Experimental Section

5-Nitro-3-dialkylaminomethylindoles.—These compounds were prepared from 5-nitroindole by the general procedure of Cavallini and Ravenna,[7] who prepared 5-nitrogramine. The amines were separated from accompanying polymeric material either by extraction into hot 6 *N* HCl, followed by precipitation with ammonia, or by recrystallization from toluene or ethanol.

5-Amino-3-dialkylaminomethylindoles.—The corresponding aminogramines were prepared by low-pressure hydrogenation of the nitro compounds over platinum oxide in ethanol. The amino compounds were somewhat unstable and difficult to obtain pure. For this reason not all of the nitrogramines were successfully converted to their amino analogs. The 5-amino compounds were recrystallized from benzene and cyclohexane mixtures. No tendency towards hydrogenolysis of the dialkylamino moiety was observed as evidenced by the consumption of only 3 equiv. of hydrogen in each case.

Melting points, yields, and analytical data for all the new compounds prepared are presented in Tables I and II.

Acknowledgment.—We wish to thank Dr. Samuel Ferguson and his staff for the mouse behavioral data and toxicities.

Substitution in the Hydantoin Ring. III. Bicyclo[3.3.0]octane Derivatives

MELDRUM B. WINSTEAD, FREDERICK R. SCHOLER, JR., AND KENNETH H. WILDRICK

Department of Chemistry, Bucknell University, Lewisburg, Pennsylvania

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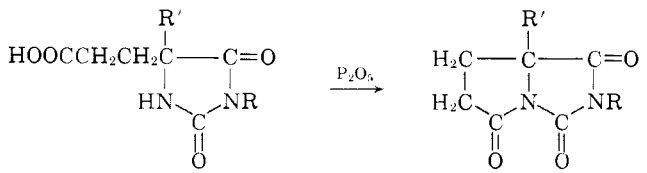
The dehydration of 4-methyl- and 4-phenyl-2,5-dioxo-4-imidazolidinepropionic acid (Ia[1] and Ib) with phosphorus pentoxide produced the cyclized products 7,7a-dihydro-7a-methyl- and 7a-phenyl-1H-pyrrolo[1,2-*c*]-imidazole-1,3,5(2H,6H)-trione (IIa and IIb). That



Ia, R = H; R′ = CH_3
b, R = H; R′ = C_6H_5

IIa, R = H; R′ = CH_3
b, R = H; R′ = C_6H_5
c, R = $CH_2NHC_6H_5$; R′ = CH_3
d, R = $CH_2NHC_6H_5$; R′ = C_6H_5

the cyclization occurred at the N-1 amide and not the N-3 imide position of the hydantoin ring was suggested by several observations. (a) IIa and IIb were readily soluble in dilute sodium hydroxide. (b) IIa showed a bathochromic shift in the ultraviolet spectrum upon changing from a neutral or acidic medium to an alkaline one.[2] Thus, in methanol or acidified methanol, the ultraviolet absorption maximum occurred at 207 mμ, whereas in alkaline methanol this maximum occurred at 223 mμ. (c) Ia and Ib each exhibited two pK_a values attributable to the carboxylic acid and imide functional groups, whereas IIa and IIb each exhibited one pK_a value attributable to the imide functional group. (d) IIa and IIb readily underwent condensation with formaldehyde and aniline to form IIc and IId, respectively. The condensation of hydantoins which are unsubstituted in the N-3 imide position with formaldehyde and amines to produce N-3 aminomethyl derivatives has received extensive investigation recently.[3-5] (e) Dehydration of 2,5-dioxo-4-imidazolidinepropionic acid has previously been shown to occur at the amide position to produce 7,7a-dihydro-1H-pyrrolo[1,2-*c*]imidazole-1,3,5(2H,6H)-trione.[6]

Pharmacology.—Several chemotherapeutic and pharmacologic tests on compounds IIa and IIb were conducted by Merck Sharp and Dohme Research Laboratories, Division of Merck and Co., Inc. The *Escherichia coli in vitro* assay, tests for effects on the nervous system in mice, and antiinflammatory activity were determined as described previously.[7] In the *E. coli in vitro* assay the compounds were inactive at 1 mg./ml. No significant effects on the nervous system were observed. In the antiinflammatory test, IIb was inactive at 100 mg./kg.

Experimental Section[8]

7,7a-Dihydro-7a-methyl-1H-pyrrolo[1,2-*c*]imidazole-1,3,5-(2H,6H)-trione (IIa).—Three grams (0.02 mole) of Ia[1] was dissolved in 50 ml. of hot 1,1,2,2-tetrachloroethane, and 1.5 g. (0.01 mole) of P_2O_5 was added quickly. The mixture was refluxed 2 hr. and then filtered from the charred residue. Upon cooling the filtrate, IIa precipitated in 85% yield. After recrystallization from tetrachloroethane and vacuum drying, it melted at 210.5°. The product was very soluble in dilute NaOH and somewhat soluble in water and ethanol; $\lambda_{max}^{CH_3OH}$ 207 mμ (ε 10,593), $\lambda_{max}^{CH_3OH-HCl}$ 207 mμ (ε 10,392), $\lambda_{max}^{CH_3OH-KOH}$ 223 mμ (ε 12,998). Potentiometric titration of IIa (4.15 mg./100 ml. of water) with 0.005 *N* NaOH gave a pK_a of 6.80. The starting reagent, Ia (53.5 mg./100 ml. of water and titrated with 0.025 *N* NaOH), had pK_{a_1} = 4.40 and pK_{a_2} = 8.55.

Anal. Calcd. for $C_7H_8N_2O_3$: C, 50.00; H, 4.80; N, 16.66; mol. wt., 168.2. Found: C, 50.40; H, 4.96; N, 16.64; mol. wt., 167.6.

Attempts to effect the cyclization in either dilute HCl or $POCl_3$ in dioxane resulted only in the recovery of unreacted hydantoin. Cyclization was effected in polyphosphoric acid, but the yield of IIa was low.

2,5-Dioxo-4-phenyl-4-imidazolidinepropionic Acid (Ib).—The preparation of Ib from benzoylethene has been described previously.[9] In this study the compound was prepared from 3-benzoylpropanoic acid, $(NH_4)_2CO_3$, and KCN in 60% ethanol in a manner similar to that described by Goodson.[10] The reaction medium was maintained at 60° for 24 hr. The product was recrystallized from ethanol; yield 80%, m.p. 216–217.5°. Additional recrystallizations raised the melting point to 219° (lit.[9] m.p. 215°).

7,7a-Dihydro-7a-phenyl-1H-pyrrolo[1,2-*c*]imidazole-1,3,5-(2H,6H)-trione (IIb).—Ib (2 g., 0.008 mole) and 1 g. (0.007 mole) of P_2O_5 were suspended in 50 ml. of *m*-xylene and refluxed for 3 hr. The insoluble substances were filtered hot and the residue was washed with aqueous $NaHCO_3$ to give IIb (78%). It was insoluble in water but was soluble in dilute NaOH. After recrystallization from ethanol it melted at 265.5–266.5°; $\lambda_{max}^{CH_3OH}$ 263, 258.5 mμ (ε 276, 405); $\lambda_{max}^{CH_3OH-HCl}$ 262.5, 258.5 mμ (ε 424, 553).

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(8) Melting points were determined in a Mel-Temp apparatus and are corrected. Infrared and ultraviolet spectrograms of the compounds reported appear in the "Sadtler Standard Spectra Catalog," Sadtler Research Laboratories, Inc., Philadelphia, Pa., 1965. Nitrogen analyses are by the semimicro Kjeldahl method. Other analyses were determined by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Potentiometric titrations were conducted with a Beckman Zeromatic pH meter using a glass electrode and a reference electrode saturated with potassium chloride.

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Potentiometric titration of IIb (0.1668 g./100 ml. of 50% ethanol) with 0.0995 N NaOH gave a pK_a of 6.75. The starting reagent, Ib (36.2 mg./100 ml. of water, titrated with 0.025 N NaOH), had $pK_{a_1} = 4.36$ and $pK_{a_2} = 8.92$.

Anal. Calcd. for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17; mol. wt., 230.2. Found: C, 62.34; H, 4.49; N, 12.17; mol. wt., 232.8.

The cyclization reaction was repeated as above except that 40 ml. of 1,1,2,2-tetrachloroethane was used as the solvent and the refluxing time was reduced to 2 hr. This was the preferable method of preparation, as IIb was more soluble in the boiling solvent and crystallized in 76% yield upon filtering and cooling. Cyclization was effected also in polyphosphoric acid, but the yield of IIb was low.

2-(Anilinomethyl)-7,7a-dihydro-7a-methyl-1H-pyrrolo-[1,2-*c*]imidazole-1,3,5(2H,6H)-trione (IIc).—This compound was prepared by condensing IIa with equivalent quantities each of formaldehyde and aniline according to the procedure described previously[3]: yield 65%. After recrystallization from ethanol, it melted at 152–152.5°; $\lambda_{max}^{CH_3OH}$ 287, 240.5 mμ (ε 1757, 11,410); $\lambda_{max}^{CH_3OH-HCl}$ 282, 239.5 mμ (ε 796, 7871); $\lambda_{max}^{CH_3OH-KOH}$ 286.5, 238 mμ (ε 1782, 12,708).

Anal. Calcd. for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53. Found: C, 61.73; H, 5.70.

In a similar manner **2-(anilinomethyl)-7,7a-dihydro-7a-phenyl-1H-pyrrolo[1,2-*c*]imidazole-1,3,5(2H,6H)-trione** (IId) was prepared from IIb in 86% yield. After recrystallization from ethanol, it melted at 150–151°; $\lambda_{max}^{CH_3OH}$ 287, 267, 240 mμ (ε 1871, 1073, 13,414); $\lambda_{max}^{CH_3OH-HCl}$ 281.5, 267, 263, 239.5 mμ (ε 547, 471, 584, 5165); $\lambda_{max}^{CH_3OH-KOH}$ 287, 231 mμ (ε 1841, 21,228).

Anal. Calcd. for $C_{19}H_{17}N_3O_3$: C, 68.05; H, 5.11. Found: C, 68.25; H, 5.10.

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Synthesis and Biological Activity of 9-β-D-Ribofuranosyl-6-hydroxylaminopurine[1]

ALFREDO GINER-SOROLLA, LILLIAN MEDREK, AND AARON BENDICH

Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, and Sloan-Kettering Division, Graduate School of Medical Sciences, Cornell University Medical College, New York, New York 10021

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The simple derivative of adenine, 6-hydroxylaminopurine,[2] has been found to induce mitotic inhibition and nuclear degeneration of mouse Sarcoma 180 cells, but not normal embryo skin fibroblasts, over a concentration range of 10^{-6} to 10^{-4} M.[3] When administered intraperitoneally to rats and mice at a single dose of 500 mg./kg., or at lower dosages over prolonged periods, 6-hydroxylaminopurine proved to be toxic (LD_{50} for a single dose, 470 mg./kg.).[4]

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Since 6-hydroxylaminopurine was inhibitory to several mouse tumors and leukemias (Table I) and was toxic, 9-β-D-ribofuranosyl-6-hydroxylaminopurine (II) was synthesized. It was found to induce a complete inhibition of mouse leukemia P815, but was ineffective on solid tumors. Mice survived the daily administration of 500 mg. of II for 2 weeks and a dose of 250 mg. for 3 weeks.

TABLE I

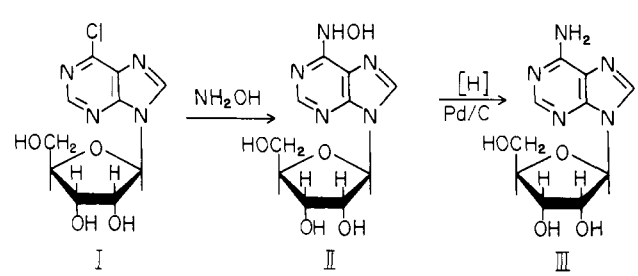
EFFECT OF SOME HYDROXYLAMINOPURINE DERIVATIVES ON MOUSE TUMORS AND LEUKEMIA[a]

	Dose, mg./kg./day	Result
6-Hydroxylaminopurine		
Sarcoma 180	125	−
Mouse carcinoma E0771	125	+
Mouse carcinoma E0771	250	+
Mouse carcinoma C1021	125	+
Ridgway osteogenic sarcoma	125	−
Ridgway osteogenic sarcoma	250	±
Mouse leukemia P815	250	+
Mouse leukemia P815 resistant to 6-mercaptopurine	150	±
Mouse leukemia P815 resistant to 6-mercaptopurine	40	−
9-β-D-Ribofuranosyl-6-hydroxylaminopurine (II)		
Sarcoma 180	62.5	−
Mouse carcinoma E0771	125	−
Ridgway osteogenic sarcoma	125	−
Mouse leukemia B82	125	±
Mouse leukemia B82	62.5	−
Mouse leukemia P815	150	+++
Mouse leukemia P815 resistant to 6-mercaptopurine	135	+
Mouse leukemia P815 resistant to 6-thioguanine	90	+
Mouse leukemia P388 resistant to 6-thioguanine	90	+

[a] The agents were administered intraperitoneally in physiological saline containing 0.5% carboxymethylcellulose for 2 weeks. −, no effect; ±, slight inhibition; +, moderate inhibition; +++, complete inhibition. Data courtesy of Dr. K. Sugiura and C. C. Stock of the Division of Experimental Chemotherapy.

The synthesis of 9-β-D-ribofuranosyl-6-hydroxylaminopurine (II) was achieved in 90% yield by treatment of 9-β-D-ribofuranosyl-6-chloropurine (I) with an excess of hydroxylamine in ethanol. Compound II was catalytically hydrogenated to adenosine (III) in 93% yield (Scheme I). Attempts to prepare II from either

SCHEME I



6-mercapto- or 6-methylmercaptopurine riboside upon interaction with hydroxylamine were unsuccessful; hypoxanthine or inosine were the reaction products.