that the purification of this substance is not as readily accomplished as would be expected. That this is obviously the case, can be concluded from the recent studies on the chemical composition of technical DDT by Haller, Bartlett, Drake, Newman and associates, and reported melting point data by these and other investigators,[2] which values range from 105 to 109°. Moreover the fact that three grades of DDT are recognized by the War Production Board and the armed forces of this country is a further suggestion of difficulties involved in its purification. The writers have found that a simple extraction or washing process of technical or laboratory prepared DDT specimens will provide a grade of purity at least equal to the minimum limit prescribed for aerosol quality (m. p. 103°) and, in most instances a value of 105° or better. The details of the experimental purification procedure of a technical DDT specimen procured from a well-known supply distributor are given as follows.

One hundred grams of the technical product which melted at 60° is treated with 50 ml. of 95% ethyl alcohol to form a thick paste and then diluted with 300 ml. of water. This mixture is filtered on a Buchner, transferred to a 600 ml. beaker and treated with 250 ml. of 95% ethyl alcohol. After chilling in an ice–salt brine it was filtered and washed with an additional 100-ml. portion of cold alcohol. The alcohol extracted product is treated similarly with (30–60°) petroleum ether and after filtration, washing and drying, 65 g. of partially purified DDT m. p. (block value) 106–107° is obtained. The latter fraction on recrystallization from 800 ml. hot 95% ethanol gave 60 g. of product, m. p. 109.5–110° (cor.). This upon two additional and alternate petroleum ether extractions, and alcohol recrystallizations under conditions as described above, yielded 46 g. pure product m. p. 110–110.5° (cor.) in a Roth apparatus. Further purification attempts did not change this value. Needless to say in ordinary purification work, the products from the previous steps were not completely dried inasmuch as the efficiency of subsequent extractions is believed to be lowered due to decreased wetting effect of the solvent; furthermore less time is required in the purification process. Thus, the technical specimen referred to above contains approximately 67% DDT, and approximately 70% of the actual DDT is recovered. Cautious evaporation of the several extraction filtrates to remove the last traces of solvent reveals evidence in the residues of progressively decreasing quantities of contaminants together with some pure DDT.

Laboratory specimens prepared by a modification of the chlorosulfonic acid condensation,[3] and similarly purified as previously described, melted at 110–110.5° (cor.). Mixed melting point of the laboratory purified product and that isolated from technical DDT, showed no depression.

In order to establish further that the compound with m. p. 110–110.5° was pure DDT and not DDD[4] (1,1-di-*p*-chlorophenyl-2,2-dichloroethane), which melts also at 110.5–111°, a mixed melting point determination of both substances showed a marked depression. Additional evidence for the identification of our product (m. p. 110–110.5° cor.) as DDT is given in the following.

Anal.[5] Calcd. for $C_{14}H_9Cl_5$: C, 47.43; H, 2.56; Cl, 50.01. Found: C, 47.06; H, 2.76; Cl, 49.93.

A dinitro derivative[2] of our product melted at 148–148.5° (cor.). This value is in agreement with that reported by Haller and associates, and higher than the 143° value reported by Zeidler. In ordinary recrystallization operations in which large quantities of partially purified DDT are involved acetone is superior to alcohol inasmuch as less acetone is required and the contaminants are more soluble. For example, with 354.5 g. (1 mole) of partially purified DDT (m. p. 105–107°) dissolved in 1600 ml. acetone at room temperature, filtered and on slow addition of 400 ml. water with stirring, a product was obtained which upon drying weighed 335 g. (94.7% recovery); m. p. 109.5–110° (cor.). With alcohol in place of acetone as the solvent, at least two recrystallizations each with a solvent-volume, solute-weight ratio of approximately 10 to 1 were found necessary to achieve the same degree of purity. It should be noted, further, that the above described purification procedure is not intended to cover commercial dusting powders or artificially blended spray preparations containing DDT as one of the ingredients. Moreover, as a future primary reference standard for entomological and pharmacological studies, the product which melts at 110–110.5° (cor.) is recommended for acceptance.

(2) Haller, Bartlett, Drake, Newman and co-workers, THIS JOURNAL, **67**, 1591 (1945); Gooden, *ibid.*, **67**, 1617 (1945); Zeidler, *Ber.*, **7**, 1180 (1874); Bailes, *J. Chem. Ed.*, **22**, 122 (1945), and Gunther, *ibid.*, **22**, 238 (1945). The latter states that one to two recrystallizations from excess ethanol or isopropanol usually yields a m. p. of 105° and to secure a very pure product it is usually necessary to decolorize the material at least once with charcoal, followed by four or five recrystallizations.

(3) Rueggeberg and Torrans, *Ind. Eng. Chem.*, **38**, 211 (1946).

(4) Sample kindly supplied by Dr. William A. Mosher of Pennsylvania State College.

(5) Duplicate analyses were made and the average values for C, H and Cl reported here. Microanalyses by Dr. Carl Tiedcke.

KNIGHT CHEMICAL LABORATORY
UNIVERSITY OF AKRON
AKRON, OHIO

RECEIVED NOVEMBER 23, 1945

Specificity of the Action of Urease

BY CLARA L. DEASY

Urease catalyzes the hydrolysis of urea, but the enzyme has been shown to be without effect on a number of derivatives of urea.[1] This study was made to determine whether urease can catalyze the hydrolysis of guanylurea, or whether the action of urease on urea can be inhibited by guanylurea.

Experimental.—Guanylurea was used in the form of the commercially available sulfate,[2] which was recrystallized after treatment with bone charcoal. The urease solution was prepared according to the Folin–Wu method,[3] except that the concentration of the solution was increased by using five times the amount of jack bean meal.

The urea was used in 3% solution (30 mg./ml.); 5 ml. of water and 1 ml. of urease solution were added in each determination. The mixtures were incubated for fifteen minutes at 45–50°. Analysis of extent of hydrolysis was made according to the method of Marshall,[4] by titration with standard hydrochloric acid (0.09306 *N*) with methyl orange as indicator. Blanks were run on the urease, urea, guanylurea sulfate and guanylurea sulfate + urea, each incubated for fifteen minutes at 45–50° with 5 ml. of water. The necessary blanks were subtracted from the volume of standard hydrochloric acid used in each determination to give the corrected volume (Column 4, Table I). Each experiment is an average value of 4 determinations.

In experiment 1 the blanks for guanylurea sulfate and for urease exceeded by 0.03 ml. the volume of standard hydro-

(1) See, for example, Armstrong and Horton, *Proc. Roy. Soc.* (London), **B85**, 109 (1912); Cajori, *Proc. Soc. Exptl. Biol. Med.*, **30**, 184 (1932); Bonnet and Razafimahery, *Enzymologia*, **1**, 55 (1936).

(2) Supplied through the courtesy of American Cyanamide and Chemical Corp.

(3) Peters and Van Slyke, "Quantitative Clinical Chemistry," Vol. II, Williams and Wilkins Co., Baltimore, Md., 1932, p. 545.

(4) Marshall, *J. Biol. Chem.*, **14**, 283 (1913).

TABLE I

No.	Guanylurea sulfate, mg.	Urea added, mg.	HCl, ml. cor.	Urea found, mg.
1	100	0	−0.03	..
2	0	30.0	10.68	29.8
3	100	30.0	10.62	29.7
4	100	30.0	10.36	28.9
5	100	30.0	10.39	29.0

chloric acid required for an end-point when guanylurea sulfate and urease were incubated together. Similar results were obtained when the incubation time for the mixture was increased to thirty minutes. In experiment 3 the urease was added to a mixture of guanylurea sulfate and urea. To determine whether the action of urease on urea would be influenced by previous treatment with guanylurea sulfate, the urease was added to the guanylurea sulfate in Expts. 4 and 5 and kept in contact with it at room temperature for five and for fifteen minutes, respectively, before the urea was added.

Results.—The experiments indicate that urease does not catalyze the hydrolysis of guanylurea sulfate. A solution of urease which has first been treated with guanylurea sulfate gives somewhat low values in the determination of urea; this effect is, however, very slight.

TEMPLE UNIVERSITY
SCHOOL OF MEDICINE
PHILADELPHIA 40, PA. RECEIVED APRIL 25, 1946

Picrolonates of Bufotenine, Bufotenidine and Dehydrobufotenine

BY VENANCIO DEULOFEU AND BLANCA BERINZAGHI

Following our work[1] on the basic constituents of the venom of some South American toads, we have prepared the picrolonates of the indolic bases present in toad venom.

The picrolonates of bufotenidine and dehydrobufotenine can be prepared and purified more easily than bufotenine picrolonate. Dehydrobufotenine can be isolated more readily as the picrolonate than as its picrate, from the final residual mother liquors of the toad venom when the bases are extracted according to our procedure.[1] Dehydrobufotenine picrolonate, however, has too high a melting point to be of ready use in identification.

Bufotenine Picrolonate.—Amorphous bufotenine from *B. arenarum* was dissolved in ethanol and treated with an small excess of picrolonic acid. By heating the acid dissolves and when the solution was cooled crystals were obtained melting not sharply at 110°. By recrystallizing from ethanol, small, yellow prisms, melting 120–121° were obtained.

Anal. Calcd. for $C_{12}H_{16}ON_2 \cdot C_{10}H_8O_5N_4$: N, 17.94. Found: N, 18.34.

Bufotenidine Picrolonate.—Bufotenidine iodide was obtained from amorphous bufotenine and methyl iodide according to Wieland, Konz and Mittasch,[2] and melted at 210°. The iodide was dissolved in a little amount of water and treated with a slight excess of picrolonic acid. This was dissolved by heating, and on cooling yellow prisms melting 253–255° were obtained. Recrystallized from 50% ethanol, the fine yellow needles melted 255°.

Anal. Calcd. for $C_{13}H_{18}ON_2 \cdot C_{10}H_8O_5N_4$: N, 17.42. Found: N, 17.14.

Dehydrobufotenine picrolonate was obtained by treating a water solution of dehydrobufotenine hydrochloride with an excess of picrolonic acid, heating to dissolution and cooling. The picrolonate precipitates and after crystallization from ethanol (50%) yellow prisms melting above 300° and darkening from 275° (quick heating) were obtained.

Anal. Calcd. for $C_{12}H_{14}ON_2 \cdot C_{10}H_8O_5N_4$: N, 18.02. Found: N, 17.99.

A similar picrolonate was obtained from the solution of crude bases of *B. arenarum* after separation of bufotenine.

FACULTAD DE CIENCIAS EXACTAS F. Y. N.
LABORATORIO DE QUÍMICA ORGÁNICA
BUENOS AIRES, ARGENTINA RECEIVED MARCH 20, 1946

(1) V. Deulofeu and E. Duprat, *J. Biol. Chem.*, **153**, 459 (1944).
(2) H. Wieland, W. Konz and H. Mittasch, *Ann.*, **513**, 1 (1934).

The Use of Liquid Phase Oxidation for the Preparation of Nuclearly Substituted Styrenes. II. *p*-Vinylphenyl Acetate

BY WILLIAM S. EMERSON, JOSEF W. HEYD, VICTOR E. LUCAS, WILLIAM B. COOK, GRAFTON R. OWENS AND ROBERT W. SHORTRIDGE

In a previous paper[1] we have shown that methyl *p*-ethylbenzoate is smoothly oxidized to methyl *p*-acetylbenzoate by air in the presence of chromium oxide and calcium carbonate. While the oxidation of *p*-ethylphenyl acetate to *p*-acetylphenyl acetate is a great deal more difficult, we have accomplished it successfully (24% conversion and 79% yield) by means of oxygen in the presence of a chromium oxide–cobalt hydrate–calcium carbonate catalyst. Any free phenol in the reaction mixture inhibits the oxidation altogether, so that its presence must be rigorously avoided.

The *p*-acetylphenyl acetate was smoothly hydrogenated in the presence of copper chromite to *p*-(α-hydroxyethyl)-phenyl acetate. Distillation of the latter compound from potassium bisulfate yielded 48% of *p*-vinylphenyl acetate based on this carbinol.

The authors are grateful to Dr. G. F. Deebel and Messrs. C. E. Wheelock, E. L. Ringwald and R. P. Haase for the preparation of considerable quantities of *p*-ethylphenol.

Experimental

***p*-Ethylphenol** was prepared essentially according to the method of Hartman.[2] One hundred fifty-two grams (58%) was obtained from the fusion of 450 g. of sodium *p*-ethylbenzenesulfonate with 300 g. of potassium hydroxide and 750 g. of sodium hydroxide. It boiled at 95–101° (10 mm.) (218.5–219.5°).[3]

***p*-Ethylphenyl Acetate.**—*p*-Ethylphenyl acetate was prepared by refluxing for six hours 713 g. of *p*-ethylphenol with 1 liter of acetic anhydride containing 100 g. of sodium acetate. The reaction mixture was diluted with water and benzene, the layers separated and the benzene layer dis-

(1) Emerson, Heyd, Lucas, Chapin, Owens and Shortridge, THIS JOURNAL, **68**, 674 (1946).
(2) Hartman, "Org. Syntheses," Coll. Vol. I, p. 175.
(3) Béhal and Choay, *Bull. soc. chim.*, [3] **11**, 209 (1894).