which separated were collected and crystallized from ethanol–water (4:1) to give 110 mg of needles of compound **21**, mp 223–227°; $[\alpha]^{29}D$ +42.5° (*c* 0.86, $CHCl_3$); λ_{max} 220 mμ (ε 5250); ν_{max} (Nujol) 3640, 1730, and 1695 cm^{-1}.

Anal. Calcd for $C_{32}H_{48}O_6$: C, 72.69; H, 9.15. Found: C, 72.33; H, 8.84.

Dimethyl 2β,3β-Dihydroxy-13,27-cyclooleanene-23,28-dioate (22). Compound **21** (90 mg) was added to prereduced PtO_2 (100 mg) in acetic acid (10 ml) and stirred under a hydrogen atmosphere. After 10 min, 3.6 ml of hydrogen (at 26°) was absorbed. After 30 min, the mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue crystallized from methanol–water as needles (72 mg), mp 221°, then 237°; $[\alpha]^{28.5}D$ +58.5° (*c* 0.40, $CHCl_3$); ν_{max} (Nujol) 3700, 1735, and 1695 cm^{-1}; τ 10.01, 9.49 (2 H, AB pattern, J_{AB} = 5 cps (cyclopropyl protons)), 9.10 (9 H, singlet (3 C-methyls)), 8.80, 8.66, (3 H each, singlets (2 C-methyls)), 6.32, 6.28 (3 H each, singlets ($2COOCH_3$)). Though this compound showed one spot on tlc; the pmr spectrum indicated a small amount of a contaminant probably due to the 1,4-addition product.

Anal. Calcd for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50. Found: C, 72.36; H, 9.36.

Dimethyl 2β,3β-Dihydroxy-Δ^{12}-oleanene-23,28-dioate (Dimethyl Medicagenate) (26). A solution of 54 mg of compound **22** in a mixture of 10 ml of acetic acid and 1.6 ml of concentrated hydrochloric acid was heated at reflux for 1 hr. Dilution of the reaction mixture with water afforded a crystalline solid which showed the presence of partially acetylated materials by tlc ($CHCl_3$–ethyl acetate, 3:1). The solid was heated in a solution of 100 mg of potassium carbonate in 10 ml of methanol and 4 ml of water for 1 hr. Upon cooling, crystals separated (42 mg). Crystallization from methanol afforded needles of dimethyl medicagenate, mp 229–237°; $[\alpha]^{26.5}D$ +88.5° (*c* 0.45, $CHCl_3$); ν_{max} (Nujol) 3540, 3470, 1735, 1720, and 1690 cm^{-1}; identical with an authentic sample[18] in all respects (mixture melting point, infrared spectra, and tlc) (Figure 1).

Anal. Calcd for $C_{32}H_{50}O_6$: C, 72.41; H, 9.50. Found: C, 72.57; H, 9.55.

Dimethyl 2β,3b-Acetoxy-Δ^{12}-oleanene-23,28-dioate (Dimethyl Medicagenate Diacetate) (27). A sample of 10 mg of dimethyl medicagenate (**26**), obtained by degradation of presenegenin, was acetylated at room temperature in a mixture of 0.5 ml of pyridine and 0.25 ml of acetic anhydride. After 72 hr, the mixture was poured into ice water. The product which separated crystallized from methanol as rhombs (6 mg), mp 238–241°, ν_{max} (Nujol) 1760 (with inflection at 1770), 1730, 1740 cm^{-1}, identical with a sample of dimethyl medicagenoate diacetate prepared from authentic medicagenic acid diacetate.[18c]

Catalytic Hydrogenation of Dimethyl 2β,3β-Isopropylidenedioxy-13,27-cyclo-Δ^{11}-oleanene-23,28-dioate (20). The title compound (55 mg) was shaken with 100 mg of prereduced PtO_2 in 10 ml of acetic acid for 1 hr. The hydrogen uptake amounted to 1.6 equiv. The catalyst was removed by filtration and evaporation of the solvent *in vacuo* gave an oily residue, which was heated in a mixture of acetic acid (10 ml) and hydrochloric acid (1.6 ml) for 1 hr under reflux. The mixture was extracted with ether, the extract was evaporated, and the residue was heated for 40 min on a steam bath with a solution of 100 mg of potassium carbonate in 10 ml of 90% methanol. Dilution with water gave an amorphous substance. Tlc (benzene–ethyl acetate, 4:1) showed one major and one minor spot, separated on preparative tlc to give fraction A (31 mg) and fraction B (6 mg). Fraction A is an oily substance (**23**), ν_{max} (film) 3650 and 1740 cm^{-1}; τ 9.27 (3 H), 9.08 (6 H), 8.93, 8.87, 8.84, 8.78, 8.73, 6.37, 6.28 (3 H each), 4.70 (1 H, triplet).

Fraction B crystallized from methanol–water as needles, mp *ca.* 221°, but was not purified further.

Acknowledgment. We thank Professors C. Djerassi and R. J. Morris for kindly providing authentic samples of medicagenic acid derivatives.

Purine Nucleosides. XIV. Unsaturated Furanosyl Adenine Nucleosides Prepared *via* Base-Catalyzed Elimination Reactions of 2′-Deoxyadenosine Derivatives[1]

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Contribution from the Department of Chemistry, University of Utah, Salt Lake City, Utah 84112. *Received December 13, 1965*

Abstract: 3′-O-Tosyl-2′-deoxyadenosine (I) has been treated with sodium methoxide in dimethylformamide to provide the first reported synthesis of 2′,3′-dideoxy-2′,3′-didehydroadenosine (II). Studies have been made which support a simple E2 mechanism for the general introduction of a 2′,3′ double bond from the corresponding 3′-O-tosyl-2′-deoxyadenosine derivative under these conditions. In the presence of potassium *t*-butoxide in dimethyl sulfoxide further elimination occurs with 5′-S-ethyl-3′-O-tosyl-5′-thio-2′,5′-dideoxyadenosine (V) to yield 9-(5′-methyl-2′-furyl)adenine (X) as a final product. A mechanism has been proposed for the formation of X consistent with the present work. The preparation of these unsaturated adenine nucleosides has provided a new route to the synthesis of 2′,3′-dideoxyadenosine (III) and 2′,3′,5′-trideoxyadenosine (VIII) by direct hydrogenation procedures. The direct utilization of these novel unsaturated nucleoside derivatives as reaction intermediates offers a unique opportunity for future synthetic studies.

The interest in the synthesis of 2′,3′-unsaturated nucleoside derivatives has been stimulated by the proposal that unsaturation at the 2′,3′ position is an intermediate step in the biosynthesis of 2′-deoxyribonucleotides.[2–4] Additional interest in unsaturated nucleosides has resulted from the recent elucidation of the structure of the antibiotic blasticidin S as a 2′,3′-unsaturated pyranosyl derivative of cytosine.[5] Decoy-

(1) Supported by Research Grant CA-08109 from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) P. Reichard, *J. Biol. Chem.*, **237**, 3513 (1962).

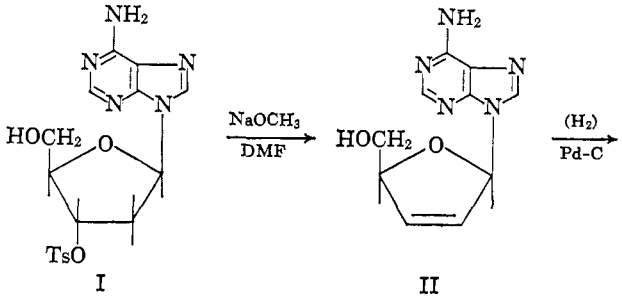
(3) A. Larsson *ibid.*, **238**, 3414 (1963).

(4) (a) A. Larsson, *Biochemistry*, **4**, 1984 (1965); (b) S. Fukui, Y. Tamao, T. Kato, and S. Shimizu *J. Vitaminol.* (Kyoto), **11**, 81 (1965).

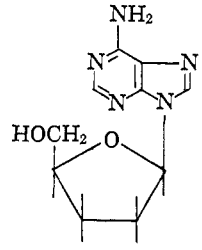
(5) N. Otake, S. Takeuchi, T. Endo, and H. Yonehara, *Tetrahedron Letters*, 1411 (1965).

nine (angustmycin A) has recently been shown to be an adenine nucleoside derivative possessing a furanose ring with a 4′,5′-exocyclic double bond.[6] Synthetic procedures for the introduction of a 2′,3′ double bond have recently been reported for pyrimidine nucleosides[7,8] based largely on the intermediate formation of a 2,3′-anhydronucleoside or an oxetane ring to provide a suitable leaving group in a base-catalyzed elimination reaction. Since the formation of a 2,3′-anhydro linkage is peculiar to the pyrimidine nucleosides, Goodman and co-workers[9] have made an attempt to prepare the 2′,3′-unsaturated derivative of adenosine (II) by the general procedure of Corey and Winter[10] for introduction of unsaturation *via* a 2′,3′-O-thiocarbonato derivative. Although adenosine 2′,3′-O-thiocarbonate was prepared, attempts to convert this derivative to the desired 2′,3′-dideoxy-2′,3′-didehydroadenosine (II) were unsuccessful.[9] The direct synthesis of 2′,3′-dideoxy-2′,3′-didehydropyranosylpurine nucleosides in our laboratory has been rather successful utilizing various glycal derivatives.[11] This procedure, however, is limited to the pyranose ring due to the general unavailability and high reactivity of the requisite "furanal."[12]

The recent preparation of 3′-O-*p*-toluenesulfonyl-2′-deoxyadenosine[13,14] (I) from 2′-deoxyadenosine has provided a readily available starting material to study base-catalyzed elimination reactions in the purine nucleoside series.

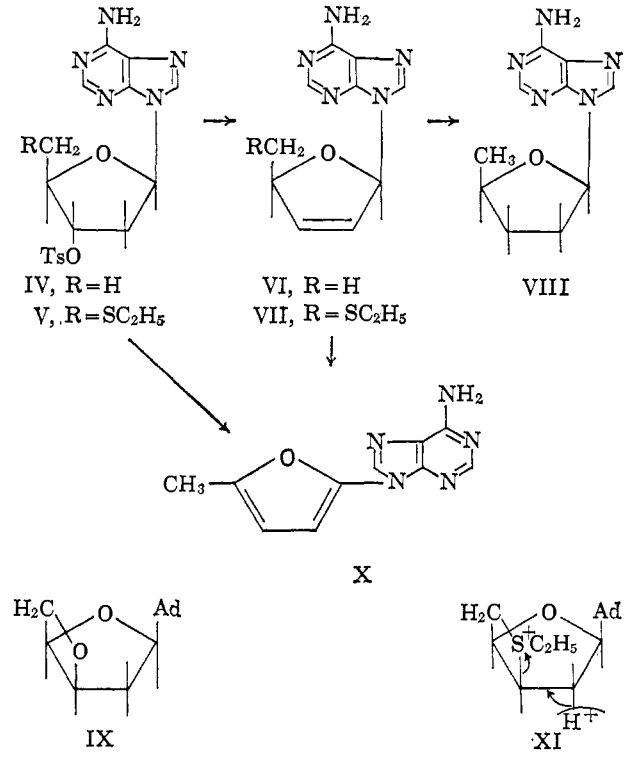


I II



III

When I was treated with an excess of sodium methoxide in dimethylformamide at room temperature 6-amino-9-(2′,3′-dideoxy-2′,3′-didehydro-β-D-*glycero*-pentofuranosyl)purine, (2′,3′-dideoxy-2′,3′-didehydroadenosine) (II) was isolated in above 60% yield. The pmr spectra of II showed two adjacent vinyl protons



centered at δ 6.5 and 6.2, respectively. The anomeric proton occurred at δ 7.1 with a coupling constant of 2 cps (spectra determined in deuterated dimethyl sulfoxide).

The position of the double bond was further established by hydrogenation of II with palladium-on-carbon catalyst to give a 67.2% yield of 2′,3′-dideoxyadenosine (III).[9,13,14] This provides a new and convenient synthesis for 2′,3′-dideoxyadenosine. The generality of the present procedure for introduction of unsaturation at position 2′,3′ in a purine nucleoside was examined further for compound V, 5′-S-ethyl-3′-O-*p*-toluenesulfonyl-5′-thio-2′,5′-dideoxyadenosine.[14] When V was treated with sodium methoxide in dimethylformamide, 6-amino-9-(5′-S-ethyl-5′-thio-2′,3′,5′-trideoxy-2′,3′-didehydro-β-D-*glycero*-pentofuranosyl)purine (VII) was isolated in above 50% yield. Treatment of VII with Raney nickel in refluxing ethanol gave 2′,3′,5′-trideoxyadenosine (VIII) previously prepared by an unambiguous route.[14]

It was possible that the synthesis of II had occurred *via* a 3′,5′-epoxy intermediate such as IX which has been shown to give rise to a 2′,3′ double bond in the case of thymidine.[6] Similarly one could envisage a thietane intermediate such as XI which might be involved as a reaction intermediate in the synthesis of VII. To investigate further the mechanism of this elimination it was decided to study the case of 3′-O-tosyl-2′,5′-dideoxyadenosine (IV), since in this case the participation of an oxygen or sulfur at position 5′ was impossible. The synthesis of 3′-O-tosyl-2′,5′-dideoxyadenosine (IV) was accomplished directly from 2′,5′-dideoxyadenosine.[14] Treatment of IV with sodium methoxide in dimethylformamide as in the case of I readily gave the corresponding 2′,3′-unsatu-

(6) H. Hoeksema, G. Slomp, and E. E. van Tamelen, *Tetrahedron Letters*, 1787 (1964).
(7) J. P. Horwitz, J. Chua, I. L. Klundt, M. A. DaRooge, and M. Noel, *J. Am. Chem. Soc.*, **86**, 1896 (1964).
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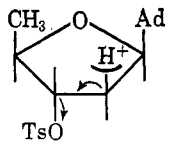
(9) G. L. Tong, W. W. Lee, and L. Goodman, *J. Org. Chem.*, **30**, 2854 (1965).
(10) E. J. Corey and R. E. A. Winter, *J. Am. Chem. Soc.*, **85**, 2677 (1963).
(11) W. A. Bowles and R. K. Robins, *ibid.*, **86**, 1252 (1964).
(12) R. K. Ness and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 435 (1963).
(13) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **86**, 3585 (1964).
(14) M. J. Robins, J. R. McCarthy, Jr., and R. K. Robins, *Biochemistry*, **5**, 224 (1966).

rated derivative 2′,3′,5′-trideoxy-2′,3′-didehydroadenosine (VI) in over 55% yield. The structure of VI was verified by pmr spectra (see Figure 1) and by hydrogenation to the known 2′,3′,5′-trideoxyadenosine[14] (VIII). It thus appears that it is unnecessary to invoke an oxetane or thietane ring as an intermediate to account for the introduction of unsaturation at positions 2′,3′ in the present study. The mechanism is visualized as a simple E2 elimination reaction as illustrated in the case of IV. Additional support for this conclusion is the fact that with I or V in the presence of dimethylformamide and sodium methoxide, the products II or VI, respectively, were the only products detected by paper chromatography after 1 min of reaction time.



From the treatment of 5′-S-ethyl-3′-O-tosyl-5′-thio-2′,5′-dideoxyadenosine (V) with sodium methoxide in dimethylformamide a small amount of another compound was isolated which indicated a second double bond had been introduced into the carbohydrate moiety. This compound was assigned the structure X, since the pmr spectra indicated that the anomeric proton was missing, a methyl group was present, and the protons characteristic of a 2,5-substituted furan were found at δ 6.7 and 6.37, respectively (deuterated DMSO). When V was treated with potassium *t*-butoxide in dimethyl sulfoxide, 9-(5′-methyl-2′-furyl)-adenine (X) was isolated in 20% yield. In considering the synthesis of X from V under these strongly basic conditions, VII was considered to be a possible intermediate in the aromatization of the furan ring. Accordingly 6-amino-9-(5′-S-ethyl-5′-thio-2′,3′,5′-trideoxy-2′,3′-didehydro-β-D-*glycero*-pentofuranosyl)-purine (VII) was treated with potassium *t*-butoxide in dimethyl sulfoxide to give X. The preparations of X in these instances were accompanied by substantial amounts of adenine. The synthesis of X is postulated to occur from V *via* a series of base-catalyzed eliminations as shown in reaction Scheme I. Extraction of a proton from $C_{2'}$ compound V gives a good yield of VII which then loses a second proton (at $C_{4'}$) in the presence of stronger base to yield an anion which can become stable by elimination of the adenine base or by elimination of the ethyl mercaptide ion. The latter path is postulated to give an exocyclic methylene derivative (XII) which rapidly loses a proton at C_1 to give the stable 9-(5′-methyl-2′-furyl)adenine (X).

The extension of the use of base-catalyzed elimination reactions for the synthesis of nucleosides possessing an exocyclic methylene group is a problem presently under active investigation in our laboratory. The present studies provide ample evidence that purine nucleosides unsaturated in the carbohydrate moiety can be readily prepared from simple nucleoside derivatives. The availability of these unsaturated purine nucleosides suggests that these compounds may well serve as novel intermediates in the synthesis of new and unusual purine nucleosides of considerable biochemical interest.

Scheme I

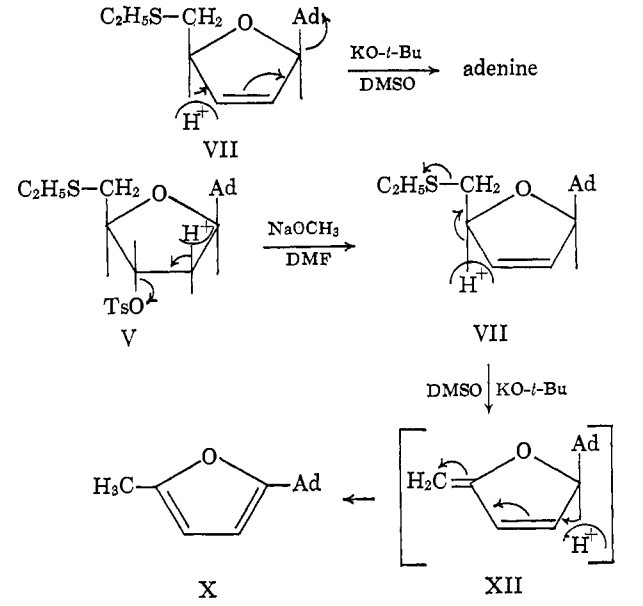


Table I gives the R_{ad} values by which the described nucleosides may be readily identified.

Table I. R_{ad} Values for Adenine Nucleoside Derivatives[a]

Compound	R_{ad} solvent systems[b] A	B	C	D
2′,3′-Dideoxy-2′,3′-didehydroadenosine (II)	1.8	1.2	1.1	1.6
6-Amino-9-(5′-S-ethyl-5′-thio-2′,3′,5′-trideoxy-2′,3′-didehydro-β-D-*glycero*-pentofuranosyl)purine (VII)	2.8	1.5	1.4	2.6
9-(5′-Methyl-2′-furyl)adenine (X)	2.8	1.4	1.2	2.9
3′-O-*p*-Toluenesulfonyl-2′,5′-dideoxyadenosine (IV)	3.3	1.5	1.3	3.4
2′,3′,5′-Trideoxy-2′,3′-didehydroadenosine (VI)	2.3	1.3	1.2	2.5
2′,3′-Dideoxyadenosine (III)	1.9	1.3	1.2	1.6
2′,3′,5′-Trideoxyadenosine (VIII)	2.4	1.4	1.2	2.7

[a] All data are $R_{adenine}$ values run descending on Whatman No. 1 paper. [b] Solvent systems are: A, DMF–*i*-PrOH–concd aq NH_3 (25:65:10); B, *i*-PrOH–concd aq NH_3–H_2O (12:1:5); C, EtOH–H_2O (7:3); D, *n*-BuOH–acetone–concd aq NH_3–H_2O (40:50:3:15) (all proportions v:v); R_f adenine: A, 0.25; B, 0.59; C, 0.67; D, 0.25.

Experimental Section

6-Amino-9-(2′,3′-dideoxy-2′,3′-didehydro-β-D-*glycero*-pentofuranosyl)purine (2′,3′-Dideoxy-2′,3′-didehydroadenosine) (II). 3′-O-*p*-Toluenesulfonyl-2′-deoxyadenosine[13,14] (I, 1.00 g, 0.00247 mole) was dissolved in 5 ml of dimethylformamide and added to a solution of sodium methoxide (0.54 g, 0.01 mole) in 20 ml of dimethylformamide. The light yellow solution was protected from moisture and allowed to stir for 45 min at room temperature. The solution was then diluted with 200 ml of methanol, 10 ml of Amberlite IRC-50 (H^+ form) was added, and the mixture was stirred for 1 hr until the solution was neutral. The resin was then removed by filtration and the filtrate was evaporated to dryness *in vacuo* (bath temperature 25°). The resulting light yellow solid was extracted with four 70-ml portions of boiling acetone. The combined acetone extracts were concentrated to 40 ml and allowed to cool to room temperature. The 2′,3′-dideoxy-2′,3′-didehydroadenosine (II) which crystallized (0.36 g, 62.6%) was collected by filtration. Recrystallization from acetone gave a product, mp 187–190° dec (when placed on a melting point block preheated to

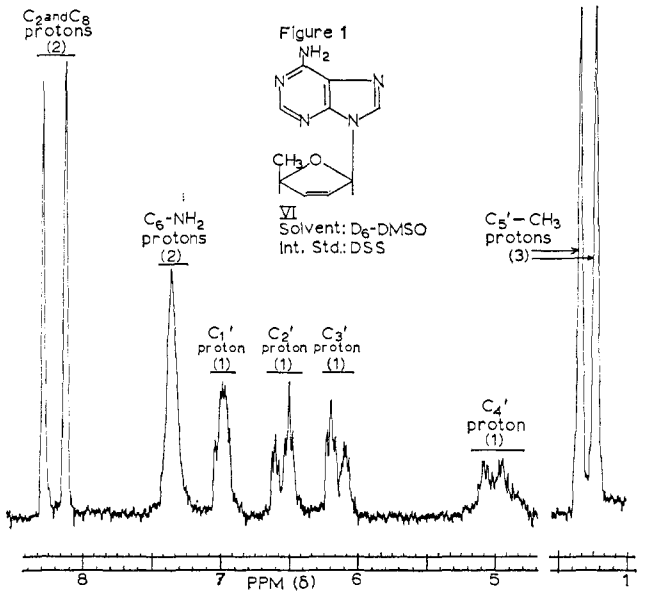


Figure 1.

180° and heated rapidly). The melt rapidly resolidified and did not remelt below 300°; ultraviolet spectra λ_{max}^{MeOH} 259.5 mμ (ε 15,500), $[\alpha]^{23}D$ +19.1° (*c* 1.00, methanol).

Anal. Calcd for $C_{10}H_{11}N_5O_2$: C, 51.5; H, 4.7; N, 30.0. Found: C, 51.3; H, 4.7; N, 29.8.

6-Amino-9-(5'-S-ethyl-5'-thio-2',3',5'-trideoxy-2',3'-didehydro-β-D-*glycero*-pentofuranosyl)purine (VII). 5'-S-Ethyl-3'-O-*p*-toluenesulfonyl-5'-thio-2',5'-dideoxyadenosine[14] (V, 3.65 g, 0.0081 mole) was added to a solution of sodium methoxide (2.38 g, 0.044 mole) in 60 ml of dimethylformamide at room temperature. The dark orange solution was protected from moisture and allowed to stir for 45 min. The solution was cooled to 0° and maintained at this temperature during neutralization to pH 7 by the dropwise addition of 6 *N* acetic acid. The solvent was removed *in vacuo* (bath temperature 30°) to give a solid which was extracted with four 100-ml portions of boiling ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness *in vacuo* yielding 3.0 g of solid. This crude solid was dissolved in 8 ml of chloroform and applied to a column of neutral alumina (150 g). The column was washed with chloroform (300 ml) and ethyl acetate (200 ml) and these washes were discarded. The column was then eluted with ethanol and 50-ml fractions were collected. All fractions with appreciable absorption at 260 mμ were combined and evaporated to dryness. Recrystallization of the solid from a chloroform and hexane mixture provided 6-amino-9-(5'-S-ethyl-5'-thio-2',3',5'-trideoxy-2',3'-didehydro-β-D-*glycero*-pentofuranosyl)purine (VII, 1.20 g, 53.3%) as white needles, mp 136–137°, λ_{max}^{MeOH} 259.5 mμ (ε 14,900); pmr spectral data (deuterated DMSO) indicated the presence of an ethyl group and sharp doublet at δ 2.85 due to $-CH_2-$. The spectra for C_1', C_2', and C_3' protons were found to be virtually identical with that observed for VI (see Figure 1).

Anal. Calcd for $C_{12}H_{15}N_5OS$: C, 52.0; H, 5.4; N, 25.3. Found: C, 51.9; H, 5.3; N, 25.5.

9-(5'-Methyl-2'-furyl)adenine (X). **Method 1.** 5'-S-Ethyl-3'-O-*p*-toluenesulfonyl-5'-thio-2',5'-dideoxyadenosine[14] (V, 2.50 g, 0.00556 mole) was dissolved in 50 ml of dimethyl sulfoxide at room temperature and added to a solution of potassium *t*-butoxide (5 g, 0.075 mole) in 150 ml of dimethyl sulfoxide. The dark-colored solution was protected from moisture and allowed to stir at room temperature 30 min. Fifty milliliters of ethanol was added and the solution was cooled to 0°. This temperature was maintained during the neutralization of the solution with 6 *N* acetic acid. The solution was then evaporated to dryness *in vacuo* (oil pump) at 50° (bath temperature). The resulting solid was extracted four times with 100 ml of warm ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness *in vacuo*, 50 ml of ethanol was added, and the mixture was again evaporated to dryness. This procedure was repeated and the resulting residue was dissolved in 50 ml of hot ethanol. The dark colored solution was cooled to 0° overnight and the tan crystals were filtered to yield 0.250 g (20.9%) of X. The product was recrystallized from ethanol to yield 0.16 g of pure material, mp 236–237°. Ultraviolet absorption showed λ_{max}^{MeOH} 249 mμ (ε 20,000), $\lambda_{max}^{pH\ 11}$ 251 mμ (ε 19,100), $\lambda_{max}^{pH\ 1}$ 252 mμ (ε 22,700); pmr showed a sharp singlet at δ 2.4 (three protons), a sharp doublet centered at 6.7 (one proton), and a sharp doublet (with small secondary splitting) centered at 6.3 (one proton), a broad singlet at 7.3 (two protons, NH_2), two sharp singlets at 8.3 and 8.36 (two protons C_2 and C_8) (pmr spectra determined in deuterated dimethyl sulfoxide, DDS as standard).

Anal. Calcd for $C_{10}H_9ON_5$: C, 55.8; H, 4.2; N, 32.6. Found: C, 55.7; H, 4.0; N, 32.3.

Method 2. Continued elution with ethanol of the alumina column employed in the preparation of VII yielded fractions absorbing at 250–254 mμ. These fractions were combined and concentrated to 5 ml. The solution was cooled to −15° overnight to yield 0.055 g of 9-(5'-methyl-2'-furyl)adenine (X) which was collected by filtration. This product melted at 236–237°. A mixture melting point with a sample prepared by method 1 was undepressed. The ultraviolet absorption spectral data further confirmed the identity of X.

Method 3. 6-Amino-9-(5'-S-ethyl-5'-thio-2',3',5'-trideoxy-2',3'-didehydro-β-D-*glycero*-pentofuranosyl)purine (VII, 2.50 g, 0.0090 mole) was treated with potassium *t*-butoxide–dimethyl sulfoxide at room temperature as in method 1 to yield 0.43 g of a tan solid. Recrystallization from ethanol gave X, mp 235–237°, which was shown to be identical with the product prepared by methods 1 and 2.

3'-O-*p*-Toluenesulfonyl-2',5'-dideoxyadenosine (IV). 2',5'-Dideoxyadenosine[14] (0.800 g, 0.0034 mole) was dissolved in 10 ml of dry pyridine. After cooling the pyridine to room temperature, *p*-toluenesulfonyl chloride (0.810 g, 0.00425 mole) was added. The yellow solution was allowed to stand at room temperature for 4 days. The brown solution was cooled to 0° and a mixture of sodium bicarbonate (0.43 g, 0.00513 mole), ice (100 g), and water (100 ml) was added with stirring. The chloroform layer was separated from the aqueous solution and the aqueous layer was extracted again with 30 ml of chloroform. The combined organic phase was washed with water and dried over sodium sulfate. The solvent was filtered and removed *in vacuo* to give a light yellow foam. The foam was dissolved in 50 ml of acetone and streaked on two 8 × 10 in. glass plates spread with a 2-mm layer of SilicAR™ TLC-7GF.[15] The thin layer plates were placed in a tank (ascending) using acetone as the solvent system. The ultraviolet absorbing band with R_f 0.42–0.72 (first main band) was removed from the plate and the absorbent was extracted with boiling acetone (two 100-ml portions). The acetone extracts were evaporated to dryness *in vacuo*, leaving a solid, white foam. This foam was dissolved in 50 ml of chloroform and added dropwise to 1 l. of petroleum ether (bp 60–90°). 3'-O-*p*-Toluenesulfonyl-2',5'-dideoxyadenosine (IV, 0.500 g, 38%) was collected by filtration. The material was dissolved in 60 ml of boiling methanol and filtered through a charcoal Celite pad, and the methanol was evaporated to dryness *in vacuo*. Reprecipitation as previously described afforded analytically pure material (0.35 g), mp 119–120.5°. Spectral data showed λ_{max}^{MeOH} 227 mμ (ε 14,000) and 259 mμ (ε 15,600), $\lambda_{max}^{pH\ 11}$ 230 mμ (ε 13,900) and 259 mμ (ε 16,700), strong infrared band at 1170 cm^{-1} (OTs).

Anal. Calcd for $C_{17}H_{19}N_5O_4S$: C, 52.4; H, 4.9; N, 18.0. Found: C, 52.7; H, 5.0; N, 17.81.

2',3',5'-Trideoxy-2',3'-didehydroadenosine (VI). 3'-O-*p*-Toluenesulfonyl-2',5'-dideoxyadenosine (IV, 0.31 g, 0.00080 mole) was dissolved in 5 ml of dimethylformamide and added to a solution of sodium methoxide (0.216 g, 0.004 mole) in dimethylformamide (15 ml). The light yellow solution was stirred for 45 min at room temperature, then diluted with 150 ml of methanol, and 6 ml of Amberlite IRC-50 (H^+ form) was added. The mixture was stirred for 1 hr. The resin was removed by filtration and washed with 150 ml of warm methanol (40°), and the filtrate was evaporated to dryness *in vacuo* at 25° (bath temperature) to yield a light yellow solid. This solid was extracted with three 40-ml portions of boiling benzene. The combined benzene extracts were heated to boiling and filtered through a charcoal Celite pad. The filtrate was concentrated to 25 ml and allowed to cool to room temperature to yield long, colorless needles (0.100 g, 57.8%) of 2',3',5'-trideoxy-2',3'-didehydroadenosine (VI), mp 175–177° dec (when placed on a melting point block preheated to 170° and heated rapidly). Ultraviolet absorption spectra showed λ_{max}^{MeOH} 259 mμ (ε 14,800). The pmr spectrum of VI is shown in Figure 1.

(15) Purchased from Mallinckrodt Chemical Co.

Anal. Calcd for $C_{10}H_{11}N_5O$: C, 55.3; H, 5.06; N, 32.25. Found: C, 55.51; H, 5.12; N, 32.02.

2',3'-Dideoxyadenosine (III). 2',3'-Dideoxy-2',3'-didehydroadenosine (VI) (0.28 g, 0.0012 mole) analytically pure, was dissolved in 60 ml of ethanol and the solution was hydrogenated at atmospheric pressure for 1 hr, at room temperature, in the presence of 0.15 g of 5% palladium on carbon. The catalyst was removed by filtration and washed with 50 ml of boiling ethanol. The colorless solution was evaporated to dryness *in vacuo* (oil pump) at 30° (bath temperature). The resulting white solid was recrystallized from ethanol to yield 2',3'-dideoxyadenosine (III), 0.19 g, 67.2%, mp 183–185°, $[\alpha]^{23}D$ −26.8° (*c* 1.00, H_2O) (lit[13] $[\alpha]^{25}D$ −25.2° (*c* 1.01, H_2O)), λ_{max}^{MeOH} 259.5 mμ (ε 14,600).

Anal. Calcd for $C_{10}H_{13}N_5O_2$: C, 51.1; H, 5.5; N, 29.8. Found: C, 51.3; H, 5.7; N, 30.1.

2',3',5'-Trideoxyadenosine (VIII). Method 1. 6-Amino-9-(5'-S-ethyl-5'-thio-2',3',5'-trideoxy-2',3'-didehydro-β-D-*glycero*-pentofuranosyl)purine (VII, 0.900 g, 0.00325 mole) was dissolved in 80 ml of ethanol and W-7 Raney nickel (14 g) was added. The mixture was refluxed for 6.5 hr. The catalyst was removed by filtration and washed with 500 ml of boiling ethanol. Evaporation of the combined filtrate *in vacuo* gave a solid which was extracted with three 15-ml portions of boiling benzene. The combined benzene extracts were concentrated to 10 ml and cooled to room temperature overnight to yield 0.071 g of 2',3',5'-trideoxyadenosine (VIII), mp 156–159°. A mixture melting point with an authentic sample of 2',3',5'-trideoxyadenosine[14] was undepressed.

Anal. Calcd for $C_{10}H_{13}N_5O$: C, 54.8; H, 5.9; N, 32.0. Found: C, 54.5; H, 5.8; N, 32.2.

Method 2. 2',3',5'-Trideoxy-2',3'-didehydroadenosine (VI, 0.035 g) was dissolved in 10 ml of ethanol and the solution was hydrogenated at atmospheric pressure for 30 min in the presence of 0.02 g of 5% Pd–C. The filtrate was chromatogramed in four solvent systems (Table I) against an authentic sample of 2',3',5'-trideoxyadenosine.[14] Both spots were found to run identical in the four solvent systems. The colorless filtrate was evaporated to dryness *in vacuo* at 30° (bath temperature) and the resulting solid was recrystallized from benzene to yield pure VI, mp 155–159°. A mixture melting point with an authentic sample[14] was undepressed. The infrared spectrum was identical with that of 2',3',5'-trideoxyadenosine.[14]

Evidence for the Biological Incorporation of Radioactivity from Benzoate-1-C^{14} into the Quinone Carbons of Coenzyme Q_9

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Abstract: 3',6'-Diacetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid (obtained by ozonolysis of the diacetate of coenzyme Q_9 hydroquinone) was converted to the lactone of 3',6'-dihydroxy-4',5'-dimethoxy-2'-methylphenylacetic acid, by acid hydrolysis. On treatment of the latter with hydrogen peroxide in alkaline solution, the major acidic products were malonic acid and the epoxide of α-methyl-α,β-dihydroxytricarballylic acid. When coenzyme Q_9 biosynthesized from benzoate-1-C^{14} in the rat was subjected to the above degradation, it was found to contain radioactivity only in one or both of its quinone carbon atoms.

We have previously shown that benzoate-1-C^{14} (*i.e.*, benzoate with the label on the ring carbon atom which carries the carboxyl group) is incorporated into coenzyme Q_9 (CoQ_9, **1**) in the rat.[1] When the radioactive CoQ_9 was degraded by ozonolysis of the diacetate of CoQ_9 hydroquinone, as previously described,[2] all of the radioactivity was in the benzoquinone moiety obtained as 3',6'-diacetoxy-4',5'-dimethoxy-2'-methylphenylacetic acid (**2**). To gain further information about the conversion of benzoate to the benzoquinone nucleus, it was necessary to locate the labeled atoms in the substituted phenylacetic acid (**2**). We have therefore investigated the hydrolysis and alkaline peroxide oxidation of **2**. From these reactions, two products have been characterized. The first is a novel, epoxytricarboxylic acid (**4**) which contains virtually all of the radioactivity found in CoQ_9 biosynthesized from benzoate-1-C^{14}. The second is malonic acid, which is devoid of radioactivity. These findings, together with other relevant data, lead to the conclusion that the label from benzoate-1-C^{14} is incorporated biologically into one or both of the quinone carbons of CoQ_9.

(1) R. E. Olson, R. Bentley, A. S. Aiyar, G. H. Dialameh, P. H. Gold, V. G. Ramsey, and C. M. Springer, *J. Biol. Chem.*, **238**, 3146 (1963). For a review of recent work, see R. E. Olson, *Federation Proc.*, **24**, 85 (1965).

(2) R. Bentley, V. G. Ramsey, C. M. Springer, G. H. Dialameh, and R. E. Olson, *Biochemistry*, **4**, 166 (1965).

Results and Discussion

In this work, sufficient quantities of the substituted phenylacetic acid **2** were obtained by ozonolysis of the diacetate of 2,3-dimethoxy-5-methyl-6-phytylbenzohydroquinone. When **2** was allowed to stand at room temperature with concentrated hydrochloric acid, the acetyl groups were removed and the initial product lost water to form the lactone of 3',6'-dihydroxy-4',5'-dimethoxy-2'-methylphenylacetic acid (**3**). This compound was insoluble in sodium bicarbonate and was not oxidized to a quinone with silver oxide in ether.

In oxidation experiments, the lactone **3** was treated with 30% hydrogen peroxide solutions in the presence of KOH. The temperature of the reaction was initially 0° and was raised slowly to 60–65° until all of the peroxide was consumed. On acidification of the mixture, CO_2 was released in amounts averaging 2 moles/mole of lactone. In examinations for neutral materials, small amounts of methanol could be identified by the chromotropic acid test; a small amount of a neutral material obtained by ether extraction was shown not to