of relatively insoluble "acid" salts when solutions of phosphonic acids are partially neutralized.

Because of the low energy content of the hydrogen bond and the low activation energy involved in its formation and rupture, most types of molecules that are associated by hydrogen bonds dissociate rapidly when dissolved in polar solvents such as water, acetic, and formic acids.[5] For example, carboxylic acids, which are associated in the solid and vapor state and in non-polar solvents, exist in the monomeric form in acetic acid solution.[5] Unless the hydrogen bonds present in organophosphorus acids are extraordinarily strong, one would expect these compounds also to be present in polar solvents as single molecules.

After we found that many phosphonic and phosphinic acids are easily soluble in acetic acid, we used this solvent for cryoscopic molecular weight determinations of a number of aromatic, aliphatic, and alicyclic compounds. The results obtained are listed in Table I. It is seen that all the compounds

TABLE I

CRYOSCOPIC MOLECULAR WEIGHT DETERMINATIONS IN ACETIC ACID

Compound	Δt, °C.	Molecular Weight Calc'd	Molecular Weight Found
RPO_3H_2			
Ethylphosphonic acid[a]	0.393	110.0	110.6
n-Butylphosphonic acid[b]	.350	138.1	137.9
Cyclohexylphosphonic acid[c]	.251	164.1	165.9
Phenylphosphonic acid[d]	.266	158.1	164.1
p-Tolylphosphonic acid[d]	.375	172.1	171.4
R_2PO_2H			
Dicyclohexylphosphinic acid[c]	.352	230.3	227.7
Diphenylphosphinic acid[d]	.287	218.2	216.9
Phosphafluorinic acid[e]	.102[f]	216.2	198.7
Other compounds			
Phenylphosphinic acid[g]	.241	142.1	146.0
Triphenylphosphine oxide[h]	.290	272.2	289.7
Benzenearsonic acid[i]	.429	202.0	209.5
Benzoic acid[j]	.288	122.1	117.7

[a] Prepared by refluxing diethyl ethylphosphonate with concentrated hydrochloric acid for 24 hours and evaporating the resulting solution to dryness. [b] Prepared by refluxing dibutyl butylphosphonate with concentrated hydrochloric acid for 48 hours, evaporating the resulting solution to dryness, and recrystallizing the residue from benzene. [c] Prepared as described by Freedman, Doak and Petit, *J. Am. Chem. Soc.*, **77**, 4262 (1955). [d] Prepared as described by Doak and Freedman, *J. Am. Chem. Soc.*, **73**, 5658 (1951). [e] Prepared as described by Freedman and Doak, *J. Org. Chem.*, 21, 238 (1956). [f] Because of the limited solubility of phosphafluorinic acid in cold acetic acid, a higher value of Δt could not easily be obtained. [g] Obtained from the Victor Chemical Works and recrystallized from benzene. [h] Prepared as described by Gilman and Brown, *J. Am. Chem. Soc.*, 67, 824 (1945). [i] Prepared by the Bart reaction. [j] Reagent grade material from Merck and Co., Inc.

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[1] The term "phosphinic acid" is used to include both monosubstituted compounds of the type RPO_2H_2 and disubstituted compounds of the type R_2PO_2H. This usage is in accord with the nomenclature proposed by the Organic Division's Advisory Committee on the Nomenclature of Organic Phosphorus Compounds; *cf. Chem. Eng. News*, **30**, 4515 (1952).

[2] (a) Daasch and Smith, *Anal. Chem.*, **23**, 853 (1951); (b) Bellamy and Beecher, *J. Chem. Soc.*, 1701 (1952).

[3] (a) Kosolapoff and Powell, *J. Am. Chem. Soc.*, **72**, 4291 (1950); (b) Kosolapoff and Powell, *J. Chem. Soc.*, 3535 (1950) (c) Ashby and Kosolapoff, *J. Am. Chem. Soc.*, **75**, 4903 (1953).

[4] Kosolapoff, *J. Am. Chem. Soc.*, **74**, 3427 (1952).

[5] Lassettre, *Chem. Revs.*, **20**, 259 (1937).