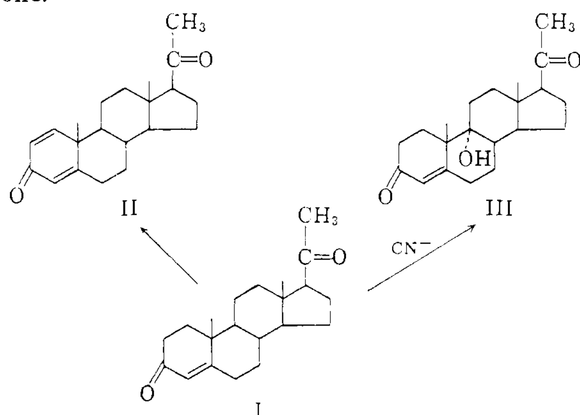
they aid in resolving the sequence of microbial degradation. Several methods have been used to study these intermediate transformations: (a) a genetic mutant which has a metabolic block is employed; (b) the nutrition of the organism is carefully studied in order to limit a particular factor required for a metabolic process during degradation; (c) the environment (*p*H, temperature, aeration) is changed.

The present communication deals with an alternate method using a metabolic inhibitor. Since the organism can oxidize an organic compound to carbon dioxide and water, it was thought that by slowing down the electron transport system of the organism, the rate of oxidative processes would also be decreased with the possible accumulation of new intermediate compounds. Evidence for this mode of inhibition was obtained by the following observations:

Cells of a *Nocardia sp.* were grown in a medium previously described.[1] After 48 hours of growth, progesterone (I) dissolved in dimethylformamide was added to the fermentation medium to give a concentration of 500 λ/ml. of medium; a total of one gram was fermented. After six hours, the culture broth was filtered, and the filtrate (2500 ml.) extracted three times with 500 ml. portions of chloroform. The combined chloroform extracts were concentrated to dryness. An aliquot of the extract was chromatographed on Whatman No. 1 paper and developed in a toluene–propylene glycol system[2] for 3 hours. A major product was observed having a R_f of 0.90 of progesterone which corresponds to the mobility of 1-dehydroprogesterone (II). By fractional crystallization, 200 mg. of a compound with the constants m.p. 150–151°, λ_{max}^{EtOH} 244 mμ (ϵ = 16,500), was obtained. The infrared spectrum in Nujol was identical to that of an authentic sample of 1-dehydroprogesterone.



When the steroid was left in contact with the organism for 24 hours, the substrate completely disappeared without any apparent detectable products. When potassium cyanide at a $10^{-3}M$ concentration was added at the time of steroid addition, however, a new product (30% yield), having a mobility of 0.55 of progesterone in the toluene–propylene glycol system, was observed. It is important that the cyanide be added at the same time as the steroid or no conversion takes place. Also, the inhibitor must be added to a culture that is about 48 to 72 hours old.

A 1-g. preparative fermentation was carried out under the conditions described above. The product was isolated by chromatographing a portion of the steroid mixture on a cellulose powder column (1.5 × 12 cm.) using propylene glycol as the stationary phase; the mobile phase consisted of a gradient system of methylcyclohexane and toluene. Three and one-half milliliter fractions were collected every three minutes. Fractions 8–55 were combined and the solvent removed *in vacuo*. The residue was taken up in 100 ml. of chloroform and washed twice with 50-ml. portions of water to remove any propylene glycol. The chloroform solution was dried over sodium sulfate, concentrated to dryness, and the residue recrystallized from acetone–hexane yielding 149 mg. of 9α-hydroxyprogesterone[3] (III), m.p. 190–191°, $[\alpha]_D$ +188° (chloroform), λ_{max}^{EtOH} 242 mμ (ϵ = 15,100), λ_{max}^{Nujol} 2.97 μ, 5.88 μ, 6.10 μ, 6.22μ.

One might suspect that cyanide ion inhibits the 1-dehydrogenation reaction. Using partially purified Δ^1-dehydrogenase[4] from this organism, however, we observed no inhibition by cyanide. A more plausible explanation is that, under normal conditions, the organism carries out both of the reactions required for degradation of the steroid, but the 1-dehydrogenation reaction operates at a faster rate than the hydroxylation reaction. On addition of cyanide, a known inhibitor of hematin cytochrome oxidases, the terminal electron transport systems are slowed down so that more reduced pyridine nucleotide, which is essential for hydroxylation reactions,[5] is available. Thus, in the presence of cyanide, the hydroxylation reaction proceeds at a faster rate than the 1-dehydrogenation reaction with the accumulation of 9α-hydroxyprogesterone.

(1) C. J. Sih and R. E. Bennett, *Biochim. et Biophys. Acta*, **38**, 378 (1960).

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(3) D. Perlman, J. D. Dutcher, J. Fried and E. O. Titus, U. S. Patent 2,840,578 (1958).

(4) C. J. Sih and R. E. Bennett, to be published.

(5) (a) J. K. Grant, *Biochem. J.*, **64**, 559 (1956); (b) G. M. Tomkins, J. F. Curran and P. M. Michael, *Biochim. et Biophys. Acta*, **28**, 449 (1958).

THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH
NEW BRUNSWICK, NEW JERSEY
CHARLES J. SIH
FRANK L. WEISENBORN

RECEIVED APRIL 8, 1960

A NEW SYNTHESIS OF CHLOROPURINE RIBONUCLEOSIDES[1]

Sir:

Chloropurine nucleosides have served as valuable synthetic intermediates for the preparation of a number of important purine nucleoside derivatives.[2–9]

(1) This investigation has been supported by Contract No. SA-43-ph-1928 with the Cancer Chemotherapy National Service Center of the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(3) L. Goldman, J. W. Marsico and R. B. Angier, THIS JOURNAL, **78**, 4173 (1956).

(4) H. M. Kissman and M. J. Weiss, *J. Org. Chem.*, **21**, 1053 (1956).

In each instance the synthesis of the requisite chloropurine nucleoside has been accomplished by introduction of the chlorine into the purine ring prior to attachment of the sugar. The high reactivity of the chlorine atom has led to a number of experimental difficulties. The removal of various blocking groups on the sugar moiety has in certain cases been impossible without concomitant replacement of the chlorine atom.[10,11] Thus, it would be advantageous to be able to introduce the chlorine atom into the purine nucleus at the final stage of synthesis.

This has now been achieved successfully and is the subject of the present communication. The recently reported[12] preparation of 6-chloropurine from 6-methylthiopurine prompted us to investigate the preparation of 6-chloro-9-β-D-ribofuranosylpurine (I) from the readily available 9-β-D-ribofuranosylpurine-6-thiol (II).[13]

Chlorine gas was bubbled slowly into a suspension of five grams of II in methanol at $-10°$. During the reaction, 9-β-D-ribofuranosylpurine-6-thiol gradually went into solution. Methanolic ammonia then was added carefully to neutralize the excess acid, and 6-chloro-9-β-D-ribofuranosylpurine (I) was isolated from the reaction mixture in above 80% yield. Treatment of 6-methylthio-9-β-D-ribofuranosylpurine (III)[13] (10 g.) in a similar manner gave I in 90% yield.

The present clinical interest in 6-chloropurine in cancer chemotherapy[14] has increased the need for large quantities of 6-chloro-9-β-D-ribofuranosylpurine (I) for preclinical evaluation. This preparation of I was found to be readily adaptable to large-scale synthesis since 125 g. of II gave 60.0 g. of I after recrystallization from methanol–water. This product decomposed at 168–170°[15] and was chromatographically pure. The ultraviolet absorption spectrum was identical with that previously recorded.[11] The product (I) was found to exhibit an infrared spectrum identical with that of an authentic synthetic sample.[15,16] The specific rotation was found to be $[\alpha]^{28}D$ -45.2 (0.796% in water) which is the same as that previously recorded.[11] *Anal.* Calcd. for $C_{10}H_{11}ClN_4O_4$: C, 41.9; H, 3.87; N, 19.5; Cl, 12.4. Found: C, 41.9; H, 3.82; N, 19.5; Cl, 12.4.

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(11) B. R. Baker, K. Hewson, J. J. Thomas and J. A. Johnson, Jr., *J. Org. Chem.*, **22**, 954 (1957).
(12) C. W. Noell and R. K. Robins, THIS JOURNAL, **81**, 5997 (1959).
(13) J. J. Fox, I. Wempen, A. Hampton and I. L. Doerr, *ibid.*, **80**, 1669 (1958). The 9-β-D-ribofuranosylpurine-6-thiol for this investigation was kindly supplied by the Cancer Chemotherapy National Service Center.
(14) R. R. Ellison, D. A. Karnofsky and J. H. Burchenal, *Blood*, **13**, 705 (1958). See also *Cancer Chemotherapy Reports*, December, 1959, National Cancer Institute.
(15) Brown and Weliky (ref. 2) record a decomposition temperature of 170–171° and state that the decomposition point varies with the rate of heating and is not a satisfactory criterion of purity for this compound.
(16) Kindly supplied by Dr. Alexander Hampton of the Sloan–Kettering Institute, New York, N. Y.

The recently reported synthesis of 2-amino-6-chloropurine[17] from 2-amino-6-methylthiopurine suggested the synthesis of the previously unreported 2-amino-6-chloro-9-β-D-ribofuranosylpurine (IV). This synthesis proceeded readily from 2-amino-6-methylthio-9-β-D-ribofuranosylpurine[18] (10 g.) and chlorine gas in methanol at $-10°$. The product was isolated after carefully adjusting the *p*H to 9 with methanolic ammonia so that the internal temperature was maintained below $-10°$. After recrystallization first from water and then twice from absolute methanol, IV was obtained in 51% yield. When heated rapidly from room temperature, IV melted with decomposition at 171–172°. 2-Amino-6-chloro-9-β-D-ribofuranosylpurine (IV) in the ultraviolet exhibited: $\lambda_{max}^{pH\ 1}$ 310, 246, 221 mμ, ε 7,450, 7,300, 24,600; $\lambda_{min}^{pH\ 1}$ 267, 236 mμ, ε 1,450, 6,500, $\lambda_{max}^{pH\ 11}$ 308, 246 mμ, ε 7,950, 8,250; $\lambda_{min}^{pH\ 11}$ 267, 235 mμ, ε 1,860, 7,150. The specific rotation was found to be $[\alpha]^{26}D$ -27.7 (0.614% in water). *Anal.* Calcd. for $C_{10}H_{12}ClN_5O_4$: C, 39.9; H, 4.0; N, 23.2; Cl, 11.8. Found: C, 39.7; H, 4.0; N, 23.5; Cl, 11.8.

This relatively mild method of introduction of a halogen atom into a nitrogen heterocyclic system is presently under further investigation.

Acknowledgment.—The author wishes to thank Dr. Howard W. Bond of the Cancer Chemotherapy National Service Center for his suggestion of applying this reaction to the synthesis of 6-chloro-9-β-D-ribofuranosylpurine (I).

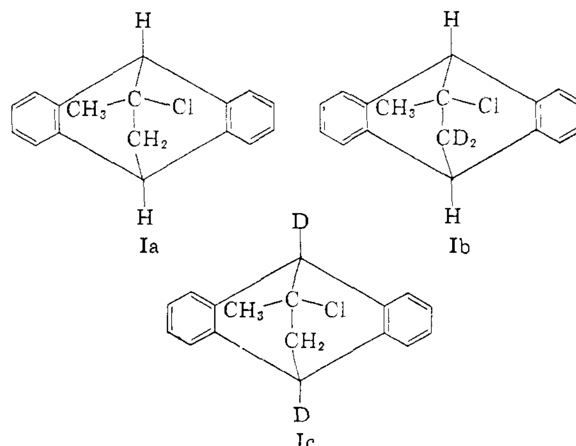
(17) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel and A. G. Beaman, THIS JOURNAL, **82**, 2633 (1960).
(18) The preparation of 2-amino-6-methylthio-9-β-D-ribofuranosylpurine was accomplished from 2-amino-9-β-D-ribofuranosylpurine-6-thiol[13] and will be reported later.

DEPARTMENT OF CHEMISTRY
ARIZONA STATE UNIVERSITY
TEMPE, ARIZONA ROLAND K. ROBINS
RECEIVED MARCH 19, 1960

STERIC INHIBITION OF A SECONDARY DEUTERIUM ISOTOPE EFFECT[1]

Sir:

I would like to report the synthesis and solvolysis rate constants of compounds Ia,b,c.



(1) National Science Foundation Senior Postdoctoral Fellow and Alfred P. Sloan Research Fellow.