requirements for animals of the age and body weight of these 2 subjects (*9*). On both the stock diet and Purina Chow these experimentals showed audiogenic seizures consistently. Of the sporadically susceptible experimentals, one animal failed to have sound-induced seizures while on the self-selected diet. This rat was found to have an average daily intake of magnesium chloride that amounted to 3 mg. Compared with the magnesium requirements of rats of the age and body weight of this subject, this amount can be considered excessive. The animals continued to show fits sporadically when placed on either stock or Purina diet. Three of the consistently susceptible controls and one of the sporadically susceptible control rats failed to have audiogenic fits during the 42 days on the self-selected diet.

In every instance the controls whose seizures were alleviated by the self-selected diet showed excessive intakes of thiamine hydrochloride. The approximate average amount of this vitamin consumed per rat per day amounted to 5.0 mg. In general, in the cases where self-selection alleviated seizure susceptibility, the rats failed to show the seizures after an average of 8 days on the diet.

References

1. MORGAN, C. T., and MORGAN, J. D. *J. Comp. Psychol.*, **27**, 505 (1939).
2. FINGER, F. W. *Am. J. Psychol.*, **55**, 68 (1942).
3. PATTON, R. A., KARN, H. W., and KING, G. C. *J. Comp. Psychol.*, **32**, 543 (1941).
4. CHICK, H., EL SADR, M. M., and WORDEN, A. N. *J. Biol. Chem.*, **34**, 595 (1940).
5. KRUSE, H. D., ORENT, E. R., and MCCOLLUM, E. V. *J. Biol. Chem.*, **96**, 519 (1932).
6. GRIFFITHS, W. J., JR. *J. Comp. Psychol.*, **38**, 65 (1945).
7. RICHTER, C. P., HOLT, L. E., JR., and BARELARE, B. *Am. J. Physiol.*, **122**, 734 (1938).
8. GRIFFITHS, W. J., JR. *J. Comp. Physiol. Psychol.*, **42**, 303 (1949).
9. GRIFFITH, J. Q., and FARRIS, E. J. *The Rat in Laboratory Investigation.* Philadelphia: J. B. Lippincott (1949).

Phenyl Phenaceturate from the Decomposition of Penicillin in the Presence of Phenol

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Aqueous solutions of potassium penicillin G containing sodium citrate as buffer and phenol as a preservative may be kept under refrigeration for relatively long periods of time without undergoing serious deterioration. However, at room (25° C) or higher temperatures such solutions have been observed to deposit a colorless crystalline precipitate. This precipitate has been identified as phenyl phenaceturate.

A solution containing 31.6 g of crystalline potassium penicillin G, 2.5 g of phenol, and 2.5 g of sodium citrate in 1 liter of water was kept at 37° C. After 24 hr a slight crystalline precipitate was present and after 48 hr about 2 g of the precipitate had separated. No more appeared to be formed on longer standing at 37° C. The long needlelike crystals were collected and air-dried. They were insoluble in water, sparingly soluble in ether, readily soluble in acetone, and easily recrystallized from alcohol, mp 132°–133° C. Analysis showed the presence of 71.39% carbon, 5.85% hydrogen, and 5.49% nitrogen. Calculated values for phenyl phenaceturate ($C_{15}H_{15}NO_3$) are 71.36% carbon, 5.62% hydrogen, and 5.20% nitrogen. A sample of the compound was saponified by warming with dilute alcoholic sodium hydroxide solution. After evaporation of the alcohol and acidification of the residue with hydrochloric acid, there was present a strong odor of phenol, and phenaceturic acid crystallized from the solution. It was identified by mp 142°–143° C and mixed melting point with an authentic sample. The phenaceturic acid was also characterized by the identity of its x-ray powder diagram with that of an authentic sample.

The phenyl phenaceturate obtained from penicillin was further identified by finding that its x-ray powder diagram and infrared absorption spectrum were identical with those of a synthetic sample of phenyl phenaceturate. The synthetic sample was prepared as follows: Phosphorus tribromide, 7.1 g (0.026 mole), was added to a solution of 5.5 g (0.028 mole) of phenaceturic acid in 50 ml of dry dioxane. The resulting crystalline precipitate of 2-benzyl-4(5)-oxazalone hydrobromide (*1*) was centrifuged and washed with anhydrous ether. To the solid was added 5.3 g (0.056 mole) of phenol, and the mixture was heated at 80°–90° for 1 hr, after which it was poured into ice water. The oil that separated eventually crystallized. This was dissolved in ethyl acetate; the solution was washed with aqueous sodium bicarbonate solution, dried, and evaporated in vacuum until a crystalline precipitate separated. The product, phenyl phenaceturate, was recrystallized from alcohol—yield, 1.25 g (20%); mp 132°–133°—and mixed with the product obtained from penicillin as described above, mp 132°–133°.

It is not surprising to find derivatives of phenaceturic acid resulting from the decomposition of penicillin G (*2*), nor is it particularly surprising that the azlactone ring of penicillin is apparently opened by phenol. The azlactone ring is similarly opened by methanol, ethanol, and other alcohols, which form the corresponding esters of penicilloic acid (*3*). However, it was surprising to find that phenyl phenaceturate was formed from penicillin G and phenol in aqueous solution only when a buffer was present. In the absence of a buffer the solutions turned yellow on long standing or warming, but deposited no precipitate. Potassium phosphate, as well as sodium citrate, as described above, was effective in promoting the formation of phenyl phenaceturate in solutions containing phenol and penicillin G.

References

1. CLARK, JOHNSON, and ROBINSON, Eds. *The Chemistry of Penicillin*, p. 781. Princeton, N. J.: Princeton Univ. Press (1949).
2. *Ibid.*, p. 56.
3. *Ibid.*, p. 544.