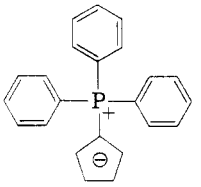
involved bromination of cyclopentadiene[5] in methylene chloride solution, followed by addition of two molar equivalents of triphenylphosphine. Addition of two molar equivalents of aqueous sodium hydroxide afforded II. A comparison of the proper-



II

ties—in particular the ultraviolet absorption spectrum—of phosphinemethylene II and acyclic analogs is of considerable significance; the stability of II, however, limits its use in the preparation of olefins. Whether conditions can be found for the reaction of II with aldehydes or specially constituted ketones (*i.e.*, fluorenone) is under investigation.

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(5) D. Lloyd and J. Sneezum, *Chemistry & Industry*, 1221 (1955).

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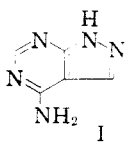
4-Hydroxy-6-Aminopyrazolo [3,4-*d*]-Pyrimidine, A Potential Guanine Antagonist

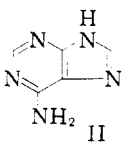
Sir:

Earlier synthetic studies conducted in this laboratory have resulted in the synthesis of the potential adenine antagonist, 4-aminopyrazolo-[3,4-*d*]pyrimidine[1] (I). The recently detected anti-



I

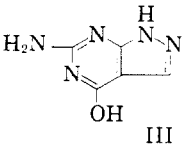
4-Aminopyrazolo[3,4-*d*]pyrimidine



II

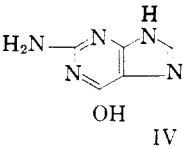
Adenine

tumor activity[2] and growth-inhibiting properties[3] of 4-aminopyrazolo[3,4-*d*]pyrimidine (I) have stimulated interest in the preparation of the corresponding guanine analog, 4-hydroxy-6-aminopyrazolo[3,4-*d*]pyrimidine (III).[3a] Interest in III is further simulated by its structural relationship to 5-amino-7-hydroxy-1-*v*-triazolo[*d*]pyrimidine (V)



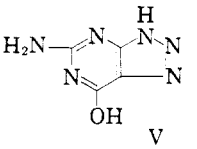
III

4-Hydroxy-6-aminopyrazolo [3,4-*d*]pyrimidine



IV

Guanine

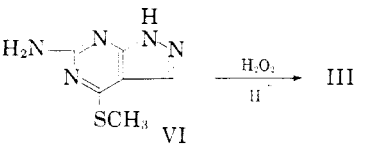


V

5-Amino-7-hydroxy-1-*v*-triazolo[*d*]pyrimidine (8-azaguanine)

(8-azaguanine), a known anti-tumor agent.[4] Although preliminary attempts to prepare III were unsuccessful,[1] the synthesis of this compound has now been realized and is the subject of the present communication. This compound has been prepared in several steps from 6-hydroxy-4-mercaptopyrazolo[3,4-*d*]pyrimidine[1] as follows:

6-Hydroxy-4-methylmercaptopyrazolo[3,4-*d*]-pyrimidine was prepared by methylation of 6-hydroxy - 4 - mercaptopyrazolo[3,4 - *d*]pyrimidine. Treatment of 6-hydroxy-4-methylmercaptopyrazolo[3,4-*d*]pyrimidine with phosphorus oxychloride and dimethylaniline gave 6-chloro-4-methylmercaptopyrazolo[3,4-*d*]pyrimidine which was further converted to 6-amino-4-methylmercaptopyrazolo-[3,4-*d*]pyrimidine (VI) with ammonium hydroxide.



VI

Treatment of VI with hydrogen peroxide in an acidic solution resulted in replacement of the methylmercapto group to give the desired 4-hydroxy-6-aminopyrazolo[3,4-*d*]pyrimidine (III). III was isolated from the acidic solution by carefully neutralizing the hot solution with ammonium hydroxide to *p*H 8. The product appeared as a fine precipitate which was filtered and washed with water and dried at 130° for analysis. *Anal.* Calc'd for $C_5H_5N_5O$: C, 39.8; H, 3.3; N, 46.4. Found: C, 39.8; H, 3.5; N, 46.2. A small amount was dissolved in a large volume of hot dilute sulfuric acid. This solution when cooled deposited white needles of the sulfate. This compound was washed and dried at room temperature. *Anal.* Calc'd for $C_5H_5N_5O \cdot {}^1/_2H_2SO_4 \cdot H_2O$: N, 32.0. Found: N, 31.5. This salt when dried in the oven at 130° still retained a small amount of water. *Anal.* Calc'd for $C_5H_5N_5O \cdot {}^1/_2H_2SO_4$: C, 29.9; H, 3.0. Found:

(1) Robins, *J. Am. Chem. Soc.*, **78**, 784 (1956).

(2) Skipper, Robins, and Thomson, *Proc. Soc. Exp. Biol. and Med.*, **89**, 594 (1955).

(3) Hsu, Robins, and Cheng, *Science* (in press), Studies on 4-APP (4-Aminopyrazolo(3,4-*d*)pyrimidine) I. Differential Cellular Damage on Tissues in Culture.

(3a) Note added in proof. S. Graff, *J. Mt. Sinai Hosp.* (New York) **19**, 313 (1952) mentions the biological testing of III which he refers to as "5-amino-7-hydroxypyrazolo [3,4-*d*]pyrimidine"; however, no reference is given for the synthesis of this compound.

(4) Kidder, Dewey, Parks, and Woodside, *Science*, **109**, 511 (1949).

C, 29.5; H, 3.5. The ultraviolet absorption spectra of III at *p*H 1 showed $\lambda_{max.}$ 252 mμ, E 5,800, at *p*H 11 $\lambda_{max.}$ 270 mμ, E 8,000.

A complete description of the experimental procedure involved in the stepwise synthesis of III will appear in a forthcoming publication involving other related work. 4-Hydroxy-6-aminopyrazolo-[3,4-*d*]pyrimidine (III) is currently being tested in several laboratories for anti-tumor action. Dr. L. W. Law and Dr. S. E. Reaume of the Leukemia Studies Section of the National Cancer Institute in preliminary studies have found III to show activity of varying extent against three lymphocytic neoplasms of the mouse, L-1210, L-5178, and L-4946. These results when completed will be reported elsewhere.

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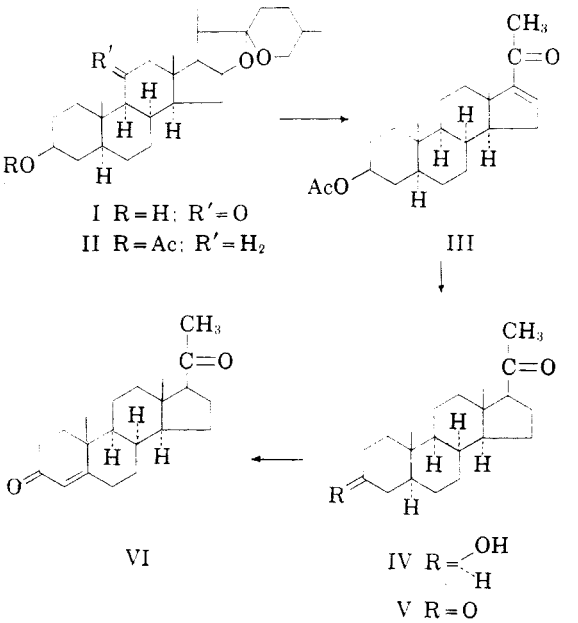
8-Isoprogesterone

Sir:

Much of the recent emphasis in the steroid field has been concerned with the synthesis of hormone analogs which could fulfill one of the following functions: (a) increased hormonal activity, (b) hormone antagonism or (c) separation of certain hormonal from other physiological effects (*e.g.*, nonandrogenic anabolic agents). Our own efforts in this field have centered on the preparation of certain isomers which differ from the natural hormones only in the stereochemistry of a relatively inaccessible center and on determining the consequences of such a subtle change upon biological activity. The stereoselective preparation of certain 11-oxygenated sapogenins[1] differing only in the orientations of C-8 and/or C-14 has led us to examine some derived steroids and the synthesis of 8-isoprogesterone (VI) is recorded herewith. This isomer of progesterone was considered to be a particularly appropriate test case since progestational activity is known[2] to be extremely specific and dependent upon precise structural and stereochemical features.

Modified Wolff-Kishner reduction[3] of 22a,25a,-5α,8α-spirostan-3β-ol-11-one (I)[1] followed by acetylation led to 22a,25a,5α,8α-spirostan-3β-ol acetate (8-isotigogenin acetate) (II), m.p. 186–189°, $[\alpha]_D$ −10° (all rotations in chloroform); *Anal.* Calc'd for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 75.73; H, 10.33. Application of the standard side chain degradation produced Δ^{16}-8α-allopregnen-3β-ol-20-one acetate (III) (m.p. 178–180°, $[\alpha]_D$ +118°, λ_{max}^{EtOH} 240 mμ, log ε 4.00; *Anal.* Calc'd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.94; H, 9.40) which was hydrogenated (palladized charcoal catalyst in ethyl acetate solution) and saponified[4] to yield 8α-allopregnan-3β-ol-20-one (IV) (m.p. 184–187°, $[\alpha]_D$ +149°; *Anal.* Calc'd for $C_{21}H_{34}O_2$: C, 79.19; H, 10.76. Found: C, 79.14; H, 10.76). Chromium trioxide oxidation of IV furnished 8α-allopregnane-3,20-dione (V) (m.p. 173–176°, $[\alpha]_D$ +144°; *Anal.* Calc'd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.90; H, 10.31), while the subsequent introduction of the 4,5-double bond was patterned after that employed for the synthesis of 11α-hydroxyprogesterone.[5]



The resulting 8-isoprogesterone (VI) (m.p. 144–147°, $[\alpha]_D$ +130°, $\lambda_{max}^{CHCl_3}$ 5.86, 5.98, and 6.12 μ, λ_{max}^{EtOH} 241 mμ, log ε 4.18; *Anal.* Calc'd for $C_{21}H_{30}O_2$: C, 80.21; H, 9.62. Found: C, 79.95; H, 9.67) was subjected to biological testing in rabbits using suitable controls treated with progesterone. In the standard Clauberg test,[6] 8-isoprogesterone (VI) by subcutaneous injection in a total of 12 animals exhibited between one-half to one-fourth the progestational activity, while local injection (into the uterine lumen) by the McGinty technique[7] in 12 animals indicated the same order of activity

(1) Djerassi and Thomas, *Chemistry & Industry*, 1228 (1954). Djerassi, Frick, Rosenkranz and Sondheimer, *J. Am. Chem. Soc.*, **75**, 3496 (1953).

(2) *Cf.* Djerassi, Miramontes, and Rosenkranz, *J. Am. Chem. Soc.*, **75**, 4440 (1953); Gunthard, Beriger, Engel, and Heusser, *Helv. Chim. Acta*, **35**, 2437 (1952); Ehrenstein, *Chem. Revs.*, **42**, 457 (1948).

(3) Barton, Ives, and Thomas, *J. Chem. Soc.*, 2056 (1955).

(4) That no isomerization had occurred during the alkali treatment was established by re-acetylation to the original acetate.

(5) Mancera, Romo, Sondheimer, Rosenkranz, and Djerassi, *J. Org. Chem.*, **17**, 1066 (1952).

(6) *Cf.* Emmens, *Hormone Assay*, Academic Press, New York, 1950.

(7) McGinty, Anderson, and McCullough, *Endocrinology*, **24**, 829 (1939).