hand, is activated considerably, the increase in activity amounting to about 200 %. As the neutral pyrophosphatase is also strongly activated by magnesium it seems essential to demonstrate the mutual independence of the pyrophosphatase 5 and the neutral one. This was tried on rat liver homogenates by fractional precipitation with acetone at 0° C. Comparison between the original activities and the activities of the proteins precipitated by 40 to 75 % acetone demonstrates the requested independence as appears from Table 1. The activity ratio may be changed in opposite directions by slight changes in pH etc. This means that the two enzymatic activities are differently affected, *i. e.* a selective partial inactivation occurs.

This is further evidence for the existence of an acid pyrophosphatase with optimum at pH 5—5.5 in addition to the three earlier known isodynamic enzymes. The enzyme seems to be present not only in rat liver but also in the liver of guinea pig, hamster, rabbit and cat.

This investigation has been supported by funds from the State Medical Research Council. I am indebted to P. O. Bethge for technical assistance.

1. Norberg, B. *Acta Chem. Scand.* **4** (1950) 601.
2. Swanson, M. A. Personal communication.
3. Plumel, M. *Bull. soc. chim. biol.* **30** (1948) 129.
4. Bamann, E., and Gall, H. *Biochem. Z.* **293** (1937) 1.

Received April 2, 1951.

New Salts of Benzylpenicillin with Organic Bases

LARS NATHORST WESTFELT

Centrallaboratoriet, Astra, Södertälje, Sweden

It is known that benzylpenicillin forms crystalline well defined salts with several organic bases, and salts with secondary¹ and tertiary¹⁻⁴ as well as primary^{1,5-7} amines have been described.

In an attempt to find bases, which might be useful for the isolation of penicillin, a further series of organic amines have been investigated. The penicillin salts were all prepared in essentially the same way; namely, by the addition of a slight excess of the base, dissolved in acetone to a solution of pure free benzylpenicillin in amyl acetate-acetone. Some of the salts separated in crystalline form, others as oils, as shown in the following table.

Base	Reaction product with benzylpenicillin
N-Monomethylethylene-diamine ⁸	oil
N-Monoethylethylene-diamine ⁸	»
2-Methylimidazoline ⁹	crystals
2-Ethylimidazoline ⁹	oil
2-Propylimidazoline ⁹	crystals
2-Amylimidazoline ⁹	oil
2-Benzylimidazoline	»
2,4(5)-Dimethylimidazoline	»
2-Ethyl-4(5)-methylimidazoline	crystals
2-Propyl-4(5)-methylimidazoline	»

The preparation and properties of the dialkylated imidazolines referred to will be described in a forthcoming communication in this Journal.

Only one example of the preparation of the benzylpenicillin salts will be given in detail.

The 2-propylimidazoline salt of benzylpenicillin. 5.0 g of sodium benzylpenicillin was dissolved in 20 ml of water at 0—5° C, 40 ml of cold amyl acetate was added, and then, with vigorous shaking, 4.5 ml of 60 % phosphoric acid. The aqueous layer was separated and extracted with an additional 10-ml portion of cold amyl acetate. The combined

cold amyl acetate solutions were dried with sodium sulphate, filtered, and diluted with 50 ml of dry acetone. To this solution was added, with stirring, during a period of about 15 minutes, a 10 % solution of 2-propylimidazoline in dry acetone, in amount sufficient to produce a marked green colouration of bromthymol blue indicator. During the addition, the 2-propylimidazoline salt of benzylpenicillin began to crystallize. The mixture was set aside in the refrigerator overnight, then the salt was collected, washed with small amounts of acetone, and dried *in vacuo* at 20°. Yield: 6.2 g of colourless, small needle-like crystals, $[\alpha]_D^{20} + 231^\circ$ ($c = 1$ in water); m. p. 114° (decomp.); pH of aqueous solution, 5.25; biological assay 1 290 U/mg.

The compound is hygroscopic and very soluble in water and ethanol, soluble in chloroform.

The 2-methylimidazoline salt of benzylpenicillin. 5.0 g of sodium benzylpenicillin yielded 5.8 g of colourless, rod-like crystals in rosettes, $[\alpha]_D^{30} + 242^\circ$ ($c = 1$ in water); m. p. 80°; pH of aqueous solution, 4.98; biological assay 1 350 U/mg.

The salt is more hygroscopic than the 2-propylimidazoline salt and it has not been possible to obtain in a completely pure state, as is evident from the analytical figures. The compound is very soluble in water, ethanol and chloroform.

The 2-ethyl-4(5)-methylimidazoline salt of benzylpenicillin. 5.0 g of sodium benzylpenicillin yielded 5.3 g of colourless, prismatic crystals, $[\alpha]_D^{30} + 232^\circ$ ($c = 1$ in water); m. p. 141° (decomp.); pH of aqueous solution, 5.75; biological assay 1 270 U/mg.

The salt relatively non-hygroscopic and very soluble in water and ethanol, soluble in chloroform.

The 2-propyl-4(5)-methylimidazoline salt of benzylpenicillin. 5.0 g of sodium benzylpenicillin yielded 6.3 g of colourless, rod-like crystals, $[\alpha]_D^{30} + 224^\circ$ ($c = 1$ in water); m. p. 132° (decomp.); pH of aqueous solution, 6.15; biological assay 1 240 U/mg.

The salt is relatively non-hygroscopic and very soluble in water and ethanol, soluble in chloroform.

1. Clarke, H. T., *et al.* *The chemistry of penicillin*. Princeton, New Jersey (1949) 89.
2. Leigh, T. Brit. Pat. 607 004. *C. A.* **43** (1949) 3982, Anslow, W. K. Brit. Pat. 626 027. *C. A.* **44** (1950) 288, Rigler, N. E. Brit. Pat. 629 662. *C. A.* **44** (1950) 5550.
3. Florey, H. W., *et al.* *Antibiotics*. London (1949) 745.
4. Bide, A. E., *et al.* Brit. Pat. 604 563. *C. A.* **43** (1949) 1158.
5. Cooper, D. E. U. S. Pat. 2 504 182. *C. A.* **44** (1950) 5918.
6. Goldberg, M. W., and Teitel, S. U. S. Pat. 2 483 382. *C. A.* **44** (1950) 5063.
7. Smith, E. L., and Bide, A. E. Brit. Pat. 606 482, *C. A.* **43** (1949) 1158; Brit. Pat. 610 731, *C. A.* **43** (1949) 2742.
8. Aspinall, S. R. *J. Am. Chem. Soc.* **63** (1941) 852.
9. Chitwood, H. C., and Reid, E. E. *Ibid.* **57** (1935) 2424.

Received March 31, 1951.