bined with the filtrate. The solution was made alkaline, evaporated *in vacuo* to about 25 ml., and acidified. The precipitate thus obtained was recrystallized from water; colorless prisms, 0.8 g., m.p. and mixed m.p. with benzoic acid, 121–122°.

Anal. Calcd. for $C_7H_6O_2$: C, 84.07; H, 6.05. Found: C, 83.88; H, 6.25.

The filtrate was made alkaline, evaporated to half its volume, neutralized with 10% sodium hydroxide, then acidified with one drop of dilute hydrochloric acid. To this solution 1 g. of phenacyl bromide and 10 ml. of ethanol were added. After refluxing for two hours, the product was recrystallized from ethanol; colorless needles, 0.4 g., m.p. and mixed m.p. with an authentic sample of diphenacyl glutarate, 103–104°.

Anal. Calcd. for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47. Found: C, 68.95; H, 5.24.

Oxidation of the Dimer with Permanganate.—To a solution of 3 g. of the substance in 40 ml. of chloroform a few drops of a solution of 9 g. of potassium permanganate in 400 ml. of water was added at room temperature under mechanical stirring. The purple color did not fade after 4 hours. Upon the addition of 20 ml. of 10% sulfuric acid, however, the color was bleached out within a few minutes. The permanganate solution (400 ml.) was then added (100 ml. each 30 min.). The manganese dioxide was filtered off. The product when treated as described above did not yield either benzoic acid or a phenacyl ester. The chloroform phase was evaporated to dryness and recrystallized from ethanol. Unoxidized dimer (1 g.) was recovered which was identified by its m.p. and ultraviolet spectrum.

Interconversion of the Dimer and 2,6-Dibenzylidenecyclohexanone.—When an ethanol or benzene solution of either 2,6-dibenzylidenecyclohexanone or its dimer was refluxed for 24 hours, a mixture of the compounds resulted. When a preparation obtained from the dimer was recrystallized from ethanol, first colorless and then yellow crystals separated out. The latter showed m.p. and mixed m.p. (with 2,6-dibenzylidenecyclohexanone), 117–118°; yield 5%.

Anal. Calcd. for $C_{20}H_{18}O$: C, 87.56; H, 6.61. Found: C, 87.35; H, 6.59.

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COMMUNICATIONS TO THE EDITOR

THE SYNTHESIS OF GUANOSINE-5′-PHOSPHATE USING A NEW METHOD OF PHOSPHORYLATION

Sir:

The isolation of guanosine-5′-mono-, di- and triphosphates, from both yeast and animal tissues,[1] recently has been reported from different laboratories and the biochemical importance of these compounds has been established.[2] However, these new nucleotides remain highly inaccessible and efficient chemical syntheses of these important substances are being attempted in this laboratory. Despite the efforts of earlier workers,[3] using several methods of phosphorylation, no satisfactory synthesis of guanosine-5′-monophosphate (GMP), the key substance in the projected synthesis of the higher phosphates, has emerged. Michelson and Todd[3b] have reported a 20% yield of GMP by the phosphorylation of 2′,3′-isopropylidene guanosine (IV) with phosphorus oxychloride in a mixture of pyridine and dimethylformamide. In our hands, however, the yield of GMP by this method was even lower and extensive paper and ion exchange chromatography demonstrated the concomitant formation of hitherto unidentified phosphorus-containing products. We now wish to record the preparation of GMP in excellent yield by the use of a new method of phosphorylation.

Invariably side products were formed when pyridine was present in the phosphorylation mixtures. This fact led us to conclude that the solution of the problem lay in the use of a powerful, monofunctional reagent which would not require basic catalysis for the phosphorylation of the 5′-hydroxyl group in IV. Tetra-*p*-nitrophenyl pyrophosphate[4,5] (III), prepared *in situ* by the reaction of di-*p*-tolyl carbodiimide (II) with two equivalents of di-*p*-nitrophenyl phosphate (I) in dioxane, has been found to satisfy these requirements. This reagent[5] was allowed to react with (IV) in the presence of one equivalent of the free acid (I) and after a reaction period of 15 hours at 20°, 2′,3′-isopropylidene guanosine-5′-di-*p*-nitrophenyl phosphate (V) was isolated in nearly quantitative yield as an amorphous powder. After crystallization from acetonitrile, the product showed a transition point at 161–163° and dec. 264°. *Anal.* Calcd. for $C_{25}H_{24}N_7O_{12}P\cdot 1H_2O$: C, 45.28; H, 3.95; N, 14.80; P, 4.67. Found: C, 44.95; H, 3.99; N, 15.00; P, 4.80.

The neutral ester (V) was suspended in 50% aqueous acetonitrile and converted to 2′,3′-isopropylidene guanosine-5′-*p*-nitrophenyl hydrogen phosphate (VI) under mildly alkaline conditions.[6] A solution of (VI) in *tris*-hydroxymethyl amino-

(1) Selected references are: H. Schmitz, R. B. Hurlbert and V. R. Potter, *J. Biol. Chem.*, **209**, 11 (1954); H. Schmitz, *Naturwiss.*, **5**, 120 (1954); *Biochem. Z.*, **325**, 555 (1954); R. Bergkvist and A. Deutsch, *Acta. Chem. Scand.*, **8**, 1889 (1954).

(2) P. Ayengar, D. M. Gibson and D. R. Sanadi, *Biochem. Biophys. Acta*, **13**, 309 (1954); D. R. Sanadi, D. M. Gibson and P. Ayengar, *ibid.*, **14**, 434 (1954).

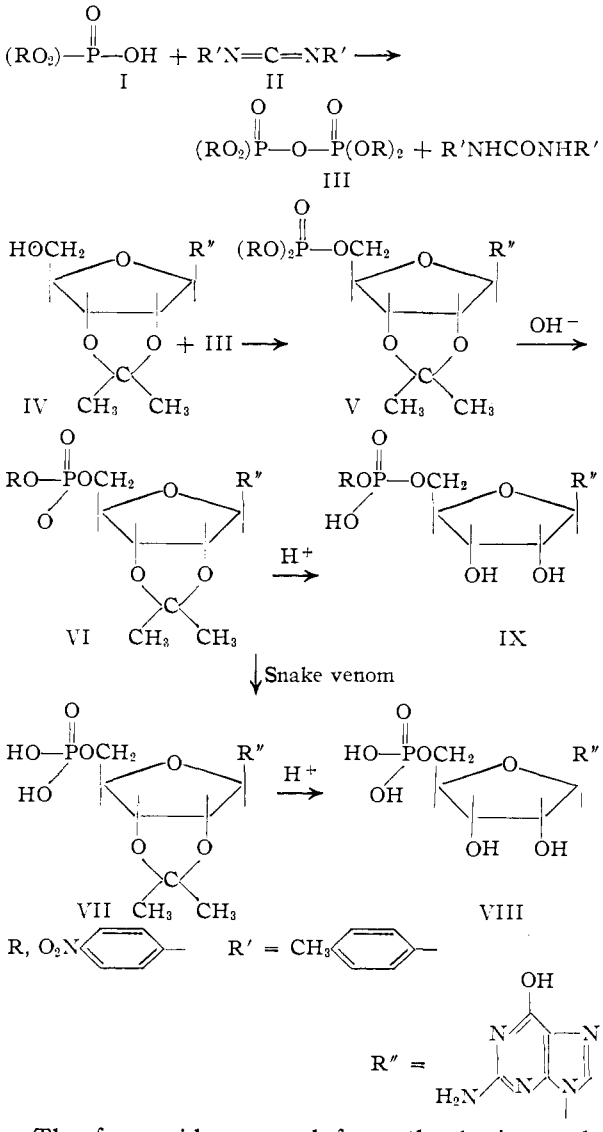
(3) (a) J. M. Gulland and G. I. Hobday, *J. Chem. Soc.*, 746 (1940); H. Bredereck and E. Berger, *Ber.*, **73**, 1124 (1940); (b) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 2476 (1949).

(4) H. G. Khorana and A. R. Todd, *ibid.*, 2257 (1953).

(5) A detailed study of this and related pyrophosphates as chemical phosphorylating agents will be reported (J. G. Moffatt and H. G. Khorana, forthcoming publication).

(6) The *p*-nitrophenol liberated was estimated spectrophotometrically at 440 mμ.

methane buffer, *p*H 8.8, was subjected directly to the action of crude rattlesnake venom[7] to remove the second *p*-nitrophenyl group,[8] and the isopropylidene group was then removed by mild acid treatment to yield GMP (VII), isolated as its barium salt. On a 1-g. scale, the over-all yield of GMP from (III) was 72%.[8]



The free acid prepared from the barium salt travelled as a single spot on paper chromatograms developed in five solvent systems and had R_f values identical with those of a sample obtained from natural sources.[9] The ion exchange behavior of the acid was identical with that of another synthetic sample[10] which had full biological activity. The two synthetic samples gave, furthermore, identical ultraviolet and infrared spectra.

It is of interest to point out that the 5′-nucleotidase which is also present in the crude venom was inactive toward VI but was fully active against guanosine 5′-*p*-nitrophenyl phosphate (IX). Because of this specificity of the 5′-nucleotidase and the relative lack of specificity of the phosphodiesterase, the crude venom can be used directly for the conversion of VI to VII.

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(7) *Crotalus adamenteus* venom obtained from Ross Allen's Reptile Farm, Florida.

(8) The yield isolated very probably can be improved since on the basis of the total *p*-nitrophenol liberated the over-all yield of GMP is 85%.

(9) This sample was prepared by Dr. D. R. Sanadi by the enzymatic dephosphorylation of a sample of guanosine 5′-pyrophosphate isolated from yeast. We are grateful to Dr. Sanadi for this sample and for carrying out enzymatic tests on a synthetic sample[10] of GMP.

(10) This sample was prepared by another method which will be described in a later communication.

(11) Life Insurance Medical Research Postdoctoral Fellow.

4-HYDROXY-5-METHYL-6-PHENYL-7-H-1,2-DIAZEPINE; A NOVEL HETEROCYCLIC COMPOUND

Sir:

We wish to describe the formation and some of the properties of a unique heterocyclic compound obtained by the treatment of 3-diazoacetyl-3-methyl-4-phenyl-Δ^1-pyrazoline (I) with acetic acid. The product, a highly colored, enolic compound, $C_{12}H_{12}ON_2$, has been formulated as 4-hydroxy-5-methyl-6-phenyl-7H-1,2-diazepine (III), presumably arising *via* an intermediate ion such as II. The most important characteristic of this diazepinol is the facility with which it undergoes rearrangement reactions. Under a variety of conditions, products have been obtained from III which appear to be representative of at least four different heterocyclic systems.

The pyrazoline I, m.p. 90–91° (*Anal.* Calcd. for $C_{12}H_{12}ON_4$: C, 63.14; H, 5.30; N, 24.55. Found: C, 63.34; H, 5.09; N, 24.50), obtained by the action of diazomethane on α-methylcinnamoyl chloride, on warming (70°) with acetic acid furnishes III in 70% yield (*Anal.* Calcd. for $C_{12}H_{12}ON_2$: C, 71.97; H, 6.04; N, 13.99. Found: C, 71.82; H, 6.70; N, 14.23). The product crystallizes from alcohol in orange-red prisms, m.p. 150–151°; λ_{max}^{EtOH} 220 mμ (ϵ 17,500), 312 mμ (5,000), 401 mμ (2,960); I.R. bands (μ) at 3.01 (s), 6.08 (vs), 6.37 (w), 6.62 (m), 7.45 (m); it is weakly acidic, dissolving in aqueous alkali and precipitating unchanged on acidification; $pK'_A > 12$. Acetylation of III with acetic anhydride–pyridine furnishes an acetate (IIIa), yellow prisms, m.p. 89° (*Anal.* Calcd. for $C_{14}H_{14}O_2N_2$: C, 69.40; H, 5.82; N, 11.56. Found: C, 69.40; H, 6.05; N, 11.95); λ_{max}^{EtOH} 221 mμ (19,200), 315 mμ (6,400), 390 mμ (2,600); I.R. bands (μ) at 5.87 (s), 5.99 (m), 6.30 (w), 8.49 (s). Although the frequency of the carbonyl band is markedly lower than that of typical enol acetates, the structure of IIIa is supported by the very rapid hydrolysis in 1% alkali to quantitatively regenerate III. A benzoate (IIIb) is similarly obtained by treatment with benzoic anhydride in pyridine, yellow plates, m.p. 147° (*Anal.* Calcd. for $C_{19}H_{16}O_2N_2$: C, 74.98; H, 5.30; N, 9.21. Found: C, 74.91; H, 5.34; N, 9.43). Catalytic or chemical reductions of III lead