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PART I

REACTIONS INVOLVING DIAZOMETHANE AND CERTAIN STEROIDAL KETONES

PART II

CONFORMATIONAL STUDIES OF PURINE NUCLEOSIDES BY NUCLEAR MAGNETIC RESONANCE

by ROBERT A. SANDMANN

(Under the supervision of Associate Professor Phillip A. Hart)

PART I

The alumina-catalyzed addition of diazomethane to several steroidal ketones showed a specificity for epoxide formation that was apparently a combined function of the catalyst as well as the A-B ring fusion.

When 5β-androstan-17β-ol-3-one was treated with excess distilled diazomethane in ether in the presence of alumina a three-component mixture resulted. The principal component isolated by silica gel chromatography was A-homo-5β-androstan-17β-ol-4-one, However, when 5α-androstan-17β-ol-3-one was treated in the same manner with diazomethane in the presence of alumina the main component was the terminal 3-epoxide. Also when 5α-androstan-3-one was treated as above the terminal epoxide was again obtained.

To determine differences between alumina and methanol catalysis, 5α-androstan-3-one was allowed to react with diazomethane in methanol solution. A complex mixture

containing at least eight components was obtained, with nmr and ir data indicating the presence of both ketones and epoxides. When 5β-androstan-17β-ol-3-one was treated in the same way, a complex mixture was again obtained with one component, A-bis-homo-5β-androstan-17β-ol-ξ-one, comprising a major part of the mixture.

The results of this study indicate that control of diazomethane reactions with steroidal ketones is best achieved by use of aluminum oxide as catalyst. In most cases the product mixture was easily separated and the respective reactions' products isolated and characterized. It is apparent that the alumina-catalyzed reactions give less complex mixtures than conventional procedures as well as different product distributions which are dependent on the configuration of the ring junction.

PART II

Information regarding the possible conformations of purine nucleosides is required in order to evaluate the various models being proposed for the DNA molecule. It was the intent of this work to further add to the information concerning the possible conformations of purine nucleosides by the use of nmr as a method to determine the favored conformations of the purine nucleosides in various solvents and at various temperatures.

Appropriate models representing both the <u>syn</u> and <u>anti</u> conformations of purine nucleosides were synthesized and

spectral data obtained. It was proposed that the anomeric proton of these nucleosides would be somewhat shielded by the purine ring in an anti conformation with a decrease in shielding as the molecule approaches the syn conformation. The spectral data obtained on the model compounds supported this proposal.

The chemical shifts of the isopropylidene derivatives of the natural nucleosides guanosine and adenosine were compared to the data for the syn and anti models to determine the conformations of these natural purine nucleosides. This comparison indicated that the natural nucleosides occurred in a predominately anti conformation since the chemical shifts for the anomeric protons of the natural nucleosides were closer to those values obtained from the model compounds in an anti conformation. However, it was not possible to predict quantitatively the specific conformation of these nucleosides.

Temperature studies indicated that some conformational change occurred as the temperature of the sample was decreased. However, these data were not conclusive enough to predict any conformer population.

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PART I

REACTIONS INVOLVING DIAZOMETHANE AND CERTAIN STEROIDAL KETONES

PART II

CONFORMATIONAL STUDIES OF PURINE NUCLEOSIDES BY NMR

by

ROBERT A. SANDMANN

A thesis submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

(Pharmaceutical Chemistry)

at the

UNIVERSITY OF WISCONSIN

1979

TO

C. FRANKLIN BAUER

for his help and constant
interest in my education

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation and gratitude to Professor Phillip A. Hart for suggesting these problems and for his encouragement and guidance throughout my graduate studies at Wisconsin.

I am indebted to the National Institutes of Health for financial support in the form of a General Research Support Grant, and to the Wisconsin Alumni Research Foundation.

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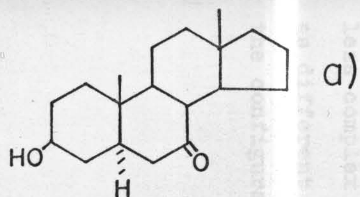

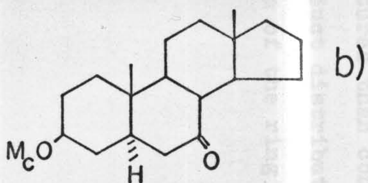

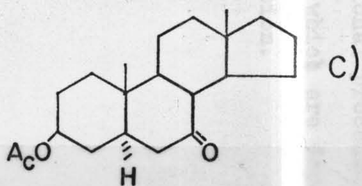

5.0

a

b

0

-5.0

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-10.0

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PART I

REACTIONS INVOLVING DIAZOMETHANE AND CERTAIN STEROIDAL KETONES

INTRODUCTION

The reaction between cyclic ketones and diazomethane to produce ring enlarged ketones and/or oxides, Figure I, is undoubtedly influenced by both steric and electronic factors. Investigations have been carried out which indicate that both factors exert controls at two stages in the reaction: the initial attack of diazomethane at the carbonyl group and the rearrangement of the resulting intermediate (1,2,3).

Many times when the need of a specific ring enlarged ketone was apparent, diazomethane was employed, only to find that the specific compound wanted was not consistently obtained or was in a mixture that was difficult to separate (4,5). Attempts to standardize the conditions for reaction of diazomethane with ketones have been made (4), with the result that concentration of diazomethane, solvents, temperature and various bases all have been found to affect the direction of, as well as the extent of reaction. Whether diazomethane was generated _in situ_ or _ex situ_ also seemed to have an effect on the direction of the reaction with ketones.

Exploration of the reaction of diazomethane with steroidal ketones has also yielded hard-to-resolve mixtures and inconsistent results (6,7). Isolation of the possible epoxides formed has not been accomplished as these appeared primarily as by-products in the reaction.

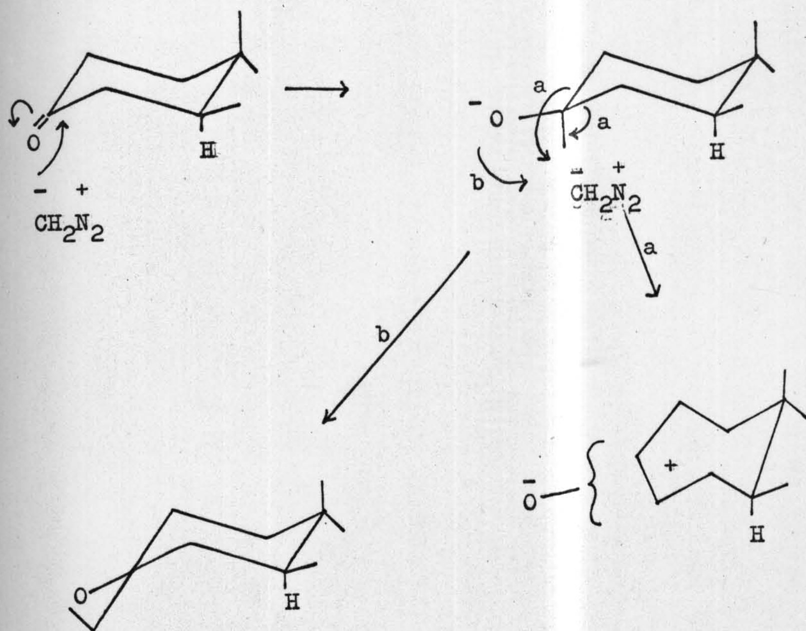


Figure I. Mechanism of reaction of diazomethane with a ketone.

Certain of these A- and B-homosteroids would make interesting models for conformational analysis of seven-membered rings as well as possible candidates for testing as modified estrogens, progestogens and androgens. Therefore, it was the intent of the following work to investigate the reaction involving steroidal-ketones and diazomethane with the hope of controlling its direction to give more consistent and less complex reactions.

PREVIOUS STUDIES INVOLVING DIAZOMETHANE AND POLYCYCLIC KETONES

One of the most complete studies of reaction conditions involving diazomethane and polycyclic ketones was that of Gutsche and Peter (4). The investigation was concerned primarily with temperature, concentration, solvent and base effects on the reaction of diazomethane with _trans_-α-decalone. Also a study involving the _cis_-α-decalone was carried out to show a comparison between steric isomers and to evaluate the differences, if any, caused by stereochemistry.

It was found that under comparable conditions, _trans_-α-decalone showed a greater tendency to form the oxide than did _cis_-α-decalone, the ratio of ketone to oxide being about 2:1 for the _trans_ isomer and greater than 20:1 for the _cis_ isomer. The effect of changing solvents and temperature seemed only to be in degree and to not affect the product ratios. However, when the concentration of reactants was changed by dilution with the respective solvents, differences in product ratios were observed.

Nelson and Schut (6) investigated the reaction of cholestan-3-one with excess diazomethane generated _in situ_ and found the major product to be A-homo-cholestan-4-one (45%) with about 10% of A-homo-cholestan-3-one and 5% of a compound presumed to be a bis-homo-cholestanone product.

No mention of oxide formation was made. However, purification was difficult in that after the several necessary recrystallizations only 27% of the pure A-homo-cholestan-4-one was recovered.

In the preparation of A-homosteroids for investigation of steroidase activity, Jones and Price (7) found the direction of diazomethane ring enlargement of 17β-hydroxy-5α-androstan-3-one (10) appeared to be controlled by the long range effect of the 17β substituent and proceeded with migration of the C_3—C_4 bond to give the A-homo-3-one (VII) in contrast to Nelson and Schut's previous findings of C_2—C_3 bond migration to give A-homo-4-one (VIII) in the cholestanone series. That the change in direction of ring enlargement was due to the effect of the hydroxyl group alone, and not in some way associated with the differences between the androstane and cholestane skeletons, was established by the observation that for 5α-androstan-3-one diazomethane ring enlargement proceeded mainly with C_2—C_3 bond migration, as in the cholestanone series, to give 80% of A-homo-5α-androstan-4-one.

Catalytic control of the reaction of diazomethane with cyclic ketones was attempted by Müller and Bauer (8). A series of Lewis acids was used along with diazomethane in an ether solution in enlarging cyclohexanone to mixtures of seven-, eight- and nine-membered ring ketones. It was felt by Müller (8) and also by von E. Doering (5) that if one

used Lewis acids, the carbonyl would be bound and prevent epoxide formation. Accordingly they did not report isolation of any oxides either in the cyclohexanone series by Müller or in the ring enlargement of barbaralone by von E. Doering. The best yields of ring enlarged products were achieved when aluminum chloride was used as catalyst in the reaction with cyclohexanone while von E. Doering found alumina to be more effective in the barbaralone series. However, von E. Doering found that often the enlargement failed for no explainable reason.

RESULTS AND DISCUSSION

It was thought that control of the reaction of diazomethane with steroidal ketones could best be accomplished by catalyzing the reaction with Lewis acids in the presence of an inert solvent. In addition, some of the steroidal ketones were also allowed to react with diazomethane in solution with methanol used as solvent-catalyst to compare the products formed by both reaction systems.

It was of interest in this study to investigate the reaction of 5α-androstan-3-one (I) with diazomethane in the presence of each solvent system. The reaction of (I) with diazomethane prepared *in situ* in methanol was reported (7,9) to give 80% conversion to A-homo-5α-androstan-4-one (II). These results were contrasted in this study by the reaction of (I) with diazomethane which, when prepared *ex situ* in the presence of methanol, gave a complex mixture containing at least three products (tlc). The mixture was chromatographed on silica gel but separation of each component was not achieved. However, it was thought that the mixture contained an oxide, A-homo-5α-androstan-3-one (II) and A-bishomo-5α-androstan-4-one (IX) based on spectral data of the partially purified eluates containing these compounds.

In comparison, the reaction of (I) with diazomethane in an ether suspension of aluminum oxide (activity I)

yielded one product (tlc). This compound was separated from starting material by column chromatography on silica gel. A white crystalline material, mp 96-97.5° was obtained in good yield. The infrared (ir) spectrum showed the absence of carbonyl function and indicated a terminal epoxide at 10.88 and 11.09 μ. The nuclear magnetic resonance (nmr) spectrum showed a two proton singlet at 2.59 δ which was assigned to the oxymethylene protons of a terminal epoxide.

The product of the alumina-catalyzed reaction was proposed to be a terminal epoxysteroid based on the ir and nmr data and was assigned the name spiro-3β-oxiranyl-5α-androstane (III) (Figure III). This represents the first report of conclusive terminal epoxide formation by the reaction of a steroidal ketone with diazomethane. This result was unanticipated since Müller (8) and von Doering (5) indicated that oxide formation in this type of reaction could be prevented by the binding of the carbonyl group to a Lewis acid such as alumina. However, recognizing that binding of a Lewis acid to a carbonyl group is an equilibrium process, it may be proposed that the following sequence is a possible explanation for the terminal epoxide formation (Figure II). The complex (IV) would rapidly dissociate to give the free oxygen anion which in turn would attack the methylene carbon expelling nitrogen to yield the epoxide.

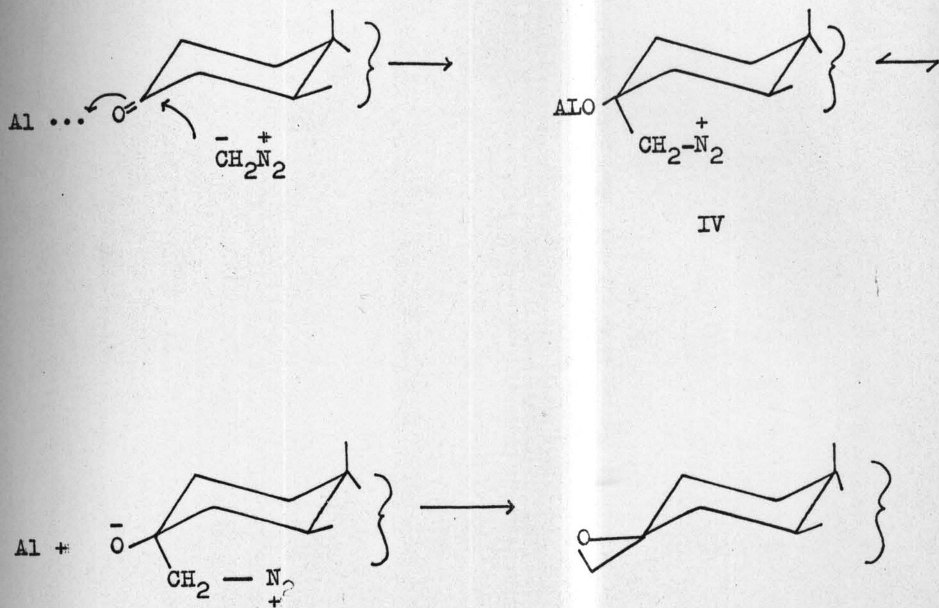
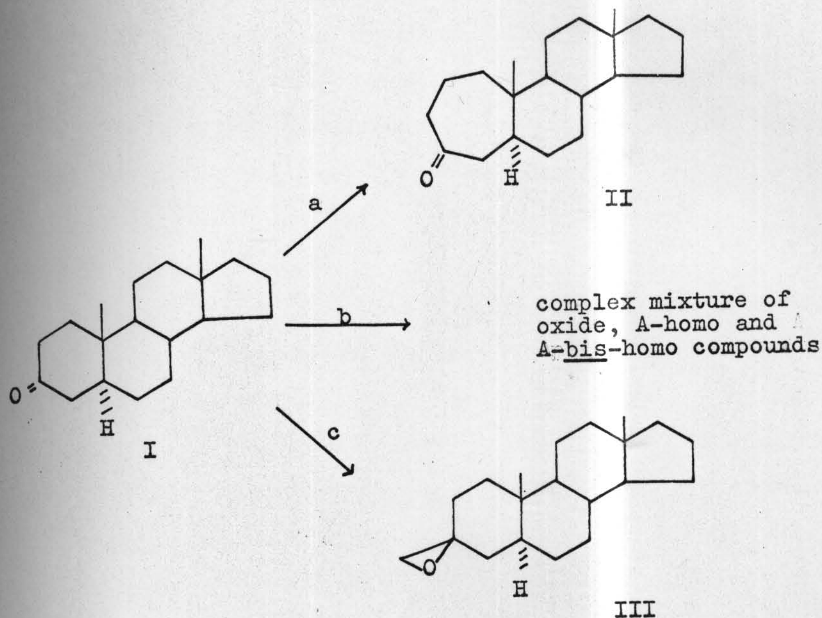


Figure II. Reaction sequence showing formation of the aluminum oxide complex and collapse to give a terminal epoxide.



a) CH_2N_2/CH_3OH <u>in situ</u>

b) CH_2N_2/CH_3OH <u>ex situ</u>

c) CH_2N_2/Al_2O_3

Figure III. Scheme showing reaction of 5α-androstan-3-one (I) with diazomethane.

An investigation of the effect of the stereochemistry of the A/B ring junction on the reaction of steroidal ketones with diazomethane was carried out using 5α-androstan-17β-ol-3-one (IV) and 5β-androstan-17β-ol-3-one (V), (Figure IV). The 5β isomer was prepared in 85% yield by the reduction of testosterone in basic media (10). Preparation of the 5β isomer in 60% yield was achieved by a lithium-ammonia reduction of testosterone (11).

Reaction of (IV) with diazomethane in the presence of aluminum oxide gave two products (tlc). Separation of the products was achieved by column chromatography on silica gel. The major product was isolated as a white crystalline solid, mp 173-174°. The ir spectrum showed the absence of carbonyl absorption and the presence of a shoulder at 3.30 μ and a peak at 7.9 μ which were assigned to the methylene of a terminal epoxide (13). Absorption at 10.88 μ and 11.09 μ was also thought to be caused by the methylene protons of a terminal epoxide. The nmr spectrum indicated a two proton singlet at 2.61 δ which together with the ir spectral data indicated the compound was spiro-2β-oxiranyl-5α-androstan-17β-ol (VI). This compound had previously been prepared by Wall, <u>et al</u>. (12) using a dimethylsulfoxonium methylide reaction.

The minor product was isolated and infrared analysis showed a carbonyl absorption at 5.89 μ. The nmr spectrum of this compound showed two three-proton singlets at

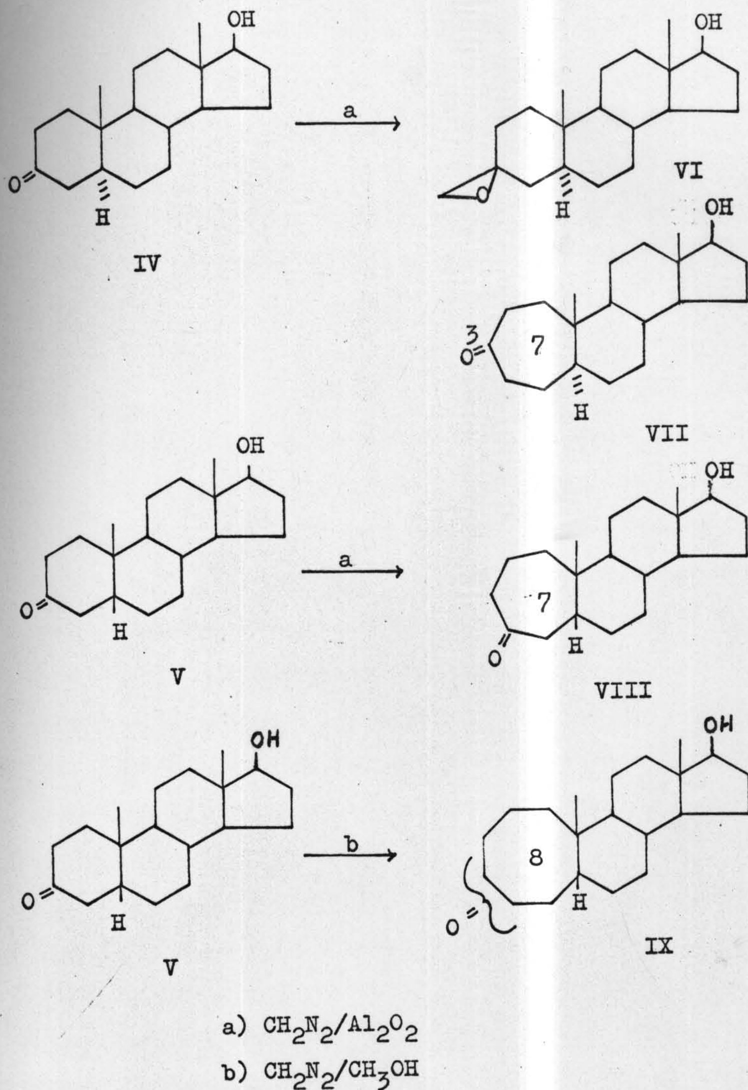


Figure IV. Reaction of 5α-androstan-17β-ol-3-one (IV) and 5β-androstan-17β-ol-3-one (V) with diazomethane.

0.74 δ and 0.83 δ which were assigned to the C-18 and C-19 angular methyl protons respectively. A one proton multiplet was observed at 3.67 δ. These data compared well with the compound A-homo-5α-androstan-17β-ol-3-one (VII) reported by Price (7).

The aluminum oxide catalyzed reaction of V with diazomethane gave three products (tlc). The major reaction product was isolated by column chromatography and recrystallization from n-hexane gave white crystals, mp 170-171°. The ir spectrum showed a peak at 5.89 μ which was attributed to seven-membered ring carbonyl absorption. A comparison of the ord curve with that of A-homo-5β-androstan-17β-ol-4-one (VIII) (7) showed the curves to be superimposible. On the basis of these data the major product was assigned the structure (VIII).

The two minor products could not be purified, but ir and nmr spectral data of the chromatographic fractions containing these compounds were consistent with structures corresponding to an epoxide and two different enlarged-ring ketones.

As a part of this study it was considered desirable to react (V) with diazomethane in the presence of methanol. One major compound was obtained and the spectral evidence for this product indicated that it was an enlarged ring ketone. The ir spectrum showed carbonyl absorption at 5.90 μ. Two seven-membered A-ring ketones were considered as possible structures for the reaction product. These

were VIII and A-homo-5β-androstan-17β-ol-3-one (X). The ord curves for VIII and X are shown in Figure V. Compound VIII exhibited a negative Cotton effect while compound X showed a positive Cotton effect. The ord curve for the reaction product is shown in Figure VI. While it shows a positive Cotton effect, the curve is not superimposible with the ord curve of X. Further evidence that neither VII nor X was the correct structure was obtained from ir data. Both seven-membered ring ketones exhibited carbonyl absorption at 5.89 μ as compared to 5.90 μ for the compound under investigation.

On the basis of the evidence discussed as well as nmr data and physical constants the compound was proposed to be A-bis-homo-5β-androstan-17β-ol-ξ-one (IX) (mp 184-185°), the position of the carbonyl function being undetermined.

General applicability of the aluminum oxide catalyzed reaction of diazomethane with steroidal ketones was investigated by attempting the reaction with 5α-androstan-3β-ol-acetate-7-one (XI). A very rapid reaction (complete in 30 min) was followed by tlc which indicated two products were formed. One very nonpolar substance was formed in small yield (less than 10%) together with a very polar compound which was the principal product. The mixture was chromatographed on silica gel and the major component was isolated and crystallized to give white needles, mp 141-142.5°. The nmr spectrum showed two three-proton singlets

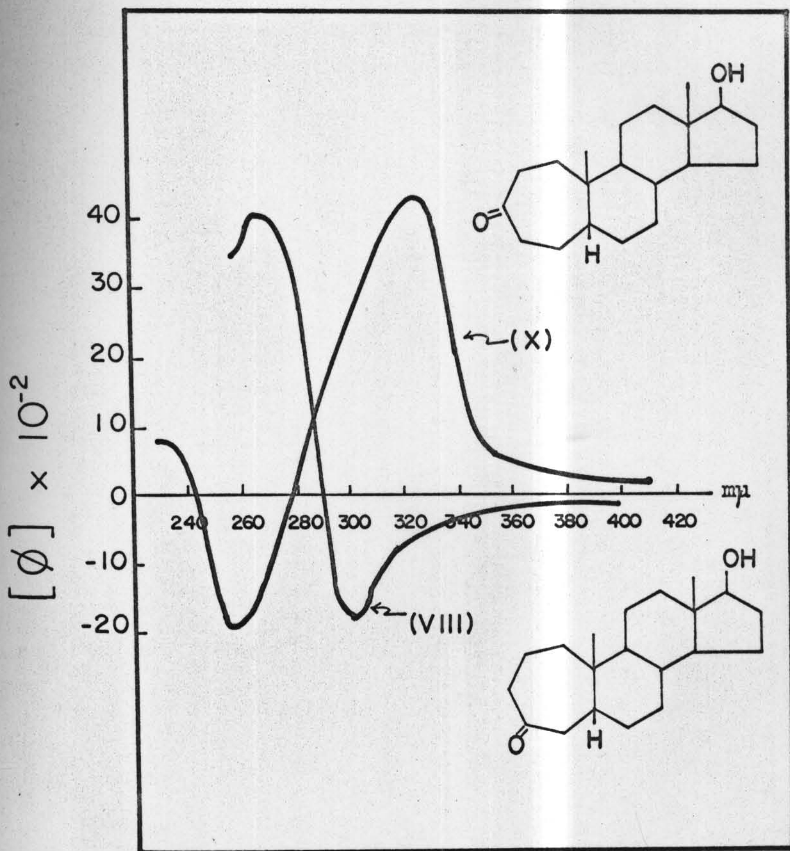


Figure V. Optical Rotatory Dispersion Curve of A-Homo-5β-androstan-17β-ol-4-one (VIII) and A-Homo-5β-androstan-17β-ol-3-one (X).

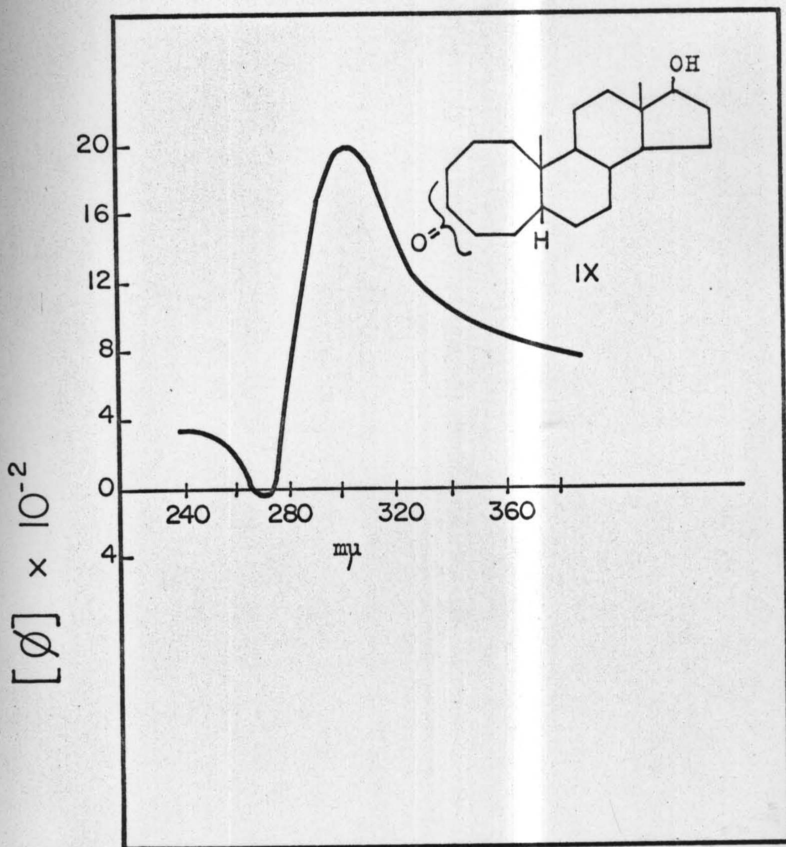


Figure VI. Optical Rotatory Dispersion Curve of A-bis-homo-5β-androstan-17β-ol-ξ-one (IX).

at 0.70 δ and 1.10 δ which were assigned to be the C-18 and C-19 angular methyl protons respectively. A one-proton multiplet was observed at 3.57 δ which disappeared when the sample was exchanged with deuterium oxide. This evidence indicated that hydrolysis of the 3-acetate had occurred yielding 5α-androstan-3β-ol-7-one (XII). The melting point found for the isolated compound was not in agreement with the recorded literature values for XII (16,17). The nmr and ir spectrum of (XII) obtained by direct hydrolysis in ethanolic potassium hydroxide was identical with that of the isolated compound and the melting point was not depressed upon admixture of the two samples. Therefore, it was concluded that hydrolysis had occurred during the reaction of (XI) with diazomethane under extremely mild reaction conditions.

Further reactions involving aluminum oxide catalyst, ethereal diazomethane solutions which had been dried over potassium hydroxide, solutions which were not dried, and 5α-androstan-3β-ol-acetate-7-one indicated the hydrolysis was due to an equilibrium between the acetate and free alcohol in basic ethereal diazomethane solution. This equilibrium was driven toward the free alcohol by esterification of the acetate anion with excess diazomethane.

The minor, nonpolar product isolated from the reaction of XI with diazomethane crystallized with difficulty giving

waxy light brown crystals, mp 86-87.5°. The nmr spectrum indicated C-18 and C-19 methyl absorption and a three-proton singlet at 3.35 δ. This line position corresponded to that for the protons of a methyl ether (14). The ord curves of XI, XII, and the minor product (Figure VII) showed negative Cotton effects of identical shape but of different amplitude. On the basis of the evidence presented the structure of the minor product was probably 3β-methoxy-5α-androstan-7-one (XIII).

Formation of the methyl ether (XIII) of a secondary alcohol in the presence of diazomethane and aluminum oxide may be explained by a mechanism similar to that reported by Neiman, et al. (15) who found that many sterols could be methylated in the presence of fluoboric acid and diazomethane by the formation of a relatively long-lived diazonium cation complex that would react with an alcohol as the nucleophile. They found this complex could also be formed with Lewis acids such as boron tri-fluoride etherate and effectively alkylate sterols. It would not be unreasonable to assume formation of an aluminum oxide diazonium cation complex which could alkylate a sterol albeit at a slower rate.

The reaction of diazomethane with 5α-androstan-3β-ol-7-one (XII) further demonstrated the unreactivity of the 7-ketone, there being no reaction of any kind in that case.

The results of this study indicate that control of diazomethane reactions with steroidal ketones is best

Figure VII. Optical rotatory dispersion curves of XI, XII and XIII.

achieved by the use of aluminum oxide as catalyst. In most cases the product mixture was easily separated and the respective reaction products isolated and characterized. It is apparent that the alumina-catalyzed reactions give less complex mixtures than conventional procedures as well as different product distributions which are dependent on the configuration of the ring junction.

EXPERIMENTAL

Melting points were determined on a Kofler block. Infrared absorption spectra were recorded, unless otherwise specified, in chloroform solution on a Beckman Model IR-5A double beam infrared recording spectrometer. Nmr spectra were determined on a Varian A-60A spectrometer in deuterochloroform (unless otherwise stated), tetramethylsilane as internal standard. Coupling constants, J, are recorded in cps, while chemical shifts are recorded in δ values (ppm). Optical rotations and optical rotatory dispersion curves were determined in anhydrous methanol (unless otherwise stated) in a strain free 0.1 dm cell on a Cary 60 Spectropolarimeter. The specific rotation values are approximated to the nearest degree. Microanalyses were performed by Mr. J. F. Alicino, P.O. Box 267, Metuchen, N.J. Skellysolve B refers to a petroleum ether fraction boiling at 60-68°. Column chromatography was carried out with silica gel and thin layer chromatography (tlc) was carried out with silica gel G (unless stated otherwise), Brinkmann Instruments, Inc. Tlc plates were sprayed with 4% ceric sulfate solution in 4 N sulfuric acid and were heated to locate the spots.

Preparation of Reactants for Diazomethane Reactions

Preparation of 5α-androstan-3β-ol-17-one. To a solution of Δ^5-androsten-3β-ol-17-one (15 g) in 95% ethanol

(360 ml) was added 10% palladium on carbon (1.5 g). Hydrogenation (18) was carried out using a Parr apparatus at approximately 45 psi and room temperature for 4 hr. The catalyst was filtered off and evaporation of the filtrate under reduced pressure gave a solid residue. Recrystallization from aqueous ethanol gave 5α-androstan-3β-ol-17-one as white crystals, mp 172-173°.

Wolff-Kishner reduction of 5α-androstan-3β-ol-17-one. A solution of 5α-androstan-3β-ol-17-one (15 g) in diethylene glycol (225 ml), *n*-butanol (75 ml) and hydrazine hydrate (85% in water) (75 ml) was refluxed for 2 hr. After cooling, potassium hydroxide (30 g) was carefully added. The alkaline solution was distilled and the distillate collected until the temperature of the solution in the distilling flask was 210°. The solution was refluxed for 4 hr, cooled and poured into water, yielding a precipitate. Recrystallization from aqueous methanol gave 5α-androstan-3β-ol (11 g), mp 151-152°.

5α-Androstan-3-one (I). A solution of 5α-androstan-3β-ol (7 g) in reagent acetone (500 ml) was treated dropwise while stirring with 8 N chromic acid (Jones' reagent (19)) until an orange color persisted for 3 min. An excess of anhydrous magnesium sulfate was added and the suspension filtered. The filtrate was concentrated under reduced pressure and poured into water (125 ml). The resulting precipitate was removed by filtration and dried

under vacuum. Crystallization from aqueous ethanol gave (I) (5.6 g), mp 103-104° (lit. (18), mp 104-105°); $\lambda_{max}^{CHCl_3}$ 5.81 µ, 7.25 µ.

Preparation of Δ^5-androsten-3β-ol. A solution of Δ^5-androsten-3β-ol-17-one (15 g) in diethylene glycol (200 ml), n-butanol (80 ml) and 85% hydrazine hydrate (60 ml) was refluxed for 2 hr (11). The reaction procedure for the reduction of 5α-androstan-3β-ol-17-one was followed using 25 g of potassium hydroxide and 800 ml of water. The precipitate was collected by suction filtration, washed liberally with water and dried under vacuum. Recrystallization from aqueous methanol gave Δ^5-androsten-3β-ol (12 g), mp 134-136°; $\lambda_{max}^{CHCl_3}$ 2.8 µ, 2.95 µ, 7.27 µ.

Preparation of Δ^5-androsten-3β-ol-acetate. Acetic anhydride (120 ml) was rapidly added dropwise to a stirred solution of Δ^5-androsten-3β-ol (12 g) in anhydrous pyridine (250 ml). Analysis of the reaction mixture by tlc showed the esterification to be complete in 4 hr. The unreacted acetic anhydride was hydrolyzed by the slow addition of 120 ml of water. The resulting heavy precipitate was filtered and the filter cake washed with water in a 400 ml beaker. The suspension was filtered and the precipitate dried under vacuum. Recrystallization from acetone-water gave Δ^5-androsten-3β-ol-acetate,

mp 93-95.5°; $\lambda_{max}^{CHCl_3}$ 5.9 μ, 8.1 μ.

Preparation of Δ^5-androsten-3β-ol-acetate-7-one. To 20 ml of hot carbon tetrachloride was added Δ^5-androsten-3β-ol-acetate (2.56 mmole) (806 mg) and N-bromosuccinimide (2.8 mmole) (450 mg). Bis-azoisobutyronitril (1 mgm) was added as a catalyst and the mixture refluxed. The reaction was initiated after 20 min and was complete after 40 min as evidenced by the presence of succinimide on the surface of the solution. The mixture was cooled and the succinimide removed by filtration. To the filtrate was added Merck Reagent Aluminum Oxide (7 g) and the suspension was stirred for 2 hr at room temperature, then filtered. The alumina was washed thoroughly with carbon tetrachloride and the combined filtrates evaporated to dryness under reduced pressure. The oily residue was suspended in glacial acetic acid (80 ml) and treated dropwise during 10 min with a solution of chromic acid (182 mg) in glacial acetic acid (5 ml). The mixture was stirred for 48 hr and then poured into ice water (250 ml). The aqueous solution was extracted three times with ether, twice with 5% sodium bicarbonate solution, and dried over anhydrous magnesium sulfate. The ether was evaporated in vacuo leaving a white crystalline solid (0.499 g) which was recrystallized from aqueous methanol to give Δ^5-androsten-3β-ol-acetate-7-one (0.390 g), mp 177-178° (lit. (20), mp 179-180°); $\lambda_{max}^{CHCl_3}$ 2.8-2.95 μ (broad carbonyl), 6.05 μ, 7.25 μ, 8.1 μ.

5α-Androstan-3β-ol-acetate-7-one (XI). To a solution of Δ^5-androsten-3β-ol-acetate-7-one (1 g) in 95% ethanol (85 ml) was added 10% palladium on carbon (250 mg). Hydrogenation was carried out at atmospheric pressure and room temperature for 35 min. The volume of hydrogen consumed was 77 ml. Filtration of the catalyst and evaporation of the solvent under reduced pressure yielded a viscous oil which showed two spots on tlc (10% ethylacetate in benzene), the lower spot corresponding to the reduced compound and the upper spot to a small amount of the reduced ketone. The mixture was dissolved in a minimum amount of 50% ethyl acetate in benzene and chromatographed on 65G of silica gel packed in benzene. Fractions of 9 ml were collected. Fractions 47-50 were combined (0.810 g) and recrystallized from aqueous methanol to give XI, mp 131.5-132°, (lit. (16), mp 130-132°); $\lambda_{max}^{CHCl_3}$ 5.81-5.9 μ (broad carbonyl), 7.26 μ, 8.1 μ.

5α-Androstan-3β-ol-7-one (XII). To a solution of potassium hydroxide (200 mg) in 95% ethanol (5 ml) was added XI (100 mg) and the mixture warmed to 40° for 30 min. Analysis of the reaction by tlc (10% ethyl acetate in benzene) showed the hydrolysis to be complete. The solution was poured into water, filtered and the precipitate collected, rinsed with water and finally dried. Recrystallization from acetone-Skellysolve B yielded 55 mgm of white crystalline material (XII), mp 140-142°,

(lit. (16), mp 128-129.5°, (17), mp 130-131°); nmr singlet at 0.708 δ (C-18 angular methyl protons), singlet at 1.18 δ (C-19 angular methyl protons), multiplet at 3.57 δ (C-3 proton).

5α-Androstan-17β-ol-3-one (IV). A solution of testosterone (500 mg) in 50% dioxane-ether (20 ml) was added dropwise to a solution of lithium (50 mg) in anhydrous liquid ammonia (50 ml) with rapid stirring (11). The reaction mixture was stirred for 15 min and the blue color discharged by the addition of ammonium chloride (2.5 g). The ammonia was allowed to evaporate. The solution was extracted twice with ether (150 ml) and the combined extracts were washed twice with water (60 ml) and dried over anhydrous magnesium sulfate. Evaporation of the ether solution under reduced pressure yielded a viscous oil which appeared as three spots on tlc (20% ethanol-benzene). The highest R_f spot was the major component, the middle one was a minor component and the lower spot was starting material. The mixture was dissolved in a minimum amount of 15% ethyl acetate in benzene and chromatographed on 30 g of silica gel packed as a slurry in benzene. The highest R_f component (on tlc) was eluted with 5% ethyl acetate in benzene (yield, 0.300 mg, 60%) and was identified as IV, mp 178-180°, (lit. (7), mp 181-182°); $[\alpha]_D$ +11° (c, 1.15); $\lambda_{max}^{CHCl_3}$ 2.77 μ (s) (monomeric hydroxyl), 2.90 μ (w) (polymeric hydroxyl),

5.86 μ (s) (carbonyl); nmr singlet at 0.78 δ (C-18 angular methyl protons), singlet at 1.04 δ (C-19 angular methyl protons), multiplet at 3.66 δ (C-17 proton).

5β-Androstan-17β-ol-3-one (V). To a solution of testosterone (10 g) in 95% ethanol saturated with barium hydroxide (75 ml) was added 10% palladium on carbon (500 mg). Hydrogenation (10) was carried out at atmospheric pressure and room temperature for 30 min. The volume of hydrogen consumed was 920 ml (theoretical, 887 ml). The catalyst was removed by filtration and the filtrate evaporated to dryness under reduced pressure, to yield an oily residue. Analysis of the oil by tlc showed two spots which corresponded to (V) and (IV) in the ratio 80:20. The oily mixture was chromatographed on 400 g of silica gel packed as a slurry in benzene. Elution was carried out using 5% ethyl acetate in benzene and 5 ml fractions were collected. Fractions 80-200 were shown to contain (IV). Fractions after 270 containing V were combined (4.5 g) and recrystallized twice from aqueous methanol to give white crystals of V, mp 140-141° (lit. (7), 141-142°); $[\alpha]_D$ + 36° (c, 0.85); $\lambda_{max}^{CHCl_3}$ 2.77 μ (monomeric hydroxyl), 2.90 μ (polymeric hydroxyl), 5.85 μ (carbonyl); nmr singlet at 0170 δ (C-18 angular methyl protons), singlet at 1.10 δ (C-19 angular methyl protons), multiplet at 3.60 δ (C-17 proton).

General Procedures for Diazomethane Reactions

A solution of diazomethane in ether was prepared *ex situ* by a standard method (21) and dried over potassium hydroxide for a minimum of two hours.

Procedure A: Reaction of diazomethane with steroidal ketones using aluminum oxide as catalyst. The steroidal ketone was dissolved in a minimum amount of methylene chloride. To this solution was added an equivalent weight of Woelm neutral alumina (activity grade I) and the suspension cooled to -5°C in an ice-salt bath. The diazomethane solution was added dropwise to the cooled, rapidly stirred, suspension until a 13 to 1 mole excess of diazomethane was obtained. Stirring was continued as the ice-salt bath slowly equilibrated with the room temperature and for 4 to 6 hours thereafter. The alumina was removed by filtration and the filtrate evaporated to yield viscous yellow-brown oils.

Procedure B: Reaction of diazomethane with steroidal ketones in the presence of methanol. The steroidal ketone was prepared as a 10% solution in methanol. To this rapidly stirred solution which had been cooled to -5°C in an ice-salt bath was added dropwise a previously prepared diazomethane solution until a 13 to 1 molar excess of the diazomethane was obtained. Stirring was continued as the ice-salt bath slowly equilibrated with room temperature and for 4 to 6 hours thereafter. The solution was

evaporated under reduced pressure to yield a viscous yellow-brown oil.

Diazomethane Reactions with Steroidal Ketones

Reaction of 5α-androstan-3-one (I) with diazomethane. A solution of (I), 1 g, in dried methylene chloride (25 ml) was treated with diazomethane according to procedure A. The resulting oil (1.4 g) appeared as two spots on tlc (10% ethyl acetate in benzene). The higher R_f spot corresponding to product and the lower to starting material. The oil was chromatographed on 75 g of silica gel slurried in benzene. Elution was carried out with a solution of 10% ethyl acetate in benzene and 8 ml fractions were collected. Fractions 60 to 120 were combined and the solvent removed under reduced pressure to yield an oily residue, 355 mg. Crystallization from aqueous methanol gave a white, crystalline solid which was characterized as 5α-androstan-3,3-methylene oxide (III), mp 96-97.5°; $[\alpha]_D^{25}$ +18° (c, 0.49); $\lambda_{max}^{CHCl_3}$ 7.22 μ (methyl), 11.05 μ and 10.85 μ (terminal epoxide); nmr singlet at 0.71 δ (C-18 angular methyl protons), singlet at 0.82 δ (C-19 angular methyl protons), singlet at 0.59 δ (two oxy-methylene protons).

Anal. Calcd for $C_{20}H_{32}O$ (288.48): C, 83.25; H, 11.2. Found: C, 83.14; H, 10.93.

Reaction of 5α-androstan-3β-ol-acetate-7-one (XI) with diazomethane. A solution of (XI) (1 g) in dried methylene chloride (25 ml) was reacted with diazomethane solution (60 ml) according to procedure A. Analysis of the reaction after 1 hour showed two spots on tlc (10% ethyl acetate in benzene), one corresponding to starting material and the other to the reaction product. An additional 60 ml of the diazomethane solution was added. Analysis by tlc after 30 minutes revealed two products. A very nonpolar material was present as a minor component while a very polar material was present as the major product. The resulting oil was chromatographed on silica gel slurried in benzene and eluted with 5% ethylacetate in benzene. After 200 10-ml fractions were collected the polarity of the eluate was increased to 20% ethyl acetate in benzene. The major reaction product being eluted completely after 350 fractions. Evaporation of the fractions containing this product yielded a solid residue which was recrystallized from acetone-water to give fine white needles (840 mg), mp 141.0-142.5°; nmr singlet at 0.70 δ (C-18 angular methyl protons), singlet at 1.10 δ (C-19 angular methyl protons), multiplet at 3.57 δ (C-3 proton).

A comparison of this product with (XII) showed these two compounds to be identical. The mixed mp with (XII) was undepressed. The ir spectra of the two samples in chloroform were superimposible.

Reaction of 5α-androstan-17β-ol-3-one (IV) with diazomethane. A solution of (IV) (500 mg) in anhydrous methylene chloride (20 ml) was treated with diazomethane solution as in procedure A. The oily residue appeared as two spots in the approximate ratio of 3:1 on tlc.(20% ethyl acetate in benzene). The higher R_f spot corresponded to the major reaction product and the lower R_f spot to the minor. The mixture was chromatographed on 30 g silica gel slurried in benzene. Elution was carried out with a 10% ethylacetate solution in benzene and 5 ml fractions were collected. Fractions up to 95 were combined and evaporated to yield 250 mg of a solid residue. Recrystallization from aqueous methanol gave a crystalline compound which was characterized to be 5α-androstan-3,3-methyleneoxy-17β-ol (VI); mp 173.0-174.0°; $\lambda_{max}^{CHCl_3}$ 2.77 μ (monomeric hydroxyl), 2.90 μ (polymeric hydroxyl), 10.88 μ, 11.09 μ (terminal epoxide); nmr singlet at 0.76 δ (C-18 methyl protons), 0.87 δ (C-19 angular methyl protons), singlet at 2.61 δ (two oxymethylene protons), multiplet at 3.63 δ (C-17 proton).

Anal. Calcd for $C_{20}H_{32}O_2$ (304.48): C, 78.89; H, 10.59: Found: C, 77.10; H, 10.68.

Subsequent elution was carried out and fractions 96 to 105 were combined and evaporated to dryness. An oily residue (30 mg) was obtained which failed to crystallize. A comparison of the nmr spectrum of this compound and the spectrum of A-homo-5α-androstan-17β-ol-3-one (7) showed

these compounds to be identical.

Reaction of 5β-androstan-17β-ol-3-one (V) with diazomethane. A solution of (V) (500 mg) in anhydrous methylene chloride (25 ml) was reacted with diazomethane solution (200 ml) according to procedure A. The resulting yellow oil was shown to be three compounds on tlc (20% ethylacetate in benzene). The major compnent was the lowest R_f spot. The mixture was chromatographed on 30 g silica gel and elution carried out using 5% ethylacetate in benzene. Five ml fractions were collected and fractions 60 through 70, 71 through 85, and 86 through 95 were combined and showed two spots on tlc. Fractions 96 through 115 were combined and evaporation in vacuo yielded a solid residue. Recrystallization from n-hexane gave a crystalline compound which was characterized to be A-homo-5β-androstan-17β-ol-4-one (VIII), mp 170-171°, (lit., mp 172.5-173.0°) (7); $\lambda_{max}^{CHCl_3}$ 2.76 μ (monomeric hydroxyl), 5.89 μ (seven-membered ring carbonyl); nmr singlet at 0.96 δ (C-18 angular methyl protons), singlet at 1.08 δ (C-19 angular methyl protons), 1.07 δ (hydroxyl proton), multiplet at 3.66 δ (C-17 proton); and (Figure IV) (c, 0.11 in CH_3OH) 25°; $[\phi]_{390}$ -11.1°; $[\phi]_{320}$ -730°; $[\phi]_{280}$ +201°.

Reaction of 5β-androstan-17β-ol-3-one (VI) with diazomethane. A solution of (V) (500 mg) in anhydrous methanol (50 ml) was reacted with diazomethane solution according to procedure B. The yellow-brown viscous oil was

shown to be essentially one spot on tlc.(20% ethylacetate in benzene). The oil was chromatographed on 30 g of silica gel. Elution was carried out with 4% ethylacetate in benzene and the major component eluted after 350 ml of eluate had been collected. Recrystallization from acetone-Skellysolve B yielded a compound characterized to be A-bis-homo-5β-androstan-ξ-one (IX), white plates, mp 184-185°; $[\alpha]_D^{25}$ + 65°; $\lambda_{max}^{CHCl_3}$ 2.77 μ (monomeric hydroxyl), 5.90 u (eight-membered ring ketone), 7.23 μ; nmr singlet at 0.74 δ (C-18 angular methyl protons), 0.90 δ (C-19 angular methyl protons), multiplet at 3.03 δ (C-17 proton).

Anal. Calcd for $C_{21}H_{34}O_2$ (318.51): C, 79.19; H, 10.76. Found: C, 78.56; H, 10.58.

REFERENCES

1. Kohler, E. P., Tishler, M., Potter, M. and Thompson, H. T., J. Amer. Chem. Soc., 61, 1057 (1939).

2. Adamson, D. W. and Kenner, J., J. Chem. Soc., 181 (1939).

3. Mousseron, M. and Manon, G., Bull. Soc. Chim. France, 392 (1949).

4. Gutsche, C. D. and Peter, H. H., J. Amer. Chem. Soc., 77, 5971 (1955).

5. Doering, W. von E., personal communication to P. A. Hart.

6. Nelson, N. A. and Schut, R. N., J. Amer. Chem. Soc., 81, 6486 (1959).

7. Jones, J. B. and Puce, P., Canadian J. Chem., 44, 999 (1966).

8. Müller, E. and Bauer, M., Ann., 654, 92 (1962).

9. Jones, J. B., Pander, J. Malcolm and Price, P., J. Amer. Chem. Soc., 89, 1, 94 (1967).

10. Prelog, V., Ruzicka, L., Meister, P. and Wieland, P., Helv. Chim. Acta, 28, 618 (1945).

11. Bauers, A., Ringold, H. J. and Denot, E., J. Amer. Chem. Soc., 80, 6115 (1958).

12. Cook, C. E., Corley, R. C. and Wall, M. E., Tetrahedron Letters, no. 14, 891 (1965).

13. Nakanishi, K., "Infrared Absorption Spectroscopy; Practical," Holden-Day, Inc., San Francisco, 1962, p. 155.

14. Bhacca, N. S. and Williams, D. H., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, 1964, pp. 39-40.

15. Nieman, M., Caseris, M. C., Roberts, J. D. and Johnson, W. S., Tetrahedron, 6, 36 (1958).

16. Williams, D. H., Bhacca, N. S. and Djerassi, C., J. Amer. Chem. Soc., 85, 2810 (1963).

17. Heard, R. D. H. and McKay, A. F., J. Biol. Chem., 165, 677 (1946).

18. Shapiro, R. H., Williams, D. H., Budzikiewicz, H. and Djerassi, C., *J. Amer. Chem. Soc.*, *86*, 2837 (1964).

19. Bowden, K., Heilbron, I. M., Jones, E. R. H. and Weedon, B. C. L., *J. Chem. Soc.*, 39 (1946).

20. Williams, D. H., Bhacca, N. S. and Djerassi, C., *J. Amer. Chem. Soc.*, *85*, 2810 (1963).

21. Moore, J. A. and Reed, D. E., *Org. Syntheses*, *41*, 16 (1961).

PART II

CONFORMATIONAL STUDIES OF PURINE NUCLEOSIDES BY NUCLEAR MAGNETIC RESONANCE

INTRODUCTION

Information regarding the possible conformations of purine nucleosides is required in order to evaluate the various models being proposed for the DNA molecule. In a survey of the number of ways in which the four bases of DNA could form hydrogen-bonded pairs with one another Donohue (17) determined that 24 different pairings were possible. Models for the DNA helix have been proposed by Pauling and Corey (18), Watson and Crick (19), and Spencer (2). These models required different dimensions for the bases and base pairs, but all proposed an adenine-thymine guanine-cytosine pairing and a two chain helical structure for DNA. It is apparent that data regarding the stability of the various required conformations and information on the energy barriers between them is extremely important in determining which models are the most acceptable.

The proposal of an adenine-guanine pairing (1) which fits into the Watson-Crick structure of DNA emphasizes the need for additional structural studies on the various parts of the DNA molecule, since with the data now available it is not possible to assert that this pairing cannot become a part of the Watson-Crick DNA helix. Spencer (2) has proposed that the alternating A-T G-C pairing in the DNA structure may not

be possible because the required conformations about the C_{sugar}---N_{base} bonds in nucleosides and nucleotides have not been observed in the X-ray crystallographic studies to date. However, even though much of the evidence on possible base pairing and the DNA structure is inconclusive, most of the indirect evidence tends to favor the Watson-Crick proposed pairing.

It is the intent of this work to further add to the information concerning the possible conformations of purine nucleosides by the use of nuclear magnetic resonance spectroscopy (nmr) as a method to determine the favored conformations of the purine nucleosides in various solvents and at various temperatures. Conclusive data on this subject would be a valuable addition to the study of the total structure of the DNA molecule.

PREVIOUS WORK ON CONFORMATIONAL ANALYSIS OF NUCLEOSIDES

Although studies to deduce conformations of polynucleotides have been conducted intensively in the last six years, primarily using optical rotatory dispersion (ord), little work was done on their monomeric components. As a result of this lack of information on the monomeric units and also because the interaction between adjacent residues in a chain was not understood, an interpretation of the conformation of polynucleotides from the ord curves obtained was not possible.

Recently studies of oligo- and polynucleotides and the interpretation of the ord of diadenylic acid in terms of the interaction between the two bases (3) has provided a basis for the understanding of the ord data of these substances (4). However, a more complete knowledge of the preferred conformations of the nucleosides and nucleotides is needed to properly evaluate the data from the polynucleotides.

This problem has been considered by Donohue and Trueblood (1) who defined a torsion angle (ϕ_{CN}) as the angle formed by the C-1'-O bond and the plane of the purine or pyrimidine ring. The angle is taken as zero when C_2 of the base is antiplanar to the carbohydrate ring oxygen; positive angles are those measured in a clockwise direction from this zero point while viewing

from C to N. Two extreme conformations are possible, one corresponding to ϕ_{CN} = -30° (anti) and the other to ϕ_{CN} = 150° (syn), (Figure I).

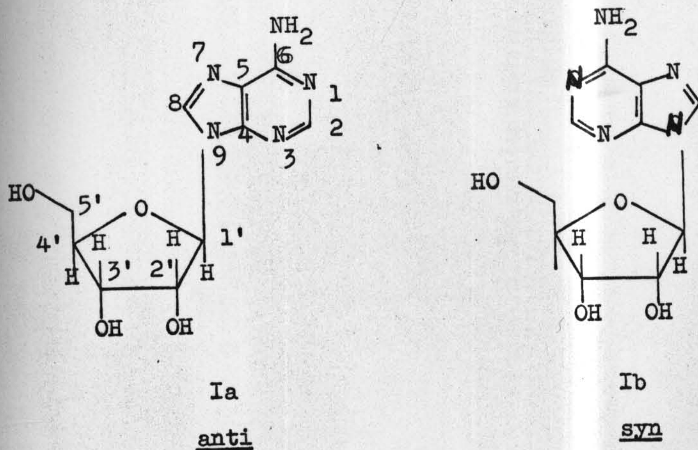


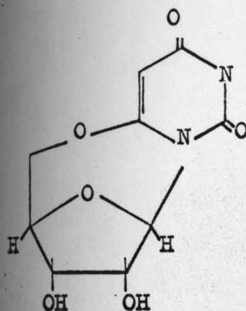
Figure I

Ulbricht, et al. (5) after studying a series of about forty pyrimidine nucleosides, including derivatives of uracil, cytosine and thymine, as well as

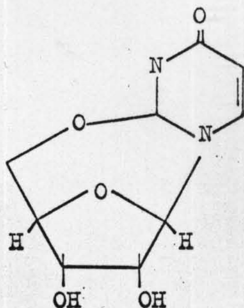
cyclonucleosides, aza-pyrimidine nucleosides and pseudouridines, attempted to determine what factors affect the long wavelength Cotton effect produced by these compounds. He found that the sign of the Cotton effect was influenced by the following parameters:

a. the anomeric configuration at C-1'
b. the position of substitution of the glycosidic residue on the pyrimidine ring
c. substitution of N for C-H in the heterocyclic nucleus
d. formation of a third ring between C-5' and N-3.

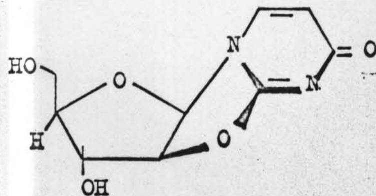
This latter effect together with the data obtained from a series of cyclonucleosides (II-IV) led Ulbricht to the conclusion that the β-pyrimidine nucleosides, which are normal constituents of nucleic acids have the _anti_-conformation in aqueous solution. The _syn_-conformation was thought to be less stable due to the high-energy interactions of the C-2 oxygen of the pyrimidine moiety with the furanose ring.



II



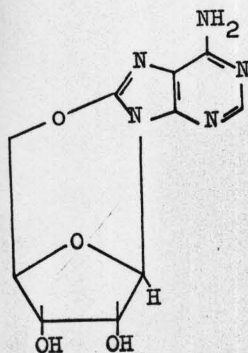
III



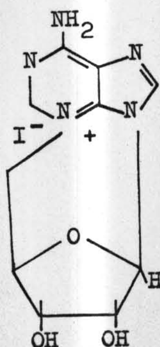
IV

Haschemeyer and Rich (22), using van der Waal's radii and bond lengths and angles determined by X-ray measurements, calculated preferred conformations for the pyrimidine nucleosides. He found that rotation about the glycosidic bond is strongly hindered by the substituent ring atoms C-2 and H-6. On the basis of the number of close contacts between atoms, the syn form in pyrimidine nucleosides was thought to be sterically less favorable than the anti conformation. It was pointed out however that even though the barriers to interconversion of the syn and anti conformations are quite high these could be reduced by changes in bond angles. Thus one could not rule out the possible occurrence of the syn conformation in a pyrimidine nucleoside. The necessary stabilization for such a structure may be achieved in some circumstances in a polynucleotide chain as it is in the cycloneucleosides.

Ulbricht and co-workers (6) also investigated purine nucleoside conformation. They found that the ord curves of these nucleosides in aqueous solution were more difficult to obtain than the curves of the pyrimidine nucleosides. This was due to the fact that the purines absorb even more intensely in the ultraviolet necessitating the use of very dilute solutions and also due to the small optical rotations exhibited by these compounds. For all of the noncyclic purine nucleosides studied, a negative Cotton effect was observed. The cyclic nucleoside, 8,5'-cycloadenosine (V), in which the _anti_ conformation is fixed by a ring linking C-5' of the sugar to C-8 in the imidazole ring was found to exhibit a negative Cotton effect. These data suggested that a negative Cotton effect is associated with an _anti_ conformation.



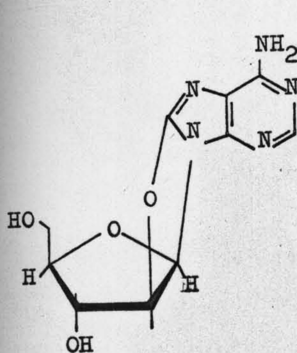
V



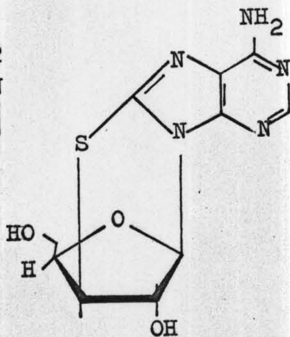
VI

In contrast, 2',3'-isopropylidene-N-3,5'-cycloadenosine iodide (VI), which necessarily has the <u>syn</u> conformation, was found to have a small positive Cotton effect. Ulbricht proposed, then, that the purine nucleosides, in aqueous solution, exist in the <u>anti</u> conformation. However, the difficulty in obtaining reliable ord data along with the high absorbance characteristic of the purine moiety must be taken into consideration when evaluating this work, and the possibility that a change in the chromophore may have influenced the sign of the Cotton effect cannot be overlooked.

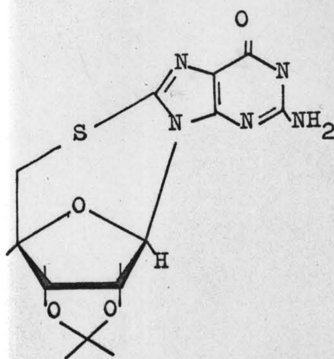
Recently, Ikehara, <u>et al</u>. (7) in determining the ord of a variety of cyclonucleosides, including (VII)-(IX), all of which were in an <u>anti</u> conformation, found the Cotton effect to be positive in all instances.



VII



VIII



IX

They felt that in consideration of these structures together with the fact that purine nucleosides generally have a negative Cotton effect the free nucleosides in aqueous solution must have a <u>syn</u> conformation.

Eyring, <u>et</u> <u>al</u>. (23) demonstrated the dependence of optical rotation of the purine nucleosides on the nature of substituents, the sugar-base torsion angle, and changes in the puckered conformations of the ribose residue. He felt that while an analysis of the preliminary work indicated that an <u>anti</u> range of the torsion angle is preferred in adenine nucleosides, conclusive evidence was not available until more suitable model nucleosides have been investigated. Also Klee and Mudd (24) prefer to leave the question of conformation in the purine riboside series open following ord studies of a fairly wide variety of models.

Thus, interpretation of the conformational data on purine nucleosides can only lead to a conclusion that, for the compounds considered, a <u>syn</u> conformation is favored for some nucleosides and an <u>anti</u> for others. Indeed this may be the case since calculations by Haschemeyer and Rich (22) have shown that two stable isomers would occur for the purine nucleosides. One was in the <u>anti</u> range (Φ_{CN} = -83° to -60°) and the other in the <u>syn</u> range (Φ_{CN} = +110° to +115°) with only low energy barriers to interconversion.

RESULTS AND DISCUSSION

Since the work of Ulbricht (8) and Ikehara (7) could not resolve the controversy on conformation of purine nucleosides in solution, using data from ord studies, it was felt that a method other than ord must be used to facilitate this investigation. Further it was felt that nuclear magnetic resonance (nmr) would provide a useful analytical tool for conformation studies. The spectral line positions of the protons (chemical shifts) in an nmr spectrum are sensitive to their chemical locations because the surrounding electrons which constitute the chemical environment "shield" the nucleus from the applied magnetic field (Ho). The applied magnetic field sets up current circulations in the electron orbitals that surround the nucleus and these in turn produce at the nucleus a slight field, δH, which is in opposition to the incident field Ho. In different chemical environments, this effect occurs to differing extents.

One factor that would cause changes in the shielding of the nucleus and thus in its chemical shift is the electron density around the nucleus (10). The inductive effects of atoms or functional groups in close proximity to the nucleus contribute to the electron density around it and help determine the applied field strength necessary to cause the precession of the nucleus. It follows that

strongly electronegative atoms would reduce the electron density about the nucleus or effectively "deshield" the nucleus. The applied field necessary to promote energy absorption would then be lower than the field necessary if the nucleus was shielded by a more electropositive group.

The electronegativity of neighboring groups is not the only factor determining chemical shifts. Fields resulting from currents induced in the high electron concentrations associated with certain adjacent groups also contribute to the magnetic field acting at the proton. These fields generally oppose the applied magnetic field. If the electrons tend to circulate more freely around one axis than another, the material is said to have an anisotropy of diamagnetic susceptibility. This anisotropy effect occurs in systems such as benzene, ethylene and carbonyl functions.

The anisotropy of the carbonyl group arises from paramagnetic circulations induced by the component of the applied field in the plane of the trigonal carbon atom. It was concluded (26) that the shielding associated with the carbonyl group is positive in conical regions extending above and below the plane of the double bond and negative elsewhere (Figure I).

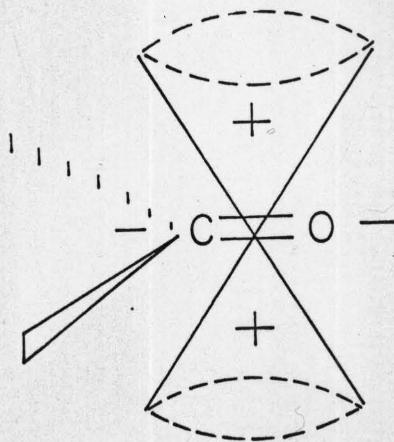


Figure I. Long range shielding of the carbonyl group.

Thus the deshielding of protons will be greatest if they lie in the plane of the carbonyl group.

For compounds containing aromatic systems the effect of the induced field which arises from diamagnetic circulations of the π electrons may be felt by protons as far removed as 5 or 6 Å from the center of the

system (27). The approximate nature of the induced field for benzene is illustrated in Figure II.

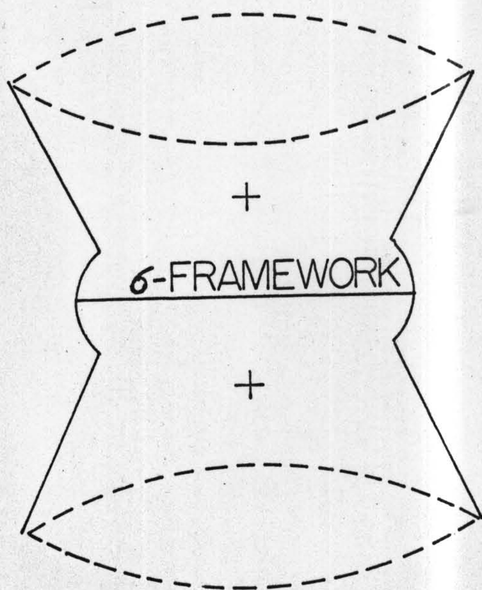
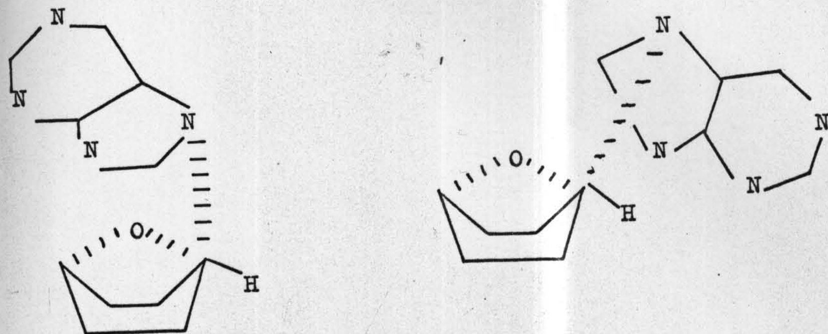
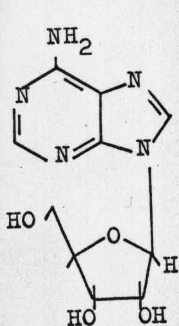


Figure II. Long range shielding of the benzene ring.

Although an anisotropic effect for the purine ring had not been demonstrated, one could propose that conformational changes about the N-9 · · · C-1' bond in purine nucleosides would produce different chemical shifts for H-1' of the sugar moiety. One could predict as an analogy to the anisotropy of benzene, that the H-1' proton of a purine nucleoside should exhibit an increased shielding by the aromatic system of the purine ring in certain conformations of the nucleoside. According to intramolecular measurements of purine nucleosides using Framework Molecular Models, the distance from H-1' to the center of the purine ring approaches 3.3 Å (Figure III) as the purine ring assumes the anti conformation. Since this distance is well within the 6 Å found as the maximum effective distance of shielding for other aromatic systems it could be assumed that H-1' would be somewhat shielded by the purine ring in an anti conformation. In the syn conformation the shielding effect of the purine ring on H-1' would be decreased by the increase in distance between H-1' and the purine ring.

In order to observe the effect of different conformations on the chemical shift of H-1' of purine nucleosides it was necessary to obtain model compounds which adequately fit the syn and anti definitions. All of the following compounds were then synthesized in this laboratory with the exception of 8,2'-anhydro-9-arabinofuranosyl adenine (XVI). The nmr data for (XVI) were





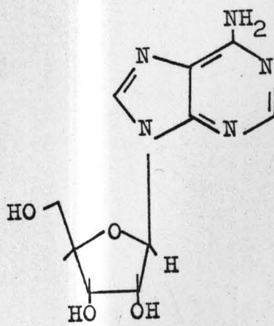
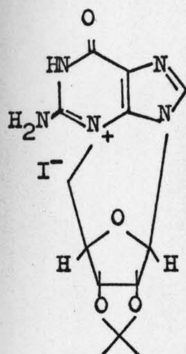
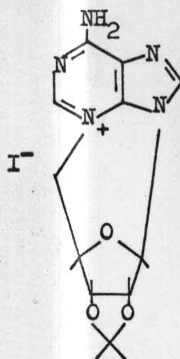


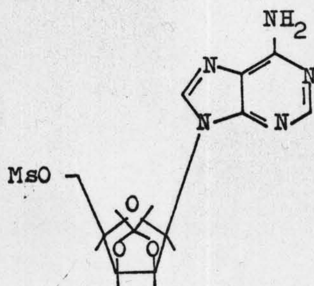
Figure III. Relation of the purine ring to H-1' in purine nucleosides.



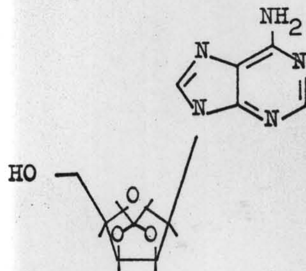
2',3'-isopropylidene-
3,5'-cycloguanosine
iodide (X)



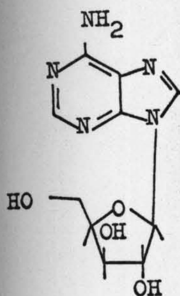
2',3'-isopropylidene-
3,5'-cycloadenosine
iodide (XI)



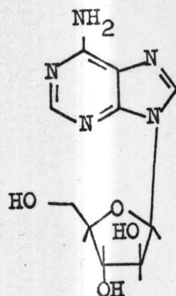
9-(2',3'-O-isopropylidene-5'-
o-methylsulfonyl-β-D-lyxo-
furanosyl)adenine (XII)



9-(2',3'-o-isopropylidene-
β-D-lyxofuranosyl)adenine
(XIII)



9-(β-D-xylofuranosyl)-
adenine (XIV)



9-(β-D-arabinofuranosyl)-
adenine (XV)

graciously supplied by Mario Ikehara (25). The models chosen for the _syn_ conformation were 2',3'-isopropylidene-3,5'-cycloguanosine iodide (X) and 2',3'-isopropylidene-3,5'-cycloadenosine iodide (XI). The presence of the N-3 . . . C-5' bond in these compounds provided the required _syn_ conformation.

The model chosen for the _anti_ conformational studies was 9-(2',3'-O-isopropylidene-β-D-lyxofuranosyl) adenine (XIII). Preparation of the cyclic derivative (_syn_ conformation) has not been reported whereas Reist, _et al_. (12) found that 9-(2',3'-O-isopropylidene-5'-O-methylsulfonyl-β-D-lyxofuranosyl) adenine (XII) to be extremely resistent toward nucleophilic displacement necessary to form the respective 3,5'-cyclonucleoside. Rotation about the N-9 . . . C-1' bond would seem to be prevented due to the steric hindrance of the isopropylidene methyl groups. Therefore, it could be assumed that the lyxofuranoside (XIII) was an adequate model for the _anti_ conformation.

Goodman (11), _et al_. reported they had prepared 9-(β-D-xylofuranosyl) adenine (XIV) by a fusion of xylosetetraacetate and acylamido purine but did not include experimental detail. Reist (12), _et al_. had reported conversion of (XIV) to 9-(β-D-lyxofuranosyl) adenine (XIII) which was thought to be the model of choice for an _anti_ conformation of purine nucleosides. However, this conversion used (XIV) prepared by a coupling of the blocked xylofuranosyl bromide and chloromercuri-6-benzamido

purine (13). An adaptation of these two preparations (Figure IV) yielded the required lyxofuranoside (XIII).

All models previously mentioned had the same configuration of the hydroxyl group at C-2' and C-3'. Investigation of the lyxofuranoside (XIII) (having a different hydroxyl configuration at both C-2' and C-3') as a model for the <u>anti</u> conformation showed changes in the chemical shift of H-1' as was expected. It was felt that configurational changes at C-2' would affect the chemical shift of H-1' however it was assumed that any change in configuration of the C-3' hydroxyl would have little effect on the chemical shift of H-1'. In order to determine the effect of configurational change at C-2' on the chemical shift of H-1', 9-(β-D-arabinofuranosyl) adenine (XV) was studied. The magnitude of the contribution due to the configurational differences at C-2' was found to be 0.34 ppm when the arabino-nucleoside was compared to the ribose-nucleoside adenosine. This value was subtracted from the observed chemical shift of H-1' of XIII to give the corrected value (Table II).

It can be seen from Table I that the anomeric protons of the cyclonucleosides (<u>syn</u> conformation) have marked downfield shifts (δ = 6.6 ppm or greater) when compared to the model for the <u>anti</u> conformation (XIII) with a corrected H-1' of δ = 5.78 ppm. For the adenosine series the "pure" <u>syn</u> conformation has H-1' centered at 6.73 ppm, while the

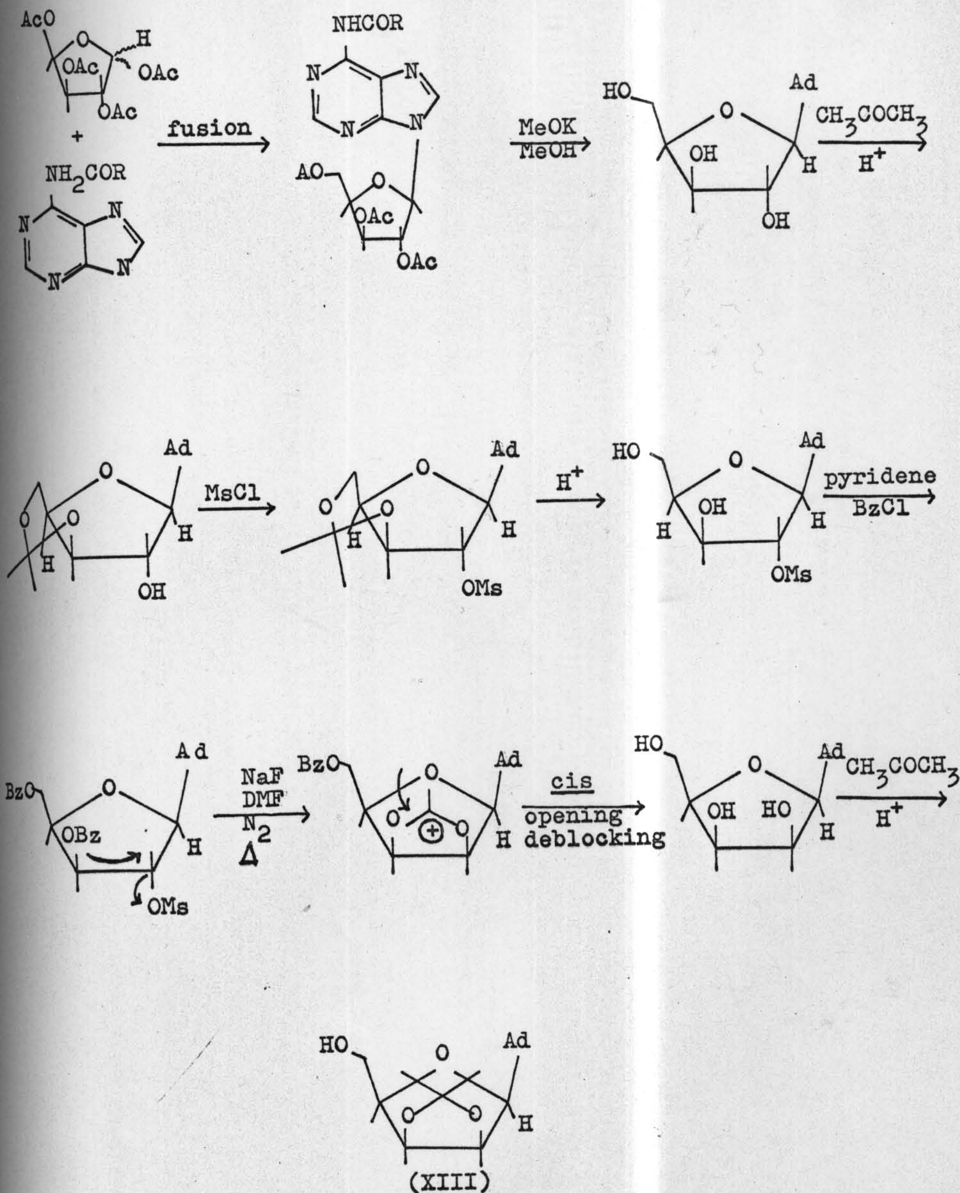


Figure IV

TABLE I

Chemical Shifts (in ppm) at 39° in $DMSOd_6$ (TMS)

	H_8	H_2	H_1'	H_2'	H_3'	H_4'	H_5' (2)	Isopropylidene methyls
Adenosine	8.38	8.18	5.93	4.65	4.22	4.01	3.67	
2',3'-O-Isopropylidene adenosine	8.36	8.19	6.16	5.38	4.99	4.25	3.6 3.53	1.56, 1.33
2',3'-Isopropylidene-3,5'-cycloadenosine iodide (XI)	8.71	8.56	6.73	5.01	4.55	4.43	5.01	1.47, 1.22
Quanosine	7.95		5.74	4.45	4.16	3.92	3.60	
2',3'-O-Isopropylidene quanosine	7.92		5.95	5.25	4.99	4.14	3.60 3.51	1.53, 1.33
2',3'-O-Isopropylidene-3,5'-cycloguanosine (X)	8.15		6.59	5.11	4.64		4.84	1.48, 1.35
Sodium guanosine-5'-monophosphate	8.19		5.94				4.10 4.03	
9-(β-D-xylofuranosyl) adenine (XIV)	8.28	8.06	6.00					

TABLE I - Cont.

	H_8	H_2	H_1'	H_2'	H_3'	H_4'	H_5' (2)	Isopropylidene methyls
9-(3',5'-O-Isopropylidene-β-D-xylofuranosyl) adenine	8.37	8.23	6.05					1.46, 1.28
9-(2',3'-O-Isopropylidene-β-D-lyxofuranosyl) adenine	8.24	8.51	6.12					1.44, 1.23
2',3'-O-Isopropylidene adenosine-5'-acetate (in $CDCl_3$)	7.95	8.33	6.16	5.55	5.09			1.64, 1.42 [Acetate Me-- 2.01]

TABLE II

Comparison of the Anomeric Proton Chemical Shifts in the Adenosine Series (Relative to the Cycloadenosine Iodide)

	Conformation	H-1	Δδ
2',3-Isopropylidene-3,5'-cycloadenosine iodide (XI)	syn	6.73	
Adenosine	natural[c]	5.93	-0.80
2',3'-Isopropylidene adenosine	natural[c]	6.16	-0.57
2',3'-Isopropylidene adenosine, 5-acetate (XVI)	natural[c]	6.16	-0.57
9-(2',3'-O-Isopropylidene-β-D-lyxofuranosyl) adenine (XIII)	anti	6.12[a] 5.78[b]	-0.61
9-(β-D-Arabinofuranosyl) adenine	natural[c]	6.27	-0.46

[a]observed value

[b]value corrected for change in C-2' hydroxyl configuration

[c]natural conformation is that achieved by the free nucleoside in solution

value for the anti conformation is centered at 5.78 ppm. Thus the difference in chemical shifts of H-1' between the syn and anti conformation for the adenosine series is equal to 0.95 ppm. For the guanosine series this difference in chemical shifts of H-1' decreases somewhat to 0.81 ppm, but is still significant and follows the predicted pattern of decreased shielding of H-1' with a syn conformation.

When the chemical shifts of H-1' of the isopropylidene derivatives of the natural nucleosides, guanosine and adenosine are compared to the data for the syn and anti models (Table IV) it becomes evident that the natural nucleosides occur in a predominately anti conformation since the values of the H-1' protons for the natural nucleosides are closer to the values found for the anti models. However, it is not possible as yet to predict quantitatively the specific conformation of these nucleosides.

In a private communication, M. Ikehara (25) reported that 8,2'-anhydro-9-arabinofuranosyl adenine (XVI) had a chemical shift for H-1' of 6.50 ppm. When this value is corrected for the arabino-configuration it becomes 6.16 ppm which is in close agreement to the values obtained in this study for the anti conformation. Particularly when one considers the model of the anhydro derivative it becomes apparent that the conformation is

TABLE III

Comparison of the Anomeric Proton Chemical Shifts in the Guanosine Series (Relative to the Cycloguanosine)

	Conformation	H-1	$\Delta\delta$
2',3'-Isopropylidene-3,5'-cycloguanosine (X)	*syn*	6.59	
Guanosine	natural[a]	5.74	-0.85
2',3'-Isopropylidene guanosine	natural[a]	5.95	-0.64
Sodium guanosine-5'-monophosphate	natural[a]	5.94	-0.65

[a]natural conformation is that achieved by the free nucleoside in solution

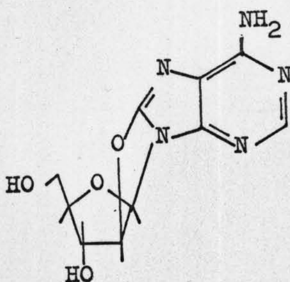
TABLE IV

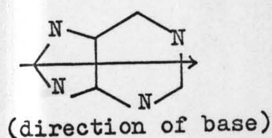
Comparison of Adenosine and Guanosine Series

Adenosine Series	H-1'	Conformation	H-1'	Guanosine Series
9-(2',3'-Isopropylidene-β-D-lyxofuranosyl) adenine	5.78 (corrected for C-2' hydroxyl configuration)	anti		
2',3'-O-Isopropylidene adenosine	6.16	natural[a]	5.95	2',3'-O-Isopropylidene guanosine
2',3'-Isopropylidene-3,5'-cycloadenosine iodide	6.73	syn	6.59	2',3'-O-Isopropylidene-3,5'-cycloguanosine

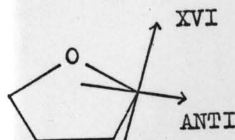
[a]natural conformation is that achieved by the free nucleoside in solution

not pure anti and therefore H-1' should be somewhat less shielded than in the model for the pure anti conformation (Figure 10).





(direction of base)



Schematic representation of the position of the base in XVI.

8,2'-Anhydro-9-arabinofuranosyl adenine (XVI)

The effect of temperature on the spectra of the nucleosides was investigated since it was felt that if an energy barrier between the _syn_ and _anti_ conformations of purine nucleosides existed it may be possible to observe this with change in temperature of the sample.

Raising the temperature to as high as 83°C had no effect on the spectra of the free nucleosides, either in position of absorption or in coupling constant (Table V). Since one could not lower the temperature of the nucleoside-DMSO-d_6 solutions below 16°C due to the limitation of the freezing point of the solvent, a solution of the isopropylidene derivatives of adenosine and guanosine were prepared in dimethylformamide. It was possible to decrease the temperature of this solution to -40°C before freezing occurred. At this temperature H-1' of isopropylidene adenosine and isopropylidene guanosine was shifted downfield by approximately 0.1 ppm as compared to their position in DMF solution at probe temperature of 39°C. Attempts to further lower the temperature again required changing the solvent system. However, the isopropylidene derivatives were not soluble in any of the potential nmr solvents with freezing points less than -40°C.

Therefore, 2',3'-O-isopropylidene adenosine-5'-acetate (XVII) was prepared since this was thought to offer a compound which would have a wider range of organic solvent

TABLE V

Temperature Studies on Isopropylidene Adenosine-5-Acetate

Solvent	Temp.	Me_1	Me_2	AcO-	H-1'	H_8	H_2
CDCl+20% $(CD_3)_2$	39°	1.42	1.63	2.00	6.22	8.09	8.34
$CDCl_3$	39°	1.42	1.63	2.01	6.16	7.95	8.33
$CDCl_3$+5% MeOD	39°	1.42	1.63	2.01	6.15	7.98	8.30
$CDCl_3$	-20°	1.47	1.69	2.08	6.28	8.15	8.45
$CDCl_3$	-40°	1.47	1.69	2.07	6.27	8.11	8.39
$CDCl_3$	-60°	1.47	1.67	2.06	6.28	8.11	8.37
$CDCl_3$+5% MeOD	-74°	1.51	1.71	2.08	6.36	8.23	8.44

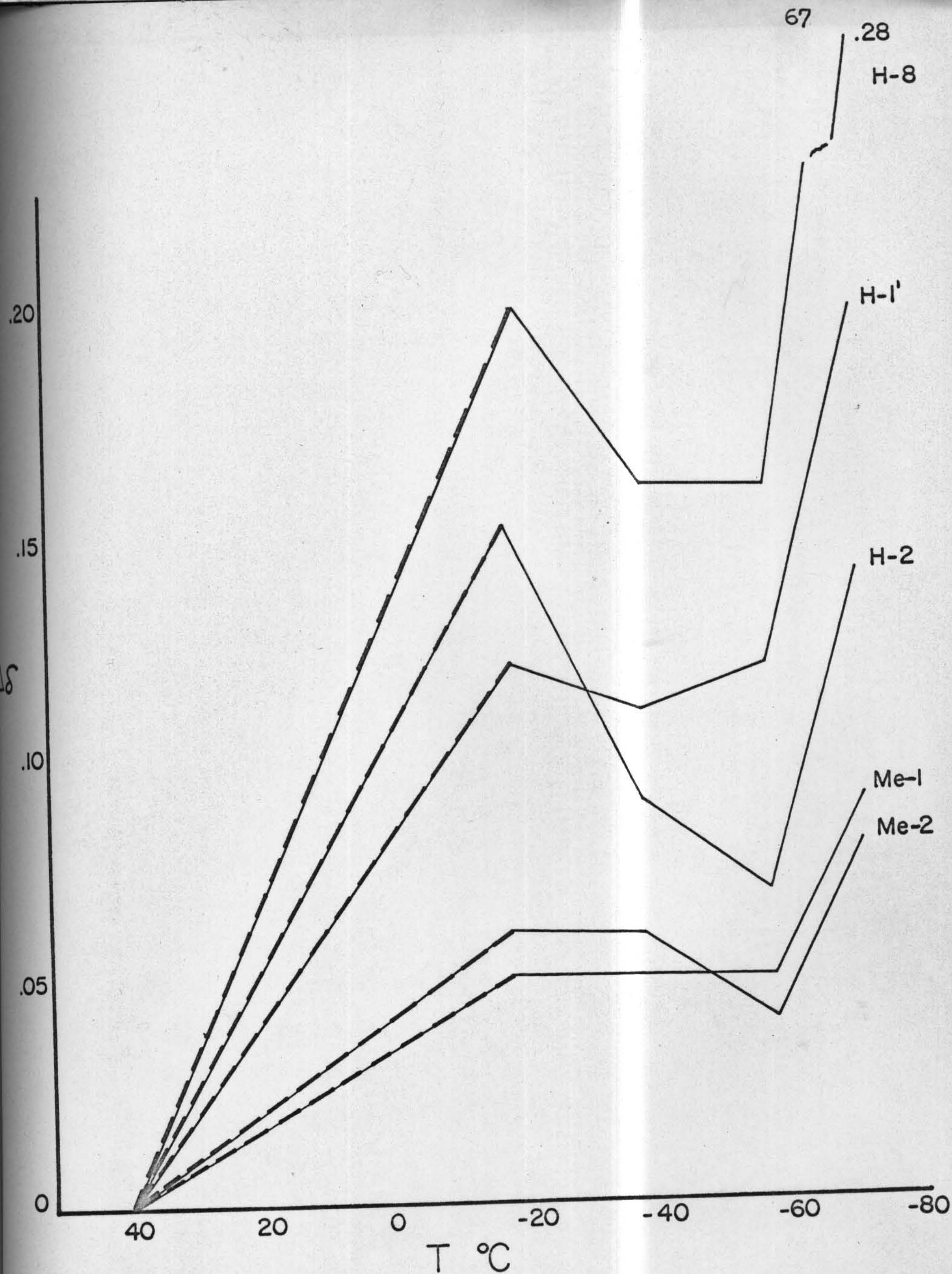


Figure V. Relation of change in chemical shift to change in temperature for 2',3'-isopropylidene-adenosine-5'-acetate.

solubilities and thus could yield spectra at even lower probe temperatures. This could only be partially accomplished as it was possible to take a solution of isopropylidene-adenosine-5'-acetate (XVII) in 5% methanol-d_4/chloroform-d to -74°C before freezing occurred. Attempts to dissolve the nucleoside in pure methanol-d_4 and a series of Freons® was unsuccessful at these temperatures. The spectrum at -74°C did indicate an even greater shifting of H-1' at -74°C (0.21 ppm) than at -40°C. Together with the shift of H-1', it can be seen from Table V and Figure V that the entire spectrum begins to shift downfield as the temperature becomes lower with the protons H-2 and H-8 being particularly affected. Also with this shifting it was noted that the entire spectrum began to lose resolution with the exception of the tetramethylsilane (TMS) signal. This can be interpreted to indicate the beginning of "freezing out" of a conformation or might also indicate self-association of the nucleoside molecules. However, the solution was prepared at a concentration such that self-association should have occurred even at normal probe temperatures.

These experiments indicate that there is some conformational change but are too inconclusive to predict any conformer population. It is apparent that more work is required to establish the equilibrium composition of the possible conformations in solution.

EXPERIMENTAL

Boiling point determinations and infrared absorption spectra were obtained as in Part I. Nmr spectra were determined on a Varian A-60A spectrometer and Varian HA 100 mgc NMR spectrometer. Spin-spin decoupling was achieved using the spin-decoupler (Model V-6058A) accessory on the A-60A model. Variable temperature measurements were carried out with a variable temperature unit (V-6040 NMR). For low temperature studies the probe was cooled by flow of gaseous nitrogen which had been cooled by passing through a heat exchanger submerged in liquid nitrogen. Various deuterated solvents were used and are specified where appropriate. Both tetramethylsilane and tetramethylsilanepropionic acid were used as internal standards. Ultraviolet analysis of the chromatographic fractions was obtained on a Cary 14 Recording Spectrometer. Paper chromatography was employed using Whatman No. 1 paper by a descending method. The spots were located by visual examination with an ultraviolet lamp. The solvent system used was n-butanol saturated with water. Thin layer chromatograms were run on silica gel F_{254} (E. Merck A.-G., Darmstadt). The spots were detected by visual examination under ultraviolet light (254 mμ). Solvent system for nucleoside chromatograms was benzene-methanol (70:20).

Preparation of Diacetone Xylose. To one liter of reagent anhydrous acetone were added, in the following order, concentrated sulfuric acid (5 ml), powdered anhydrous copper sulfate (100 g) and xylose (50 g). This solution was mechanically stirred for 16 hr. The copper sulfate and unchanged xylose were filtered off, 12.5 ml of water added and the solution neutralized with powdered calcium hydroxide. After filtering the mixture the solution was concentrated under reduced pressure to a thick syrup (55 g) which was considered pure enough to be used in the following reaction.

Preparation of Monoacetone Xylose from Diacetone Xylose. The crude syrup of diacetone xylose (55 g) was dissolved in 0.16% hydrochloric acid solution (900 ml) and stirred at 24° for 2 1/3 hr. This solution was then immediately neutralized with lead carbonate and concentrated under reduced pressure. The insoluble inorganic salts were removed by filtration and a thick syrup was obtained. This syrup was dissolved in an equal volume of acetone and a crystalline material precipitated. The crystalline material was removed by filtration and characterized by nmr as being xylose. The mother liquor was evaporated to yield 47 g of monoacetone xylose as a viscous syrup, nmr singlet at 1.3 δ (isopropylidene methyl protons), singlet at 1.47 δ (isopropylidene methyl protons), doublet at 5.95 δ (anomeric proton).

Preparation of Diacetyl Monoacetone Xylose. Monoacetone xylose (47 g) was dissolved in anhydrous pyridine (300 ml) and to this solution was added 51 ml of acetic anhydride (2.2 moles). After 12 hr at room temperature water (10 ml) was added to the solution and after an additional 30 min the crude mixture was concentrated under reduced pressure (8 mm Hg) at 40° to a volume of about 75 ml. This mixture was dissolved in water (100 ml) and extracted twice with chloroform (100 ml). The chloroform extracts were combined, dried over anhydrous magnesium sulfate and evaporated under reduced pressure (8 mm Hg) at 40° to yield 55 g of diacetyl monoacetone xylose as a viscous syrup. $\lambda_{max}^{CHCl_3}$ 5.7 μ (carbonyl), 7.25 μ (methyl), 8.0 μ-8.2 μ (broad acetate C-O-C), 9.3 μ-9.7 μ (broad ketal C-O-C); nmr singlet at 1.32 δ (isopropylidene methyl protons), 1.51 δ (isopropylidene methyl protons), 2.06 δ (acetate methyl protons), 2.10 δ (acetate methyl protons), 5.95 δ (anomeric proton).

Preparation of 1,2,3,5-tetra-O-acetyl-D-xylose. To a solution of 3,5-di-O-acetyl-1,2-O-isopropylidene-D-xylo-furanose (40.3 g) in glacial acetic acid (300 ml) and acetic anhydride (56 ml) was added concentrated sulfuric acid (25 ml) dropwise with stirring and sufficient cooling to maintain a temperature of about 5°. After the addition was complete the reaction was left at room

temperature for 18 hr and then poured slowly into ice water (400 ml) while stirring. The aqueous layer was extracted four times with chloroform (200 ml). The chloroform extracts were combined and stirred vigorously with 600 ml of 5% aqueous sodium bicarbonate solution. The chloroform layer was separated from the aqueous layer, washed once with 200 ml of water and dried over anhydrous magnesium sulfate. The solution was filtered and evaporated to dryness under reduced pressure to yield 1,2,3,5-tetra-O-acetyl-D-xylose (50 g). Nmr, 2.1 δ (acetate methyl protons), 6.12 δ (anomeric singlet), 6.41 δ (anomeric doublet).

Preparation of n-octylamidoadenine. Adenine (10 g) was dissolved in anhydrous pyridine (50 ml) and cooled to 0°. This cooled, stirring solution was treated dropwise with n-octyl chloride (12.25 g). This solution was refluxed for 2 hr, then poured into ice water (100 ml). The resulting suspension was made just acid with dilute hydrochloric acid and the precipitate filtered and recrystallized from ethanol to give n-octylamidoadenine (11.2 g) as crystals, mp 187.5-188.5° (lit. (16) 184-186°). Nmr, triplet 0.78 δ (terminal methyl), broad singlet 1.23 δ (methylene protons), 8.46 and 8.67 δ (ring protons), 11.08 δ (N-9 proton).

Coupling reaction of xylosetetraacetate and 6-n-octylamidopurine. A mixture of xylosetetraacetate (3.49 g) and 6-*n*-octylamidopurine (1.1 g) was heated on an oil bath to 160° under a water aspirator vacuum for 5 min with stirring to mix and dry the reactants. After bubbling had ceased the mixture was cooled to room temperature, the vacuum released and *p*-toluenesulfonic acid monohydrate (100 mg) was added. The flask was re-evacuated using a water aspirator and the mixture heated to 185° to achieve a good melt and maintained at 170° for 45 min. During this time a slow bubbling of the melt occurred. The mixture was then cooled to room temperature, the vacuum released and the residue dissolved in chloroform (60 ml). The precipitate was removed by filtration and the filtrate washed first with 5% aqueous sodium bicarbonate (30 ml), then with water (30 ml), and dried over anhydrous magnesium sulfate. The solution was filtered and evaporated to dryness under reduced pressure to yield a brown tar (508 mg). This residue was dissolved in anhydrous methanol (60 ml) and a solution of 1 N potassium methoxide in methanol was added until a pH of 9 was obtained as shown on moist pHydrion paper. After refluxing for 30 min the solution was neutralized with acetic acid and evaporated to dryness. The residue was dissolved in a minimum amount of water and chromatographed on a Dowex 1-X2 (50-100 mesh) column (20 x 60 cm) which had been regenerated by 1 N sodium

hydroxide and rinsed with distilled water until the eluate was neutral. Elution was carried out using 30% methanol in water until 1000 ml of eluate were collected, then the eluting solvent was changed to 60% methanol in water and 6 ml fractions were collected. Evaporation of fractions 275-400 gave 9-(α-D-xylofuranosyl)adenine (22 mg) as a clear foam. Nmr doublet (J = 3.2 cps) 6.46 δ (anomeric proton), singlets 8.16 δ and 8.24 δ (purine protons). Evaporation of fractions 401-625 yielded 9-(β-D-xylofuranosyl)adenine as a clear foam, (103 mg). Nmr, doublet (J = 3.0 cps) 6.0 δ (anomeric proton), 8.06 δ and 8.28 δ (purine protons). The yield of 9-(β-D-xylofuranosyl)adenine, based on the unrecovered acylamidopurine, was 15%.

Preparation of 9-(3',5'-O-isopropylidene-β-D-xylofuranosyl)adenine. A solution of 9-(β-D-xylofuranosyl)-adenine (0.9661 g) in anhydrous reagent acetone (100 ml) and ethane sulfonic acid (1.5 ml) was stirred at room temperature for 16 hr and then poured into a solution of sodium bicarbonate (3.2 g) in water (60 ml). This solution was extracted four times with chloroform (60 ml) and the combined extracts washed once with water (60 ml) and dried over anhydrous magnesium sulfate. The chloroform solution was evaporated to dryness under reduced pressure to yield crude 9-(3',5'-O-isopropylidene-β-D-xylofuranosyl)adenine (1.087 g). Recrystallization

from methanol gave crystals (66% yield), mp 205-207° (lit. (11), mp 207-208.5°). Nmr singlet 6.05 δ (anomeric proton), 8.23 δ and 8.37 δ (purine protons), 1.28 δ and 1.46 δ (methyl protons).

Preparation of 9-(2'-O-methylsulfonyl-β-D-xylofuranosyl)adenine. A solution of 9-(3',5'-O-isopropylidene-β-D-xylofuranosyl)adenine (which had been dried at 50° under vacuum for 9 hr) (732 mg) in anhydrous pyridene (50 ml) was cooled to 5°. Methanesulfonyl chloride (0.8 ml) was added dropwise with stirring. The solution was allowed to stand for 24 hr at room temperature and then poured slowly into ice water. The cold mixture was extracted with four portions of chloroform (30 ml). The combined extracts were washed with 5% aqueous sodium bicarbonate (25 ml), then with water (25 ml) and dried over magnesium sulfate. The chloroform solution was evaporated to dryness under reduced pressure to yield a yellow foam. The residue was dissolved in 90% aqueous acetic acid (25 ml) and the solution maintained at 50° for 8 hr and then evaporated to dryness to give the crude mesylate, 511 mg, which was used for the following reaction.

Preparation of 9-(β-D-xylofuranosyl)adenine. A solution of the crude mesylate, (511 mg), in anhydrous pyridine (10 ml) was cooled to 0° in an ice bath. Benzoyl chloride (1.05 ml) was added dropwise with

stirring and continued cooling. After the addition was complete, the reaction was maintained at 5° for 18 hr and then poured into ice water (25 ml). The mixture was extracted three times with chloroform (30 ml). The combined extracts were washed with 5% aqueous sodium bicarbonate, dried over magnesium sulfate and evaporated to dryness under reduced pressure. The residue was taken up in toluene (10 ml) and the solvent removed under reduced pressure. This procedure was repeated again to remove the last traces of pyridine. The brown oily residue was chromatographed on silica gel packed as a slurry in chloroform. Elution was carried out with chloroform and 5 ml fractions were collected. The benzoylated derivative was eluted immediately and evaporation of the eluate yielded the crude product (820 mg) which was used in the next step.

A mixture of the crude benzoylated product (820 mg) and sodium fluoride (1 g) was dried at 40° at 10 mm Hg for 5 hr and then dissolved in 40 ml of dried dimethylformamide. This solution was heated under a nitrogen atmosphere at 140° for 24 hr on an oil bath. To the cooled solution was added 3 ml of water and the mixture stirred at 60° for 1 hr. The dark suspension was poured into water (80 ml) and extracted twice with chloroform (50 ml). The combined extracts were washed with 5% aqueous sodium bicarbonate, dried over anhydrous magnesium sulfate and evaporated to dryness under reduced

pressure to yield a dark oil. The oily residue was dissolved in methanol (20 ml), which had been saturated previously at 0° with ammonia, and the solution maintained at room temperature for 18 hr. The solution was then evaporated to dryness under reduced pressure and the residue partitioned between chloroform (25 ml) and water (25 ml). The aqueous phase was evaporated to dryness under reduced pressure to give 310 mg of a mixture of nucleosides. A solution of this mixture (310 mg) in a minimum volume of water was chromatographed on a Dowex 1-X2 (50-100 mesh) column (1.5 x 40 cm) which had been regenerated by 1 N sodium hydroxide and rinsed with distilled water until the eluate was neutral. Elution was carried out using 20% methanol in water and 5 ml fractions were collected. Fractions 100-140 were found to contain the desired product as shown by nmr and uv analysis at 260 mμ. Evaporation of the solvent yielded crude 9-(β-D-lyxofuranosyl)adenine (75 mg) as a clear oil.

<u>Preparation of 9-(2',3'-O-isopropylidene-β-D-lyxofuranosyl)adenine</u>. A solution of the crude 9-(β-D-lyxofuranosyl)adenine (75 mg) in ethane sulfonic acid (0.15 ml) and 10 ml of anhydrous acetone was stirred at room temperature for 22 hr, then poured into a cold (0°) solution of sodium bicarbonate (70 mg) in water (15 ml).

The solution was stirred to room temperature and then evaporated to dryness under reduced pressure. The white residue was continuously extracted with chloroform for 24 hr. The chloroform was evaporated to dryness under reduced pressure to give 107 mg of a mixture of the 2',3'- and 3',5'-isopropylidene derivatives. This mixture was chromatographed on a Dowex 1-X2 (50-100 mesh) column (1.5 x 40 cm) and 5 ml fractions were collected. Elution was carried out with 20% methanol in water and 35 mg of the crude 2',3'-isopropylidene derivative were obtained. Nmr singlets at 1.23 δ and 1.44 δ (methyl protons), doublet at 6.12 δ (J = 2.4 cps) (anomeric proton), singlets at 8.15 δ and 8.24 δ (purine protons).

<u>Preparation of 5'-deuterohydroxyl-6,6-dideutero-amino-2',3'-isopropylidene adenosine</u>. To a solution of 2',3'-isopropylidene adenosine (500 mg) in dimethyl-formamide (2 ml) was added deuterium oxide (0.5 ml). This solution was distilled until the distilling temperature reached 110°. Deuterium oxide (0.5 ml) was then added to insure complete exchange of replaceable hydrogen with deuterium and distillation continued until the distilling temperature was again 110°. This solution was used directly for low temperature studies in dimethylformamide.

Preparation of 6,5'-dideuterohydroxyl-2,2-dideuteroamino-2',3'-isopropylidene guanosine. To a solution of 2',3'-isopropylidene guanosine (500 mg) in dimethylformamide (2 ml) was added deuterium oxide (0.5 ml) and the same procedure followed as for the above.

Preparation of 2',3'-isopropylidene-3,5'-cycloguanosine. 2',3'-Isopropylidene-3,5'-cycloguanosine iodide (0.2 g) was added with stirring to water (2 ml). The pH of the solution was adjusted to pH 7 by the addition of 2 drops of concentrated ammonium hydroxide. The mixture was cooled at 10° for 12 hr and the precipitate filtered to yield 2',3'-isopropylidene-3,5'-cycloguanosine (25 mg) as white needles, mp 145° d (lit. (14), mp 150° d); nmr singlets at 1.28 δ and 1.49 δ (methyl protons), doublet at 4.64 δ (H-3'), doublet at 5.11 δ (H-2'), singlet at 6.59 δ (anomeric proton), singlet at 8.15 δ (purine proton).

Preparation of 2',3'-isopropylidene adenosine-5'-acetate. 2',3'-Isopropylidene (0.98 g) was dried in the Abderhalden for 12 hr, then added to anhydrous pyridene (25 ml) and acetic anhydride (3 ml) was added. The solution was stirred for 12 hr at room temperature. Ethanol (10 ml) was added and the solution was allowed to stand for 1 1/2 hour and was then evaporated to dryness under reduced pressure. The residue was dissolved in chloroform (30 ml) and washed twice with

5% aqueous sodium bicarbonate (10 ml) and then twice with water (10 ml) and finally dried over anhydrous magnesium sulfate. The drying agent was filtered off and solution evaporated to yield a colorless resin which appeared to be two products in the ratio 3:1 (nmr). The resin was suspended in ether and chloroform added just to the point of solvation. The suspension was cooled at 10° for 36 hr and the precipitate filtered to yield 2',3'-isopropylidene-5'-acetate (415 mg) as white crystals, mp 164°, (lit. (15), mp 167°); nmr singlets at 1.42 δ and 1.64 δ (ketal methyl protons), singlet at 2.01 δ (acetate methyl protons), quartet at 5.09 δ (H-3'), doublet (J = 2.0 cps) 6.16 δ (anomeric proton), singlets at 7.95 δ and 8.23 δ (purine protons).

REFERENCES

1. Donohue, J. and Trueblood, K. E., J. Molec. Biol., 2, 363 (1960).

2. Spencer, M., Acta Cryst., 12, 59, 66 (1959).

3. Ularstiao, M. W., Bush, C. A. and Tirioco, I., Jr., Biochem. Biophys. Res. Commun., 18, 633 (1965).

4. Ulbricht, T. L. V., Ann. Rept. Chem. Soc., 62, 402 (1965).

5. Ulbricht, T. L. V., Emerson, T. R. and Swan, R. J., Biochem. Biophys. Res. Commun., 19, 643 (1965).

6. Emerson, T. R., Swan, R. J. and Ulbricht, T. L. V., Biochem. Biophys. Res. Commun., 22, 505 (1966).

7. Ikehara, M., Kaneko, M., Muneyama, K. and Tanaka, H., Tetrahedron Letters, 40, p. 3977 (1967).

8. Emerson, T. H., Swan, R. J. and Ulbricht, T. L. V., Biochemistry, 6, 843 (1967).

9. Gatler, L. and Davis, J. C., Jr., J. Amer. Chem. Soc., 84, 4464 (1962).

10. Mathieson, D. W., "Nuclear Magnetic Resonance for Organic Chemists," Academic Press, 1968, pp. 1-41.

11. Lee, W. W., Martinez, A. P., Tong, G. L. and Goodman, L., Chem. Ind., 2007 (1967).

12. Reist, E. J., Calkins, D. F. and Goodman, L., J. Org. Chem., 32, 169 (1967).

13. Baker, B. R. and Hewson, K., J. Org. Chem., 22, 966 (1957).

14. Holmes, R. E. and Robins, R. K., J. Org. Chem., 28, 3483 (1963).

15. Brown, D. M., Haynes, L. J. and Todd, A. R., J. Chem. Soc., 3299 (1950).

16. Ueda, T., Kato, S. and Toyoshima, S., Japan, 9773 (1957), Chem. Abstr., 52, 15599b (1958).

17. Donohue, J., Proc. Natl. Acad. Sci., 42, 60 (1956).

18. Pauling, L. and Corey, R. B., Arch. Biochem. Biophys., 65, 164 (1956).

19. Watson, J. D. and Crick, F. H., Nature, 171, 737 (1958).

20. Spencer, M., Acta Cryst., 12, 66 (1959).

21. Samejima, T. and Uang, J. T., Biochemistry, 3, 613 (1964).

22. Haschemeyer, A. E. V. and Rich, A., J. Molec. Biol., 27, 369 (1967).

23. Miles, D. W., Hahn, S. J., Robins, R. K., Robins, M. J. and Eyring, H., J. Phys. Chem., 73, 1483 (1968).

24. Klee, W. A. and Mudd, S. M., Biochemistry, 6, 988 (1967).

25. Ikehara, M., private communication to Dr. P. A. Hart.

26. Pople, J. A., Proc. Roy. Soc., A239, 541 (1957).

27. Jackman, L. M., "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, Inc., New York, 1959, p. 112.

APPENDIX

THE ALUMINA-CATALYZED ADDITION OF DIAZOMETHANE TO STEROIDAL KETONES

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