dialkylphosphinates and 1230-1238 cm^{-1} for phosphinates of the type ROP(O)(R')(R'') in which R, R', R'' = mixed alkyl and aryl.

Acknowledgment.—The author wishes to express his appreciation to Drs. Y. F. Shealy and J. A. Montgomery for encouragement in this work, and to the members of the Analytical Section of Southern Research Institute who performed the spectral and most of the microanalytical determinations reported under the direction of Dr. W. J. Barrett. Some of the analyses reported were performed by Galbraith Laboratories, Knoxville, Tenn.

Notes

Nucleosides from Homoribose[1]

JOHN A. MONTGOMERY AND KATHLEEN HEWSON

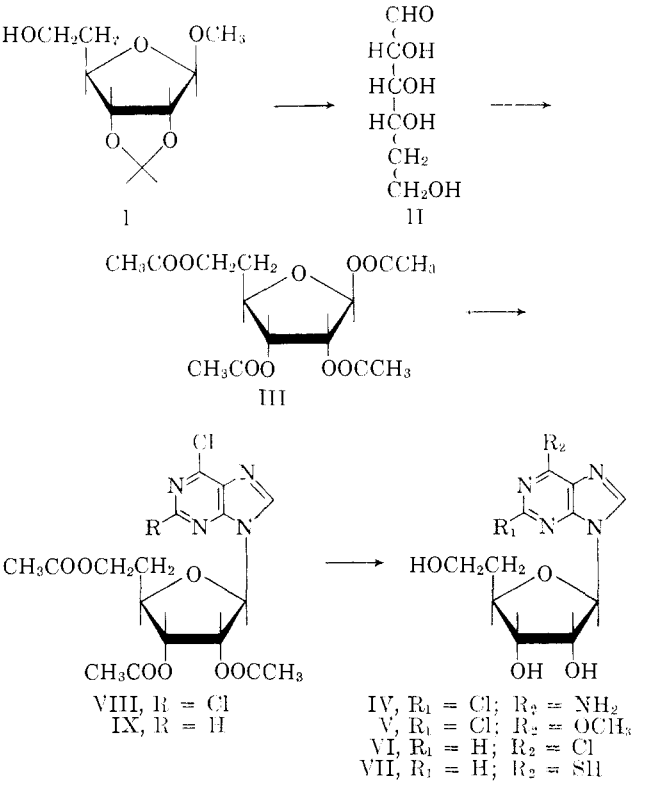
Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama

Received August 2, 1965

In a previous paper[2] we described the synthesis of homoribose (5-deoxy-D-*ribo*-hexose) from methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside and its proof of structure by proton magnetic resonance spectroscopy. We have now prepared some purine nucleosides from homoribose.[3]

Methyl 5-deoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside (I)[2] was hydrolyzed in a mixture of dilute hydrochloric acid and ethanol, but concentration of the reaction mixture caused the resulting 5-deoxy-D-*ribo*-hexose (II) to condense with itself. Treatment of this material with acetic anhydride resulted in a low yield of impure tetra-*O*-acetyl-5-deoxy-D-*ribo*-hexose (III). Neutralization of the acid hydrolysis media with ion-exchange resin before concentration did not prevent self-condensation, but the use of dilute sulfuric acid followed by neutralization with barium hydroxide and then freeze-drying gave a high yield of II, which was readily converted to the tetraacetate III, a light yellow oil (see Scheme I). The β-configuration was assigned to III on the basis of the comparison of its proton magnetic resonance spectrum with that of tetra-*O*-acetyl-β-D-ribofuranose.[5] Furthermore the fact that the absorption due to the proton at C-1 appears as a singlet ($J \leq 1$ cps)[6] also indicates the β-configuration.[6] Compound III was allowed to react with 2,6-dichloropurine by the fusion technique using *p*-toluenesulfonic acid as catalyst.[7] From this reaction a 34% yield of 9-(2,3,6-tri-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranosyl)-2,6-dichloropurine (VIII) was isolated as a crystalline solid. The β-configuration was assigned to this nucleoside on the basis of the comparison of its proton magnetic resonance spectrum with that of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (X).[8] The striking similarity of the

SCHEME I



spectra of these two nucleosides (See Figure 1) provides the best available evidence for this anomeric assignment. Although attempts have been made to relate the coupling constant for the proton at C'-1 ($J_{1'2'}$) of purine nucleosides to the anomeric configuration at C'-1 by use of the Karplus equation,[9] these attempts have unfortunately been entirely unsuccessful.[10-12] Lemieux and Lineback[13] have pointed out that in a furanose ring the coupling constant for vicinal *cis* protons can vary from 3.5 to 8.0 cps and for vicinal *trans* protons from 0 to 8.0 cps. Consequently, the coupling constant $J_{1'2'}$ for VIII, 5.0 cps, provides no definitive information concerning its anomeric configuration; however, from our investigation of the proton magnetic resonance spectra of a number of 9-β-ribofuranosylpurines we have found the chemical shift of the C'-1 hydrogen to be character-

(1) This work was supported by the C. F. Kettering Foundation and by the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH-43-64-51.

(2) J. A. Montgomery and K. Hewson, *J. Org. Chem.*, **29**, 3436 (1964).

(3) Ryan, *et al.*,[4] have described the synthesis of homoribose and homoadenosine by a different route.

(4) K. J. Ryan, H. Arzoumanian, E. M. Acton, and L. Goodman, *J. Am. Chem. Soc.*, **86**, 2503 (1964).

(5) H. Zinner, *Ber.*, **83** 153 (1950); **86**, 817 (1953).

(6) K. L. Rinehart, Jr., W. S. Chilton, M. Hicheus, and W. von Phillipsborn, *J. Am. Chem. Soc.*, **84**, 3216 (1962).

(7) T. Sato, T. Shimadate, and Y. Ishido, *Nippon Kagaku Zasshi*, **81**, 1440 (1960).

(8) J. A. Montgomery and K. Hewson, *J. Heterocyclic Chem.*, **1**, 213 (1964).

(9) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(10) L. Goldman and J. W. Marsico, *J. Med. Chem.*, **6**, 113 (1963).

(11) N. J. Leonard and R. A. Laursen, *J. Am. Chem. Soc.*, **85**, 2026 (1963).

(12) J. A. Montgomery and H. J. Thomas, *ibid.*, **87**, 5442 (1965).

(13) R. U. Lemieux and D. R. Lineback, *Ann. Rev. Biochem.*, **32**, 155 (1963).

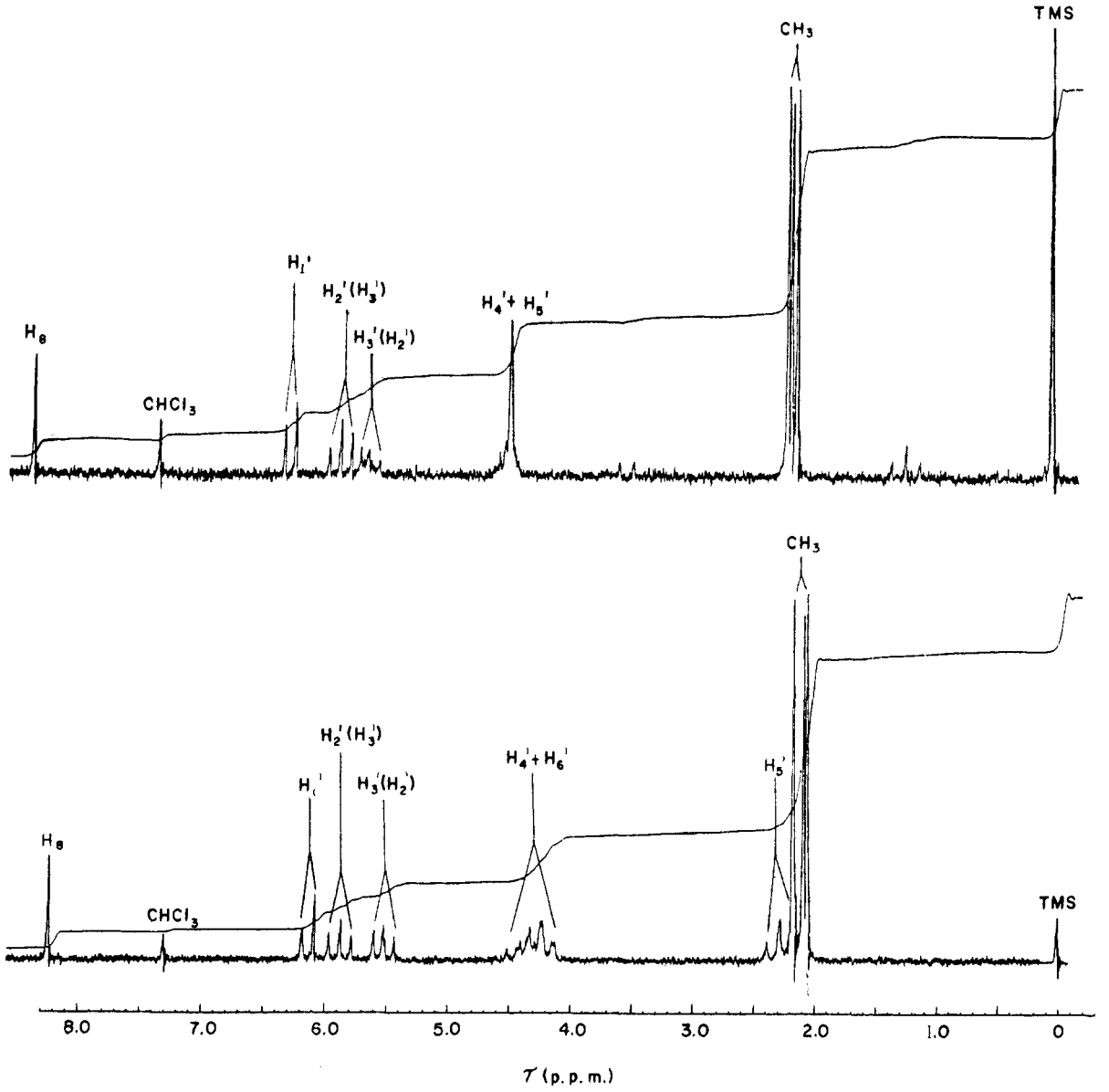


Figure 1.—Proton magnetic resonance spectra of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine (top) and VIII (bottom).

istic (4.0–4.2 ppm) and the coupling constant $J_{1'2'}$ to lie between 5.1 and 5.7 cps. In the present work the values for the deacetylated nucleosides derived from VIII and IX fall in these ranges (see Experimental Section).

Treatment of VIII with methanolic ammonia at 5° for 1 week resulted in removal of the acetyl groups from the sugar hydroxyls with concomitant displacement of the chlorine atom at C-6 of the purine moiety, but in contrast to the results obtained with 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-2,6-dichloropurine,[8] the product was a mixture of 2-chloro-9-(5-deoxy-β-D-*ribo*-hexofuranosyl)adenine (2-chlorohomoadenosine, IV) and 2-chloro-6-methoxy-9-(5-deoxy-β-D-*ribo*-hexofuranosyl)purine (V). The identity of V was firmly established from its ultraviolet, infrared, and proton magnetic resonance spectra. The potent nucleophilicity of the methoxide ion has been observed before.[14]

Reaction of III with 6-chloropurine as described above gave a 46% yield of 9-(2,3,6-tri-*O*-acetyl-5-deoxy-β-D-*ribo*hexofuranosyl)-6-chloropurine (IX) as a glass. This material was deacetylated in the usual manner to give 9-(5-deoxy-β-D-*ribo*-hexofuranosyl)-6-chloropurine (VI), also obtained as a glass. Reaction of VI with sodium hydrosulfide in methanol gave 9-(5-deoxy-β-D-*ribo*-hexofuranosyl)purine-6(1H)-thione (VII), a homolog of 6-mercaptopurine ribonucleoside.

Biological Activity.—The toxicity of KB, HEp-2/S, and HEp-2/MP cells in culture of the homoribonucleosides, 2-chlorohomoadenosine (IV) and 6-mer-

TABLE I

	ED_{50}[a]		
Compd	KB	HEp-2/S	HEp-2/MP
2-Chloroadenine	11		
IV	>100	>100	100
6-Mercaptopurine	0.25	0.25	>100
VII	3.1	2.8	>100

[a] ED_{50} is that concentration of compound in μg/ml inhibiting the growth of cells to 50% of controls. Cells were grown on glass and growth was measured by determination of protein content [V. I. Oyama and H. Eagle, *Proc. Soc. Exptl. Biol. Med.*, **91**, 305 (1956)] after 4 days growth in the presence of the compound.

(14) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961).

captopurine homoribonucleoside (VII), are compared with those of the purines from which they are derived in Table I. The cytotoxicity of VII to KB and HEp-2/S cells appears to be due to its cleavage by phosphorylases to 6-mercaptopurine, since it is not cytotoxic to cells resistant to 6-mercaptopurine (HEp-2/MP); however, IV must not be cleaved to any significant extent to 2-chloroadenine, since it (IV) is not cytotoxic to any of the cell lines, whereas 2-chloroadenine is.

Experimental Section

The melting points reported were determined on a Kofler Heizbank and are corrected. The ultraviolet spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer. The infrared spectra were determined in KBr disks with a Perkin-Elmer Model 521 spectrophotometer. The proton magnetic resonance spectra were determined on 10% (w/v) solutions in $CDCl_3$ (IV, V, and VII in DMSO-d_6) with a Varian A-60 spectrometer. The optical rotations were determined in the solvents specified with a Rudolph Polarimeter Model 80.

1,2,3,6-Tetra-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranose (III).—A solution of methyl 5-deoxy-2,3-*O*-isopropylidene-β-D-*ribo*-hexofuranoside (I, 5.2 g, 23.8 mmoles) in 0.04 *N* H_2SO_4 (50 ml) was heated for 1 hr at 95°. After cooling to room temperature the solution was neutralized with 0.1 *N* $Ba(OH)_2$ (20 ml) and the $BaSO_4$ that precipitated was removed by filtration. The filtrate was decolorized before it was concentrated *in vacuo* (0.5 mm, 20–25°) to 0.1 vol. Freeze-drying of the concentrate followed by overnight drying *in vacuo* (0.03 mm) at room temperature gave 3.8 g (100% yield) of 5-deoxy-*ribo*-hexose as an oil suitable for use as an intermediate. Thin layer chromatography on silica gel H (Merck) using chloroform–methanol (1:1) as the eluent indicated a minor impurity and the absence of polymeric material.

To a solution of dry 5-deoxy-*ribo*-hexose (3.8 g, 23.4 mmoles) in anhydrous pyridine (40 ml) cooled in an ice bath was added acetic anhydride (16 ml). The resulting reaction solution was refrigerated overnight and then allowed to stand at room temperature for 2 hr before it was poured onto 300 ml of water and ice. The aqueous mixture was extracted four times with chloroform (200 ml total volume). The combined $CHCl_3$ extracts were washed successively with cold water, excess cold saturated $NaHCO_3$, and cold water before they were dried ($MgSO_4$) for 4 hr. After removal of the $MgSO_4$ by filtration, the solution was evaporated to dryness *in vacuo* and the resulting oil was extracted with six 200-ml portions of petroleum ether. The combined extracts were evaporated to dryness *in vacuo*. The residue was dissolved in diethyl ether and the solution decolorized. Evaporation of the ether solution to dryness *in vacuo* gave the purified tetraacetyl-*ribo*-hexofuranose as an oil; yield 4.5 g (58%). Thin layer chromatography on silica gel H (Merck) using $CHCl_3$–ethyl acetate (9:1) indicated two minor impurities. The chromatographically homogeneous sample used for spectral analyses was obtained by elution of the product area developed on thick thin layer plates under the above conditions: $\bar{\nu}$ (in cm^{-1}), 2980–2920 (CH), 1750 (C=O), 1110–1000 (COC); τ (in ppm), 8.05 multiplet (C^5H_2), 7.96, 7.94, 7.92, and 7.88 (CH_3), 5.78 (multiplet (C^4H and C^6H_2), 4.75 t and 4.67 (C^2H and C^3H), 3.85 (C^1H). The pmr spectrum of III is as follows: τ (in ppm), 7.93, 7.90, 7.88, and 7.83 (CH_3), 5.67 multiplet (C^5H_2 and C^4H), 4.60 multiplet (C^2H and C^3H), 3.80 (C^1H). The similarity of the chemical shifts of the protons at C^1, C^2, C^3, and C^4 of these two sugars confirm that the stereochemical relationships of these protons are the same in the two and therefore III must have the β-configuration at C^1.

2-Chloro-9-(5-deoxy-β-D-*ribo*-hexofuranosyl)adenine (IV).—A methanolic NH_3 solution (40 ml of absolute methanol saturated with dry NH_3 at 5°) of 9-(2,3,6-tri-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranosyl)-2,6-dichloropurine (VIII, 636 mg, 1.4 mmoles) was kept at 4° for 1 week. The reaction solution was evaporated to dryness *in vacuo* and the resulting semisolid residue was triturated with water (5 ml). The insoluble solid was collected by filtration, washed with small portions of water, and dried *in vacuo* to give 167 mg (38%) of essentially pure product. Recrystallization of this product from water gave analytically pure material, yield 137 mg (31%), mp 214°, $[\alpha]^{25}D$ no observable rotation (concentration: 0.5 g/100 ml of methanol). Thin layer chromatography on silica gel H (Merck) using $CHCl_3$–methanol (4:1) as the eluent showed a single spot which gave positive Schiff-metaperiodate test; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)], pH 1—263 (14.3), pH 7—263 (14.6), pH 13—264 (15.0); $\bar{\nu}_{max}$ (in cm^{-1}), 3420–3100 (OH, NH), 2950, 2900 (CH), 1660 (NH), 1595, 1570 (C=C, C=N), 1080, 1060, 1040 (COC); τ (in ppm), 8.13 q (C^5H_3), 6.47 q (C^6H_2), 5.92 multiplet (C^2H and C^3H), 5.52 multiplet (C^4OH), 5.42 multiplet (C^2H), 4.83 d and 4.52 d (C^2OH and C^3OH), 4.17 d (C^1H), 2.20 (NH_2), 1.64 (C^8H); $J_{1'2'} = 5.3$ cps.

Anal. Calcd for $C_{11}H_{13}ClN_5O_4$: C, 41.84; H, 4.48; N, 22.18. Found: C, 41.78; H, 4.57; N, 22.00.

Evaporation of the combined filtrate and washings (from the isolation of the purified 2-chloroadenosine analog) to dryness after $CHCl_3$ extraction of the aqueous solution gave a 1:1 mixture of two products identified by thin layer chromatography on silica gel H (Merck), using $CHCl_3$–methanol (4:1) as the eluent, as additional 2-chlorohomoadenosine and another Schiff-metaperiodate positive material. A chromatographically homogeneous sample of the unknown product was isolated after two recrystallizations of the mixture from boiling ethanol: mp 180°. Spectral data identified the product as 2-chloro-6-methoxy-9-(5-deoxy-β-D-*ribo*-hexofuranosyl)purine (V): λ_{max} [in mμ ($\epsilon \times 10^{-3}$)], pH 1—258 (11.7), 265 (sh), pH 7—258 (11.7), 265 (sh), pH 13—258 (12.5), 265 (sh); $\bar{\nu}_{max}$ (in cm^{-1}), 3400–3200 (OH), 2950, 2930, and 2890 (CH), 1598 and 1575 (C=C, C=N), 1065 and 1045 (COC); τ (in ppm), 8.04 t (C^5H_2), 6.48 q (C^6H_2), 5.87 s over multiplet (OCH_3, C^3H and C^4H), 5.53 t (C^6OH), 5.31 t (C^2H), 4.78 d and 4.53 d (C^3OH and C^2OH), 4.08 d (C^1H), 1.39 (C^8H); $J_{1'2'} = 5.2$ cps.

6-Chloro-9-(5-deoxy-β-D-*ribo*-hexofuranosyl)purine (VI).—A methanolic NH_3 solution (70 ml of absolute methanol saturated with dry NH_3 at 5°) of 9-(2,3,6-tri-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranosyl)-6-chloropurine (IX) (2 g) was kept at 4° for 2 days. The reaction solution was evaporated to dryness *in vacuo* and the resulting residue was dissolved in water. The aqueous solution was extracted with chloroform, treated with Norit, and filtered. The filtrate was concentrated *in vacuo* and extracted in a liquid–liquid extractor with ethyl acetate. Evaporation of the ethyl acetate extract to dryness gave the purified product; yield 660 mg (48%). Thin layer chromatography on silica gel H (Merck) using $CHCl_3$–methanol (4:1) as the eluent indicated a single major spot which gave a positive Schiff-metaperiodate test; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)], pH 1, 7, 13—263 (6.5). The several chromatographic impurities observed were present in low concentration.

9-(5-Deoxy-β-D-*ribo*-hexofuranosyl)purine-6(1H)-thione (VII).—Sodium hydrosulfite solution (2 ml of 1 *N* sodium methoxide saturated with H_2S) was added to an anhydrous solution of 6-chloro-9-(5-deoxy-β-D-*ribo*-hexofuranosyl)purine (VI, 320 mg, 1 mmole) in methanol (7 ml), and the resulting solution was refluxed for 20 min before it was evaporated to dryness *in vacuo*. The residue was dissolved in water (4 ml) and filtered through dry Celite, and the filtrate was acidified with glacial acetic acid. The insoluble solid that deposited was collected by filtration, washed with water, and dried *in vacuo* to give 100 mg (35%) of essentially pure product, mp 240°. Water recrystallization of this product gave an analytically pure sample, yield 70 mg (24%), mp 245°, $[\alpha]^{25}D$ −45.7 ± 0.8° (concentration: 1.02 g/100 ml of 0.1 *N* NaOH). Thin layer chromatography on silica gel H (Merck) using $CHCl_3$–methanol (4:1) as the eluent showed a single spot; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)], pH 1—224 (9.5), 322 (23.9), pH 7—226 (10.3), 318 (23.5), pH 13—232 (15.0), 310 (23.0); $\bar{\nu}_{max}$ (in cm^{-1}), 3440–3350 (OH), 3100 3040, 2950–2660 (CH and acidic NH), 1600, 1570, 1530, (C=C, C=N), 1080, 1040 (COC); τ (in ppm), 8.17 q (C^5H_2), 6.49 t (C^6H_2), 5.96 multiplet (C^3H and C^4H), 5.41 multiplet (C^2H and OH), 4.67 broad (2 OH), 4.12 d (C^1H), 1.73 (C^8H), 1.52 (C^9H); $J_{1'2'} = 5.7$ cps.

Anal. Calcd for $C_{11}H_{14}N_4O_4S$: C, 44.28; H, 4.74; N, 18.79. Found: C, 44.16; H, 4.58; N, 18.89.

9-(2,3,6-Tri-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranosyl)-2,6-dichloropurine (VIII).—A mixture of 2,6-dichloropurine (1.3 g, 6.6 mmoles) and 1,2,3,6-tetra-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranose (III, 2 g, 6.0 mmoles) was fused *in vacuo* (25 mm) at 130° with *p*-toluenesulfonic acid catalyst (75 mg) for 15 min. The resulting clear amber melt was cooled to room temperature and dissolved in $CHCl_3$ (4 ml). The $CHCl_3$ solution was washed ($NaHCO_3$, water), dried ($MgSO_4$), and evaporated to dryness. The residue was dissolved in diethyl ether, decolorized with

Norit, and evaporated to dryness to give a yellow oil which redissolved in warm ethanol. The crystals that formed were collected by filtration, washed with ethanol, and dried *in vacuo* to give essentially pure product, yield 950 mg (34%), mp 121°. Thin layer chromatography on silica gel H (Merck) using $CHCl_3$–ethyl acetate (4:1) as the eluent showed 2,6-dichloropurine as the only contaminant. Recrystallization of a sample of the isolated material from boiling ethanol gave the pure product: mp 123°; λ_{max} [in mμ ($\epsilon \times 10^{-3}$)], pH 1, 7—252 (7.3), 273 (13.2), 280 (sh), pH 13—255 (sh), 258 (15.0), 265 (sh), 280 (sh); $\bar{\nu}_{max}$ (in cm^{-1}), 3115, 3060, 3050–3000 (CH), 1755, 1740, 1725 (C=O), 1595, 1560 (C=C, C=N), 1240, 1205 (COC).

Anal. Calcd for $C_{17}H_{18}Cl_2N_4O_7$: C, 44.37; H, 3.94; N, 12.18. Found: C, 44.25; H, 3.99; N, 12.12.

6-Chloro-9-(2,3,6-tri-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranosyl)purine (IX).—A mixture of 6-chloropurine (1.5 g, 9.7 mmoles) and 1,2,3,6-tetra-*O*-acetyl-5-deoxy-β-D-*ribo*-hexofuranose (III, 3.4 g, 10.0 mmoles) was fused *in vacuo* (25 mm) at 130° with *p*-toluenesulfonic acid catalyst (75 mg) for 25 min. The resulting dark melt was cooled to room temperature, dissolved in $CHCl_3$ (10 ml), and filtered to remove unreacted 6-chloropurine. The filtrate was washed ($NaHC_3O_3$, water), dried ($MgSO_4$), and evaporated to dryness. The residue was triturated with ethanol and filtered to remove additional 6-chloropurine, and the filtrate was decolorized with Norit before it was evaporated to dryness *in vacuo*. Petroleum ether extraction of this residue partially removed the blocked sugar contaminant from the insoluble oily product, which was then dried *in vacuo;* yield 2.0 g (46%). Thin layer chromatography on silica gel H (Merck) using $CHCl_3$–ethyl acetate (3:1) indicated the material was suitable for use as an intermediate.

Acknowledgment.—The authors are indebted to Dr. W. J. Barrett and members of the Analytical Section of Southern Research Institute who performed the spectral and analytical determinations, to Dr. W. C. Coburn, Jr., and Mrs. M. Thorpe for their interpretation of the proton magnetic resonance spectra, and to Miss E. A. Dulmage for the cytotoxicity data reported herein.

Preparation and Antitumor Activity of Olivacine and Some New Analogs[1]

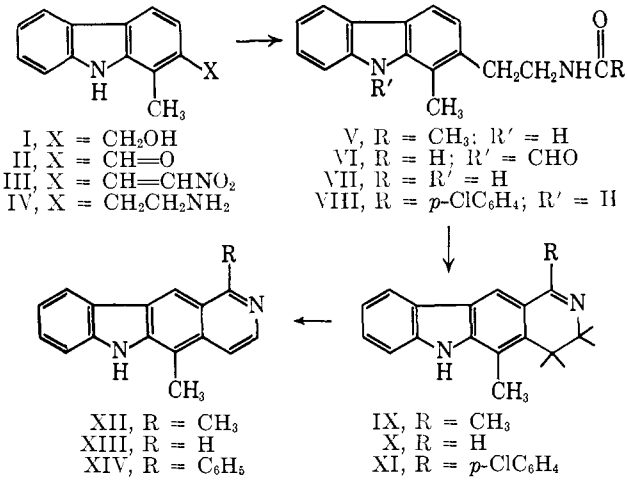
CAROL W. MOSHER, OSBORNE P. CREWS, EDWARD M. ACTON, AND LEON GOODMAN

Life Sciences Research, Stanford Research Institute, Menlo Park, California

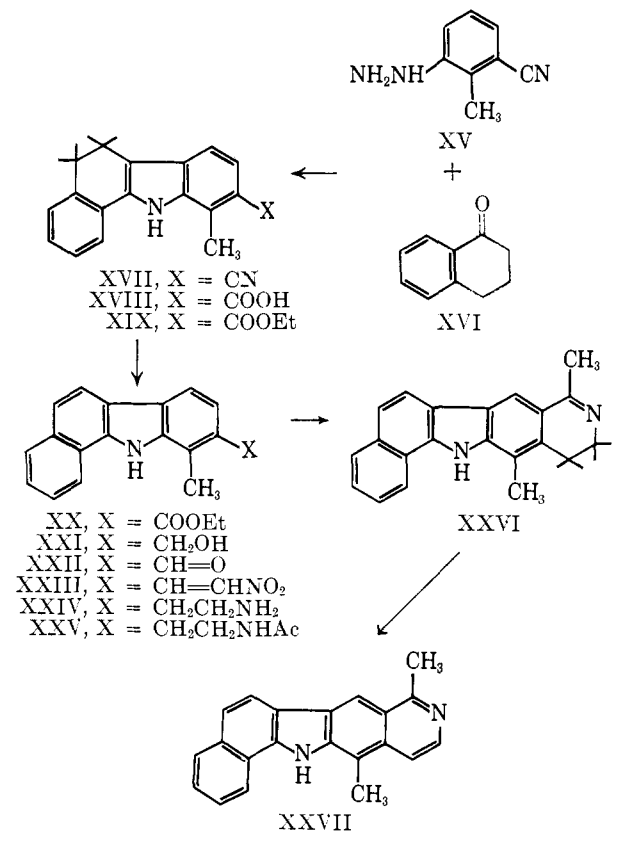
Received September 7, 1965

Preliminary reports from the Cancer Chemotherapy National Service Center[1] of potentially useful antitumor activity with the alkaloid olivacine (XII) necessitated the synthesis of large quantities for further biological testing. This has been accomplished (Scheme I) by revising a previous synthesis[2] to reduce the number of steps and avoid the use of diazomethane. The two previous syntheses[2,3] were useful mainly for the small amounts required for structure confirmation of XII. Structural requirements for activity in substituted pyridocarbazoles were studied briefly by the preparation of several analogs of XII. A demethyl derivative XIII of olivacine was easily accessible by Scheme I; this compound (XIII) is also a demethyl derivative of the alkaloid ellipticine (the 5,11-dimethylpyridocarbazole isomeric with olivacine). A recent synthesis[4] of ellipticine, but in very low yield, is similar in outline to the sequence in Scheme I which is convenient for quantities of XII and XIII. Preparation of a *p*-chlorophenyl derivative of olivacine was undertaken, because of the often encountered activity enhancement with this moiety, but the chlorine was lost in the final dehydrogenation and a simple phenyl derivative XIV resulted. A similar sequence (Scheme II) was used to prepare the benzoolivacine XXVII.

SCHEME I



SCHEME II



Biological Data.[5]—On the basis of incomplete testing results, the alkaloids related to olivacine and the corresponding dihydro compounds seemed to be poten-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. PH-43-64-500. The opinions expressed in this paper are those of the authors and not necessarily those of the Cancer Chemotherapy National Service Center.

(2) J. Schmutz and H. Wittwer, *Helv. Chim. Acta*, **43**, 793 (1960).

(3) E. Wenkert and K. G. Dave, *J. Am. Chem. Soc.*, **84**, 94 (1962).

(4) T. R. Govindachari, S. Rajappa, and V. Sudarsanam, *Indian J. Chem.*, 1, 247 (1963).

(5) The compounds were screened under the auspices of the Cancer Chemotherapy National Service Center according to its protocols, outlined in *Cancer Chemotherapy Rept.*, **25**, 1 (1962).