theoretically required for complete esterification. Heating of the reaction mixture often occurred at this point. The mixture was allowed to stand at room temperature for twenty-four hours after all of the sugar was in solution. Stirring was helpful in accelerating the rate of solution of the sugar in certain cases, as with sucrose, in which an exothermic reaction does not occur. The reaction mixture was then concentrated *in vacuo* with a bath temperature of 50–80°. If the derivative was known to be crystalline, the sirupy residue was induced to crystallize by scratching or seeding. The crystals were then dried in a vacuum desiccator over solid sodium hydroxide and concentrated sulfuric acid and finally in a vacuum oven. When the ester was a sirupy liquid it was either purified directly by distillation or freed of traces of pyridine by drying in thin layers in a vacuum oven.

Acyl analyses, with the exception of those on the esters of D-glucose, were carried out by saponification at room temperature in acetone solution with either aqueous or alcoholic potassium hydroxide. The glucose esters were analyzed by a slight modification of the method of Elek and Harte.[4]

Acknowledgment.—Mr. C. H. Van Etten performed several of the acyl analyses listed in the table.

STARCH AND DEXTROSE DIVISION
NORTHERN REGIONAL RESEARCH LABORATORY
PEORIA, ILLINOIS[5] RECEIVED JULY 7, 1945

(4) Elek and Harte, *Ind. Eng. Chem., Anal. Ed.*, **8**, 267–269 (1936).

(5) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

The Species Specificity of Heparin

BY M. L. WOLFROM,[1] J. V. KARABINOS,[1] C. S. SMITH,[2] P. H. OHLIGER,[2] J. LEE[3] AND O. KELLER[3]

Charles and Scott[4] demonstrated that a blood anticoagulant, presumably heparin, was widely distributed in various tissues of beef. It was found in greatest quantities in muscle, liver and lung; smaller quantities were obtained from heart, thymus, spleen and blood. Charles and Todd[5] proved the chemical and biological identity of the crystalline barium acid heparinate isolated from beef lung and beef liver. Jaques, Waters and Charles[6] isolated crystalline barium acid heparinate from the lungs of pork and sheep and from the liver of dogs. These workers found no significant chemical differences in the product from these sources and from beef but they claimed a wide variation in biological activity, the blood anticoagulant potencies being in the order 10:5:2:1 = dog:beef:pork:sheep.

Species specificity is the rule with protein material but is unusual for carbohydrate principles. The heparin from dog liver, being reputedly of the highest activity, was of greatest interest and was accordingly prepared. The crude heparin (sodium heparinate) was isolated from the excised dog livers according to the general procedure of Charles and Scott.[7] The crude product was purified through the benzidine salt and converted into the amorphous sodium salt. This substance showed an anticoagulant potency[8] of 577 Roche anticoagulant units (ACU) per mg. (dry basis). The sodium salt was transformed into the crystalline barium acid heparinate which showed a potency of 600 Roche ACU per mg. (dry basis). These potencies are entirely within the normal range for beef heparin prepared by the same procedures. Had the potencies of the dog heparin been twice that of beef, values in the range of 1200 Roche ACU per mg. should have been found.

Our results therefore do not support the claim of Jaques and co-workers that a species variation exists between dog and beef heparin. It is possible that the variation in potencies found by these workers is to be ascribed to the sensitivity of the crystalline barium acid salt and the ease with which it is inactivated by mild acidity.[9]

(1) Department of Chemistry, The Ohio State University.

(2) Department of Physiological Chemistry, The Ohio State University.

(3) Scientific Department, Hoffmann-La Roche, Inc.

(4) A. F. Charles and D. A. Scott, *J. Biol. Chem.*, **103**, 431 (1933).

(5) A. F. Charles and A. R. Todd, *Biochem. J.*, **34**, 112 (1940).

(6) L. B. Jaques and E. T. Waters, *J. Physiol.*, **99**, 454 (1941); L. B. Jaques, *Science*, **93**, 488 (1941); L. B. Jaques, E. T. Waters and A. F. Charles, *J. Biol. Chem.*, **144**, 229 (1942).

(7) A. F. Charles and D. A. Scott, *Biochem. J.*, **30**, 1927 (1936).

(8) The bioassays were performed according to the procedure described by R. H. K. Foster, *J. Lab. Clin. Med.*, **27**, 820 (1942). We are indebted to Dr. R. H. K. Foster for the bioassays.

(9) M. L. Wolfrom and W. H. McNeely, THIS JOURNAL, **67**, 748 (1945).

NUTLEY, NEW JERSEY
COLUMBUS, OHIO RECEIVED JULY 7, 1945

NEW COMPOUNDS

p-Acetamino-(β-chloro-*t*-butyl)-benzene

The monoacetamino derivative of neophyl chloride was prepared by means of the general procedure previously described.[1] Recrystallization from dilute alcohol yielded nacreous flakes, m. p. 155–156°.

Anal. Calcd. for $C_{12}H_{16}ONCl$: Cl, 15.72. Found: Cl, 15.59.

No diacetamino derivative was isolated, presumably because of hindrance by the chlorobutyl group of nitration in the ortho position.

(1) V. N. Ipatieff and L. Schmerling, THIS JOURNAL, **59**, 1056 (1937); **60**, 1476 (1938).

RESEARCH LABORATORIES
UNIVERSAL OIL PRODUCTS COMPANY V. N. IPATIEFF
RIVERSIDE, ILLINOIS LOUIS SCHMERLING
RECEIVED JULY 19, 1945

N,N-Dimethylphthalamidic Acid

The reaction of phthalic anhydride with dimethylamine gave a 71–75% yield of N,N-dimethylphthalamidic acid. In a 3-liter, two-necked, round-bottomed flask fitted with a reflux condenser and an inlet tube were placed 296.2 g. (2.0 moles) of phthalic anhydride and 1000 ml. of *dry* benzene. The mixture was heated to boiling and 90 g. (2.0 moles) of liquid dimethylamine was allowed to evaporate in a separate flask and bubble slowly through the inlet tube. (The dry amine was previously obtained by the