by weight of substrate) at 40 atmospheres of pressure. The applicability and high efficiency of the recently developed noble metal synthetic high polymer catalysts, described in previous papers[2,3,4,5] suggested that hydrogenation be attempted employing this type of catalyst. A palladium-polyvinyl alcohol (Pd-PVA) catalyst was prepared wherein the palladium was 0.5% by weight of substrate and cyanoacetic ethyl ester was successfully hydrogenated at atmospheric pressure.

Procedure

The Pd-PVA catalyst was composed of 30 mg. of Pd and 500 mg. of PVA in 100 cc. of 50% alcohol–water mixture which was 0.5 *N* in hydrochloric acid. The purpose of the high acidity was to minimize formation of secondary amines.[6] Six and four-tenths g. of cyanoacetic ester was introduced into the vessel with the catalyst and the whole shaken for twenty hours at room temperature and ordinary pressure. At the end of this time 1822 cc. of a theoretical 2580 cc. of hydrogen had been adsorbed. The rate of adsorption was very slow at this point, so the isolation of β-alanine was started.

The colloidal catalyst was flocculated by addition of about 500 cc. of ethanol. After filtering, the liquid was concentrated. Hereafter, a separation into two layers was noticed, the lower layer containing some unreduced starting material, which is insoluble in water. The latter was drawn off and to assure complete removal of unreduced cyanoacetic ethyl ester, the remaining aqueous layer was extracted twice with ether. The aqueous solution was then taken to dryness under reduced pressure. The residual salt was twice extracted with 35-cc. portions of hot 95% ethanol, filtered while hot and allowed to cool. On cooling crystallization of ammonium chloride, which had been extracted along with the β-alanine ethyl ester hydrochloride, occurred. This was filtered off and discarded. The filtrate was again evaporated to dryness *in vacuo*. The residual salt was extracted with 35-cc. portions of absolute ethanol and filtered. The filtrate was concentrated *in vacuo* and then diluted with about 20 cc. of water and hydrolyzed under reflux. After concentrating under reduced pressure to remove the excess hydrochloric acid, the residue was dissolved in water and made up to a volume of 30 cc. A 2-cc. portion was withdrawn for determination of total halide.[6] A suspension of silver oxide prepared from 10% more than the equivalent quantity of silver nitrate was added to the solution to bring about complete precipitation of halide.

After standing for some time, the precipitate was filtered, the filtrate concentrated and treated with hydrogen sulfide. The silver sulfide is centrifuged off, the solution treated with norite and filtered. The crystals of β-alanine may be obtained by concentrating the filtrate in a vacuum desiccator until crystallization begins. Upon recrystallization from water a product of m. p. 194–196° was obtained with the analysis calculated: N, 15.73; found: N, 15.56; yield, 15%.

No attempt was made to increase this yield as the primary purpose of the experiments was to demonstrate the ability of the colloidal noble metal synthetic high polymer catalysts to serve in the reduction of the nitrile group.

Acknowledgment.—These hydrogenations were carried out with the aid of grants of the Bache Fund of the National Academy of Sciences and the Penrose Fund of the American Philosophical Society. Analyses were performed by Mr. Joseph Alicino of the Squibb Institute for Medical Research, New Brunswick, N. J.

(2) Louis D. Rampino and F. F. Nord, THIS JOURNAL, **63**, 2745, 3268 (1941).

(3) Rampino and Nord, *ibid.*, **65**, 429 (1943).

(4) Kevin E. Kavanagh, *ibid.*, **64**, 2721 (1942).

(5) Kevin E. Kavanagh and F. F. Nord, *ibid.*, **65**, 2121 (1943).

(6) "Organic Syntheses," Vol. 16, John Wiley and Son, Inc., New York, N. Y., 1936, p. 1.

COMMUNICATION NO. 36 FROM THE
DEPARTMENT OF ORGANIC CHEMISTRY
FORDHAM UNIVERSITY
NEW YORK 58, N. Y. RECEIVED SEPTEMBER 22, 1944

Chlorination of Fluorene with Sulfuryl Chloride

BY ANDREW STREITWIESER

Sulfuryl chloride is being used increasingly as a chlorinating agent because of the ease with which it reacts and the high yields obtained. In 1939, Kharasch and Brown[1] treated fluorene with this reagent and reported that the fluorene was chlorinated in the nucleus. However, they did not report the position taken by the entering chlorine atom, nor did they give any yields or procedures. The purpose of this work was to determine the position of the entering chlorine and to find out whether this method is a convenient one for preparing the chloro compound.

The fluorene was chlorinated in ether solution with a slight excess of sulfuryl chloride. The product melted at 95–96°.[2] This compares favorably with the 2-chlorofluorene prepared simultaneously by Chanussot[3] and Courtot and Vignati,[4] m. p. 96–97°. Authentic 2-chlorofluorene was prepared by the method of Chanussot[3] from 2-aminofluorene by the Sandmeyer reaction. A mixed melting point determination showed the two to be identical. The following procedure is recommended as a quick, convenient method for preparing 2-chlorofluorene.

Experimental

2-Chlorofluorene from SO_2Cl_2.—Sixteen grams of fluorene and 120 cc. of anhydrous ether was placed in a distilling flask. Not all of the fluorene dissolved. The flask was stoppered with a rubber stopper carrying a separatory funnel containing 8 cc. (13.4 g.) sulfuryl chloride. The latter was added rapidly to the solution. When all was added, the ether was distilled off, and the residue recrystallized from alcohol. It can also be purified by steam distillation: yield, 85% of a white crystalline powder, m. p. 95–96°.

The author wishes to acknowledge the assistance of Mr. L. Friedman and Mr. E. Kosower in this work.

(1) Kharasch and Brown, THIS JOURNAL, **61**, 2149 (1939).

(2) All melting points corrected.

(3) Chanussot, *Añales asocn. quim. Argentina*, **15**, 216 (1927).

(4) Courtot and Vignati, *Compt. rend.*, **184**, 1479 (1927).

80-29 169TH STREET
JAMAICA 3, N. Y. RECEIVED OCTOBER 13, 1944

The Preparation of N-Alkyl Derivatives of *p*-Aminobenzoic Acid[1]

BY A. R. SURREY AND H. F. HAMMER

It has been reported that procaine hydrochloride and other local anesthetics which are

(1) Presented before the Division of Medicinal Chemistry, A. C. S., Cleveland, Ohio, April, 1944.

TABLE I

PROPERTIES AND ANALYSES OF COMPOUNDS

	Alkyl	Yield, %	M. p., °C.	Formula	Nitrogen,[d] % Calcd.	Found	Nitroso deriv. M. p., °C.	Nitrogen, % Calcd.	Found
				Methyl *p*-Alkylaminobenzoates					
I	Ethyl[a]	41	138–139	$C_{10}H_{13}NO_2$	7.82	7.93	72–73	13.48	13.22
II	*n*-Propyl	30	61–62	$C_{11}H_{15}NO_2$	7.25	7.36	52–53	12.61	12.56
III	*n*-Butyl	47	104–105	$C_{12}H_{17}NO_2$	6.76	6.51	.....	...	...
				Ethyl *p*-Alkylaminobenzoates					
IV	Ethyl	50	72–73	$C_{11}H_{15}NO_2$	7.25	7.43	55–56	12.61	12.74
V	*n*-Propyl[b]	45	68–69	$C_{12}H_{17}NO_2$	6.76	6 72	54–55	11.88	12.14
VI	*n*-Butyl[c]	80	68–69	$C_{13}H_{19}NO_2$	6.33	6.49	.....	.....	...
				p-Alkylaminobenzamides					
VII	Ethyl	39	144–145	$C_9H_{12}N_2O$	17.08	16.90	187–188	21.76	21.90
VIII	*n*-Propyl	54	137–138	$C_{10}H_{14}N_2O$	15.73	15.95	177–178	20.28	20.59
IX	*n*-Butyl	60	111–112	$C_{11}H_{16}N_2O$	14.58	14.60	179–180	19.00	19.15

[a] Mentioned in U. S. Patent 2,073,100 (1937). [b] Described in German Patent 431,166, m. p. 70°. [c] Described in U. S. Patent 1,889,645 (1932), m. p. 69–70°. [d] Microanalyses were carried out in these Laboratories by Miss Esther Bass and Miss Patricia Curran.

esters of *p*-aminobenzoic acid have antisulfonamide action.[2] Dimethylaminoethyl *p*-butylaminobenzoate (Pontocaine), another common local anesthetic, a derivative of *p*-butylaminobenzoic acid, also was reported to show this behavior. In the course of an investigation to determine whether N-substitution in general affects antisulfonamide action, a series of *p*-alkylaminobenzoic acid esters and amides was prepared; these compounds are described in Table I.

These alkyl derivatives were all prepared from the corresponding amino compounds. The N-ethylaminobenzoic esters were prepared by ethylation with ethyl sulfate. Ethyl iodide was used to prepare the N-ethylaminobenzamide. This amide and the ethyl ester of *p*-ethylaminobenzoic acid were separated from the reaction mixture and purified by means of their nitroso derivatives. The N-propyl and *n*-butyl derivatives were prepared by reaction of the amino compound with the appropriate aldehyde in a reducing medium.[3] Attempts to prepare the amides from the alkylaminobenzoic acids by fusion and treatment with ammonia gave only the corresponding alkylanilines. The same results were obtained by heating the amino acids at 210–220°. These results can be explained when the alkylaminobenzoic acids are considered as vinylogs of carbamic acid.

Bacteriological investigations indicate that whereas local anesthetics derived from *p*-aminobenzoic acid show marked antisulfonamide activity, the N-alkylamino derivatives, including Pontocaine, have little or no such effect. Details of this work will be published elsewhere from these Laboratories.

Experimental

Methyl *p*-Ethylaminobenzoate.—A mixture of 25 g. (0.165 mole) of methyl *p*-aminobenzoate, 25.7 g. (0.165 mole) of ethyl sulfate and 82.5 cc. (0.165 mole) of 2 *N* sodium hydroxide was refluxed gently for twenty minutes. The solid which separated on cooling was filtered off and washed with water. The product was purified by recrystallizations from methyl alcohol.

Methyl *p*-Propylaminobenzoate.—Eleven and one-half grams (0.198 mole) of propionaldehyde was added dropwise over a period of one and one-half hours to a well stirred refluxing mixture of 25 g. (0.165 mole) of methyl *p*-aminobenzoate, 42.5 g. (0.65 mole) of zinc dust, 40 g. (0.67 mole) of glacial acetic acid, and 130 cc. of benzene. After an additional one and one-half hours of refluxing, the benzene solution was filtered hot and dilute sodium hydroxide solution was added until the aqueous layer remained alkaline. The benzene layer was separated, dried over sodium sulfate, the benzene removed by distillation, and the residue was vacuum distilled. The product was collected at 143–145° (1 mm.). It was recrystallized first from dilute alcohol and then from benzene and "Skellysolve A." Compounds III, V and VI were prepared by this procedure.

Ethyl *p*-Ethylaminobenzoate.—A mixture of 82.5 g. (0.5 mole) of ethyl *p*-aminobenzoate, 77 g. (0.5 mole) of ethyl sulfate and 250 cc. of 2 *N* sodium hydroxide was refluxed for one-half hour. The oily layer was separated and dissolved in 400 cc. of dilute hydrochloric acid. To this solution, with ice cooling and stirring, was added a solution of 35 g. of sodium nitrite in 100 cc. of water. The nitroso derivative which separated was filtered off, washed with dilute hydrochloric acid and then water and dried. The product was dissolved in 600 cc. of absolute alcohol and saturated with dry hydrogen chloride. It was then refluxed for thirty minutes and most of the alcohol was distilled under reduced pressure. The hydrochloride of ethyl *p*-ethylaminobenzoate separated. A sample was recrystallized from a mixture of alcohol and ether; m. p. 139–140°.

Anal. Calcd. for $C_{11}H_{16}NO_2Cl$: N, 6.14. Found: N, 6.29.

Water was added to the alcohol residue to dissolve the hydrochloride completely and the solution was filtered with charcoal. The base was precipitated with sodium carbonate and recrystallized from dilute alcohol.

Butylaniline.—Twenty-nine grams of *p*-butylaminobenzoic acid was heated in a flask at 220° for two hours, and 40 cc. of benzene was added. After cooling, a small amount of unchanged acid was filtered off. The benzene in the filtrate was removed and the residue was vacuum distilled; b. p. 68° (1 mm.). The hydrochloride was recrystallized from ethyl acetate; m. p. 114–115°.

Anal. Calcd. for $C_{10}H_{16}NCl$: N, 7.55; Cl, 19.15. Found: N, 7.81; Cl, 18.92.

(2) Kelch, Baker, Krahl and Clowes, *Proc. Soc. Exptl. Biol. Med.*, **47**, 533 (1941).

(3) Lockemann, *Frdl.*, **16**, 356 (1927); German Patent 491,856.

p-**Ethylaminobenzamide.**—A mixture of 6.8 g. (0.05 mole) of *p*-aminobenzamide, 7.8 g. (0.05 mole) of ethyl iodide and 5 g. of sodium bicarbonate in 75 cc. of 35% aqueous alcohol was refluxed on the steam-bath for eight hours. Most of the solvent was distilled off, water added, and the solid filtered off. The product was dissolved in 70 cc. of dilute hydrochloric acid, cooled in ice water and to it, with stirring, was added a solution of 3 g. of sodium nitrite in 15 cc. of water. The nitroso derivative was filtered off, washed with water and dried; crude yield, 5.5 g. It was dissolved in 180 cc. of hot absolute alcohol and filtered with charcoal. Dry hydrogen chloride was bubbled into the cooled and well stirred filtrate at a rapid rate until all the solid went into solution. The clear solution was stirred at room temperature for four and one-half hours, filtered with charcoal, and evaporated almost to dryness. Water was added to dissolve the hydrochloride which separated and the solution was made alkaline with ammonium hydroxide. On standing, the product separated out. It was recrystallized from hot water.

p-**Propylaminobenzamide.**—A mixture of 10.8 g. of *p*-aminobenzamide, 60 g. of zinc dust, 100 cc. of glacial acetic acid, and 200 cc. of absolute alcohol was refluxed on a steam-bath with stirring. Five grams of freshly distilled propionaldehyde was added over a period of one hour and refluxing continued for one hour longer. The solid was filtered off and the filtrate was steam distilled to remove the alcohol and acetic acid. The residue (about 750 cc.) was cooled and the solid which separated was filtered off and recrystallized from dilute alcohol. Compound IX was prepared by a similar procedure.

RESEARCH LABORATORIES
WINTHROP CHEMICAL CO., INC.
RENSSELAER, N. Y.

RECEIVED JUNE 9, 1944

COMMUNICATIONS TO THE EDITOR

THE NATURE OF CYPRIDINA LUCIFERIN

Sir:

Johnson and Eyring have recently stated[1] that "'luciferin' apparently contains both coenzyme (I or II) and a flavin prosthetic group." Ball and Ramsdell[2] are led to "suspect that flavin-adenine dinucleotide may play some role in the luminescent mechanisms of the firefly." Since *Cypridina* luciferin is oxidizable[3] reversibly[4] this latter suggestion is a plausible possibility. The more definite conclusion of Johnson and Eyring[1] is *a priori* a very attractive one because of the fundamental importance of the coenzymes and flavins in cellular oxidations and the occurrence of flavins in a number of oxidases. Unfortunately, a reëxamination of the experimental material on luciferin does not entirely confirm their conclusion.

In regard to the absorption spectrum of *Cypridina* luciferin, comparison should be made with the flavins rather than the flavoproteins. Oxidized riboflavin has maxima at about 3600 and 4500 Å.[5] while reduced riboflavin is colorless. Reduced luciferin concentrates show a maximum at about 4300 Å.[6] and hence, since this is probably due to luciferin, it is a colored compound. This band disappears after brief aeration and a new band appears at about 4700 Å. The 4700 Å. band disappears after prolonged exposure of the solution to air while a band at about 3600 Å. appears.

Johnson and Eyring report[1] that luminescence occurs after treatment of "luciferase" solution with $Na_2S_2O_4$, reduced coenzymes or riboflavin. Such an experiment is difficult to interpret if the observed luminescence was faint. It is easily possible for the partially dark adapted eye to see one millionth part of the light emitted by the luciferin from a small amount of *Cypridina*.[7] In any case if reduced riboflavin is to be regarded as a substrate in the luminescent reaction identical with or analogous to luciferin, the amount of light emitted should be large and should be related to the amount of substrate oxidized.

Indirect studies on the oxidation–reduction potential of luciferin[4] place it near toluhydroquinone and hydroquinone.[8] The oxidation reduction potential of riboflavin is 0.4 or 0.5 v. lower than this at *p*H 7.0, while that of coenzyme is still lower. It has been reported that luciferin concentrates contain no nitrogen,[9] although the sensitivity of the method in relation to the amount of partially purified luciferin was not stated. In unpublished experiments of Dr. M. Kunitz, attempts to measure coenzyme I in luciferin preparations were unsuccessful. Here the sensitivity was such that the luciferin sample could hardly have contained as much as 2% of coenzyme.

The available data, therefore, although they may not exclude the conclusion of Johnson and Eyring, certainly give it little support.

(1) Johnson and Eyring, THIS JOURNAL, **66**, 848 (1944).
(2) Ball and Ramsdell, *ibid.*, **66**, 1419 (1944).
(3) Harvey, *J. Gen. P.*, **1**, 133 (1918).
(4) Anderson, *J. Cell. & Comp. Physiol.*, **8**, 261 (1936).
(5) Warburg and Christian, *Biochem. Z.*, **298**, 150 (1938).
(6) Chase, *J. Biol. Chem.*, **150**, 433 (1943).
(7) Harvey, *Science*, **57**, 501 (1923).
(8) This suggests the desirability of investigating luciferin as a possible link between flavins and oxygen.
(9) Chakravorty and Ballentine, THIS JOURNAL, **63**, 2030 (1941).

UNIVERSITY OF MARYLAND MEDICAL SCHOOL
BALTIMORE, MARYLAND
PRINCETON UNIVERSITY ROBERT S. ANDERSON
PRINCETON, NEW JERSEY AURIN M. CHASE

RECEIVED NOVEMBER 3, 1944

COLORIMETRIC TESTS FOR DDT AND RELATED COMPOUNDS

Sir:

The revolutionary development of the insecticide DDT,[1] the major portion of the technical product being 2,2-bis(*p*-chlorophenyl)-1,1,1-tri-

(1) Annand, *J. Econ. Entomol.*, **37**, 125 (1944); Froelicher, *Soap and Sanit. Chem.*, **20** (7), 115 (1944).