before another was added (time of addition, five hours). The reaction mixture was then poured into five volumes of water and the resulting precipitate was collected. Recrystallization from methylene chloride–hexane afforded 0.69 g. (49%) of VI, m.p. 186–188° dec. (with recrystallization at 115–120°), $[\alpha]^{25}D$ +44° (1% in chloroform).

Anal. Calcd. for $C_{23}H_{31}O_5Br$: Br, 17.09. Found: Br, 17.04.

16,17-Oxido-4-pregnen-11α-ol-3,20-dione Acetate (VII).—To a solution of 0.5 g. of VI in 50 ml. of glacial acetic acid was added, under an atmosphere of carbon dioxide, a solution containing 272 mg. of semicarbazide hydrochloride, 195 mg. of anhydrous sodium acetate, 10 ml. of water and 10 ml. of glacial acetic acid. The mixture was agitated for ten minutes and there was then added 20 ml. of 1 *N* sodium acetate in glacial acetic acid. Agitation was continued for ten minutes longer, 2 ml. of pyruvic acid was added, and the mixture was refluxed for ten minutes. The cooled solution was diluted with water and extracted with methylene chloride. The extracts were washed free of acid with water, dried over magnesium sulfate and concentrated to a small volume. Hexane was then added to the point of opalescence and the solution was chromatographed on 20 g. of Florisil prepared with hexane. Elution with hexane and mixtures of hexane and ether stripped nothing from the column. From elution with ether there resulted five 50 ml. fractions containing a total of 0.103 g. (25%) of VII, m.p. 212–214°. Recrystallization from methylene chloride–hexane raised the m.p. to 217–218°, $[\alpha]^{25}D$ +112.9° (1% in chloroform).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.55; H, 8.00.

CHEMICAL RESEARCH DIVISION
SCHERING CORPORATION
BLOOMFIELD, NEW JERSEY

The Preparation of 2-C^{14}-Adenine

BY A. R. P. PATERSON AND S. H. ZBARSKY

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As a preliminary to a study of the metabolism of the purines, with especial reference to the 2-position of the ring, the synthesis of adenine labeled in the 2-position with C^{14} was undertaken. The method described by Shaw,[1] in which 4-amino-5-imidazolecarboxamidine is formylated and the product cyclized to give adenine, appeared to be suitable since by using C^{14}-formic acid for the formylation 2-labeled adenine would be obtained. An advantage of this method is that the isotope would be introduced at a late step in the synthesis, thereby minimizing losses of radioactive material. The undesirable feature of the method, however, as far as economy of radioactive material is concerned, is that the formylation is carried out with a large excess of 98% formic acid in the presence of acetic anhydride. This would necessitate the use of an inordinately large amount of C^{14}-formate in order to obtain adenine with appreciable radioactivity.

In order to avoid the use of such a large excess of formic acid, experiments were carried out to study the feasibility of formylating the carboxamidine with an aqueous solution of formic acid, since such conditions have been used to formylate other amines.[2,3] The formylation reaction was found to proceed in 6 *M* formic acid, and by using this modification it was possible to obtain 2-C^{14}-adenine in yields of 60–65%, based on the carboxamidine used. The unreacted C^{14}-formate can be recovered almost quantitatively and used for further preparations of labeled adenine.

Method.—A solution of 0.200 g. of 4-amino-5-imidazolecarboxamidine dihydrochloride[1] in 2.0 ml. of 20% formic acid was placed in a reaction tube made from the outer member of a 24/40 standard taper joint. To this solution was added 0.170 g. of potassium formate, making the solution 6.3 *M* with respect to formate. The solution was then boiled gently under reflux for 4 hours. The formamido derivative was not isolated but was cyclized to adenine by diluting the solution to 8 ml. with water, adding sufficient potassium bicarbonate to neutralize the formic acid and to make the solution 0.5 *M* in bicarbonate, and then boiling under reflux for 1 hour. An amount of hydrochloric acid slightly less than that required to neutralize the solution was added, and the solution was concentrated under reduced pressure to a volume of 2–3 ml. On placing the solution in the refrigerator for several hours crude adenine precipitated. This material was collected by centrifugation, washed 3 times with ice-cold water and dried *in vacuo*. The supernatant and wash liquids were saved for the recovery of unreacted formate. The crude material was sublimed at 220° and a pressure of 1 mm. to give 0.083 g. of pure adenine, a yield of 61% based on the carboxamidine. Yields of 40–42% were obtained when the formylation was carried out with 4.0 *M* formic acid.

Anal. Calcd. for $C_5H_5N_5$: C, 44.44. Found: C, 44.27.

The compound formed a picrate which melted with decomposition at 286–287°.[1] Admixture with picrate prepared from authentic adenine did not depress the m.p. The ultraviolet absorption spectrum and R_f values obtained by paper chromatography[4] were identical with those of authentic adenine.

2-C^{14}-Adenine was prepared by using C^{14}-potassium formate in the above procedure. In a typical experiment, adenine having a specific activity of 1.055×10^6 c.p.m. per m*M* was synthesized and the formate recovered from the reaction mixture had a specific activity of 1.025×10^6 c.p.m. per m*M*.

The unreacted C^{14}-formate in the supernatant fluid and washings after separation of the crude adenine was recovered almost quantitatively by steam distillation.[5] For further use in preparing radioactive adenine, the steam distillate was titrated with standard potassium hydroxide solution and concentrated to small volume under reduced pressure. The concentrate was then transferred to the reaction tube and evaporated to dryness. The appropriate amount of 4-amino-5-imidazolecarboxamidine dihydrochloride was added, followed by hydrochloric acid equivalent to the formate present less the amount of hydrochloric acid present as the dihydrochloride salt. The procedure outlined above was then followed for the remainder of the synthesis.

Acknowledgment.—This work was supported by grants from the National Research Council of Canada.

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DEPARTMENT OF BIOCHEMISTRY
FACULTY OF MEDICINE
THE UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER 8, BRITISH COLUMBIA, CANADA

The Tetrachlorophthalic Anhydride Derivatives of Some Alkylbenzenes

BY GEORGE F. LEWENZ[1a] AND KASPER T. SERIJAN[1b]

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In a previous note[2] the authors reported the phthalic anhydride derivatives of several substituted alkylbenzenes. In general these derivatives distinguish satisfactorily among the alkylbenzene hydrocarbons. However, it is not possible by

(1) Present addresses: (a) The Texas Co., Beacon, N. Y.; (b) Armour and Co., Chicago, Ill.
(2) G. F. Lewenz and K. T. Serijan, THIS JOURNAL, **75**, 4087 (1953).

TABLE I

THE *o*-AROYLTETRACHLOROBENZOIC ACID DERIVATIVES OF BENZENE AND VARIOUS MONO-, DI- AND TRIALKYLBENZENES

o-Aroyltetrachlorobenzoic acid	Molecular formula	Neut. equiv. Calcd.	Neut. equiv. Found	Chlorine, % Calcd.	Chlorine, % Found	Melting point, °C. Observed	Melting point, °C. Literature
Benzene	$C_{14}H_6O_3Cl_4$	364	362	38.96	38.91	[c]	200[a]
Toluene	$C_{15}H_8O_3Cl_4$	378	388	37.51	37.79	174.9–175.8	174.5[b]
Ethylbenzene	$C_{16}H_{10}O_3Cl_4$	392	400	36.17	36.55	176.3–177.1	172–173[g]
1,2-Dimethylbenzene	$C_{16}H_{10}O_3Cl_4$	392	391	36.17	36.31	182.3–182.8	177.5–178.5[g]
1,3-Dimethylbenzene	$C_{16}H_{10}O_3Cl_4$	392	386	36.17	36.38	231.1–231.7	222–224[g]
1,4-Dimethylbenzene	$C_{16}H_{10}O_3Cl_4$	392	392	36.17	36.40	245.2–247.2	244–246[g]
n-Propylbenzene	$C_{17}H_{12}O_3Cl_4$	406	415	34.92	34.76	159.2–160.0	
i-Propylbenzene	$C_{17}H_{12}O_3Cl_4$	406	394	34.92	34.71	187.9–189.0	
1,2,3-Trimethylbenzene	$C_{17}H_{12}O_3Cl_4$	406	396	34.92	34.58	208.6–209.8	
1,3,5-Trimethylbenzene	$C_{17}H_{12}O_3Cl_4$	406	412	34.92	34.65	233.3–233.8	
1,2,4-Trimethylbenzene	$C_{17}H_{12}O_3Cl_4$	406	395	34.92	34.60	[d]	
1-Methyl-2-ethylbenzene	$C_{17}H_{12}O_3Cl_4$	406	413	34.92	34.69	173.0–173.9	
1-Methyl-3-ethylbenzene	$C_{17}H_{12}O_3Cl_4$	406	405	34.92	34.60	168.8–170.1	
1-Methyl-4-ethylbenzene	$C_{17}H_{12}O_3Cl_4$	406	405	34.92	34.59	170.4–171.6	
1,2-Diethylbenzene	$C_{18}H_{14}O_3Cl_4$	420	414	33.76	33.50	164.5–165.7	
1,3-Diethylbenzene	$C_{18}H_{14}O_3Cl_4$	420	415	33.76	33.92	[e]	
1,4-Diethylbenzene	$C_{18}H_{14}O_3Cl_4$	420	404	33.76	33.57	147.0–148.9	
n-Butylbenzene	$C_{18}H_{14}O_3Cl_4$	420	420	33.76	33.16	146.5–147.5	
s-Butylbenzene	$C_{18}H_{14}O_3Cl_4$	420	412	33.76	33.11	165.3–167.0	
i-Butylbenzene	$C_{18}H_{14}O_3Cl_4$	420	414	33.76	33.15	178.7–179.4	
t-Butylbenzene	$C_{18}H_{14}O_3Cl_4$	420	431	33.76	33.24	228.9–229.5	
1,3-Dimethyl-5-ethylbenzene	$C_{18}H_{14}O_3Cl_4$	420	418	33.76	33.65	248.6–249.4	
1-Methyl-4-*i*-propylbenzene	$C_{18}H_{14}O_3Cl_4$	420	418	33.76	33.88	188.5–189.7	
1-Methyl-2-*t*-butylbenzene	$C_{19}H_{16}O_3Cl_4$	434	444	32.67	31.90	205.8–206.3	
1-Methyl-3-*t*-butylbenzene	$C_{19}H_{16}O_3Cl_4$	434	443	32.67	32.44	207.1–208.5	
1-Methyl-4-*t*-butylbenzene	$C_{19}H_{16}O_3Cl_4$	434	425	32.67	32.24	206.1–207.0	
1-Methyl-3,5-diethylbenzene	$C_{19}H_{16}O_3Cl_4$	434	431	32.67	33.10	160.3–161.7	
1,3,5-Triethylbenzene	$C_{20}H_{18}O_3Cl_4$	448	457	31.64	31.73	[f]	

[a] H. Meyer, *Monatsh.*, **25**, 1198 (1904). [b] A. Hofmann, *ibid.*, **36**, 805 (1915). [c] Two crystalline modifications were observed: (1) 190.2–191.7; (2) 203.0–203.5. [d] Two crystalline modifications were observed: transition point: 209.2: m.p. 218.2–218.8. [e] Two crystalline modifications were observed: (1) 167.3–168.5; (2) 178.8–179.3. [f] Three crystalline modifications were observed: (1) 173.4–174.1; (2) 178.4–178.8; (3) 187.0–187.9. [g] H. Underwood, Jr., and W. Walsh, THIS JOURNAL, **57**, 940 (1935).

means of the benzoylbenzoic acids to identify 1,3-dimethylbenzene and 1,4-dimethylbenzene or *s*-butylbenzene and isobutylbenzene. No phthalic anhydride derivative was obtained from 1,4-diethylbenzene and 1-methyl-4-ethylbenzene, and the derivative of isopropylbenzene could not be obtained in sufficient purity to report. Therefore, the authors investigated the tetrachlorophthalic anhydride derivatives reported in the present work. The *o*-aroyltetrachlorobenzoic acids are suitable derivatives to identify 1,3- and 1,4-dimethylbenzene, *s*- and isobutylbenzene, isopropyl- and *n*-propylbenzene, and satisfactory derivatives have been obtained for 1,4-diethylbenzene and 1-methyl-4-ethylbenzene. By means of the *o*-aroylbenzoic acids or the *o*-aroyltetrachlorobenzoic acids or a combination of the two classes, as in the case of the butylbenzenes, it is possible to identify each of the alkylbenzene hydrocarbons investigated except 1-methyl-4-ethylbenzene. This hydrocarbon did not give a derivative with phthalic acid and its derivative formed with tetrachlorophthalic anhydride is identical to that formed with 1-methyl-2-ethylbenzene.

In the present work the tetrachlorophthalic anhydride derivatives of 28 mono-, di- and trisubstituted alkylbenzenes are described. Of these 22 are reported for the first time. Melting points, neutralization equivalents and chlorine analyses or all compounds are presented in Table I. All derivatives were recrystallized until successive recrystallizations gave no significant change in melting point. The melting points available from the literature are given in Table I and are generally in good agreement with the values obtained in this work.

In those instances where the melting points of derivatives of isomeric hydrocarbons were similar, mixed melting points were determined. In this way it was found that a common derivative was obtained from the three isomeric methyl-*t*-butylbenzenes. Similarly, 1-methyl-2-ethylbenzene and 1-methyl-4-ethylbenzene gave the same halogenated keto acid, which was, however, not the same as that derived from 1-methyl-3-ethylbenzene. This fact was indicated by the observation that a mixed melting point taken of the derivative of the 1,2- with that of the 1,3- gave a 15–20° depression and that a similar depression occurred on mixing the derivatives of the 1,3- and the 1,4- substituted hydrocarbons. On the other hand, a mixture of the derivatives of the 1,2- and the 1,4- showed no depression on melting. This could either mean rearrangement into the same keto acid, or the formation of solid solutions. If the former alternative is assumed, the difference of about 2° between the melting points of the 1,2- and the 1,4- might be explained by the formation of a trace of the 1,3-form, just enough to depress the melting point 2–3°.

In the case of the tetrachlorophthalic anhydride

derivatives of 1,2,3- and 1,2,4-trimethylbenzene a mixed melting point showed a slight depression; however, the degree of depression was not sufficient to permit a definite conclusion as to whether rearrangement of the hydrocarbon had or had not occurred.

In this series as in the previously reported one, several derivatives were observed which had at least two crystalline modifications. This phenomenon was exhibited by the tetrachlorophthalic anhydride derivatives of benzene, 1,2,4-trimethylbenzene, 1,3-diethylbenzene and 1,3,5-triethylbenzene.

Experimental

The starting materials and procedures were the same as in the previous work with the following exceptions: (1) The tetrachlorophthalic anhydride used was Eastman Kodak Co. practical grade. (2) The *o*-aroyltetrachlorobenzoic acids were recrystallized by dissolving them in a small volume of hot glacial acetic acid and adding sufficient water to cause precipitation as the solution cooled. (3) The percentage chlorine was determined by burning the acid in a Parr oxygen bomb and then titrating with silver nitrate and potassium thiocyanate using ferrous ammonium sulfate as an indicator.

Acknowledgment.—The authors would like to thank Mr. A. E. Spakowski for his valuable assistance in the chlorine determinations.

LEWIS FLIGHT PROPULSION LABORATORY
NATIONAL ADVISORY COMMITTEE FOR AERONAUTICS
CLEVELAND, OHIO

The Preparation of N-Dialkylphosphorylated Glycine

BY T. LIES, R. E. PLAPINGER AND T. WAGNER-JAUREGG

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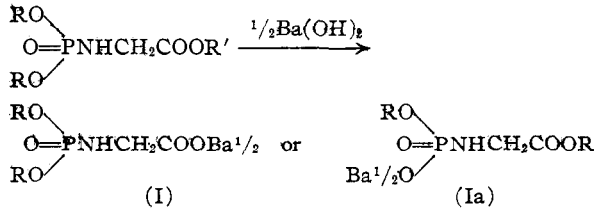
Various N-dialkylphosphorylated amino acid esters have been described recently.[1-3] Attempts to saponify the carboxylic ester group in compounds of this type have been unsuccessful.[1,3] Therefore it has been claimed that the phosphorylation of the α-amino group greatly increases the stability of the carboxylic ester linkage.[1]

By saponification of the esters of N-dialkylphosphorylglycine with one equivalent of barium hydroxide at room temperature we have been able to obtain the corresponding monobarium salts. These were transformed for further characterization into the crystalline guanidine salts.

The question arises as to whether the barium salts formed have the structure I or Ia.

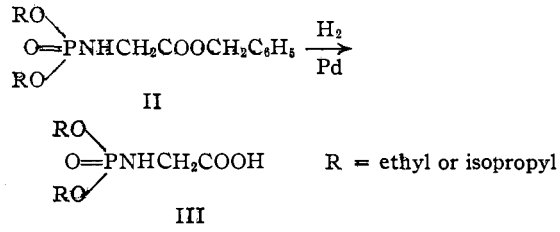


(I) (Ia)

When R and R′ are different alkyl groups (for instance R = butyl or isopropyl and R′ = methyl or ethyl) it was possible to exclude structure Ia as a possible product by elementary analysis of the guanidine salts. In the case where both R and R′ are ethyl groups the isolation of a product corresponding to structure I could be established by the absence of a positive hydroxamate test, a reaction characteristic of carboxylic acid esters.

By treatment of an aqueous solution of the guanidine salt of N-diethylphosphorylglycine with an ion-exchange resin the corresponding free acid can be liberated. Its titration curve indicates a pK_a of 3.8, which is in agreement with the expected value. The free acid corresponding to the barium salt of the structure Ia should have a lower pK_a. The aforementioned results demonstrate that the assumption of a greatly increased stability of the carboxylic ester linkage in N-dialkylphosphorylated α-amino acid esters is unjustified.[4]

N-Diethyl- and N-diisopropylphosphorylglycines (III) also were prepared from the corresponding benzyl esters II by hydrogenolysis.



II

III

No well-defined product could be obtained in the attempted hydrolysis of N-diphenylphosphorylglycine ethyl ester with barium hydroxide. Since the odor of phenol can be detected after addition of 0.5 mole of $Ba(OH)_2$ a hydrolytic attack on the O–P linkage has to be assumed.

The N-dialkylphosphorylglycine esters used for this investigation were prepared by the reaction of glycine esters with dialkylphosphoryl chlorides. N-Diethylphosphorylglycine ethyl ester (IV) is formed also during the reaction of glycine ester with tetraethyl pyrophosphate (TEPP). Previously[2] the interaction of these substances had been tentatively formulated as yielding N,N-bis-diethylphosphorylglycine ester, $H_5C_2O_2CCH_2N[PO(OC_2H_5)_2]_2$ (V). However, material presented in the Experimental Part of this paper makes it evident that a mixture of approximately two moles of IV with one mole of TEPP is obtained. This correction eliminates an apparent exception to the reaction of TEPP with amino compounds. It demonstrates that in the case of glycine ester also, the ordinary monophosphorylation takes place with a tetralkyl pyrophosphate, as originally shown by Atherton and Todd.[5]

Experimental

N-Diethylphosphorylglycine Ethyl Ester (IV).—This substance was obtained by treating glycine ester with diethylphosphoryl chloride in a manner analogous to that of the reaction of diisopropylphosphoryl chloride with amino acid esters.[2] The product obtained had a boiling point of 123–128° (0.3 mm.), $n^{30}D$ 1.4338.

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