464. *Purines, Pyrimidines, and Glyoxalines. Part X.* Synthesis of Uracil-6-phosphonic Acid, an Analogue of Orotic Acid.*

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Diethyl ethoxycarbonylacetylphosphonate (I) has been prepared by the reaction of triethyl phosphite with ethoxycarbonylacetyl chloride. With phenylhydrazine, the keto-ester gave the pyrazolone (II), and with urea and dry hydrogen chloride gave uracil-6-phosphonic acid (III).

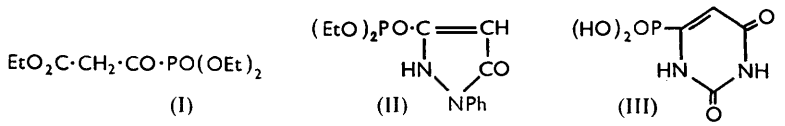
Compounds structurally related to the pyrimidines and purines of nucleic acid origin, have proved important as growth-inhibitors of bacteria, viruses, and tumours. In the pyrimidine field, most of these compounds are sterically similar to uracil, thymine, and cytosine, and have been modelled on these pyrimidines by changes such as OH ⟶ SR, OH ⟶ NR_2, CH ⟶ N and by substitution of uracil in the 5-position by such substituents as halogen, NO_2, RO, NR_2. The inhibitory properties of the compounds are generally reversed by a naturally occurring pyrimidine and, in many cases, it appears that the analogues compete with the natural pyrimidines for a place in the nucleic acid molecule. These pyrimidine analogues may be classed as antimetabolites and examples are now known of their direct incorporation into the nucleic acid molecule.[1]

* Part IX, preceding paper.

[1] " The Nucleic Acids," Ed. Chargaff and Davidson, Academic Press, New York, 1955, and references therein; Matthews and Smith, *Adv. Virus Res.*, 1955, **3**, 49; Matthews, *Virology*, 1955, **1**, 165.

Among the naturally occurring pyrimidines, orotic acid (uracil-6-carboxylic acid) is of special importance. Although it is not known to be a constituent of nucleic acids it is nevertheless more readily incorporated into these acids, where it appears as uracil, than any of the normal pyrimidine constituents. If, as appears likely, growth-inhibition in this series is related to the degree of incorporation of the compound into the nucleic acid molecule, then analogues of orotic acid assume special interest. Very recently such analogues, 5-fluoro-orotic acid,[2] uracil-6-sulphonic acid,[3] and the corresponding sulphonamide and methyl sulphone,[4] have been found to have inhibitory activity towards lactobacilli and tumours.[5]

We now report the preparation of a further analogue, uracil-6-phosphonic acid (III), in which the carboxyl group of orotic acid has been replaced by a phosphonic acid group.



Triethyl phosphite reacted smoothly with ethoxycarbonylacetyl chloride to give the keto-ester (I). This gave a red colour with alcoholic ferric chloride, and its structure was confirmed by analysis, formation of a 2 : 4-dinitrophenylhydrazone, and reaction with phenylhydrazine to give the pyrazolone (II).

Preliminary attempts to cause the keto-ester to react with urea in alcoholic or non-polar solution in the presence of sodium methoxide were unsuccessful. The ester was peculiarly unstable in hydroxylic solvents in the presence of bases. When heated with anhydrous guanidine, it reacted readily, to give a crystalline compound with an ultraviolet absorption spectrum typical of 2 : 4-pyrimid-diones, but no satisfactory structure could be assigned to this substance. The required uracil-6-phosphonic acid was eventually readily obtained by the reaction of the ester (I) with urea in acetic acid in the presence of dry hydrogen chloride, conditions which are known to de-esterify phosphonic esters; [6] the acid (III) was isolated as a disodium salt, conversion of which into the free acid proved unexpectedly troublesome. Acidification of a concentrated solution of the sodium salt with hydrochloric acid precipitated a monosodium salt, which was also obtained when solutions of the disodium salt were treated with the acid form of Zeo-Karb 225. This behaviour is reminiscent of that shown by orotic acid which is frequently precipitated as its sodium salt from alkaline solution by acid. However, addition of an excess of silver nitrate to the disodium salt solution precipitated a trisilver salt (orotic acid gives a disilver salt), and decomposition of this with hydrogen sulphide afforded the free acid (III). The ultraviolet absorption spectra of the substance in acid, neutral, and alkaline solution were similar to those of orotic acid.

Experimental

Ethoxycarbonylacetyl chloride, b. p. 44°/2·5 mm., was prepared from dry potassium ethyl malonate and thionyl chloride in ether.[7] Commercial triethyl phosphite (Eastman Kodak) was redistilled, and a fraction of b. p. 43°/9 mm. used.

Diethyl Ethoxycarbonylacetylphosphonate.—Triethyl phosphite (50·14 g., 57·3 ml.) was added during 15 min., with shaking, to ethoxycarbonylacetyl chloride (50 g.) at 0°. The solution was

[2] Heidelberger, Chaudhuri, Danneberg, Mooren, Griesbach, Duschinsky, Schnitzer, Pleven, and Scheiner, *Nature*, 1957, **179**, 663; Duschinsky, Pleven, and Heidelberger, *J. Amer. Chem. Soc.*, 1957, **79**, 4559.

[3] Greenbaum and Holmes, *ibid.*, 1954, **76**, 2899.

[4] Greenbaum, *ibid.*, 1954, **76**, 6052.

[5] Hakala, Law, and Welch, *Proc. Amer. Assoc. Cancer Res.*, 1956, **2**, 113; Handschumaker and Welch, *Cancer Res.*, 1956, 965; Heidelberger, Mooren, Griesbach, Montag, Duschinsky, Pleven, and Schnitzer, *Proc. Amer. Assoc. Cancer Res.*, 1957, **2**, 212.

[6] Freedman and Doak, *Chem. Rev.*, 1957, **57**, 479.

[7] Staudinger and Becker, 1917, **50**, 1023.

then heated on a water-bath for 30 min. Vigorous evolution of ethyl chloride occurred and the solution became bright yellow. It was set aside at room temperature overnight, then distilled *in vacuo*, to give fractions, b. p. 40—100°/0·7 mm. (15 ml.) and b. p. 100—108°/0·7 mm. (49 g.), and a high-boiling residue. The main fraction was redistilled, to give *diethyl ethoxycarbonylacetyl phosphonate* (24·5 g.), b. p. 115°/0·9 mm. (Found: C, 43·0; H, 6·65. $C_9H_{17}O_6P$ requires C, 42·85; H, 6·8%). This slowly decomposed when repeatedly distilled. A solution of the ester in ethanol gave a ruby-red colour with ferric chloride solution. The 2 : 4-*dinitrophenylhydrazone* crystallised from aqueous ethanol as yellow needles, m. p. 77—78° (Found: C, 41·65; H, 4·7; N, 12·75. $C_{15}H_{21}O_9N_4P$ requires C, 41·65; H, 4·9; N, 12·95%).

Diethyl 5-*Oxo*-1-*phenyl*-Δ^3-*pyrazolin*-3-*ylphosphonate*.—Freshly distilled phenylhydrazine (0·43 g.) and the acylphosphonate (1·1 g.) were mixed. When the initial exothermic reaction had subsided the mixture was heated on a water-bath for 1½ hr., to give a clear orange-coloured liquid. Some low-boiling material was removed *in vacuo* and the residue treated with N-sodium hydroxide; it partly dissolved. The insoluble oil was removed with ether, and the aqueous phase acidified with hydrochloric acid, to precipitate a dense orange oil. This was extracted into ether, and the solution dried, and evaporated to a gum (0·85 g.) which partly crystallised and was repeatedly crystallised from ethyl acetate–light petroleum, to give *diethyl* 5-*oxo*-1-*phenyl*-Δ^3-*pyrazolin*-3-*ylphosphonate* (0·1 g.) as colourless plates, m. p. 89—91° (Found: C, 52·55; H, 5·8; N, 9·3. $C_{13}H_{17}O_4N_2P$ requires C, 52·7; H, 5·8; N, 9·45%). An alkaline solution of the pyrazolone with benzenediazonium chloride solution gave a vivid orange colour.

Reaction of the Acylphosphonate with Anhydrous Guanidine.—The phosphonate (10 g.) and anhydrous guanidine (3·2 g.), when mixed, became hot, viscous, and bright lemon-yellow. After the initial reaction, the solution was kept at 80° (bath) for 1½ hr., a solid being precipitated. This (3·9 g.) was extracted with ethanol–ether (2 : 1) and recrystallised from an excess of water, and then from aqueous acetic acid, to give colourless needles, m. p. >350° (Found: C, 42·55; H, 4·6; N, 16·85%), $\lambda_{max.}$ 280 mμ, $\lambda_{min.}$ 241 mμ in H_2O.

Uracil-6-*phosphonic Acid*.—A solution of the acyl phosphonate (2·52 g.) and urea (0·6 g.) in dry acetic acid (4·5 ml.) was heated on a water-bath, and dry hydrogen chloride passed through the solution during 30 min. The solution was set aside at 5° for 12 hr., then evaporated *in vacuo* to a gum. This was dissolved in ethanol (10 ml.) and treated with 10N-sodium hydroxide solution till the pH was 9—10. Crystals separated which were filtered off and washed with ethanol–water (3 : 1) and ethanol. *Disodium uracil*-6-*phosphonate dihydrate* (0·55 g.) recrystallised from aqueous ethanol as needles, m. p. >300° (Found: C, 17·3; H, 2·95; N, 10·6. $C_4H_3O_5N_2PNa_2,2H_2O$ requires C, 17·65; H, 2·6; N, 10·3%). Acidification of a concentrated solution of the disodium salt with 10N-hydrochloric acid precipitated a *hydrated monosodium salt* (Found: C, 20·25; H, 2·55; N, 11·7; P, 12·5%; equiv., 238. $C_4H_4O_5N_2PNa,1\frac{1}{2}H_2O$ requires C, 19·9; H, 2·9; N, 11·6; P, 12·85%; equiv., 241). The same compound was also obtained by evaporation of a solution of the disodium salt after it had been treated with Zeo-Karb 225 (acid form). Addition of an excess of silver nitrate solution to a solution of the disodium salt precipitated a *trisilver salt* (Found: C, 9·5; H, 1·0; N, 5·45 $C_4H_2O_5N_2PAg_3,1\frac{1}{2}H_2O$ requires C, 8·9; H, 0·95; N, 5·2%). The silver salt (0·15 g.) was suspended in water and decomposed with hydrogen sulphide. Silver sulphide was filtered off, and the filtrate evaporated *in vacuo*, to give *uracil*-6-*phosphonic acid monohydrate* (0·055 g.) as needles, m. p. 270° (decomp.) (Found: C, 22·8; H, 3·5; N, 13·05. $C_4H_5O_5N_2P,H_2O$ requires C, 22·85; H, 3·35; N, 13·35%), $\lambda_{max.}$ 265 mμ (ε 5880), $\lambda_{min.}$ 238 mμ (ε 2700) in 0·1N-NaOH; $\lambda_{max.}$ 267 mμ (ε 7200), $\lambda_{min.}$ 230 mμ (ε 840) in 0·1N-HCl; $\lambda_{max.}$ 264 mμ (ε 5400), $\lambda_{min.}$ 230 mμ (ε 1430) at pH 7.

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