# Synthesis of 3′-Deoxynucleosides III

## Synthesis of 9-(3-Deoxyaldofuranosyl) Adenines Derived from 3-Deoxy-D-mannose and 3-Deoxy-D-galactose

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**The diisopropylidene derivative of 3-deoxy-D-mannose was selectively hydrolyzed to 1,2-*O*-isopropylidene-3-deoxy-D-mannofuranose. In one series of reactions, this compound was converted *via* benzoylation and acetolysis to a suitably blocked sugar, which on condensation with chloromercuri-6-benzamidopurine (using titanium tetrachloride), followed by deacylation, yielded 9-(3-deoxy-α-D-mannofuranosyl) adenine. In a second series of reactions, 1,2-*O*-isopropylidene-3-deoxy-D-mannofuranose was converted through periodate oxidation and borohydride reduction to the corresponding derivatives of 3-deoxy-D-arabinose, convertible by the above standard reactions to 9-(3-deoxy-α-D-arabinofuranosyl)adenine. Inversion of 1,2-*O*-isopropylidene-5,6-di-*O*-methanesulfonyl-3-deoxy-D-galactofuranose with sodium benzoate led to 5,6-di-*O*-benzoyl-1,2-*O*-isopropylidene-3-deoxy-L-mannofuranose from which 9-(3-deoxy-α-L-mannofuranosyl) adenine was similarly prepared.**

IN RECENT YEARS, there has been interest in the structure and stereochemistry of the sugar moiety of adenine nucleosides required for inhibitory activity. Among these are adenine compounds bearing various fraudulent sugars (1–3), including 3-deoxyaldofuranoses. In the present report are described the syntheses of 9-(3-deoxy-α-D-mannofuranosyl)adenine, 9-(3-deoxy-α-D-arabinofuranosyl) adenine, and 9-(3-deoxy-α-L-mannofuranosyl) adenine.

Acetonation of 3-deoxy-D-mannose (I, Scheme I) (4) gave the corresponding 1,2:5,6-di-*O*-isopropylidene derivative (II) as a distillable liquid in a yield of 79%. Selective hydrolysis using 0.01 *N* HCl converted II to the syrupy 1,2-*O*-isopropylidene compound (III) in a yield of 67% (based on unrecovered II). The unhydrolyzed diacetone derivative, as well as some deoxymannose produced by complete hydrolysis, could be recovered and reused. Benzoylation of the crude monoacetone compound gave the 5,6-dibenzoate (IV) as a noncrystallizing syrup, whose elemental analysis suggested it to be pure within acceptable analytical limits, although the infrared spectrum indicated that a small amount of benzoic anhydride was present. Acetolysis of the dibenzoate to the anomeric syrupy 1,2-diacetate (V) was followed by coupling with chloromercuri-6-benzamidopurine (VI) (1) in the presence of titanium tetrachloride to give the crude, blocked nucleoside (VII) as a glass. Deacylation was achieved with methanolic sodium methoxide and the crude crystalline nucleoside (VIII) rapidly separated from the cold (0°) solution in a yield of 53%. The anomeric carbon of this nucleoside was assigned the α configuration by application of the *trans* rule[1] (5). In addition, the positive rotation (+52°) is consistent with a number of furanosyl adenines of the α-D-series and is substantially more positive than would be expected for the β-D-anomer.[2]

The starting material for the related pentose nucleoside (XIV, Scheme II) was 1,2-*O*-isopropylidene-3-deoxy-D-mannofuranose (III). Oxidation of III with sodium periodate gave a colorless, mobile liquid which reduced Benedict's solution and showed infrared bands at 1,735 cm.$^{-1}$ (C=O, strong) and 2,750 cm.$^{-1}$ (C—H, weak), characteristic of an aldehyde group. Although a low carbon analysis was obtained,[3] the *aldehydo*-pentose structure (IX) is suggested for this compound. Reduction of this aldehyde with sodium borohydride gave 1,2-*O*-isopropylidene-3-deoxy-D-arabinofuranose (X) as a colorless liquid in an overall yield of 60% from the crude monoacetone hexose (III). Benzoylation of the monoacetone pentose gave an 83% yield of the 5-*O*-benzoyl derivative (XI) which was obtained crystalline. This compound

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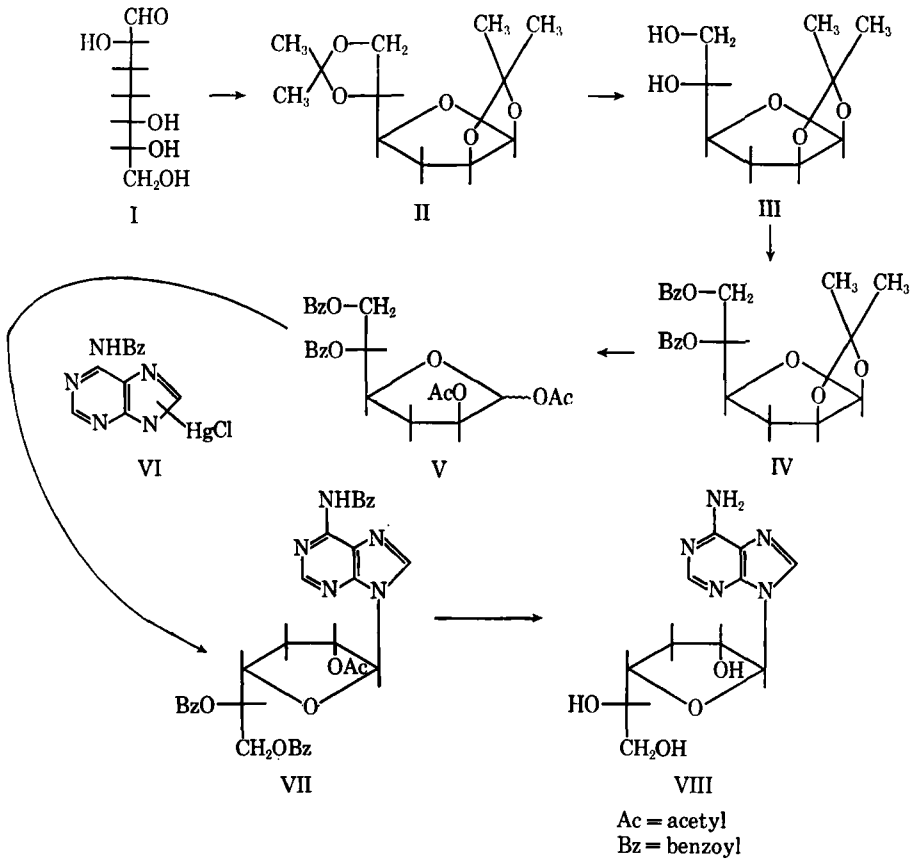
[1] The terms *cis* and *trans* as used in this paper refer to the configuration of substituents on the C1 and C2 positions of the sugar moiety. Similar condensations in this laboratory, with a variety of sugars, have shown thus far that when the *cis* nucleoside is produced, its yield is, at best, relatively small.

[2] The reader is referred to the extensive tables included in *Reference 6*.

[3] This appears to be related to the presence of impurities resulting from the oxidation of a crude sample of III (a weak hydroxyl bond was also observed in the infrared). Further purification of this compound, beyond simple distillation, was not attempted.

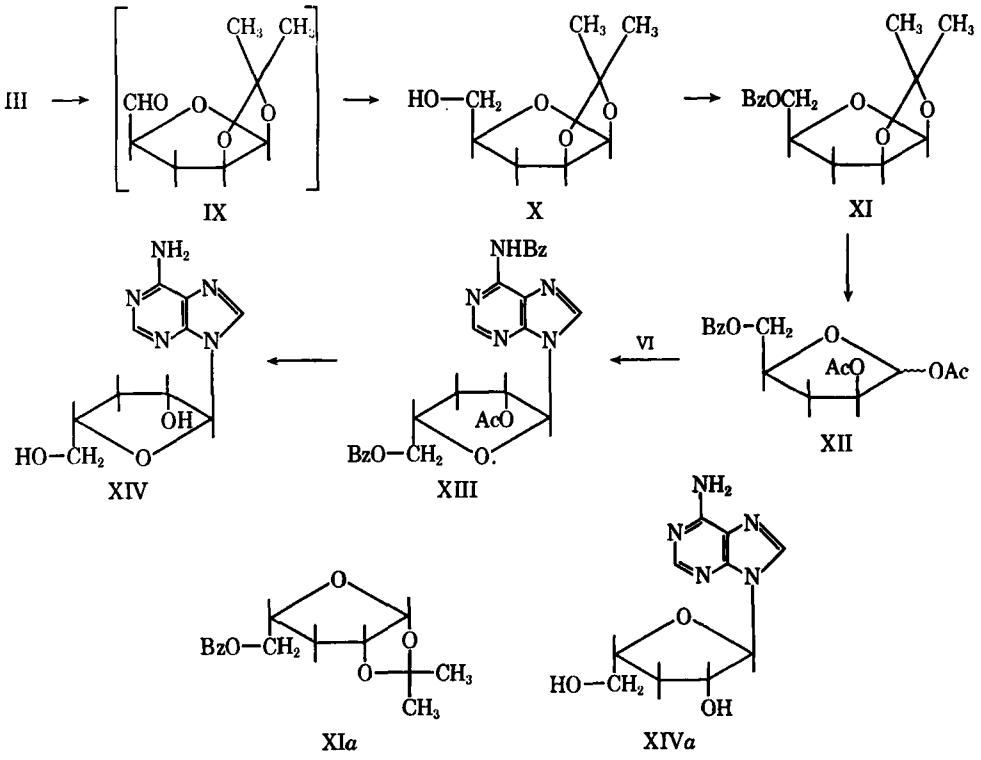



*Scheme I*

showed an infrared spectrum which was identical in all respects to that of 5-*O*-benzoyl-1,2-*O*-isopropylidene-3-deoxy-L-arabinofuranose (XI*a*) originally prepared from 1,2-*O*-isopropylidene-3-deoxy-D-galactofuranose (1). The constants shown in Table I substantiate the enantiomorphic relationship of these two pentose derivatives. Acetolysis of XI gave the syrupy 1,2-diacetate (XII) in 60% yield. This was converted to the blocked nucleoside (XIII) *via* the titanium tetrachloride procedure and removal of the blocking groups with methanolic sodium methoxide resulted in the separation of the crystalline nucleoside (XIV) in a yield of 31% (from crude XII). This nucleoside was found to be, as expected, the enantiomorph of 9-(3-deoxy-α-L-arabinofuranosyl) adenine (XIV*a*) (1). The infrared spectra of these compounds were superimposable and their constants are as shown in Table I.

In a recent publication, Rembarz (7) has formulated diisopropylidene-3-deoxy-D-mannose as a 1,2:4,6-substituted derivative, *i.e.*, as a pyranose. The optical rotation which is reported for this compound (7), as well as that of the 1,2-monoacetone derivative, closely approaches the determined values of compounds II and III, respectively. The syntheses reported in this paper are consistent throughout with the assignment of a 1,2:5,6-substituted furanose structure for compound II. There is no current explanation for this apparent discrepancy.

The route to the third nucleoside (XX) began with 1,2-*O*-isopropylidene-3-deoxy-D-galactofuranose (XV, Scheme III) (1) which, after conversion to the 5,6-di-*O*-methanesulfonyl derivative (XVI) was inverted using sodium benzoate in dimethylformamide to give syrupy 5,6-di-*O*-benzoyl-1,2-*O*-isopropylidene-3-deoxy-L-mannofuranose (XVII) in a combined yield of 13%. Acetolysis gave XVIII as a syrup which was coupled with chloromercuri-6-benzamidopurine. The acyl groups were removed from the crude blocked nucleoside (XIX) with sodium methoxide in methanol, and 9-(3-deoxy-α-L-mannofuranosyl) adenine (XX) readily crystallized from the reaction in an overall yield of 39% from XVII. The enantiomorphic retionship of this nucleoside with VIII was readily established by consideration of their constants (Table I), and their superimposable infrared spectra.




*Scheme II*

## EXPERIMENTAL[4]

**1,2:5,6-Di-O-isopropylidene-3-deoxy-D-mannofuranose (II)**—A mixture of 4.00 g. (24.4 mmoles) of finely powdered 3-deoxy-D-mannose (I) (4), 10 g. of anhydrous copper sulfate, and 100 ml. of reagent acetone containing 80 mg. of concentrated sulfuric acid was stirred continuously in a stoppered flask at room temperature for 24 hr. The mixture was filtered, the solid washed with 50 ml. of acetone, and the combined filtrate and washing was stirred for 1 hr. with 4 g. of powdered barium carbonate then filtered through diatomaceous earth.[5] After washing the cake with acetone (3 × 25 ml.), the combined filtrate and washings were evaporated to dryness *in vacuo* at 50° to give 5.69 g. (95.7%) of crude product as a pale yellow liquid of moderate viscosity. Distillation at 0.05 mm. pressure gave a clear colorless liquid with b.p. 60–61°; yield, 4.67 g. (78.5%). For analysis a sample was redistilled at 54°/0.04 mm.; $[\alpha]_D^{20}$ + 24° [c 1.46 $(CH_2Cl)_2$]; $\bar{\nu}_{max.(cm.^{-1})}^{film}$ 1,380 (*gem*-dimethyl), and no absorption at 3,400 (OH).

*Anal.*—Calcd. for $C_{12}H_{20}O_5$: C, 59.00; H, 8.25. Found: C, 59.06; H, 8.27.

**1,2-O-Isopropylidene-3-deoxy-D-mannofuranose (III)**—To 445 ml. of 50% aqueous methanol at 40°, 0.01 *N* with respect to hydrochloric acid, was added 8.87 g. (36.3 mmoles) of distilled 1,2:5,6-di-*O*-isopropylidene-3-deoxy-D-mannofuranose (II) and the solution stirred continuously at this temperature for 90 min. The reaction was neutralized with 1 *N* aqueous sodium hydroxide to the phenolphthalein end point, and evaporated *in vacuo* at 40° to a syrup, which was partitioned between 200 ml. of water and 50 ml. of chloroform. The organic phase was separated, dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* (50°) to give 0.80 g. (8.8%) of a clear colorless liquid whose infrared spectrum was identical to that of the starting material (II).

The aqueous phase was evaporated to dryness *in vacuo* at 40°, and the syrupy residue was further dried by the addition of absolute ethanol (100 ml.) followed by its removal *in vacuo* (40°). The syrupy residue was extracted with hot chloroform (3 × 30 ml.). The combined extracts were stored overnight at 5° during which time a turbidity developed. Filtration through diatomaceous earth and evaporation to dryness *in vacuo* at 50° gave the crude product as a clear colorless syrup which was sufficiently pure for the next step; yield, 4.55 g. (67.4%

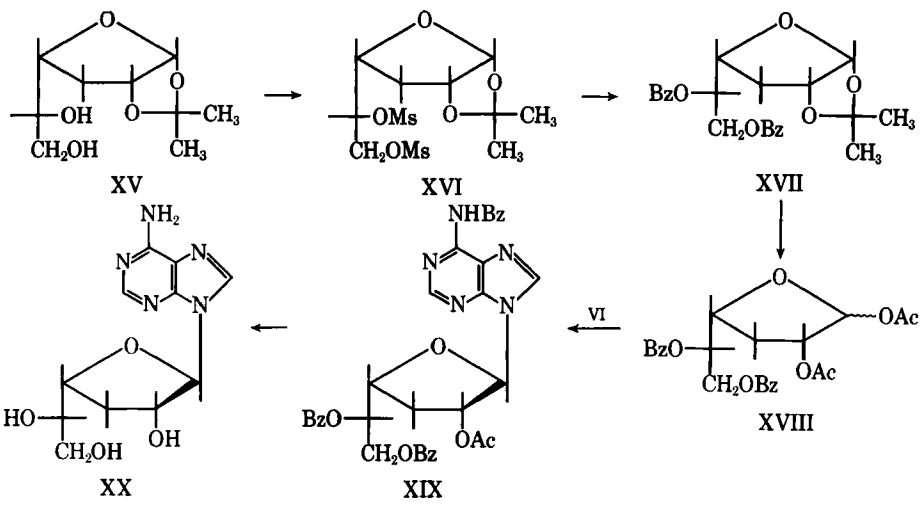
TABLE I—MELTING POINTS AND SPECIFIC ROTATIONS

| Compound | M.p., °C. | $[\alpha]_D^{20}$ ° |
|---|---|---|
| XI | 87 | +42 |
| XIa | 86.5–87[a] | −44[a] |
| XIV | 242.5–243 | +54 |
| XIVa | 242.5–243[a] | −51[a] |
| VIII | 211–212 | +52 |
| XX | 212–213 | −53 |

[a] *Reference 1.*

Melting points were determined in an oil bath and are uncorrected. Optical rotations and infrared spectra were determined using a Rudolph model 80 polarimeter and Perkin-Elmer infrared, respectively.

[5] Celite, Johns-Manville, New York, N. Y.




*Scheme III*

based on unrecovered starting material). For analysis, a sample was sublimed at 85°/0.05 mm. to give a clear, colorless syrup; $[\alpha]_D^{20}$ +24° [c 1.02 $(CH_2Cl)_2$]; $\tilde{\nu}_{max.(cm.^{-1})}^{film}$ 3,500 (OH), 1,380 (*gem*-dimethyl).

*Anal.*—Calcd. for $C_9H_{16}O_5$: C, 52.93; H, 7.90. Found: C, 52.63; H, 7.83.

The chloroform-insoluble syrupy residue (above) readily crystallized from ethanol on seeding with 3-deoxy-D-mannose.

**5,6-Di-O-benzoyl-1,2-O-isopropylidene-3-deoxy-D-mannofuranose (IV)**—To a well-stirred solution of 1.51 g. (7.39 mmoles) of crude 1,2-*O*-isopropylidene-3-deoxy-D-mannofuranose (III) in 15 ml. of reagent pyridine at 0° was added 2.22 ml. (19.2 mmoles) of benzoyl chloride dropwise, over a period of 5 min. After being stirred for an additional hour at 0°, the mixture was stored at room temperature for 24 hr. The reaction was quenched by the addition of 4 drops of water and poured slowly into 200 ml. of rapidly stirred ice water. After 45 min., the mixture was extracted with chloroform (3 × 40 ml.). The combined extracts were washed with aqueous saturated sodium bicarbonate (40 ml.) and water (40 ml.), dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 50°. The last traces of pyridine were removed by the addition of toluene (2 × 15 ml.), followed by its subsequent removal *in vacuo* (50°) to give a practically clear pale yellow syrup. The turbidity was removed by solution of the syrup in 40 ml. of 95% ethanol, filtration through a layer of diatomaceous earth and activated carbon,[6] and evaporation of the filtrate to dryness *in vacuo* (50°), finally at 0.05 mm. pressure gave the product as a clear practically colorless syrup; yield, 2.97 g. (97.4%); $[\alpha]_D^{20}$ −28° [c 2.0 $(CH_2Cl)_2$]; $\tilde{\nu}_{max.(cm.^{-1})}^{film}$ 1,725 (benzoate C=O), 1,600 (phenyl), 1,380 (*gem*-dimethyl), 1,275 (benzoate C—O—C), 710 (phenyl).

*Anal.*—Calcd. for $C_{23}H_{24}O_7$: C, 66.98; H, 5.87. Found: C, 66.82; H, 5.76.

**1,2-Di-O-acetyl-5,6-di-O-benzoyl-3-deoxy-D-mannofuranose (V)**—To a well-stirred solution of 2.80 g. (6.68 mmoles) of 5,6-di-*O*-benzoyl-1,2-*O*-isopropylidene-3-deoxy-D-mannofuranose (IV) in 27 ml. of glacial acetic acid and 2.7 ml. of acetic anhydride was added 1.50 ml. of concentrated sulfuric acid dropwise, while maintaining the temperature at 15–20°. After stirring for 1 hr., the reaction was stored at room temperature for 24 hr. The solution was poured into 150 ml. of rapidly stirred ice water and, after 75 min., the mixture was extracted with chloroform (3 × 40 ml.). The combined extracts were washed with aqueous saturated sodium bicarbonate (80 ml.) and water (80 ml.), dried over magnesium sulfate, filtered and evaporated to dryness *in vacuo* at 50°, finally at 0.05 mm. pressure, to give a clear practically colorless syrup which was sufficiently pure for the next step; yield, 2.11 g. (68.1%); $\tilde{\nu}_{max.(cm.^{-1})}^{film}$ 1,750 (acetate C=O), 1,725 (benzoate C=O), 1,600 (phenyl), 1,280 (benzoate C—O—C), 1,220 (acetate C—O—C), 710 (phenyl).

*Anal.*—Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 62.02; H, 5.67.

**9-(3-Deoxy-α-D-mannofuranosyl)adenine (VIII)**—A mixture of 1.79 g. (3.93 mmoles) of crude 1,2-di-*O*-acetyl-5,6-di-*O*-benzoyl-3-deoxy-D-mannofuranose (V), 2.33 g. (4.91 mmoles) of chloromercuri-6-benzamidopurine (VI), 2.4 g. of diatomaceous earth, and 170 ml. of ethylene dichloride was distilled until 25 ml. of distillate had been collected. To the partially cooled mixture was added a solution of 0.54 ml. (4.9 mmoles) of titanium tetrachloride in 7 ml. of ethylene dichloride, and the reaction was heated under reflux for 24 hr. After cooling, 65 ml. of aqueous saturated sodium bicarbonate was added; the mixture was stirred vigorously for 4 hr. and filtered through diatomaceous earth. After washing the cake with chloroform (3 × 20 ml.), the organic phase was separated from the filtrate and evaporated to near dryness *in vacuo* at 40°. A solution of the residue in 40 ml. of chloroform was washed with 40 ml. of 30% aqueous potassium iodide and 40 ml. of water, dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 50° to give the

[6] Nuchar, West Virginia Pulp and Paper Co., New York, N. Y.

crude blocked nucleoside (VII) as a yellow foam which hardened to a glass; yield, 2.10 g. (84.0%); $\bar{\nu}^{KBr}_{max.\ (cm.^{-1})}$ 3,300 (NH), 1,750 shoulder (acetate C=O), 1,725 (benzoate C=O), 1,690 shoulder (amide C=O), 1,600, 1,575 (C=N and C=C), 1,280 (benzoate C—O—C), 1,225 shoulder (acetate C—O—C), 1,090, 1,070, 1,025 (sugar C—O—C), 710 (phenyl).

A solution of 1.99 g. of crude blocked nucleoside (VII) in 50 ml. of 0.1 *N* methanolic sodium methoxide was heated under reflux for 2.5 hr. The cooled solution was neutralized with glacial acetic acid and stirred at 0° for 2 hr. which caused the separation of the crude nucleoside. The product was collected on a filter, washed with 2 ml. of cold methanol and dried, to give a light brown, crystalline solid; yield, 0.557 g. (53.3% from the 1,2-diacetate, V); m.p. 208–211°. For analysis, a sample was recrystallized from water with light charcoaling and again from 80% aqueous methanol to give small, white needles, m.p. 211–212°; $\lambda^{pH\ 1}_{max.\ (m\mu)}$ 257 ($\epsilon$ 14,600), $\lambda^{pH\ 13}_{max.\ (m\mu)}$ 260 ($\epsilon$ 15,000), $\lambda^{H_2O}_{max.\ (m\mu)}$ 259 ($\epsilon$ 14,700); $\bar{\nu}^{KBr}_{max.\ (cm.^{-1})}$ 3400–3100 (broad OH and NH), 1,605, 1,565 (C=C and C=N); $[\alpha]^{20}_D$ +52° (c 1.15 $H_2O$).

*Anal.*—Calcd. for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.86; H, 5.40; N, 24.72.

**1,2-O-Isopropylidene-3-deoxy-D-threo-pentodialdofuranose (IX)**—To a well-stirred solution of 2.03 g. (9.95 mmoles) of crude 1,2-*O*-isopropylidene-3-deoxy-D-mannofuranose (III) in 60 ml. of water was added 2.13 g. (9.95 mmoles) of sodium metaperiodate and the pH adjusted to between 6.0 and 6.5 (pH paper) with 0.1 *N* sodium hydroxide solution. After 1 hr., during which the pH was adjusted when necessary with sodium hydroxide solution, the solution was extracted with chloroform (5 × 60 ml.). The combined extracts were washed with 60 ml. of water, dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 40° giving the crude product as a clear, colorless, mobile liquid which was sufficiently pure for the next step; yield, 1.49 g. (87.2%). For analysis, a sample was sublimed at 60°/0.8 mm. to give a clear, colorless liquid; $\bar{\nu}^{film}_{max.\ (cm.^{-1})}$ 3,550 (OH), 2,750 (aldehyde C—H), 1,735 (aldehyde C=O), 1,380 (*gem*-dimethyl).

*Anal.*—Calcd. for $C_8H_{12}O_4$: C, 55.80; H, 7.03. Found: C, 51.63; H, 6.99.

**1,2-O-Isopropylidene-3-deoxy-D-arabinofuranose (X)**—To a stirred solution of 0.57 g. (15 mmoles) of sodium borohydride in 20 ml. of water was added a solution of 1.27 g. (7.38 mmoles) of crude 1,2-*O*-isopropylidene-3-deoxy-D-*threo*-pentodialdofuranose (IX) in 20 ml. of methanol. After 1 hr., the solution was neutralized with dilute aqueous acetic acid, then evaporated to near dryness *in vacuo* at 40°. The residue was dissolved in 25 ml. of water and extracted with chloroform (5 × 25 ml.). The combined extracts were washed with 25 ml. of water, dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 50° to give the crude product as a clear, colorless liquid; yield, 0.996 g. (77.5%). An analytical sample was prepared by sublimation at 80°/0.7 mm. to give a clear, colorless liquid; $\bar{\nu}^{film}_{max.\ (cm.^{-1})}$ 3,500 (OH), 1,380 (*gem*-dimethyl).

*Anal.*—Calcd. for $C_8H_{14}O_4$: C, 55.16; H, 8.10. Found: C, 54.98; H, 7.73.

**5-O-Benzoyl-1,2-O-isopropylidene-3-deoxy-D-arabinofuranose (XI)**—To a well-stirred solution of 0.844 g. (4.85 mmoles) of crude 1,2-*O*-isopropylidene-3-deoxy-D-arabinofuranose (X) in 12 ml. of reagent pyridine at 0° was added 0.73 ml. (6.3 mmoles) of benzoyl chloride dropwise, over a period of 4 min. After standing at room temperature for 22 hr., the mixture was poured into 130 ml. of vigorously stirred ice water. The crude product separated as a white solid which was collected on a filter, washed with cold water (5 × 10 ml.), and dried *in vacuo* over phosphorus pentoxide; yield, 1.12 g. (82.9%); m.p. 76–78°. An analytical sample was prepared by recrystallization twice from methanol–water (1:3) to give small needles; m.p. 87°; $\bar{\nu}^{KBr}_{max.\ (cm.^{-1})}$ 1,720 (benzoate C=O), 1,600 (phenyl), 1,385 (*gem*-dimethyl), 1,265 (benzoate C—O—C), 707 (phenyl); $[\alpha]^{20}_D$ + 42° (c 1.18 $CHCl_3$).

*Anal.*—Calcd. for $C_{15}H_{18}O_5$: C, 64.73; H, 6.52. Found: C, 64.62; H, 6.57.

**1,2-Di-O-acetyl-5-O-benzoyl-3-deoxy-D-arabinofuranose (XII)**—To a well-stirred solution of 0.973 g. (3.50 mmoles) of crude 5-*O*-benzoyl-1,2-*O*-isopropylidene-3-deoxy-D-arabinofuranose (XI) in 17 ml. of glacial acetic acid and 1.92 ml. of acetic anhydride was added dropwise 1.06 ml. of concentrated sulfuric acid, while maintaining the temperature between 10 and 20°. After standing at room temperature overnight, the solution was poured into 150 ml. of ice water with vigorous stirring for 40 min., then extracted with chloroform (5 × 20 ml.). The extracts were combined, washed with 100 ml. of aqueous saturated sodium bicarbonate and 50 ml. of water, dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 50° to give the crude product as a clear, colorless syrup; yield, 0.681 g. (60.2%); $\bar{\nu}^{film}_{max.\ (cm.^{-1})}$ 1,750 (acetate C=O), 1,720 (benzoate C=O), 1,600 (phenyl), 1,270 (benzoate C—O—C), 1,220 (acetate C—O—C), 710 (phenyl).

*Anal.*—Calcd. for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63. Found: C, 59.12; H, 6.08.

**9-(3-Deoxy-α-D-arabinofuranosyl) adenine (XIV)**—A mixture of 0.473 g. (1.47 mmoles) of crude 1,2-*O*-acetyl-5-*O*-benzoyl-3-deoxy-D-arabinofuranose (XII), 0.872 g. (1.84 mmoles) of chloromercuri-6-benzamidopurine (VI), 1.0 g. of diatomaceous earth, and 80 ml. of ethylene dichloride was distilled until 25 ml. of distillate had been collected. To the partially cooled mixture was added a solution of 0.20 ml. (1.8 mmoles) of titanium tetrachloride in 3 ml. of ethylene dichloride, and the mixture heated under reflux for 24 hr. While the mixture was still warm, 30 ml. of aqueous saturated sodium bicarbonate was added with vigorous stirring. After 2 hr., the mixture was filtered through diatomaceous earth and the cake washed with chloroform (3 × 10 ml.). The organic phase was separated from the filtrate and evaporated to near dryness *in vacuo* at 40°. A solution of the residue in 15 ml. of chloroform was washed with 15 ml. of 30% aqueous potassium iodide and 15 ml. of water, dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 50° to give the crude, blocked nucleoside, (XIII), as a yellow syrup; yield, 0.697 g. (94.8%); $\bar{\nu}^{film}_{max.\ (cm.^{-1})}$ 1,745 (acetate C=O), 1,720 (benzoate C=O), 1,700 (amide C=O), 1,605, 1,580 (purine ring and phenyl), 1,270 (benzo-

ate C—O—C), 1,220 (acetate C—O—C), 1,020, 1,070, 1,085 (sugar C—O—C), 708 (phenyl).

A solution of 0.675 g. of crude, blocked nucleoside (XIII) in 15 ml. of 0.1 *N* methanolic sodium methoxide was heated under reflux for 2.5 hr. The cooled solution was neutralized with glacial acetic acid, then stirred at 0° for 2 hr. which caused the product to separate as a pale brown crystalline solid. This was collected on a filter, washed with 5 ml. of methanol and dried; yield, 0.111 g. (31%) from the diacetate (XII); m.p. 240°. For analysis, a sample was recrystallized from 90% aqueous ethanol with light charcoaling, and again from 95% ethanol to give a white crystalline solid; m.p. 242.5–243°; $\lambda_{max.\ (m\mu)}^{pH\ 1}$ 257 ($\epsilon$ 15,000), $\lambda_{max.\ (m\mu)}^{pH\ 13}$ 260 ($\epsilon$ 15,200), $\lambda_{max.\ (m\mu)}^{H_2O}$ 259 ($\epsilon$ 15,000), $\tilde{\nu}_{max.\ (cm.^{-1})}^{KBr}$ 3,500–3,150 (broad OH, NH), 1,610, 1,570 (C=C and C=N); $[\alpha]_D^{20}$ + 54° (c 0.70 *N* HCl).

*Anal.*—Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.22; N, 27.88. Found: C, 47.56; H, 5.35; N, 27.78.

**1,2-O-Isopropylidene-5,6-di-O-methanesulfonyl-3-deoxy-D-galactofuranose (XVI)**—To a well-stirred solution of 2.76 g. (13.5 mmoles) of crude 1,2-*O*-isopropylidene-3-deoxy-D-galactofuranose (XV) (1) in 25 ml. of reagent pyridine at 0° was added, dropwise over a period of 10 min., 2.66 ml. (35.1 mmoles) of methanesulfonyl chloride. After being stirred for an additional hour at 0°, the mixture was stored at 5° for 24 hr. and poured into 650 ml. of vigorously stirred cold aqueous sodium bicarbonate. The crude product which separated was collected on a filter, washed with cold water (3 × 50 ml.), and dried; yield, 3.88 g. (79.8%); m.p. 115–117°. Recrystallization from 70% aqueous ethanol gave 3.20 g. of thin wiry needles, m.p. 116–117°. For analysis, a sample was recrystallized from 80% aqueous ethanol; m.p. 116–117°; $\tilde{\nu}_{max.\ (cm.^{-1})}^{KBr.}$ 1,380 (*gem*-dimethyl), 1,180 (sulfonate); $[\alpha]_D^{20}$ −62° [c 1.05 $(CH_2Cl)_2$].

*Anal.*—Calcd. for $C_{11}H_{20}O_9S_2$: C, 36.66; H, 5.59; S, 17.79. Found: C, 36.68; H, 5.53; S, 17.68.

**5,6-Di-O-benzoyl-1,2-O-isopropylidene-3-deoxy-L-mannofuranose (XVII)**—A well-stirred mixture of 3.00 g. (8.33 mmoles) of 1,2-*O*-isopropylidene-5,6-di-*O*-methanesulfonyl-3-deoxy-D-galactofuranose (XVI), m.p. 116–117°, 6.00 g. (41.7 mmoles) of sodium benzoate and 80 ml. of dimethylformamide was heated under reflux for 6 hr. The cooled dark mixture was diluted with 160 ml. of water and shaken with 120 ml. of ether to give a turbid mixture from which the two layers could not be separated. Filtration through diatomaceous earth removed the suspended material and, after washing the cake with additional ether (3 × 25 ml.), the ether phase was readily separated from the filtrate. The aqueous phase was further washed with ether (2 × 80 ml.) which was then combined with the original ether phase, washed with aqueous saturated sodium bicarbonate (100 ml.) and water (100 ml.), dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 50° to give the crude product as a brown syrup; yield, 1.30 g. (37.9%). A solution of this syrup in 10 ml. of benzene was passed through 50 g. of alumina F-20 (grade 2)[7] using benzene as the eluant. The first 200 ml. of eluate was evaporated to dryness *in vacuo* at 50° to give a light amber syrup whose infrared spectrum indicated the desired product but with some contamination. The next 600 ml. of eluate, processed in the same manner, gave a pale yellow syrup with the desired spectrum; yield, 0.556 g. (16.2%); $[\alpha]_D^{20}$ +29° [c 1.27 $(CH_2Cl)_2$]; $\tilde{\nu}_{max.\ (cm.^{-1})}^{film}$ 1,725 (benzoate C=O), 1,600 (phenyl), 1,380 (*gem*-dimethyl), 1,280 (benzoate C—O—C), 710 (phenyl). This material was not purified further but used directly in the next step.

**1,2-Di-O-acetyl-5,6-di-O-benzoyl-3-deoxy-L-mannofuranose (XVIII)**—To a well-stirred solution of 0.515 g. (1.25 mmoles) of 5,6-di-*O*-benzoyl-1,2-*O*-isopropylidene-3-deoxy-L-mannofuranose (XVII) in 5 ml. of glacial acetic acid and 0.94 ml. (10 mmoles) of acetic anhydride was added 0.28 ml. of concentrated sulfuric acid dropwise, while maintaining the temperature between 15 and 20°. After being stored overnight at room temperature, the solution was poured into 50 ml. of vigorously stirred cold 10% aqueous sodium acetate. After a further 30 min. of stirring, the mixture was extracted with chloroform (3 × 15 ml.). The combined extracts were washed with aqueous saturated sodium bicarbonate (2 × 30 ml.) and water (30 ml.), dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 50° to give a slightly turbid practically colorless syrup which was sufficiently pure for the following step; yield, 0.454 g. (79.7%); $\tilde{\nu}_{max.\ (cm.^{-1})}^{film}$ 1,750 (acetate C=O), 1,725 (benzoate C=O), 1,600 (phenyl), 1,280 (benzoate C—O—C), 1,220 (acetate C—O—C), 710 (phenyl).

**9-(3-Deoxy-α-L-mannofuranosyl)adenine (XX)**—A mixture of 0.378 g. (0.829 mmole) of crude 1,2-di-*O*-acetyl-5,6-di-*O*-benzoyl-3-deoxy-L-mannofuranose (XVIII), 0.493 g. (1.04 mmoles) of chloromercuri-6-benzamido-purine (VI), 0.5 g. of diatomaceous earth, and 55 ml. of ethylene dichloride was distilled until 25 ml. of distillate had been collected. To the partially cooled mixture was added a solution of 0.11 ml. (1.0 mmole) of titanium tetrachloride in 5 ml. of ethylene dichloride, and the mixture heated under reflux for 29 hr. While the mixture was still warm, 20 ml. of aqueous saturated sodium bicarbonate was added, the mixture stirred vigorously for 2 hr., and then filtered through diatomaceous earth. After washing the cake with chloroform (3 × 10 ml.), the organic phase was separated from the filtrate and evaporated to near dryness *in vacuo* at 40°. A solution of the residue in 15 ml. of chloroform was washed with 15 ml. of 30% aqueous potassium iodide and 15 ml. of water, dried over magnesium sulfate, filtered, and evaporated to dryness *in vacuo* at 50° to give the crude blocked nucleoside (XIX) as a yellow glass; yield, 0.396 g. (77.0%); $\tilde{\nu}_{max.\ (cm.^{-1})}^{KBr}$ 3,300 (NH), 1,750 shoulder (acetate C=O), 1,725 (benzoate C=O), 1,605, 1,580 (C=N and C=C), 1,280 (benzoate C—O—C), 1,090, 1,070, 1,025 (sugar C—O—C), 710 (phenyl).

A solution of 0.394 g. of the crude blocked nucleoside (XIX) in 10 ml. of 0.1 *N* methanolic sodium methoxide was heated under reflux for 2.5 hr. The cooled solution was neutralized with glacial acetic acid and stored at 5° overnight. The crude crystalline nucleoside, which had separated from solution, was collected on a filter, washed with cold methanol (2 × 5 ml.), and dried; yield, 0.111

[7] Alcoa Activated Alumina, Aluminum Company of America, East St. Louis, Ill.

g. (63.8%); m.p. 210–212° (dec.). For analysis, the product was recrystallized from 90% aqueous methanol with light charcoaling, and again from 80% aqueous methanol; m.p. 212–213°; $\lambda_{max.\ (m\mu)}^{pH\ 1}$ 257 (ε 15,200), $\lambda_{max.\ (m\mu)}^{pH\ 13}$ 260 (ε 15,200), $\lambda_{max.\ (m\mu)}^{H_2O}$ 259 (ε 15,000); $\bar{\nu}_{max.(cm.^{-1})}^{KBr}$ 3,400–3,100 (broad OH, NH), 1,610, 1,575 (C=C and C=N); $[\alpha]_D^{20}$ −53° (c 1.01 $H_2O$).

*Anal.*—Calcd. for $C_{11}H_{15}N_5O_4$: C, 46.97; H, 5.38; N, 24.90. Found: C, 46.84; H, 5.36; N, 25.50.

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*Keyphrases*

9-(3-Deoxyaldofuranosyl) adenines—synthesis
IR spectrophotometry—structure
Optical rotation—identity

# Determination of Caffeine in Plasma by Gas Chromatography

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**A gas chromatographic method has been developed for the rapid, precise, and specific determination of caffeine in plasma. The method overcomes the major drawbacks of previous methods for the determination of caffeine in body fluids, which were: difficult isolation from interfering materials, a substantial blank error and low sensitivity. A standard response curve relating the signal-height ratio of caffeine to that of an internal standard, hexobarbital, permits quantitation of the amount of caffeine present. The method involves extraction of caffeine from plasma with chloroform, after the aqueous phase was adjusted to pH 11.5–12.0. The chloroform extract was evaporated to dryness and the sample was redissolved in carbon disulfide. Two milliliters of plasma was used and caffeine was determined at a concentration of 0.25 mcg./ml.**

CAFFEINE, due to its widespread occurrence in beverages, is probably the drug consumed most today. There is, however, no rapid and sensitive method available for measuring this drug in biological fluids. Connors (1) reviewed many methods for the detection of xanthines, but these were generally not specific for caffeine. The older methods involved extraction of caffeine followed by a gravimetric or volumetric assay (4). These were succeeded by the colorimetric murexide reaction of Tanaka and Ohkubo (5). In addition a number of reagents that form colors have been employed by the authors and by others in an attempt to develop more specific tests for the xanthines. These were used in conjunction with paper (1–3, 13) and thin-layer chromatography (17). The reagents include mercuric chloride, potassium ferrocyanide, mercuric acetate, Dragendorff's reagent, and an alkaline phosphotungstate reagent. Visualization of the spots has also been achieved under UV light at 254 mμ using fluorescent thin-layer plates (10, 12, 17). Other methods include argentimetric (2), iodometric (1), solvent extraction (2, 8), ion exchange (2, 12), nonaqueous titrations (1), Kjeldahl (1), and spectrophotometric (2, 6–9, 11, 14, 15) methods. These have proved successful in measuring qualitatively, and in some cases

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