might suggest that *l*-tartaric acid in the aqueous phases functions in partial resolution of camphoric acid by preferential dimer formation with one of the enantiomorphs.

Experimental Section

All phases were subjected to preequilibration prior to extractions, and phase volumes of 50–100 ml were normally employed using typically 3.0 g of the racemates. Equilibrations of racemates between phases were carried out by mechanical shaking for 5–12 hr.

With the exception of *dl*-camphoric acid, the isolation of the partially resolved racemates followed similar procedures. After separation of the phases and removal of water, the residue was diluted with ligroin until precipitation of the solid was essentially complete. The diols were then crystallized to constant rotation using the following solvents: bis-4-pyridylglycol–methanol, 2,3-dibromobutane-1,4-diol–benzene, and isohydrobenzoin–methanol.

Camphoric acid was isolated by first removing the water from the separated phases, extracting the acid into 10% sodium bicarbonate, precipitating the camphoric acid by addition of hydrochloric acid, and repeating this procedure to constant rotation.

Optical rotations were measured on a Bendix automatic polarimeter Model 143A using a sodium filter and a 6.3-cm cell. Observed rotations varied from several millidegrees to over a hundred millidegrees. Although the reported sensitivity of this instrument is ±0.0002°, in determining error ranges we have assumed an accuracy in observed readings of ±0.005°. Sample concentrations were less than 200 mg/ml and rotations were taken in the following solvents: bis-4-pyridylglycol–acetic acid, 2,3-dibromobutane-1,4-diol–methanol, isohydrobenzoin–methanol, and camphoric acid–acetone. In determining the optical purity of the partially resolved compounds, the values used for the specific rotations were bis-4-pyridylglycol, 87.5°; 2,3-dibromobutanediol, 45.0°; isohydrobenzoin, 65.2°; camphoric acid, 47.7°.

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Branched Chain Sugars. The Synthesis of Adenine Nucleosides of 3-Deoxy-3-*C*-hydroxymethyl-D-erythrofuranose and 2-Deoxy-2-*C*-hydroxymethyl-D-erythrofuranose[1,2]

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Abstract: The reaction of methyl 3-amino-β-D-xylopyranoside (**5**) with nitrous acid effected a ring contraction to give methyl 3-deoxy-3-*C*-formyl-β-D-erythrofuranoside (**6**) as the main product along with small amounts of methyl 2-deoxy-2-*C*-formyl-β-D-erythrofuranoside (**16**). Reduction of the aldehyde function with sponge nickel to the diols **7** and **17** followed by esterification, acetolysis, and nucleoside condensation gave 9-(3-deoxy-3-hydroxymethyl-β-(and α)-D-erythrofuranosyl)adenine (**13**) and 9-(2-deoxy-2-hydroxymethyl-β-(and α)-D-erythrofuranosyl)-adenine (**21**). Intervention by a 1,2 ortho ester ion afforded a 5:1 ratio of **13b**:**13a**. No intervention by a 1,3 ortho ester ion was indicated by the 1:1 ratio of α and β anomers in the 2-branched series. Double-resonance nmr was used to identify the 3-branched series.

In recent years, branched chain sugars have been discovered as constituent parts of a number of antibiotics.[3] These discoveries have stimulated considerable interest in devising means of synthesis[4] of branched chain sugars. This could serve as the proof of structure of naturally occurring compounds. Also, new analogs could be made available to serve as useful intermediates for the preparation of analogs of biologically active materials.

The suggestion has been made[5] that the biological usefulness of a number of adenine nucleosides has been limited by facile conversion to the less active hypoxanthine analogs through the action of adenosine deaminase. It has been noted[6] that the replacement or substitution of the 5′-hydroxyl of adenosine or removal of the 4′-hydroxymethyl group invariably led to loss of substrate activity toward adenosine deaminase.

Recent reports[7] described the synthesis of 2′-*C*-methyl- and 3′-*C*-methyladenosine. Both compounds were

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(2) A portion of this work has been described previously. See E. J. Reist, *Chem. Ind.* (London), 1957 (1967).

(3) (a) R. U. Lemieux and M. L. Wolfrom, *Advan. Carbohydrate Chem.*, **3**, 337 (1948); (b) W. Keller-Schierlein and G. Roncari, *Helv. Chem. Acta*, **47**, 78 (1964); (c) M. Miyamoto, Y. Kawamatsu, M. Shinohara, K. Nakanishi, Y. Nakadaira, and N. S. Bhacca, *Tetrahedron Letters*, 2371 (1964); (d) H. Kawaguchi, T. Naito, and H. Tsukiura, *J. Antibiotics* (Tokyo), *Ser. A*, **18**, 11 (1965); (e) T. Naito, *Penishirin Sono Ta Koseibusshitsu*, **15**, 373 (1962); *Chem. Abstr.*, **60**, 4230*e* (1964); (f) B. P. Vaterlaus, K. Doebel, J. Kiss, A. I. Rachlin, and H. Spiegelberg, *Experientia*, **19**, 383 (1963); (g) D. J. Cooper and M. D. Yudis, *Chem. Commun.*, 821 (1967); (h) F. Shafizadeh, *Advan. Carbohydrate Chem.*, **11**, 263 (1956); (i) H. Umizawa, "Recent Advances in the Chemistry and Biochemistry of Antibiotics," Microbial Chemistry Research Foundation, Tokyo, Japan, 1964.

(4) (a) J. R. Dyer, W. E. McGonigal, and K. C. Rice, *J. Am. Chem. Soc.*, **87**, 654 (1965); (b) A. A. J. Feast, W. G. Overend, and N. R. Williams, *J. Chem. Soc.*, 7378 (1965); (c) J. S. Burton, W. G. Overend, and N. R. Williams, *ibid.*, 3433 (1965); (d) J. J. K. Novák and F. Šorm, *Collection Czech. Chem. Commun.*, **30**, 3303 (1965); (e) P. W. Austin, J. G. Buchanan, and R. M. Saunders, *Chem. Commun.*, 146 (1965).

(5) G. A. LePage and I. G. Junga, *Cancer Res.*, **25**, 46 (1965).

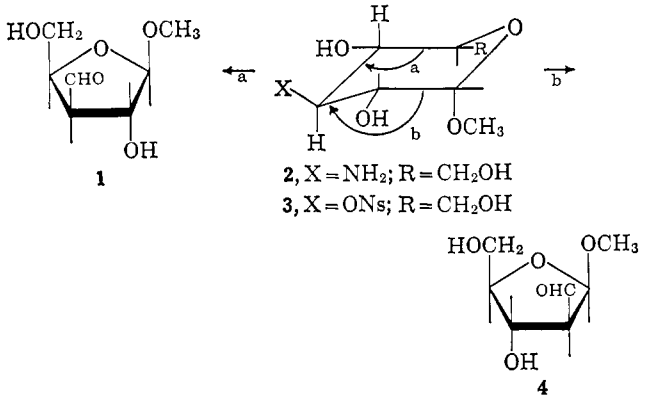
(6) A. Bloch, M. J. Robins, and J. R. McCarthy, Jr., *J. Med. Chem.*, **10**, 908 (1967).

(7) (a) E. Walton, S. R. Jenkins, R. F. Nutt, M. Zimmerman, and F. W. Holly, *J. Am. Chem. Soc.*, **88**, 4524 (1966); (b) E. W. Walton, F. W. Holly, and R. F. Nutt, Abstracts of the Winter Meeting of the American Chemical Society, Phoenix, Ariz., Jan 1966, p 37C.

reported to show cytotoxicity against KB cells in culture. In addition, 3′-*C*-methyladenosine was not measurably deaminated by adenosine deaminase while 2′-*C*-methyladenosine was deaminated at a rate $^1/_{25}$ that of adenosine. It seemed of interest to prepare other branched nucleosides for the investigation of their biological properties. The nucleosides chosen were 9-(3-deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranosyl)-adenine (**13b**) and 9-(2-deoxy-2-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenine (**21b**), both isomeric with 2′-deoxyadenosine. It is of interest to note that **13b** was regarded as a probable structure of the antibiotic cordycepin[8] until nmr studies[9] revealed it to be 3′-deoxyadenosine.

The preparation of 3-deoxy-3-*C*-substituted hexose sugars has been described by either the nitrous acid deamination of methyl 3-amino-3-deoxy-α-D-glucopyranoside[10] (**2**) or the solvolysis of methyl 3-*O*-nitrobenzenesulfonyl-α-D-glucopyranoside[4e,11] (**3**).

Although C-1 is situated in a satisfactory orientation to migrate to C-3 and give a 2-deoxy-2-*C*-formylriboside (**4**) (path b), in both cases,[10,11] the only product reported was methyl 3-deoxy-3-*C*-formyl-α-D-ribofuranoside (**1**) from the migration of C-5 to C-3. A similar type of reaction in the 3-substituted xylopyranose series might be expected to give the desired 3-deoxy-3-*C*-formylerythroside *via* path a.



1 **4**

Methyl 3-amino-3-deoxy-β-D-xylopyranoside (**5**) was prepared from D-arabinose by the procedure described by Baker, *et al.*,[12] for the preparation of methyl 3-amino-3-deoxy-β-L-xylopyranoside from L-arabinose. It had properties identical with those described by Anderson, *et al.*,[13] for this same compound. Treatment of **5** with nitrous acid effected the rapid disappearance of starting material. The syrupy reaction product proved to be aldehydic in nature since it gave a strong positive test when treated with Benedicts' reagent. Thin layer chromatography showed one main spot which gave a positive test for reducing sugar with aniline citrate. In addition, there was a trace of a second constituent which did not react to aniline citrate.

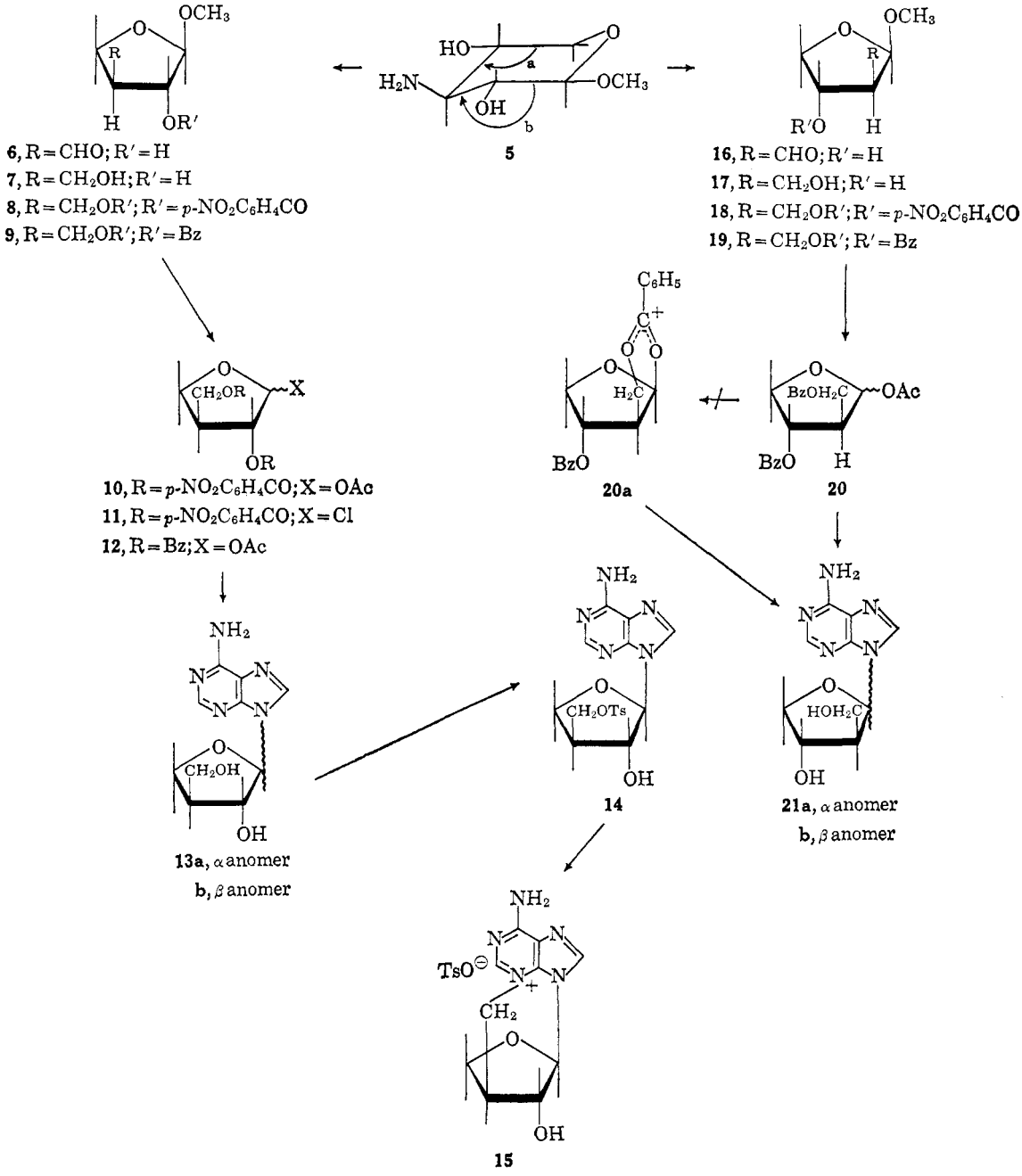
(8) K. G. Cunningham, S. A. Hutchinson, W. Manson, and F. S. Spring, *J. Chem. Soc.*, 2299 (1951).
(9) E. A. Kaczka, N. R. Trenner, B. Arison, R. W. Walker, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **14**, 456 (1964).
(10) S. Inoue and H. Ogawa, *Chem. Pharm. Bull.* (Tokyo), **8**, 79 (1960).
(11) P. W. Austin, J. G. Buchanan, and R. M. Saunders, *J. Chem. Soc., C*, 372 (1967).
(12) B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **19**, 646 (1954).
(13) C. D. Anderson, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5247 (1958).

The nmr spectrum showed three bands in the ratio 1:4:1 in the region τ 6.5–6.7, which are probably due to the methoxyl absorption of methyl glycosides. The conditions for the deamination were sufficiently mild that anomerization to an α-glycoside seemed quite unlikely so that all the reaction products probably maintained the β configuration at C-1. On the basis of the deamination studies with **2**[10,11] it seems probable that the major methoxyl peak is due to methyl 3-deoxy-3-*C*-formyl-β-D-erythrofuranoside (**6**). One of the minor methoxyl peaks may be due to methyl 2-deoxy-2-*C*-formyl-β-D-erythrofuranoside (**16**). The nonreducing contaminant which was observed on tlc could be an epoxide and probably accounts for the second minor methoxyl peak in the nmr. Hydrogenation of the crude aldehyde **6** using sponge nickel gave a syrup which was purified by distillation. The resulting product had an nmr spectrum in which H-1 occurred as a doublet with a very small coupling constant (J = 0.5 cps). The small coupling constant provided a strong indication that the main product was the branched sugar with the side chain at C-3. The alternative structure with the branch at C-2 (**17**) would have a *cis* relationship between H-1 and H-2 and would be expected to have a larger coupling constant.

Treatment of the crude hydrogenation product **7** with *p*-nitrobenzoyl chloride gave a crystalline bis-*p*-nitrobenzoate. That this material was indeed the 3-branched sugar **8** was proved by double-resonance nmr. The proton on the carbon bearing no oxygen (H-3 for structure **8**) was coupled to the branched CH_2 as well as the ring CH_2 and H-2. If the branch had occurred on C-2 (structure **18**), there could be no coupling with the ring CH_2.

Acetolysis of recrystallized methyl 3-deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranoside bis-*p*-nitrobenzoate (**8**) gave crystalline 1-*O*-acetyl-3-deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranose bis-*p*-nitrobenzoate (**10**). The nmr spectrum of the latter was completely compatible with the assigned structure. The crystalline 1-*O*-acetate **10** was converted to the glycosyl chloride **11** and condensed with chloromercuri-6-benzamidopurine in the usual fashion. The crude nucleoside obtained after deacylation with methanolic sodium methoxide was chromatographed on a column of Dowex 1 (OH form). Elution with aqueous methanol gave a 5% yield of 9-(3-deoxy-3-*C*-hydroxymethyl-α-D-erythrofuranosyl)adenine (**13a**) followed by 25% of 9-(3-deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenine (**13b**). The anomeric assignments were made on the basis of order of elution from the ion-exchange column[14] and optical rotation. That these assignments were correct was demonstrated by cyclonucleoside formation by the β anomer. Thus selective tosylation of **13b** gave a crude monotosylate (presumably **14**). When the crude tosylate **14** was heated in ethanol, cyclization occurred to give the crystalline cyclonucleoside **15** with ultraviolet absorption properties which were similar to those reported by Clark, *et al.*,[15] for the cyclonucleoside from adenosine. It should be pointed out that under the unlikely circumstance that the secondary hydroxyl of **13b** should be sulfonated in preference

(14) C. A. Dekker and J. Gin, Abstracts of 153rd National Meeting of the American Chemical Society, Miami Beach, Fla., April 1967, 37-C.
(15) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).



to the primary hydroxyl, only the β anomer would be able to cyclize to the resulting 3,2′-cyclonucleoside. Thus, cyclonucleoside formation makes the anomer assignment unequivocal.

The use of recrystallized bis-*p*-nitrobenzoate **8** as the starting material for the nucleoside condensation precluded the possibility of obtaining a nucleoside from any sugar with a branch at C-2 which might have been formed during the nitrous acid deamination reaction. In order to look for C-2-branched sugars, the mother liquors from the recrystallization of **8** were converted to nucleoside in the same fashion as the crystalline **8**; the results, however, were inconclusive. Therefore, the crude diols **7** and **17** were benzoylated to give a syrup which, after chromatography on silica gel, gave a satisfactory elemental analysis for dibenzoates **9** and **19**. Acetolysis of the dibenzoate to the 1-*O*-acetate followed by condensation with chloromercuri-6-benzamidopurine by the titanium tetrachloride method[16] gave, after deacylation with methanolic sodium methoxide a crude nucleoside mixture. Purification by ion-exchange chromatography using Dowex 1 (OH) yielded four different nucleosides. Two of them were identical with the α and β anomers of the 3-branched nucleoside **13a** and **13b** prepared using the *p*-nitrobenzoates **8**. The other two with the same elemental analysis and satisfactory spectral and chromatographic properties were assumed to be the α and β anomers of 9-(2-deoxy-2-*C*-hydroxymethyl-D-erythrofuranosyl)adenine (**21a** and **21b**). On the basis of the empirical results of Dekker,[14] the first of the two new nucleosides to be eluted from the column was assigned the α configuration, and the second of the two was given the β configuration. This assignment was consistent with optical rotation data, but is by no means conclusive. Cyclonucleoside formation could not be used as the ultimate

(16) J. Prokop and D. H. Murray, *J. Pharm. Sci.*, **54**, 359 (1965).

proof of structure. Examination of molecular models of the 2′-substituted nucleosides **21a** and **21b** showed that both were theoretically capable of forming a chemical bond between N-3 and the branched methylene group. The cyclonucleoside from the β-nucleoside has a *cis* fusion of six-membered and five-membered rings, whereas the α-nucleoside fusion of the rings is *trans*. Both nucleosides when treated with *p*-toluenesulfonyl chloride gave covalent sulfonated nucleosides which were easily converted to ionic sulfonates according to the infrared spectra, although the resulting cyclonucleosides could not be characterized.

The contrast in β:α ratios between the 2-branched nucleosides **21a** and **21b** and 3-branched nucleosides **13a** and **13b** is noteworthy. Thus, the condensation of the C-3 branched sugar **11** or **12** gives predominantly β-nucleoside, a result to be expected from the participation of the 2-*O*-acyl group. It might be expected that the branched *O*-benzoate of **20** might also participate *via* 1,3 ortho ester ion formation to control the stereochemistry. Theoretically, **20** can form either a *cis* ortho ester ion **20a** which should give the α-nucleoside **21a** or a *trans* ortho ester ion which should give the β-nucleoside **21b**. A recent report by Kovacs, *et al.*,[17] on 1,3 ortho ester ion participation of an acetoxymethyl group *cis* or *trans* to a secondary ring sulfonate described evidence for a *cis*-1,3 ortho ester ion from the *trans*-sulfonate. However, their data suggested that no *trans*-1,3 ortho ester ion was formed from the *cis*-sulfonate. By analogy, the *cis* ortho ester ion **20a** should be more important than the isomeric *trans* ortho ester ion and α-nucleoside should predominate. This was not the case, however, so it seems quite likely that 1,3 ortho ester ion formation is not significant.

Ion-exchange chromatography of complex sugar mixtures offers a useful method to analyze products from reactions such as the deamination of amino sugars. The initial preparation of adenine nucleosides provides the additional advantage of uv absorption to aid in the detection of the components as they are eluted from the ion-exchange column. It would be interesting to examine the branched reaction products from the ring contraction of the 3-substituted methyl α-D-glucopyranosides **2** and **3** in this fashion in order to determine whether any 2-branched sugar **4** was formed in addition to the 3-branched sugar **1** reported.[10,11]

Experimental Section[18]

Methyl 3-(and 2)-Deoxy-3-(and 2)-*C*-hydroxymethyl-β-D-erythrofuranoside (7 and 17). A solution of 8.0 g (49 mmol) of methyl 3-amino-3-deoxy-β-D-xylopyranoside (**5**) and 8.0 g (116 mmol) of sodium nitrite in 60 ml of water was cooled with stirring to 0°, then 58 ml (58.0 mmol) of 1.0 *N* aqueous hydrochloric acid was added dropwise with continued cooling and stirring. The reaction was stirred at 0° for 0.5 hr, then at room temperature for 3 hr. Finally it was evaporated to dryness *in vacuo* to give a yellow gummy solid. Trituration of this solid with 200 ml of cold ethyl acetate in several portions gave 6.0 g of crude methyl 3-(and 2)-deoxy-3-(and 2)-*C*-formyl-β-D-erythrofuranoside (**6** and **16**) as a yellow oil which gave a strong Benedict's test for reducing sugar.

Thin layer chromatography, using ethyl acetate–methanol (1:1) as developing solvent showed a main spot at R_f 0.55 (aniline citrate positive) together with a trace contaminant at R_f 0.2 (aniline citrate negative). There was no trace of starting material at R_f 0.3.

To a solution of 6.0 g of crude aldehydes **6** and **16** in 450 ml of 70% aqueous ethanol was added 40 g of Davison sponge nickel and the reaction was heated at reflux for 16 hr. The solution was filtered through a Celite pad, and the filtrate was evaporated to dryness *in vacuo*. The residue was triturated with four 25-ml portions of hot ethyl acetate. The ethyl acetate solution was evaporated to dryness *in vacuo* to give 4.67 g (77%) of methyl 3-(and 2)-deoxy-3-(and 2)-*C*-hydroxymethyl-β-D-erythrofuranoside (**7** and **17**) which gave a negative test for reducing sugar with Benedict's reagent.

An analytical sample was obtained by evaporative distillation of 300 mg of the above product at 90–93° (0.1 mm) to give *ca.* 250 mg of colorless distillate; $n^{28}D$ 1.4651; $[\alpha]^{23}D$ −106° (*c* 0.47, methanol).

Anal. Calcd for $C_6H_{12}O_4$: C, 48.6; H, 8.11. Found: C, 48.7; H, 8.47.

The nmr spectrum showed one peak assigned to methoxyl at τ 6.65. The H-1 absorption was a doublet at τ 5.10 (J = 0.5 cps).

Methyl 3-Deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranoside Bis-*p*-nitrobenzoate (8). A solution of 2.33 g (15.7 mmol) of crude methyl 3-deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranosides **7** and **17** in 40 ml of dry pyridine was cooled to 0° with stirring under a nitrogen atmosphere and 1.7 g (63 mmol) of *p*-nitrobenzoyl chloride was added. The reaction mixture was stored at room temperature for 3 days, then was poured slowly into 150 ml of ice water with vigorous stirring. The resulting tan-colored powder was collected and dissolved in 100 ml of chloroform. The chloroform solution was washed with two 30-ml portions of saturated aqueous sodium bicarbonate and 30 ml of water, then was dried and evaporated to dryness *in vacuo* to give a gummy solid which contained considerable quantities of *p*-nitrobenzoic anhydride, as well as the desired product, as determined by the infrared spectrum.

The crude product (*ca.* 10 g) was dissolved in 200 ml of chloroform and applied to the top of a silica gel column (140 g, 2.8 × 39 cm). Elution of the column with 1400 ml of dichloromethane gave a mixture of anhydride and *p*-nitrobenzoic acid. Subsequent elution of the column with 4 l. of dichloromethane and finally 700 ml of ether eluted the product along with some *p*-nitrobenzoic acid. The last traces of *p*-nitrobenzoic acid were removed by the partition of the mixture between chloroform and saturated aqueous sodium bicarbonate. The chloroform layer was washed with water, then dried and evaporated to dryness *in vacuo* to give 3.63 g of product **8** (52%), mp 173.0–174.5°.

The analytical sample from a previous run, was recrystallized from acetonitrile and had mp 173.5–174.5°; $[\alpha]^{22}D$ +23° (*c* 0.5, chloroform).

Anal. Calcd for $C_{20}H_{18}N_2O_{10}$: C, 53.9; H, 4.06; N, 6.28. Found: C, 54.3; H, 4.19; N, 6.48.

The nmr spectrum showed H-1 as a singlet (τ 4.68), H-2 as a doublet (τ 4.66, $J_{2,3}$ = 2 cps), H-3 as a multiplet (τ 7.13), branched methylene as a doublet (τ 5.42, J = 7 cps), H-4 as a triplet (τ 5.61; $J_{3,4}$ = 9 cps, $J_{4,4'}$ = 9 cps), and H-4′ as a quartet (τ 6.14; $J_{3,4'}$ = 7 cps, $J_{4,4'}$ = 9 cps).

Double-resonance nmr based on H-3 at τ 7.13 collapsed H-2 and the branched methylene to singlets; H-4 and H-4′ became doublets.

1-*O*-Acetyl-3-deoxy-3-*C*-hydroxymethyl-D-erythrofuranose Bis-*p*-nitrobenzoate (10). A stirred solution of 25 ml of acetic acid, 2.5 ml of acetic anhydride, and 0.93 ml of concentrated sulfuric acid was cooled to 0°, then 3.63 g (8.14 mmol) of crystalline methyl 3-deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranoside bis-*p*-nitrobenzoate (**8**) was added. The suspension was stirred at room temperature for 15 hr, then the mixture was filtered to remove the white precipitate. The precipitate was dissolved in chloroform, and the chloroform solution was washed with 40 ml of saturated aqueous sodium bicarbonate and 10 ml of water, then was dried and evaporated to dryness *in vacuo* to give 1.50 g (39%) of product as a colorless gum which crystallized on standing. The nmr spectrum showed H-1 as a singlet (τ 3.63), H-2 as a doublet (τ 4.54; J = 2 cps), H-3 as a multiplet (τ 7.1), and H-4 and H-4′ as multiplets (τ 5.5 and 6.0).

The crystalline product was purified by dissolving in 25 ml of hot glacial acetic acid, then diluting the cooled solution with 50 ml of methanol to give 1.05 g of crystals, mp 155.5–156.5°; $[\alpha]^{21}D$ +41° (*c* 0.49, chloroform).

Anal. Calcd for $C_{21}H_{18}N_2O_{11}$: C, 53.3; H, 3.83; N, 5.91. Found: C, 53.2; H, 4.11; N, 6.11.

(17) Ö. K. J. Kovács, G. Schneider, L. K. Láng, and J. Apjok, *Tetrahedron*, **23**, 4181 (1967).

(18) Melting points are corrected. Thin-layer chromatograms were run on silica gel HF (E. Merck AG, Darmstadt). Nucleoside spots were detected by visual examination under an ultraviolet light. Reducing sugars were detected by aniline citrate. All others were detected by iodine vapor.

The acetic acid–acetic anhydride filtrate obtained after the removal of the crystalline β anomer was diluted with 20 ml of water, then was extracted with three 30-ml portions of chloroform. The chloroform extracts were washed with three 80-ml portions of saturated aqueous sodium bicarbonate and two 50-ml portions of water, then were dried and evaporated to dryness *in vacuo* to give 2.86 g of product **10** as a yellow gum which could not be crystallized but which had an infrared spectrum which was satisfactory for the expected product. The nmr spectrum contained two acetate bands at τ 7.92 and 7.97 of relative intensities to suggest that the product was a mixture of β and α anomers in the ratio 5:1.

9-(3-Deoxy-3-*C*-hydroxymethyl-β-(and α)-D-erythrofuranosyl-adenine (13b and 13a). To a mixture of 3 ml of acetyl chloride, 60 ml of dry dichloromethane, and 150 ml of dry ether which had been saturated with hydrogen chloride at 0° was added 700 mg of crystalline 1-*O*-acetyl-3-deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranose bis-*p*-nitrobenzoate (**10**) and the solution was kept at 0° for 4 days. The clear colorless solution was evaporated to dryness *in vacuo*, and the last traces of acetic acid were removed by the addition and removal *in vacuo* of 20 ml of dry xylene. The residual chloro sugar was dissolved in 10 ml of dry dichloromethane, then added to an azeotropically dried suspension of 1.8 g of chloromercuri-6-benzamidopurine (64% on Celite) in 80 ml of xylene. The reaction was heated at reflux with stirring under a nitrogen atmosphere for 4 hr. The hot solution was filtered, and the filter cake was washed with chloroform. The combined filtrate and washings were evaporated to dryness *in vacuo* and the residue was dissolved in 50 ml of chloroform. The chloroform solution was washed with 30 ml of 30% aqueous potassium iodide and with 10 ml of water, then was dried and evaporated to dryness *in vacuo* to give crude blocked nucleoside as a yellow gum.

Deacylation was accomplished by heating a solution of the blocked nucleoside in 35 ml of methanol which contained 108 mg of sodium methoxide at reflux for 3.5 hr. The solution was cooled to room temperature and neutralized to pH 7 with acetic acid, then was evaporated to dryness *in vacuo*. The residue was partitioned between 25 ml each of water and chloroform. The insoluble material was removed by filtration and combined with the water fraction for chromatography. The aqueous solution was evaporated to dryness *in vacuo*. The residue, combined with the above insoluble material, was dissolved in 5 ml of warm water and applied to a column of Dowex 1X2 (OH) (20 g, 1.4 × 21 cm). Inorganic material was eluted with 100 ml of water (carbon dioxide free). Subsequent elution of methanol–water (3:7) gave two uv-absorbing peaks. The first, which contained 18 mg (5%), proved to be the α-nucleoside **13a**. The second, which contained 92 mg (25%), was the β-nucleoside **13b**.

Recrystallization of the α-nucleoside from water gave the analytical sample, mp 122.0–123.5°; $[\alpha]^{20}D$ +88° (*c* 0.30, pyridine); $\lambda_{max}^{pH\ 1}$ 257 mμ (ε 14,200); $\lambda_{max}^{pH\ 7,\ 13}$ 259 mμ (ε 14,400).

Anal. Calcd for $C_{10}H_{13}N_5O_3 \cdot 0.33H_2O$: C, 46.7; H, 5.35; N, 27.2. Found: C, 46.7; H, 5.40; N, 27.5.

Recrystallization of the β-nucleoside from 95% ethanol gave the analytical sample as a white powder, mp 212–213°; $[\alpha]^{24}D$ −72° (*c* 0.49 pyridine); $\lambda_{max}^{pH\ 1}$ 257 mμ (ε 14,000); $\lambda_{max}^{pH\ 7}$ 259 mμ (ε 14,150); $\lambda_{max}^{pH\ 13}$ 260 mμ (ε 14,400).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.8; H, 5.21; N, 27.9. Found: C, 47.8; H, 5.42; N, 27.5.

Methyl 3-(and 2)-Deoxy-3-(and 2)-*C*-hydroxymethyl-β-D-erythrofuranoside Dibenzoate (9 and 19). To a cold (0°) solution of 7.82 g (52.8 mmol) of dry methyl 3-(and 2)-deoxy-3-(and 2)-*C*-hydroxymethyl-β-D-erythrofuranoside (**7** and **17**) in 250 ml of dry pyridine was added 30.6 ml (264 mmol) of benzoyl chloride dropwise with stirring. After the addition was complete, the reaction was stirred at room temperature for 15 hr. The reaction was cooled to 0° and 2 ml of water was added dropwise with stirring, then the mixture was stirred at 0° for 1 hr. The mixture was partitioned between 120 ml of chloroform and 50 ml of water. The chloroform layer was washed with 200 ml of saturated aqueous sodium bicarbonate and 30 ml of water, then was dried and evaporated to dryness *in vacuo*. The residue was dissolved in dichloromethane and applied to the top of a column of silica gel (450 g, 4.2 × 50 cm).

Preliminary elution with dichloromethane removed most of the benzoic anhydride and benzoic acid. Ether elution gave a semicrystalline oil which contained some benzoic acid according to the infrared. The oil was dissolved in chloroform and washed with saturated aqueous sodium bicarbonate and water, then was dried and evaporated to dryness *in vacuo* to give 3.2 g of product as a yellow oil. Tlc on silica gel, using chloroform as the developing solvent, showed two spots at R_f 0.3 and 0.4. The nmr spectrum also showed indications that the product was a mixture.

Anal. Calcd for $C_{20}H_{20}O_6$: C, 67.4; H, 5.65. Found: C, 67.2; H, 5.93.

9-(2-Deoxy-2-*C*-hydroxymethyl-α-(and β)-D-erythrofuranosyl)-adenine (21a and 21b). To 2.3 g (6.7 mmol) of the above dibenzoates **9** and **19** was added a cold (10°) mixture of 20 ml of glacial acetic acid, 2 ml of acetic anhydride, and 0.77 ml of concentrated sulfuric acid. The mixture was stirred at room temperature for 16 hr, then was dissolved in 100 ml of chloroform and washed with 50 ml of water, two 50-ml portions of saturated aqueous sodium bicarbonate, and 30 ml of water, then was dried and evaporated to dryness *in vacuo* to give 1.90 g of 1-*O*-acetates **12** and **20** as a yellow oil. The nmr spectrum showed four acetate bands at τ 8, probably due to the four possible 1-*O*-acetates.

On a large scale, 9.42 g of methyl glycosides **9** and **19** gave 23.3 g of 1-*O*-acetyl dibenzoates **12** and **20** as a thick syrup.

A mixture of 19.2 g (51.2 mmol) of 1-*O*-acetyl-3-(2)-deoxy-3-(2)-*C*-hydroxymethyl-D-erythrofuranose dibenzoate (**12** and **20**) and 59 g of chloromercuri-6-benzamidopurine (64% on Celite) in 2 l. of 1,2-dichloroethane was dried by the distillation of *ca.* 150 ml of solvent. To the stirred suspension was added a solution of 7.65 g of titanium tetrachloride in 50 ml of dry 1,2-dichloroethane. The suspension was heated at reflux with stirring under a nitrogen atmosphere for 17 hr, then was cooled to room temperature and stirred with 1500 ml of saturated aqueous sodium bicarbonate. The two-phase system was filtered, the layers were separated, and the organic layer was washed with 200 ml of 30% aqueous potassium iodide and water, then was dried and evaporated to dryness *in vacuo*. The residue was deacylated with methanolic sodium methoxide in the manner described for the preparation of **13a** and **13b**. After the chloroform–water partition, the aqueous layer was concentrated to *ca.* 30 ml and applied to a column of Dowex 1X2 (OH) (*ca.* 700 g, 4.9 × 60 cm). After elution with water to remove inorganic materials, the elution was continued using methanol–water (3:7). Three main uv-absorbing fractions were eluted with this solvent mixture. The first fraction weighed 689 mg and crystallized when triturated with methanol to give white solid, mp 229–232°, that was different from the previously obtained 3-branched nucleosides **13a** and **13b** and was assumed to be α-2-branched nucleoside **21a** since it was the first eluted.[14] Recrystallization from water gave the analytical sample, mp 236.0–236.5°; $[\alpha]^{20}D$ +2° (*c* 0.25, pyridine); $\lambda_{max}^{pH\ 1}$ 257 mμ (ε 14,800); $\lambda_{max}^{pH\ 7,\ 13}$ 259 mμ (ε 15,000).

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.8; H, 5.21; N, 27.9. Found: C, 47.6; H, 5.24; N, 27.7.

The second ultraviolet-absorbing fraction (0.95 g) contained two components with R_f values of 0.56 and 0.63 on tlc using chloroform–methanol (2:1) as the developing solvent. This fraction was rechromatographed using 110 g of Dowex 1 (OH). After initial elution with water and 1 l. of methanol–water (2:8), elution with an additional 600 ml of methanol–water (2:8) gave 590 mg of 2′-branched nucleoside **21b** as a white solid which was homogeneous on tlc using chloroform–methanol (2:1) with R_f 0.63. Further elution using methanol–water (2:8) gave 300 mg of a solid which was a mixture, followed by 110 mg of solid which had R_f 0.56 and which was identical with the 3′-branched α-nucloside **23a**.

Recrystallization of **21b** from water gave the analytical sample, mp 203.5–204.5°; $[\alpha]D$ −115° (*c* 0.25, pyridine).

Anal. Calcd for $C_{10}H_{13}N_5O_3 \cdot 0.25H_2O$: C, 47.1; H, 5.28; N, 27.4. Found: C, 46.9; H, 5.15; N, 27.4.

The third ultraviolet-absorbing component (1.9 g) was identical with 3′-branched β-nucleoside **13b**.

9-(3-Deoxy-3-*C*-(*p*-tolylsulfonyloxymethyl)-β-D-erythrofuranosyl)-adenine (14) and Its Cyclization. A solution of 132 mg (0.53 mmol) of 9-(3-deoxy-3-*C*-hydroxymethyl-β-D-erythrofuranosyl)adenine (**13b**) in 4 ml of dry pyridine was cooled to 0° and 190 mg (1.03 mmol) of *p*-tolylsulfonyl chloride was added. The reaction was stirred at 0° for 1.5 hr, then was stored at 0° for 18 hr. The excess *p*-tolylsulfonyl chloride was decomposed by the addition of three drops of water at 0°. The reaction was poured into 10 ml of ice water and extracted with chloroform. The chloroform layer was washed with saturated aqueous sodium bicarbonate and water, then was dried and evaporated to dryness *in vacuo* to give crude tosylate (presumably **14**) as a yellow gum. The presence of ionic tosylate absorption bands at 9.6 and 9.9 μ in the infrared indicates that some cyclization to the cyclonucleoside **15** had occurred.

A solution of this gum in 20 ml of absolute ethanol was heated at reflux. The yellow color disappeared almost immediately to give a colorless solution. After 2 hr the solution was evaporated to dryness *in vacuo* to give a white crystalline solid. Recrystallization from 5 ml

of methanol gave the analytical sample of cyclonucleoside **15** with mp 238–239° dec; $\lambda_{max}^{pH\ 1,\ 7}$ 271 mμ (ε 14,700); $\lambda_{max}^{pH\ 13}$ 273 mμ (ε 8500).

Anal. Calcd for $C_{17}H_{19}N_5O_6S$: C, 50.4; H, 4.70; N, 17.3. Found: C, 50.9; H, 4.80; N, 17.2.

Clark, Todd, and Zussman[15] report $\lambda_{max}^{0.05\ N\ HCl}$ 272 mμ (ε 16,310) for the analogous cyclonucleoside of adenosine.

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Isomeric Dimers of Oxytocin[1]

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Abstract: Two isomeric dimers of oxytocin have been obtained as by-products in the oxidation of oxytoceine to oxytocin, the last step in the synthesis of this posterior pituitary hormone. The dimeric material was isolated by partition chromatography on Sephadex and was separated by gel filtration into two components designated as α dimer and β dimer. Determination of molecular weights gave a value of 2006 for the α dimer and 1995 for the β dimer; the calculated value for the molecular weight of a dimer of oxytocin is 2014. Both dimers exhibit low but definite oxytocic activity of the order of 1 unit/mg, compared to approximately 500 units/mg for oxytocin. Both dimeric forms are readily converted to oxytocin with full biological activity by reduction with sodium in liquid ammonia and subsequent oxidation. The reduction liberates sulfhydryl groups in an amount consistent with a dimeric structure containing two disulfide linkages in a 40-membered ring. The α dimer and the β dimer have also been obtained through disulfide interchange by treatment of oxytocin with triethylamine.

In the synthesis of oxytocin,[3] the cyclic disulfide is obtained in the last step by oxidation of the linear disulfhydryl intermediate, oxytoceine,[4] the structure of which is shown in Figure 1. When the products of oxidation of the oxytoceine are subjected to partition chromatography on Sephadex G-25 in the solvent system 1-butanol–benzene–pyridine–0.1% aqueous acetic acid (6:2:1:9) (solvent system A), the hormone emerges with an R_f of about 0.24, and some peptide material which travels slightly faster (R_f 0.32) is also obtained.[5]

In preliminary studies on the nature of this peptide material, it was submitted to countercurrent distribution[6] in 1-butanol–1-propanol–0.5% acetic acid in 0.1% aqueous pyridine (6:1:8). The material represented by the major peak with partition coefficient 0.19 was isolated and subjected to partition chromatography on Sephadex G-25 in 1-butanol–ethanol–pyridine–0.2 *N* aqueous acetic acid (8:1:1:10) (solvent system B). A single sharp symmetrical peak was obtained with an R_f of about 0.55. The isolated material possessed a molecular weight of about 2030; the calculated value for oxytocin is 1007. An acid hydrolysate of the material had an amino acid and ammonia composition identical with that of an hydrolysate of the hormone.

Although the dimeric material had behaved like a single compound in the partition chromatography employed, further efforts were made to establish whether or not it was truly homogeneous. It was finally found that gel filtration of the material on Sephadex G-25 in 0.2 *N* acetic acid resulted in the appearance of two very closely spaced peaks, provided that a long column (1.24 × 159 cm) of suitable efficiency was employed. We have designated the substance corresponding to the faster traveling peak as the α dimer and that corresponding to the slower traveling peak as the β dimer.

In order to obtain sufficient amounts of these two dimers for more complete characterization, studies were undertaken on the preparation and isolation of these compounds on a larger scale. N-Carbobenzoxy-S,S′-dibenzyloxytoceine, prepared by the stepwise *p*-nitrophenyl ester method,[7] served as the starting material. This protected nonapeptide derivative was treated with sodium in liquid ammonia for removal of the N-carbobenzoxy and S-benzyl protecting groups according to the method of Sifferd and du Vigneaud[8] as used in the synthesis of oxytocin.[3] The oxytoceine so obtained was oxidized by aeration in aqueous solution near pH 8 at a peptide concentration of about 0.75 mg/ml. After evaporation and lyophilization of the solution, the products were subjected to partition chromatography on Sephadex G-25 in solvent system A. Plotting of Folin–Lowry color values[9] gave a chromatogram containing a large peak representing oxytocin (R_f 0.24) and

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(3) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis, and S. Gordon, *J. Am. Chem. Soc.*, **75**, 4879 (1953); V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, and P. G. Katsoyannis, *ibid.*, **76**, 3115 (1954).

(4) D. Yamashiro, D. Gillessen, and V. du Vigneaud, *Biochemistry*, **5**, 3711 (1966).

(5) D. Yamashiro, *Nature*, **201**, 76 (1964).

(6) L. C. Craig, W. Hausmann, E. H. Ahrens, Jr., and E. J. Harfenist, *Anal. Chem.*, **23**, 1236 (1951).

(7) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).

(8) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(9) O. H. Lowry, N. J. Rosebrough, A. L. Barr, and R. J. Randall, *ibid.*, **193**, 265 (1951).