

NOTES

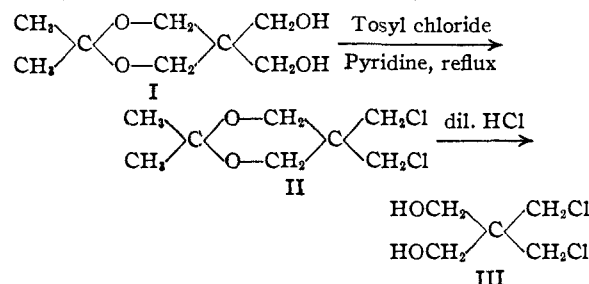
Pentaerythritol Dichloride

BY H. RAPOPORT¹

Previous workers have prepared pentaerythritol dichlorohydrin (III) from pentaerythritol, with the mono-, tri- and tetra-analogs and other products, by the action of sulfur monochloride,² concentrated hydrochloric acid in a sealed tube,³ and thionyl chloride and pyridine.⁴

During the course of some work on the chlorine substitution products of pentaerythritol, we prepared the dichlorohydrin (III) by a method which prevented the formation of any mono-, tri- or tetrachloro compound, and found that (III) melted at 79–80°, uncor. This is in close agreement with the value reported by Mooradian and Cloke⁴ (83° cor.) but differs significantly from those of Bougault² (65°) and Fecht³ (95°), who probably did not have the pure compound.

In our preparation of (III) 2,2-dimethyl-5,5-bis-(hydroxymethyl)-1,3-dioxane⁵ (I) was refluxed



in pyridine with *p*-toluenesulfonyl chloride to give 2,2-dimethyl-5,5-bis-(chloromethyl)-1,3-dioxane (II), which on warming with dilute hydrochloric acid gave (III).

Experimental⁶

2,2-Dimethyl-5,5-bis-(chloromethyl)-1,3-dioxane (II).—To a cooled solution of 13.4 g. (0.07 mole) of *p*-toluenesulfonyl chloride (purified) in 30 ml. of pyridine (dried over potassium hydroxide) was added slowly with shaking 6.2 g. (0.035 mole) of 2,2-dimethyl-5,5-bis-(hydroxymethyl)-1,3-dioxane (I), m. p. 126–127°,⁵ so that the internal temperature did not rise above 35°. After the addition was completed, the solution was refluxed for sixteen hours, cooled, the precipitated pyridinium *p*-toluenesulfonate filtered with suction and washed with 5 ml. of dry pyridine, and the combined filtrate and washings evaporated under reduced pressure. The residual magma was dissolved in 100 ml. of chloroform, and the chloroform solution was washed with dilute sulfuric acid, saturated aqueous sodium bicarbonate, and distilled water. The crystalline solid remaining after evaporation of the chloroform was recrystallized from methanol–water using decolorizing

carbon and dried over sulfuric acid. The yield was 3 g. of II, 40%. A sample, after sublimation at 35–40° at 1 mm., had a m. p. of 48–49°.

*Anal.*⁷ Calcd. for C₈H₁₄O₂Cl₂: Cl, 33.30; mol. wt., 213. Found: Cl, 33.42, 33.36; mol. wt., 203, 210 (Rast).

Pentaerythritol Dichlorohydrin (III).—A suspension of 1.5 g. (0.007 mole) of 2,2-dimethyl-5,5-bis-(chloromethyl)-1,3-dioxane (II) in 25 ml. of 0.5 *N* hydrochloric acid was heated on the steam-bath, and the effluent was bubbled through a solution of iodine in 10% sodium hydroxide in order to observe the evolution of acetone (by iodoform formation). After fifteen minutes, a heavy cloudiness appeared in the iodine solution, and after fifty minutes (total time on steam-bath) the hydrolysis appeared to be complete. The acid solution was now evaporated under reduced pressure to a sirupy residue which crystallized on cooling. Recrystallization first from water and then from benzene gave 0.5 g., 41%, of pentaerythritol dichlorohydrin (III). A sample after sublimation in high vacuum melted at 79–80°.

*Anal.*⁷ Calcd. for C₆H₁₀O₂Cl₂: Cl, 41.00; OH, 19.65. Found: Cl, 41.36, 41.10; OH, 19.5.

Purification of Pyridinium *p*-Toluenesulfonate.—The pyridinium *p*-toluenesulfonate isolated from the reaction of 2,2-dimethyl-5,5-bis-(hydroxymethyl)-1,3-dioxane (I) with *p*-toluenesulfonyl chloride in pyridine was twice recrystallized from an ethanol–ether mixture and, after drying *in vacuo* at 65°, had a m. p. of 118–119°.⁸

Anal. Calcd. for C₁₂H₁₃O₃SN: S, 12.75; neut. equiv., 251. Found: S, 12.62, 12.53, neut. equiv., 253 (by titration with 0.1 *N* sodium hydroxide).

(7) Microanalyses by Dr. Carl Tiedcke, 366 Fifth Ave., New York, N. Y.

(8) Gebauer-Fülneegg and Riesenfeld, *Monatsh.*, **47**, 185 (1926), reported m. p. 113–115° for this compound obtained by heating *p*-toluenesulfonyl chloride and pyridine in a sealed tube at 140–150°.

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The Heparin of Pig Tissue

BY WILLIAM C. RISSER

Heparin, the blood anticoagulant, has been found in most tissues and organs of the animal body.¹ Highly vascular tissues and organs such as lung and liver contain this substance in greatest quantity. Charles and Scott² first isolated crystalline barium acid heparinate from ox lung. Jaques, Waters and Charles³ have isolated the crystalline barium acid salt of heparin from the lungs of the pig and sheep and from the liver of dogs. They found that the biological activity of the heparin salts from the different species varied widely; the potencies of the acid barium salts expressed in Toronto units per mg. were reported to be: dog 240, beef 100, pig 44 and sheep 23. No significant differences in chemical analy-

(1) Present address: National Institute of Health, Bethesda, Md.

(2) Bougault, *Compt. rend.*, **123**, 187 (1896).

(3) Fecht, *Ber.*, **40**, 3888 (1907).

(4) Mooradian and Cloke, *THIS JOURNAL*, **67**, 942 (1945).

(5) Orthner, *Ber.*, **61**, 116 (1928).

(6) All melting points are uncorrected.

(1) A. F. Charles and D. A. Scott, *J. Biol. Chem.*, **102**, 431 (1933).

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

(3) L. B. Jaques and E. T. Waters, *J. Physiol.*, **99**, 454 (1941); L. B. Jaques, *Science*, **92**, 488 (1940); L. B. Jaques, E. T. Waters and A. F. Charles, *J. Biol. Chem.*, **144**, 229 (1942).

sis or rotatory power of the barium acid heparinates from the different species could be detected.

Very recently Wolfrom⁴ and co-workers have reported that the crystalline barium acid salt of heparin from dog liver has the *same* biological activity as the beef heparin barium salt, in contrast to the claims of Jaques and co-workers.

We have prepared the pure amorphous sodium salt of heparin from pig liver by a procedure similar to that of Charles and Scott.^{1,2} The biological activity of this material was determined by two different methods. The first assay method used was that of Charles and Scott² which employs freshly drawn cat's blood. This method was described in greater detail by Jaques and Charles.⁵ The second method of assay used was the excellent method of Kuizenga, Nelson and Cartland.⁶ By both methods of assay, the anticoagulant potency of the amorphous sodium salt of heparin isolated from pig liver was found to be 110–120 Toronto units per milligram,^{7,8} which is the same as the anticoagulant potency reported for the pure amorphous sodium salt of heparin isolated from beef tissue.

The pure amorphous sodium salt of heparin from pig liver was converted into the crystalline barium acid salt. This substance assayed 95 Toronto units per milligram by the cat blood method of Charles and Scott.² This, likewise, is in good agreement with the value reported for the crystalline barium acid heparinate of beef tissue.³

It has been shown that the anticoagulant potencies of the sodium salt and crystalline barium acid salt of heparin from pig liver are identical to the anticoagulant potencies of these same salts of heparin from beef tissue. Thus within the limits of the bioassay procedures, the heparin of pig liver appears to be identical with heparin from beef tissue. Furthermore, since Wolfrom⁴ has shown that heparin from dog liver is identical to heparin from beef tissue, it is apparent that the anticoagulant potencies of heparin from three different species—the dog, the pig and the ox—are identical.

These findings are therefore in disagreement with the claims of Jaques and co-workers³ that the anticoagulant potency of beef heparin is twice that of pig heparin. Wolfrom and co-workers⁴ have suggested that the observations of Jaques and co-workers on the variation of heparin po-

tency with species source may have resulted from different degrees of inactivation by acid in preparing the crystalline barium acid salts from different species.

Three samples of crystalline barium acid salt of heparin from pig tissue supplied by this Laboratory were used by Copley and Whitney⁹ in developing a colorimetric method of assay. Although it is doubtful whether reaction with toluidine blue parallels anticoagulant activity very closely, it is interesting to compare the reactivity to toluidine blue of heparin samples from pig tissue (Ba-1-PHi, Ba-3-PHi-II, Ba-2-PHi of Table V, in paper by Copley) and the remaining heparin samples, supplied by other laboratories, and of presumably different origin. No gross differences are apparent.¹⁰

(9) Alfred L. Copley and David V. Whitney, *J. Lab. Clin. Med.*, **28**, 762 (1943).

(10) The author is grateful to Dr. D. W. MacCorquodale for his interest and encouragement.

ABBOTT LABORATORIES
NORTH CHICAGO, ILLINOIS RECEIVED OCTOBER 8, 1945

2-Mercaptopyridine

BY JOHN R. THIRTLE

A recent paper¹ describing the use of mono-, di- or triethylene glycol as a reaction solvent to obviate the use of an autoclave or bomb-tube method prompts the report of a similar technique developed in this Laboratory.

2-Mercaptopyridine was prepared in 83–87% yield by the reaction of 2-bromopyridine with potassium hydrosulfide in propylene glycol. The crude product melts at 121–124° and is pure enough for most reactions. Crystallization from benzene with considerable loss raises the melting point to 128°. Previous methods made use of sodium² or potassium hydrosulfide³ or thiourea.⁴

Procedure.—Hydrogen sulfide was passed into 1000 g. (15 moles) of potassium hydroxide (reagent grade, 85%) and 250 cc. of water until saturation was reached. A little ferrous sulfide which formed was removed by filtration and the filtrate was evaporated to dryness under vacuum by heating on an oil-bath at 100–170°. The white crystalline potassium hydrosulfide was dissolved in 2500 cc. of propylene glycol. The solution was heated at 170–175° under a reflux condenser while 790 g. (5 moles) of 2-bromopyridine⁵ was added at such a rate that gentle refluxing occurred. Stirring and heating were continued for twenty hours at 150–175°. The potassium bromide was removed by filtration and washed with 1500 cc. of ethanol in portions. The filtrate, including washings, was

(1) Soffer, Soffer and Sherk, *THIS JOURNAL*, **67**, 1435 (1945).

(2) Gastel and Wibaut, *Rec. trav. chim.*, **53**, 1031 (1934).

(3) Marckwald, Klemm and Trabert, *Ber.*, **33**, 1556 (1900).

(4) Phillips and Shapiro, *J. Chem. Soc.*, 584 (1942). This procedure, requiring no special equipment, involved the reaction of 2-bromopyridine with thiourea followed by decomposition of the isothiourenium salt with ammonium hydroxide. Yields (67.5%) and quality (m. p. 116–120°) are inferior to those of the product described here.

(5) The 2-bromopyridine was made in 92% yield by the method of Craig, *THIS JOURNAL*, **56**, 231 (1934); cf. Whitmore, Mosher, Goldsmith and Rytina, *ibid.*, **67**, 393 (1945).

(4) M. L. Wolfrom, J. V. Karabinos, C. S. Smith, P. H. Ohliger, J. Lee and O. Keller, *THIS JOURNAL*, **67**, 1624 (1945).

(5) L. B. Jaques and A. F. Charles, *Quart. J. Pharm. Pharmacol.*, **14**, 1 (1941).

(6) Marvin H. Kuizenga, John W. Nelson and George F. Cartland, *Am. J. Physiol.*, **139**, 612 (1943).

(7) The author is indebted to Mr. H. C. Spruth and his co-workers, Robert T. Olsen, Marguerite Tonyan and Melle Russell Cummings for the biological assays recorded herein.

(8) Dr. E. F. Cook, Chairman of the Committee of Revision of the Pharmacopoeia of the United States, has recently supplied a provisional International Standard sodium heparin which has been given the value 130 international units per mg. (approx. 130 Toronto u./mg.) Different batches of sodium heparin from pig liver, on the dry basis, assay 120 to 140 international u./mg.