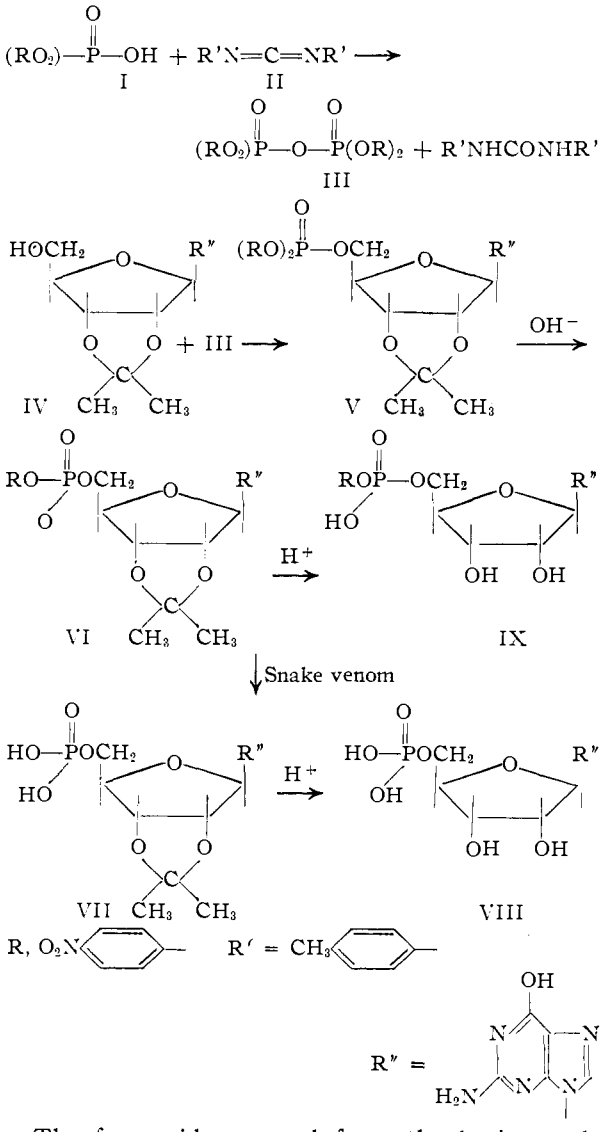
methane buffer, *p*H 8.8, was subjected directly to the action of crude rattlesnake venom[7] to remove the second *p*-nitrophenyl group,[8] and the isopropylidene group was then removed by mild acid treatment to yield GMP (VII), isolated as its barium salt. On a 1-g. scale, the over-all yield of GMP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

We wish to thank the National Research Council of Canada, Ottawa, for the financial support of this work.

ROBERT WARNER CHAMBERS[11]
BRITISH COLUMBIA RESEARCH COUNCIL J. G. MOFFATT
VANCOUVER 8, B. C., CANADA H. G. KHORANA

RECEIVED MAY 13, 1955

(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

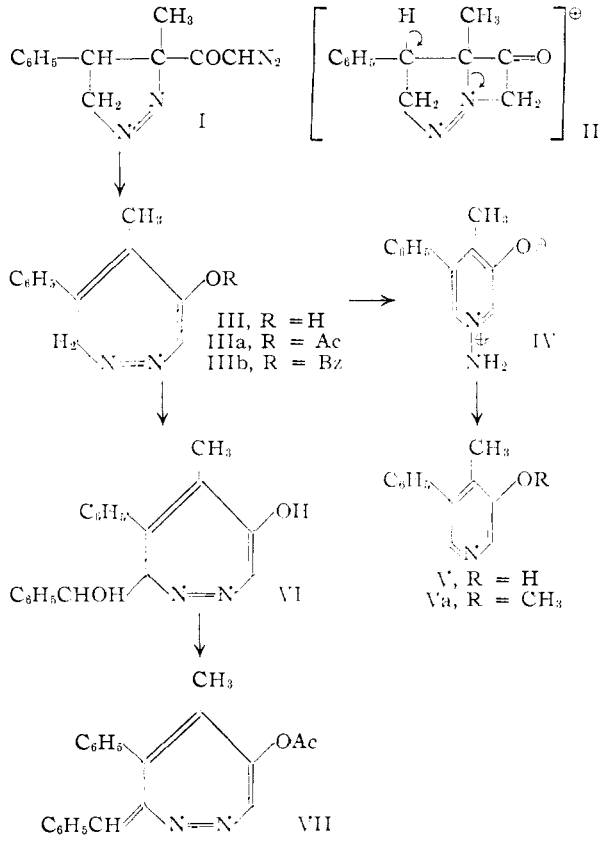
to colorless basic products which are transformed in air to dark, insoluble gums.

The presence of a reactive methylene group in the diazepinol is indicated by the reaction with benzaldehyde in the presence of sodium ethoxide to furnish a yellow *aldol product*, m.p. 145° (*Anal.* Calcd. for $C_{19}H_{18}O_2N_2$: C, 74.49; H, 5.92; N, 9.15. Found: C, 74.59; H, 6.06; N, 9.33); U.V. and I.R. spectra very similar to those of III. This product is considered to be the 7-α-hydroxybenzyl compound VI. Acetylation of VI with acetic anhydride in pyridine gives the dehydration product VII, orange-red needles, m.p. 168° (*Anal.* Calcd. for $C_{21}H_{18}O_2N_2$: C, 76.34; H, 5.49; N, 8.48. Found: C, 76.55; H, 5.40; N, 8.42); λ_{max}^{EtOH} 227 mμ (18,300), 304 mμ (23,600); I.R. 3.20 μ (w), 5.69 (s), 5.96–5.99 (vs), 6.13 (m), 6.36 (w), 6.68 (w), 7.15 (s). This acetate on acid or alkaline hydrolysis furnishes a colorless substance, $C_{19}H_{16}ON_2$, m.p. 128°; λ_{max}^{EtOH} 250 mμ (9,000); $\lambda_{max}^{EtOH-KOH}$ 291 mμ (14,500); which is clearly the product of a rearrangement. The structure of this compound cannot as yet be assigned; it forms a 2,4-dinitrophenyl hydrazone, m.p. 160° (*Anal.* Calcd. for $C_{25}H_{20}O_4N_6$: C, 64.09; H, 4.30; N, 17.94. Found: C, 64.06; H, 4.43; N, 18.03).

One of the rearrangement products of the diazepenol has been studied in some detail in an effort to adduce further evidence in support of structure III. Although the diazepinol shows no basic properties, it dissolves rapidly in warm 20% hydrochloric acid, with disappearance of the orange color. On cooling, colorless prisms of a hydrochloride $C_{12}H_{12}ON_2 \cdot HCl$, m.p. 192–195° (*Anal.* Calcd.: C, 60.88; H, 5.53; N, 11.84; Cl, 14.98. Found: C, 61.60; H, 5.74; N, 11.93; Cl, 14.94), separate in 90% yield. This salt, on treatment with one equivalent of alkali, furnishes long needles of a base, $C_{12}H_{12}ON_2$, m.p. 195–200° (dec.) (*Anal.* Found: C, 71.73; H, 6.01; N, 14.27); pK'_A 4.9; $\lambda_{max}^{H_2O}$ 228 mμ (ϵ 23,200), 320 mμ (5,700); $\lambda_{max}^{0.1\ NHCl}$ 225 mμ (21,800), 292 mμ (6,700). The free base is quite soluble in warm water, and is formulated as the zwitterion of 1-amino-3-hydroxy-4-methyl-5-phenylpyridine (IV). This constitution for the rearrangement product is established, except for the position of the methyl and phenyl substituents, by the quantitative deamination of IV with ethanolic nitrous acid to furnish V, m.p. 198° (subl.) (*Anal.* Calcd. for $C_{12}H_{11}ON$: C, 77.81; H, 5.99; N, 7.56. Found: C, 77.71; H, 6.09; N, 7.34; pK'_A 4.6, 9.5); λ_{max}^{MeOH} 283 mμ (6,600); $\lambda_{max}^{MeOH-HCl}$ 227 mμ (17,600), 289 mμ (9,050); $\lambda_{max}^{MeOH-NaOH}$ 228 mμ (22,500), 306 mμ (6,610). This deamino base is assigned a 3-hydroxypyridine structure on the basis of: positive $FeCl_3$ color, formation of an N-oxide (m.p. 285° (dec.); pK'_A 6.9) with perbenzoic acid, formation of methyl ether with diazomethane, characteristic ultraviolet spectra and dissociation constants. The spectra of IV and V, particularly the shift of maxima with pH, are practically identical with those of 3-hydroxypyridine methochloride (pK'_A 5.0) and 3-hydroxypyridine (pK'_A 4.5, 8.8), respectively. The base IV yields an acetate on brief warming with acetic anhydride, m.p. 216° (*Anal.* Found: C, 69.18; H, 5.77; N, 11.68); pK'_A 4.1, 6.3; ultraviolet spectrum virtually identical with that of IV; infrared: weak bands at (μ) 2.86, 3.17, 3.8, 5.46, 6.50 (s). Acid hydrolysis of the acetate gives IV; methylation with diazomethane gives a base, pK'_A 4.3, picrate m.p. 149–151°, which on acid hydrolysis followed by deamination furnishes the methoxypyridine Va, picrate m.p. 138°, (*Anal.* Calcd. for $C_{19}H_{16}N_4O_8$: C, 53.27; H, 3.77; N, 13.08. Found: C, 53.47; H, 3.66; N, 13.06), identical with the picrate obtained from the methylation product of the deamino base V.

One of the most interesting rearrangements of the diazepinol is encountered when III is treated with benzoyl chloride in pyridine. Two isomeric benzoates are formed, one of which is IIIb, formed exclusively by benzoic anhydride acylation. The major product (50% yield), is a colorless isomeric substance, m.p. 125° (*Anal.* Found: C, 75.07; H, 5.33; N, 9.15); λ_{max}^{EtOH} 226 (14,000), 265 mμ (8900), 331 mμ (16,600); I.R. 3.18 (w), 5.53 (s), 6.12 (s), 6.35 (w), 7.08 (s). The structure of this compound is tentatively considered to include a bicyclic system similar to that proposed for the intermediate (II).

Further reactions of III are being explored, and it is hoped that further evidence in support of the structures assigned can be presented in the full account of this work, which will be submitted for publication to THIS JOURNAL.



RESEARCH LABORATORIES
PARKE, DAVIS & COMPANY
DETROIT 32, MICHIGAN JAMES A. MOORE

RECEIVED MAY 6, 1955