Compound XIV gave a positive Benedict test only after heating on the steam-bath but did give a positive test with Tollens' reagent. Attempts were made to derivatize XIV with *p*-nitrophenylhydrazine, methylphenylhydrazine, *p*-toluenesulfonylhydrazine, aniline and *p*-nitrobenzoyl chloride but in none of the cases could solid derivatives be obtained under conditions where 2-deoxyribose gave crystalline products readily with *p*-toluenesulfonylhydrazine and aniline.

A quantitative periodate oxidation at *p*H 5 and 27–30° temperature, followed spectrophotometrically by observation of the optical density at 310 mμ[26] showed the following consumption of equivalents of periodate ion per mole of XIV as a function of time: 15 min., 0; 30 min., 0.10; 5.0 hr., 0.85. Titration with periodate under the same conditions as the spectrophotometric determination showed the consumption of 0.5 equivalent per mole of XIV at 4 hr. and 1.0 equivalent/mole at 24 hr.

2-Amino-2,3-dideoxy-D-ribose Hydrochloride (XVII).—A solution of 2.50 g. (14.3 mmoles) of crystalline XIV in 100 ml. of 4 *M* hydrochloric acid was heated at 100° for 1 hr., then cooled and evaporated *in vacuo*, finally at 1 mm., with a maximum bath temperature of 40°. Water (5–10 ml.) was added to the residue and the solution was re-evaporated *in vacuo*. After five such treatments with water, benzene (5 ml.) was added to the residue and evaporated *in vacuo*. The benzene treatment was repeated *twice* more to leave 2.67 g. (110%) of a very hygroscopic gum which gave a very diffuse infrared spectrum when run as a film or mulled in Nujol but did show the presence of amine hydrochloride as a broad band near 3.4 μ and a sharper band at 4.86 μ; there was no amide carbonyl absorption near 6.0 μ. The gum was homogeneous on paper in solvents C and E with R_{Ad} of 0.99 and 0.71, respectively, when detected by spray H, easily distinguished from XIV. For analysis a portion of the gum was dried at room temperature over phosphorus pentoxide at 0.5 mm. and had $[\alpha]^{29}D$ −48° (1.77% in water). The rotation was unchanged after 1 hr. but had changed somewhat ($[\alpha]^{25}D$ −44°) after 89 hr.

Anal. Calcd. for $C_5H_{11}NO_3 \cdot HCl$: C, 35.4; H, 7.13; N, 8.26; Cl, 20.9. Found: C, 35.6; H, 7.30; N, 8.11; Cl, 21.2.

Compound XVII gave a positive test with Fehling's solution after warming and appeared to reduce Tollens' solution, although the test was obscured by the silver chloride formed. It did not give a positive Benedict test even on warming. Attempts to prepare a solid derivative from XVII with picric acid, *p*-nitrophenylhydrazine, *p*-toluenesulfonyl chloride or acetic anhydride were unsuccessful.

Compound XVII was stable to heating in 4 *M* hydrochloric acid at steam-bath temperatures for at least 5 hr., the material being recovered unchanged from such treatment. A solution of XVII in 1 *M* aqueous sodium hydroxide, however, darkened within a few minutes at steam-bath temperature and produced a gas that gave an alkaline reaction with moist litmus paper. Similar treatment in 0.1 *M* sodium hydroxide required about 10 minutes for noticeable decomposition. Even at room temperature in 0.8 *M* sodium hydroxide, decomposition was noticeable after about 1 hr.

Titration with periodate ion at *p*H 5 and 27–30° showed the following consumption of equivalents of oxidant per mole of XVII: 30 min., 0.9; 4 hr., 1.3; 24 hr., 1.6.

Acknowledgments.—The authors wish to thank Dr. Peter Lim and his group for the infrared spectral data, paper chromatographic data, periodate data and optical rotations. They also wish to thank Mr. O. P. Crews and staff for the large-scale preparation of intermediates.

(26) G. V. Marinette and G. Rouser, *J. Am. Chem. Soc.*, **77**, 5345 (1955).

[CONTRIBUTION FROM STANFORD RESEARCH INSTITUTE, MENLO PARK, CALIFORNIA]

Potential Anticancer Agents.[1] LV. Synthesis of 3′-Amino-2′,3′-dideoxyadenosine and Related Analogs

BY WILLIAM W. LEE, ALLEN BENITEZ, CHARLES D. ANDERSON, LEON GOODMAN AND B. R. BAKER

RECEIVED NOVEMBER 10, 1960

The general chemical approach to deoxyribonucleosides by which 2′-deoxyadenosine was previously prepared has now been used to synthesize 3′- and 2′-aminodideoxyadenosine (VIII and IX, respectively) in two steps from the chloro ethylthio nucleoside I. Reaction of I with sodium azide afforded the two isomeric azido nucleosides IV and V, which were separable. Simultaneous reduction and desulfurization of IV and V gave VIII and IX, respectively. 3′-Deoxyadenosine (III) was also synthesized by desulfurization of the ethylthio nucleoside II.

Since the natural 2′-deoxyribonucleosides or their phosphates are the monomeric units of the DNA polymer, the synthesis of antimetabolites of these 2′-deoxynucleosides has been of major interest to workers in the cancer field. For example, antagonists composed of natural 2-deoxyribofuranose and such fraudulent bases as 6-azathymine,[3] 5-iodouracil[4] or 5-fluorouracil[5] have been shown to have anticancer activity. A relatively unexplored class of potential antagonists in the 2′-deoxynucleoside area are those that contain a fraudulent sugar moiety and a natural base. The fact that 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine, an analog of adenosine, has antitumor activity,[6] suggested that a corresponding analog of 2′-deoxyadenosine, namely, 3′-amino-2′,3′-dideoxyadenosine (VIII), should be synthesized and evaluated as an anticancer agent.

The first reported[7] synthesis of natural 2′-deoxyadenosine, which originated in this Laboratory, was deliberately designed to have the widest possible application for the synthesis of antimetabolites related to 2′-deoxyadenosine, rather than to be the most useful synthesis of a readily isolated and commercially available natural prod-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, see ref. 2.

(2) C. D. Anderson, W. W. Lee, L. Goodman and B. R. Baker, *J. Am. Chem. Soc.*, **83**, 1900 (1961).

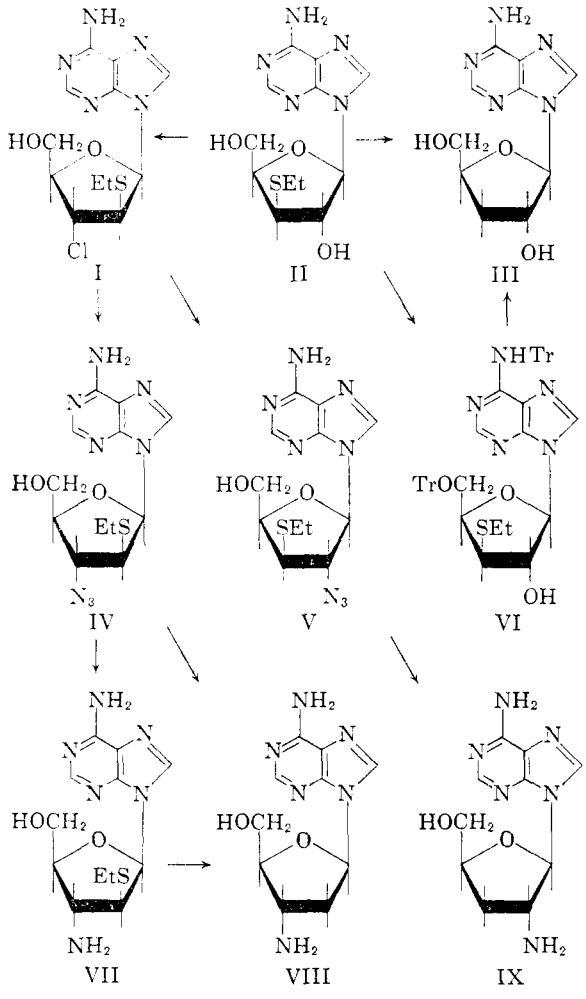
(3) A. D. Welch, W. H. Prusoff and L. G. Lajtha, *Trans. Assoc. Am. Phys.*, **68**, 112 (1955).

(4) W. H. Prusoff, *Biochim. Biophys. Acta*, **32**, 295 (1959); A. D. Welch and W. H. Prusoff, *Cancer Chemotherapy Reports*, **No. 6**, 29 (1960).

(5) P. B. Danneberg, B. J. Montag and C. Heidelberger, *Cancer Research*, **18**, 329 (1958).

(6) B. R. Baker, J. P. Joseph and J. H. Williams, *J. Am. Chem. Soc.*, **77**, 1 (1955).

(7) C. D. Anderson, L. Goodman and B. R. Baker, *ibid.*, **81**, 3967 (1959).



uct. Thus, it was anticipated[7,8] that proper modification of this episulfonium ion approach should give two classes of antimetabolites of 2′-deoxyadenosine, that is, a combination of fraudulent base-natural sugar or a combination of natural base–fraudulent sugar; subsequently reported[9,10] syntheses of 2′-deoxyadenosine could be used only for the former class of antimetabolite. Model studies on the episulfonium ion approach to nucleosides derived from 3-amino-2,3-dideoxy-D-ribofuranose are reported in the accompanying paper,[2] while the utilization of this approach for synthesis of 3′-amino-2′,3′-dideoxyadenosine (VIII) and related nucleosides is the subject of this paper.

The starting point for the synthesis of the two isomeric aminodideoxyadenosines, 9-(3′-amino-2′,-3′-dideoxy-β-D-ribofuranosyl)-adenine (VIII) and 9-(2′-amino-2′,3′-dideoxy-β-D-ribofuranosyl)-adenine (IX) was the chloro ethylthio nucleoside I[7] previously used in the synthesis of 2′-deoxyadenosine.[7] The treatment of I with sodium azide in refluxing aqueous 2-methoxyethanol for 5 hr. gave an 82% yield of a crystalline mixture of the azides IV and V, with the reaction being about 95% complete in 3 hr. Aqueous 95% ethanol was also tried as a solvent; however, it did not give complete reaction within a reasonable time.

The mixture of the azides IV and V could be separated by crystallization from ethyl acetate. Early experiments indicated that the two isomers precipitated in different crystalline forms. On a larger scale run, it was possible by one recrystallization to obtain the 3′-azido isomer IV, m.p. 212–217.5°, in 54% yield (from I). Concentration of the mother liquor to 0.4 of the original volume gave the 2′-azido isomer V, m.p. 214–215°, in 11% yield. The purity of each of these crops was over 95%. An additional 13% yield of mixed azido isomers remained in the mother liquor. The isomers IV and V each exhibited characteristic and distinctive infrared spectra and paper chromatographic behavior and showed a sharply depressed mixed melting point. The approximately 4:1 ratio of IV:V obtained in this azidolysis of I again indicated that C.3′ opening of the episulfonium ion intermediate was strongly favored.[2,11]

The 3′-azido-2′-ethylthio nucleoside IV was readily reduced to the 3′-amino-2′-ethylthio nucleoside VII in 72% crude yield by refluxing with commercial Raney nickel[12] (10:1 ratio) in dioxane for 6 hr. This method of reducing an azido group to an amino group appears to be convenient and of wide utility. Paper chromatograms indicated that very little starting material IV or desulfurized amino nucleoside VIII was formed. Recrystallization from methanol gave the analytical sample of VII, m.p. 201–202°, in 30% yield. An exploratory reduction experiment with the mixture of azides IV and V was tried. However, the results indicated that there would be no advantage in attempting to separate a mixture of amino ethylthio nucleosides (VII and its isomer) rather than the mixture of azido nucleosides IV and V.

The fact that Raney nickel treatment of IV could be stopped so cleanly at the stage of VII, without much loss of sulfur, suggested that it would be difficult to completely desulfurize VII to 3′-amino-2′,3′-dideoxyadenosine (VIII) or to simultaneously reduce and desulfurize IV to VIII. A series of small-scale desulfurizations were run, varying the catalyst type, catalyst ratio, solvent, temperature and time, and using paper chromatography in several solvent systems to compare the conditions. On the basis of the information obtained, a larger scale experiment was run with IV using Raney nickel "C" catalyst[13] in N,N-dimethylformamide at 100° for 5 hr. The resultant mixture of VII and VIII was separated on seed test paper,[14] giving approximately 30% and 44% yields of VII and VIII, respectively.

While this work was in progress, it was observed in the course of another nucleoside problem in these Laboratories[15] that the desulfurization of certain nucleosides with Raney nickel proceeded

(8) L. Goodman, A. Benitez and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 1680 (1958).

(9) R. K. Ness and H. G. Fletcher, Jr., *ibid.*, **81**, 4752 (1959).

(10) H. Venner, *Chem. Ber.*, **93**, 140 (1960).

(11) C. D. Anderson, L. Goodman and B. R. Baker, *J. Am. Chem. Soc.*, **81**, 898 (1959).

(12) Sponge nickel catalyst, Davison Chemical Co., Cincinnati, Ohio.

(13) C. D. Hurd and B. Rudner, *J. Am. Chem Soc.*, **73**, 5157 (1951).

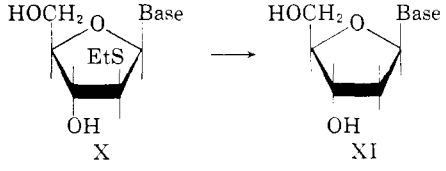
(14) H. H. Brownell, J. G. Hamilton and A. A. Cassel, *Anal. Chem.*, **29**, 550 (1957).

(15) E. J. Reist, P. A. Hart, L. Goodman and B. R. Baker, *J. Org. Chem.*, **26**, in press (1961).

very well under a hydrogen atmosphere. Accordingly, this method was applied to the conversion of IV to VIII. It was found that under a hydrogen atmosphere and in N,N-dimethylformamide, IV could be effectively converted to VIII, thereby avoiding the tedious separation of VII and VIII. A reaction time of 20 hr., with a high ratio (24:1) of commercial Raney nickel[12,16] added in two portions, gave the best results. Under these conditions, the losses of nucleoside material by adsorption were not excessive, for VIII could be obtained in 56% crude yield. This crude material was essentially free of starting material IV and intermediate VII, although small amounts of unidentified by-products were present. Crystallization from acetonitrile gave the analytical sample of 3′-amino-2′,3′-dideoxyadenosine (VIII), m.p. 185–186°. Paper chromatography results indicated that this sample contained a trace impurity which could not be removed by repeated recrystallization and was not present in sufficient amounts to adversely influence the elemental analysis. Compound VIII, although very hygroscopic in the crude amorphorus state, became easy to handle once it had been converted to the crystalline form.

When the 2′-azido-3′-ethylthio nucleoside V was desulfurized by the same procedure as that used for IV, it was readily converted to 2′-amino-2′,3′-dideoxyadenosine (IX), m.p. 179–183.5°, in 51% yield. Recrystallization from acetonitrile gave the analytical sample, m.p. 183.5–185.5°, which sharply depressed the melting point of the isomer VIII. The crude crystalline IX was not hygroscopic and was essentially free of starting material V and the amino ethylthio intermediate.

The poorest step in the 2′-deoxyadenosine (XI) synthesis[7] was the desulfurization of the 2′-ethylthio nucleoside X[7] to the 2′-deoxy nucleoside XI. This reaction resulted in severe adsorption losses in the catalyst and gave a mixture of ethylthio nucleoside X and product XI that had to be separated laboriously. Unfortunately, the desulfurization modification that employed a hydrogen atmosphere and commercial Raney nickel[12] gave similar results; *i.e.*, incomplete desulfurization and excessive loss of material and catalyst.



A comparison of the desulfurization with a highly active catalyst[13] without a hydrogen atmosphere and with commercial Raney nickel[12] in a hydrogen atmosphere was also made, using the more readily available 3′-ethylthio nucleoside II, again with very similar results to those encountered with the 2′-ethylthio nucleoside X. These studies resulted in the synthesis of the interesting compound, 3′-deoxyadenosine (III),[17] both by desulfurizing II and by deblocking the desulfurization product from the trityl nucleoside VI. Separation of the product III from the ethylthio precursor II was achieved by chromatography on seed test paper,[14] or more conveniently, by a solvent partition method. In spite of the poor yields involved in the desulfurization step, this approach appears to be more amenable to the preparation of sizable quantities of 3′-deoxyadenosine (III) than the alternative method which has been reported.[17]

The structures for the isomeric amino dideoxy adenosines VIII and IX had been assigned on the basis of analogous chemistry in the glycoside series[2]; that is, in the azidolysis of I, the reaction through the episulfonium ion intermediate[7] would preferentially give a higher ratio of 3′-to 2′-azido isomer. The assignments were unequivocally confirmed by nuclear magnetic resonance (n.m.r.) studies. This made unnecessary a complete chemical proof of structure, which would have been a sizeable task.

When the n.m.r. spectra[18] of the model compounds, 2′-deoxyadenosine (XI) and 3′-deoxyadenosine (III), were compared with those of the corresponding amino analogs, 3′-amino-2′,3′-dideoxyadenosine (VIII) and 2′-amino-2′,3′-dideoxyadenosine (IX), the correct assignment of structures for the latter compounds was quickly apparent (see Fig. 1). The key region was that in which the bands characteristic of the hydrogen atom on C.1′ appear. The center of this region, marked as region *b* in Fig. 1, varies from 405 to 374 c.p.s. for the four compounds examined.

In 3′-deoxyadenosine (III), the C.1′ hydrogen, by virtue of spin coupling to the one adjacent hydrogen on C.2′, produces the expected doublet signal centered around 386 c.p.s. For the amino analog IX, the hydrogen on C.1′ also couples with the one adjacent hydrogen on C.2′ to produce a doublet signal centered around 374 c.p.s.

For 2′-deoxyadenosine (XI), the C.1′ hydrogen is adjacent to two hydrogens on C.2′ so that the expected triplet signal appears, centered around 405 c.p.s. The amino analog, VIII, has the signal from the C.1′ hydrogen centered at 400 c.p.s. This signal shows the effect of two spin couplings of slightly different size to the two hydrogens on C.2′.[19]

(16) Desulfurization of IV with Raney nickel "C"[13] under a hydrogen atmosphere did not proceed as completely as with commercial Raney nickel, although when used without a hydrogen atmosphere, the Raney nickel "C" exhibited greater activity in desulfurization.

(17) While this manuscript was in preparation, the synthesis of 3′-deoxyadenosine by a completely different method was reported by A. Todd and T. L. V. Ulbricht, *J. Chem. Soc.*, 3275 (1960).

(18) The proton spectra were obtained with a Varian V-4300 C high resolution n.m.r. spectrometer operating at 60 mc. Samples were studied at 25° in dilute solution in heavy water with tetramethylsilane as an external reference. The positions of the various peaks are expressed in c.p.s. on the low-field side of the reference peaks. The authors are indebted to Drs. James N. Shoolery and Leroy F. Johnson at Varian Associates, Palo Alto, California, for obtaining and interpreting the spectra.

(19) Comparisons of the n.m.r. spectra of the 2′-deoxynucleosides and the analogous glycoside and of the 3′-deoxynucleosides and the corresponding glycoside reveal interesting differences in the C.1 proton resonances. Thus, the coupling of the C.1 proton with the two C.2 protons gives a triplet in 2′-deoxyadenosine (XI), a poorly resolved pair of doublets in 3′-amino-2′,3′-dideoxyadenosine (VIII) and a well resolved pair of doublets in the glycoside, methyl 3-acetamido-2,3-dideoxy-β-D-ribofuranoside (see preceding paper[2]). In the 3′-deoxy series, both the 3′-deoxynucleosides (III and IX) possess a doublet, due to spin coupling between the C.1 and C.2 protons, while the glycoside, methyl 2-acetamido-2,3-dideoxy-β-D-ribofuranoside (see preceding paper[2]) shows a single peak for the C.1 proton, which is therefore not coupled to the C.2 proton.

The other regions are relatively similar for all four compounds. Thus, the bands in region *a* are attributable to the two purine ring hydrogens. Those in region *c* are due to the HDO signal which results from interchange of the labile hydrogens with the D_2O solvent. In the portions of the spectra not shown in Fig. 1, the proton signals furthest upfield are attributable to the protons on the only carbon atom not attached to at least one hetero atom (C.2′ or C.3′, depending on the compound). These are centered around 160–180 c.p.s.

The general method developed for the chemical synthesis of 2′-deoxy-β-D-ribonucleosides[7] has now been extended successfully to the synthesis of 3′-amino-2′,3′-dideoxyadenosine (VIII). This is a fraudulent 2′-deoxyribonucleoside containing an unnatural sugar moiety and a natural base. The synthesis of other members of this class of fraudulent 2′-deoxyribonucleosides by this general approach is being investigated.

Acknowledgments.—We wish to thank Dr. Peter Lim and his staff for the chromatograms, optical rotations and interpretation of the infrared data and Mr. O. P. Crews and his staff for the large-scale preparations of certain intermediates.

Experimental[20]

9-[3′-Azido-2′,3′-dideoxy-2′-(ethylthio)-β-D-arabinofuranosyl]-adenine (IV) and 9-[2′-Azido-2′,3′-dideoxy-3′-(ethylthio)-β-D-xylofuranosyl]-adenine (V).—A mixture of 29.0 g. (0.45 mole) of sodium azide and 17.3 g. (52.4 mmoles) of 9-[3′-chloro-2′,3′-dideoxy-2′-(ethylthio)-β-D-arabinofuranosyl]-adenine[29] (I) in 290 ml. of 95% 2-methoxyethanol was refluxed (bath temperature, 135°) with stirring under nitrogen for 5 hr.[30] The dark amber reaction mixture was cooled and filtered. The filter cake was washed with a total of 80 ml. of 2-methoxyethanol and the combined filtrate and washings were evaporated to dryness *in vacuo* (40°/1 mm.). was broken up and stirred with 100 ml. of water at room temperature for 0.5 hr. The mixture was filtered and the filter cake was washed with a total of 200 ml. of water, then dried in a vacuum desiccator to give 14.4 g. (82% yield) of a crude crystalline mixture of the isomeric azido nucleosides IV and V, m.p. 191–196°.[31]

A total of 20.7 g. of isomeric nucleosides (14.4 g. from this run and 6.3 g. from another) was dissolved in 4.4 l. of hot ethyl acetate. The solution was filtered to remove a trace of dark, insoluble material and the filtrate was concentrated to 1.27 l. on a steam-bath. Crystallization occurred on cooling to room temperature. After the solution had been held for 17 hr. at 5°, the crystalline material was collected and washed with 75 ml. of cold ethyl acetate. The product, 13.6 g. (54% yield based on I) consisted of the single isomer IV, m.p. 212.5–217.5° (dec.), as indicated by the behavior in paper chromatograms and its infrared spectrum.

Successive recrystallization from ethanol and ethyl acetate gave an analytical sample of IV, m.p. 216–218°, $[\alpha]^{29}D$ −27° (0.9% in pyridine); $\lambda_{max(\mu)}^{Nujol}$ 3.06, 3.22 (OH, NH_2), 4.75 (N_3), 5.93, 6.20 (adenyl), 7.98 (SEt), 9.10 (strong, characteristic of this isomer, IV), and no absorption at 12.07 (characteristic of the isomer V); $\lambda_{max(m\mu)}^{pH\ 1}$ 258 (ε 15,000), $\lambda_{max(m\mu)}^{10\%\ of\ EtOH}$ 260 (ε 15,600), $\lambda_{max(m\mu)}^{pH\ 13}$ 260 (ε 15,600). It moved as a single spot, R_{Ad} 1.43, in solvent A (the isomer V had R_{Ad} 1.33).

Anal. Calcd. for $C_{12}H_{16}N_8O_2S$: C, 42.9; H, 4.80; N, 3.33. Found: C, 43.2; H, 4.98; N, 32.9.

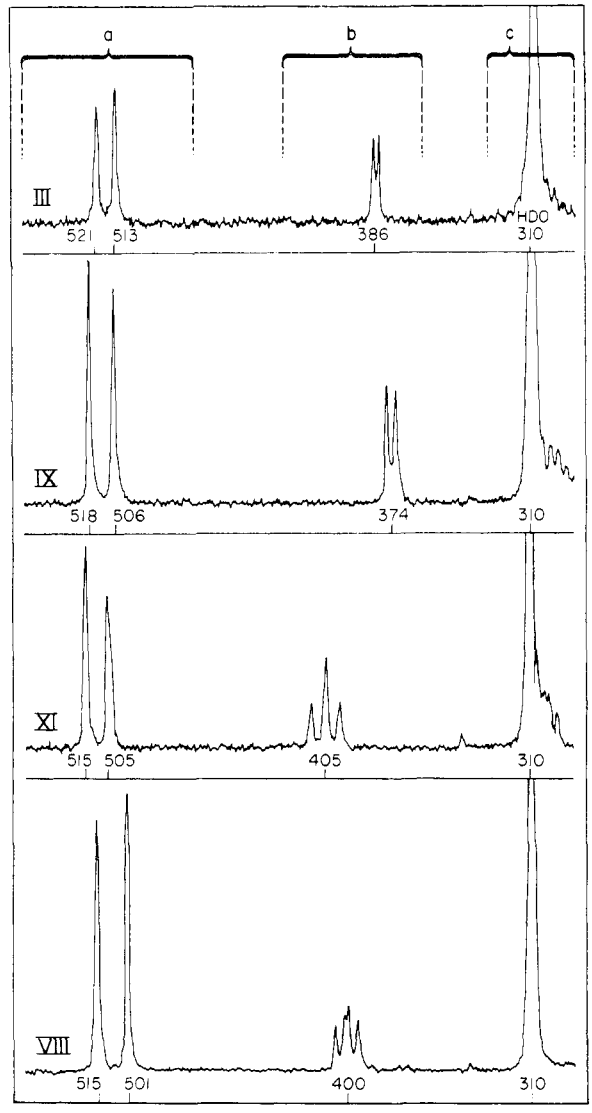


Fig. 1.—Nuclear magnetic resonance spectra of some deoxyadenosines.

(20) Melting points are uncorrected. They were obtained with the Fisher–Johns apparatus. Optical rotations were measured with a Standard Polarimeter Model D attachment to the Beckman DU spectrophotometer calibrated with standard sucrose solutions. Paper chromatograms were run by the descending technique on Whatman No. 1 paper in the following solvent systems: A,[21] benzene–water–methanol (2/1/6) on acetylated paper (either Schleicher and Schuell No. 2496 or Ederol paper); B,[22] *n*-butanol–acetic acid–water (5/2/3); C, *n*-butanol–methyl ethyl ketone–water (5/3/2); D,[23] chloroform–ethanol–water (10/10/6), lower phase; E,[24] *n*-butanol–water (saturated); F,[25] ammonium sulfate–*i*-propanol–water (2/28/70); G,[26] ethyl acetate–pyridine–water (2/1/2); H,[27] 5% Na_2HPO_4, *p*H 8.9; I,[28] *n*-butanol–acetic acid–water (4/1/5). Adenine was used as a standard (spot locations are expressed as R_{Ad} units with adenine at 1.00), and spots were detected by visual examination under ultraviolet light.

(21) Th. Wieland and W. Kracht, *Angew. Chem.*, **69**, 172 (1957).

(22) D. M. Brown, A. Todd and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

(23) T. H. Kritchevsky and A. Tiselius, *Sci.*, **114**, 299 (1951).

(24) J. G. Buchanan, *Nature*, **168**, 1091 (1951).

(25) A variant of a system first used by R. Markham and J. D. Smith, *Biochem. J.*, **49**, 401 (1951).

(26) M. A. Jermyn and F. A. Isherwood, *ibid.*, **44**, 402 (1949).

(27) C. E. Carter, *J. Am. Chem. Soc.*, **72**, 1466 (1950).

(28) R. L. M. Synge, *Biochem. J.*, **48**, 429 (1951).

(29) Synthesized by the previously described procedure[7] except that ethyl acetate was substituted for chloroform. Yields of 88–93% were obtained on runs of 10-g. size. Occasionally the product I showed a double melting point of 145–155° and 180–186°.

(30) A sample of product, removed after 3 hr., analyzed for 0.44% Cl, indicating that less than 5% of the starting material (10.8% Cl, theory) was left.

(31) Although the mixtures of IV and V obtained in different runs are of comparable quality, the observed melting points vary from ranges of 186–193° to 213–216°.

The filtrate and washings were combined and concentrated *in vacuo* to 545 ml. Crystallization occurred when the mixture was cooled to room temperature. The solution was chilled to afford 2.76 g. (11% based on I) of crystalline solid which, according to infrared analysis and paper chromatography, consisted of the single isomer V and had m.p. 215–217° (mixed m.p. with IV, 196–197.5°); $[\alpha]^{25}D$ −52° (0.4% in pyridine); $\lambda_{max(\mu)}^{Nujol}$ 3.05, 3.20 (OH, NH_2), 4.73 (N_3), 5.99, 6.24 (adenyl), 7.90 (SEt); no absorption at 9.10 and strong absorption at 12.07. It moved as a single spot in solvent A, R_{Ad} 1.33.

Anal. Calcd. for $C_{12}H_{16}N_8O_2S$: C, 42.9; H, 4.80; N, 33.3; S, 9.52. Found: C, 43.5; H, 4.46; N, 33.1; S, 9.52.

Further concentration of the washings and mother liquors to a volume of 108 ml. and 12 ml. gave two crops of solid, both consisting of mixtures of the isomers IV and V. These crops totaled 3.24 g. (13% based on I). The total amount of the 20.7 g. of crude, mixed azides recovered from the crystallizations was 19.7 g. or 95%.

In other solvent systems, the two isomers IV and V could not be separated on paper chromatograms; they both gave R_{Ad} values of 1.90 in solvent C and 1.58 in solvent D.

9-[3′-Amino-2′,3′-dideoxy-2′-(ethylthio)-β-D-arabinofuranosyl]-adenine (VII).—A mixture of 1.00 g. (2.97 mmoles) of the azido nucleoside IV, 100 ml. of 1,4-dioxane (dried over calcium hydride and freshly distilled) and 10.0 g. of commercial Raney nickel[12] (washed three times with dioxane) was heated (bath temperature 125°) and stirred for 6 hr. The reaction mixture was diluted with 50 ml. of water and filtered hot through Celite.[32] The filter cake was washed with 95% ethanol (3 × 25 ml.) and the combined filtrate and washings were evaporated to dryness *in vacuo* (40°/1 mm.). The amber gum residue, 0.67 g. (72%), consisted mainly of VII with trace amounts of starting material IV and the desulfurized product VIII, according to paper chromatograms in solvents B and C.

The crude product was recrystallized from 5 ml. of methanol with the aid of Norit, to afford 0.279 g. (30%) of white crystals, m.p. 201–202°; $[\alpha]^{32}D$ −79° (0.98% in water); $\lambda_{max(\mu)}^{Nujol}$ 2.90, 2.95, 3.05, 3.15, 3.22 (OH, NH_2), 6.01, 6.22, 6.32 (NH_2, adenyl), 7.99 (SEt), 9.00, 9.19, 9.33, 9.49, 9.80 (C–O–C and C–OH); $\lambda_{max(m\mu)}^{pH\ 1}$ 258 (ε 13,800), $\lambda_{max(m\mu)}^{pH\ 7}$ 261 (ε 14,500), $\lambda_{max(m\mu)}^{pH\ 13}$ 261 (ε 14,400). It moved as a single spot with R_{Ad} 1.08 and 1.38 in solvent systems B and C, respectively.

Anal. Calcd. for $C_{12}H_{18}N_6O_2S \cdot 1/3H_2O$: C, 45.40; H, 5.93; N, 26.48; S, 10.01; C/N 1.71. Found: C, 45.61; H, 5.80; N, 26.73; S, 9.64; C/N 1.71.

9-[3′-Amino-2′,3′-dideoxy-β-D-ribofuranosyl]-adenine (VIII).—A mixture of 120 ml. of N,N-dimethylformamide (freshly distilled), 1.00 g. (2.97 mmoles) of the azido nucleoside IV and 14.4 g. of commercial Raney nickel[12] (washed three times with the solvent) was stirred under one atmosphere of hydrogen for 15 hr. at a bath temperature of 100°. The mixture was cooled, an additional portion (8.0 g.) of Raney nickel in 6 ml. of solvent was added and the reaction continued under hydrogen at 100° for 5 more hr. The mixture was filtered hot through Celite, and the Raney nickel was washed with hot methanol (3 × 35 ml.). The combined filtrate and washings were evaporated to dryness *in vacuo* (50°/2 mm.). The amorphous VIII, 0.435 g. (56%), was hygroscopic and difficult to handle. It was essentially free of starting material IV or intermediate VII, although in some runs a small amount of VII was present. The crude VIII was dissolved in 100 ml. of boiling acetonitrile and filtered. The filtrate was concentrated to 20 ml. and chilled at 3° to precipitate 0.164 g. (21%) of crystalline VIII, m.p. 181–184°. Another recrystallization, with Norit treatment, followed by cooling the filtrate (38 ml.) to 3°, gave 0.110 g. (14%) of the analytical sample of the amino dideoxy nucleoside VIII, m.p. 184.5–186°; $[\alpha]^{26}D$ −22° (0.77% in water); $\lambda_{max(\mu)}^{Nujol}$ 3.02, 3.18 (OH, NH_2), 5.99, 6.24, 6.39 (adenyl, NH_2) 9.32, 9.46, 9.63; (C–O–C and C–OH); $\lambda_{max(m\mu)}^{pH\ 1}$ 257 (ε 14,900); $\lambda_{max(m\mu)}^{pH\ 7}$ 259 (ε 15,200); $\lambda_{max(m\mu)}^{pH\ 13}$ 261 (ε 15,200). It moved as a single spot (R_{Ad} 1.34) in solvent H and in solvent B as a main spot (R_{Ad} 0.83) with a trace spot (R_{Ad} 1.13). Solvent C is excellent for distinguishing VIII from IV and VII; however, in solvent C the spot of VIII tends to elongate and separate, almost into two spots. This may be due to salt formation by the amino group with carbon dioxide in the atmosphere, for in a number of other solvent systems containing base or an organic acid, VIII remains a single spot.

Anal. Calcd. for $C_{10}H_{14}N_6O_2$: C, 48.0; H, 5.63; N, 33.6. Found: C, 48.3; H, 5.70; N, 33.6, 33.6.

A dipicrate was prepared from crude VIII and picric acid in water and was recrystallized from water to give a solid that decomposed without melting.[33]

Anal. Calcd. for $C_{22}H_{20}N_{12}O_{16}$: C, 37.3; H, 2.85; N, 23.7. Found: C, 37.3; H, 3.48; N, 23.5.

9-(2′-Amino-2′,3′-dideoxy-β-D-ribofuranosyl)-adenine (IX).—Exactly the same procedure as that used for preparing VIII was used to prepare the isomeric nucleoside IX. From 0.240 g. (0.71 mmole) of the azido nucleoside V was obtained 0.092 g. (51%) of crude IX, m.p. 179–183.5°. Paper chromatography indicated that this contained essentially none of the starting material V or of the amino ethylthio intermediate. The mixed melting point with VIII (m.p. 183–186°) was depressed to 160–174°.

The crude IX was dissolved in 15 ml. of acetonitrile, treated with Norit, the solution filtered and the filtrate concentrated to 3.5 ml. It gave, after chilling at 3°, 0.027 g. (15%) of IX, m.p. 183.5–185.5°; $[\alpha]^{26}D$ −51° (1% in water); $\lambda_{max(\mu)}^{Nujol}$ 3.10 shoulder, 3.23 (OH, NH_2), 5.89, 6.19, 6.38, 6.60 (NH_2, adenyl), 9.00, 9.10, 9.19, 9.41, 9.55, 9.80 (C–O–C and C–OH); $\lambda_{max(m\mu)}^{pH\ 1}$ 257 (ε 14,600), $\lambda_{max(m\mu)}^{pH\ 7}$ 259 (ε 14,700), $\lambda_{max(m\mu)}^{pH\ 13}$ 261 (ε 14,800). It moved as a single spot, R_{Ad} 0.76 in solvent B and R_{Ad} 1.28 in solvent H.

Anal. Calcd. for $C_{10}H_{14}N_6O_2 \cdot 1/3H_2O$: C, 46.9; H, 5.76; N, 32.8. Found: C, 46.7; H, 5.94; N, 33.1.

3′-Deoxyadenosine (III). A. From 9-[3′-Deoxy-3′-(ethylthio)-5′-O-trityl-β-D-xylofuranosy]-6-(N-tritylamino)-purine[7] (VI).—A solution containing 0.89 g. (1.13 mmoles) of crude VI in 130 ml. of 2-methoxyethanol containing 13 g. of commercial Raney nickel[12] was refluxed with stirring for 6 hr. (protected from moisture). Absolute ethanol (30 ml.) was added and the mixture was filtered through Celite. The Celite and catalyst were washed with hot absolute ethanol (3 × 20 ml.) and the combined filtrate and washings were evaporated to dryness *in vacuo* (35°/0.5 mm.) and again reevaporated from toluene *in vacuo* to afford a pale greenish syrup. This was extracted with boiling 1:1 heptane–benzene (50 ml.) and benzene (50 ml.). The hot extracts were separately filtered and the filtrates were combined and evaporated *in vacuo* to give 1.14 g. of a mixture of crude 9-[3′-deoxy-5′-O-trityl-β-D-ribofuranosyl]-6-(N-tritylamino)-purine and starting material (VI).

A 0.81 g. portion of the above mixture was detritylated by warming in 15 ml. of 80% aqueous acetic acid for 20 minutes on the steam-bath. The hot solution was poured into 90 ml. of hot water and extracted with hot heptane (3 × 100 ml.). The aqueous phase was evaporated to dryness *in vacuo*, affording 0.27 g. of partially crystalline residue. Since direct crystallization from water afforded only impure 3′-deoxyadenosine (m.p. 165–200°), the whole crude product was chromatographed on prewashed seed test paper,[14] using solvent E. Elution of the spot due to the nucleoside II with water at room temperature afforded 45 mg. of pure II, m.p. 130–160° and 176–178° (13% recovery of II).

The 3′-deoxyadenosine spot was similarly eluted, affording 77 mg. of 3′-deoxyadenosine, m.p. 180–190°. Three recrystallizations from water yielded 30 mg. (0.12 mmole) of 3′-deoxyadenosine (III), m.p. 220–222° (11% yield based on VI, 13% yield after correction for recovered II). A final recrystallization from water afforded 25 mg. of III,[34] m.p. 222–224° which was dried for 10 hr. at 100° (0.2 mm.) for analysis. In the infrared it had $\lambda_{max(\mu)}^{KBr}$ 2.96, 3.09 (OH, H_2O, NH_2), 6.07, 6.22, 6.31 (adenyl, H_2O).

Anal. Calcd. for $C_{10}H_{13}N_5O_3 \cdot 1/4H_2O$: C, 47.0; H, 5.32; N, 27.4. Found: C, 46.9; H, 5.51; N, 27.4.

3′-Deoxyadenosine (III) could be distinguished from 2′-deoxyadenosine in solvent E where the R_{Ad} values were 0.80 and 0.69, respectively. 3′-Deoxyadenosine (III) also was chromatographically homogeneous in solvent systems F, G and water, with respective R_{Ad} values of 1.28, 1.14 and 1.51.

(32) Johns-Manville Co. diatomaceous earth.

(33) This picrate apparently decomposes on recrystallization from water, since after two recrystallizations the sample yielded poorer results for C, H and N analyses.

(34) A melting point of 212° was reported by Todd and Ulbricht.[17]

The optical rotation and ultraviolet absorption data were obtained on a chromatographically pure sample of 3'-deoxyadenosine (III), m.p. 223–225°, obtained by method B below. This sample contained more than 1/4 mole of water, as both the elemental analysis and the observed E values suggested. The observed data were $[\alpha]^{27}D$ −42° [1% (supersaturated) in water]; $\lambda_{max(m\mu)}^{pH\ 1}$ 258 (ε 13,800), $\lambda_{max(m\mu)}^{pH\ 13}$ 260 (ε 14,500), $\lambda_{max(m\mu)}^{H_2O}$ 261 (ε 14,400). The value in water was lower than that observed for either adenosine[35] or 2'-deoxyadenosine[7] [$\lambda_{max(m\mu)}$ 260 (ε 14,900)] and indicated for that sample of III an actual molecular wt. of 260 instead of 251; there was, therefore, 0.5 mole of water per mole of 3'-deoxyadenosine in that sample.

B. From the Free 3'-Ethylthio Nucleoside (II).—A mixture of the free 3'-ethylthio nucleoside (II) and 40 g. of freshly prepared Raney nickel "C"[13] (prepared by a slight modification[36]) in 175 ml. of 2-methoxyethanol was refluxed for 6 hr. and then worked up as above to give 1.15 g. of crude product.

This crude material, 1.14 g., was stirred with 20 ml. of water and 5 ml. of chloroform to give a white, crystalline precipitate, which, after filtering, water-washing (4 × 20 ml.) and drying amounted to 0.15 g. of starting material II. By paper chromatography this was found to have about 85% purity, with about 5% of product (III) and 10% of other impurities also present.

The aqueous layer and the water-washes were combined and extracted with chloroform–methanol (4:1) (4 × 50 ml.) and chloroform (4 × 25 ml.). Paper chromatography indicated that at this point the aqueous phase essentially contained only 3'-deoxyadenosine (III). Evaporation of the aqueous solution *in vacuo* gave 0.32 g. of III, m.p. 222–224°, containing about 2–3% of starting material II and no other contaminant. By infrared spectra and paper chromatographic behavior in solvent systems E (R_{Ad} 0.75), F (R_{Ad} 1.28) and G (R_{Ad} 1.14), this sample of III was shown to be identical with that obtained by Procedure A.

Evaporation of the chloroform solutions *in vacuo* gave a total of 434 mg. of a solid residue which was a mixture of 3'-deoxyadenosine (III) and starting material (II). This residue was redissolved in a known volume of 2-methoxyethanol and aliquots were removed for paper chromatography. The results indicated that this mixture consisted of about 75% 3'-deoxyadenosine (III) and 25% starting material (II).

On the basis of these results, the yield of isolated and purified 3'-deoxyadenosine (III) was 23% (corrected for unchanged starting material). The total yield of 3'-deoxyadenosine (III) including that left in the unseparated mixture was 45% (corrected for unchanged starting material)

(35) G. H. Beaven, E. R. Holiday and E. A. Johnson in "Nucleic Acids," Vol. I, ed. by E. Chargaff and J. M. Davidson, Academic Press, Inc., New York, N. Y., 1955, p. 510.

(36) The modification consisted of keeping the temperature below 10°, not only during the addition of the nickel alloy to the sodium hydroxide solution but also during the storage overnight and the subsequent rinsing and seemed to give a more active desulfurization catalyst.

[CONTRIBUTION NO. 657 FROM THE CENTRAL RESEARCH DEPARTMENT, EXPERIMENTAL STATION, E. I. DU PONT DE NEMOURS AND CO., WILMINGTON 98, DEL.]

Syntheses by Free-radical Reactions. XIII. Reactions of Thiyl Radicals with Olefins

BY E. L. JENNER AND R. V. LINDSEY, JR.

RECEIVED NOVEMBER 25, 1960

The oxidation of thiols (RSH) in the presence of butadiene (M) has been found to give a number of different types of products: RSSR, RSMH, RSMSR, RSMMSR, and RSMOH. The course of the reaction depends principally on the oxidizing agent employed. Under some conditions essentially single products are obtained and the reaction can be controlled to give good yields of the additive dimers, RSMMSR. The implications of this study on the mechanism of the oxidation of thiols to disulfides are considered.

In previous studies,[1] it was found that the generation of stoichiometric quantities of free radicals (R·) in the presence of butadiene (M) gave rise to products containing two radicals combined with two molecules of butadiene, *i.e.*, R–M–M–R. Characterization of these products, called "additive dimers," proved valuable in identifying free radicals generated in reaction systems. We have now examined additive dimerizations employing thiols as free radical sources as a means of (1) synthesizing some novel dithio compounds and (2) providing some information on the mechanism of the oxidation of thiols to disulfides.

The oxidation[2] of a mercaptan (RSH) to a disulfide (RSSR), which can be accomplished by a variety of oxidizing agents, may involve the combination of two free radicals, *i.e.*

$$RSH \xrightarrow{-e} RS\cdot$$
$$2RS\cdot \longrightarrow RSSR$$

or may occur by an ionic reaction, *e.g.*

$$RSH \xrightarrow{-2e} RS^{\oplus}$$
$$(\text{or } RSH \xrightarrow{-e} RS\cdot;\ RS\cdot \xrightarrow{-e} RS^{\oplus})$$
$$RS^{\oplus} + RSH \longrightarrow RSSR + H^{\oplus}$$

In the current study, thiols were oxidized in the presence of unsaturated compounds in an effort to capture and identify free-radical intermediates, if formed. The conditions employed differed from those typically used to effect the addition of thiols to olefins in that we employed oxidizing agents (or free-radical generators) in gross quantities as reactants rather than in catalytic amounts.

In most of this work butadiene was employed as olefin. Ethanethiol, thiolacetic acid and mercaptoacetic acid were the thiols studied and hydroxyl radicals (Fenton reagent), ceric salts, ferric chloride, ferric nitrate and potassium ferricyanide were used as oxidizing agents.

It was found that the course of the reaction varied greatly depending principally upon the oxidizing agent employed. Thus, the oxidation of thiols (RSH) in the presence of butadiene as the unsaturated compound (M) gave several types

(1) D. D. Coffman and E. L. Jenner, *J. Am. Chem. Soc.*, **80**, 2872 (1958); D. D. Coffman and H. N. Cripps, *ibid.*, **80**, 2877 and 2880 (1958).

(2) L. Michaelis and M. P. Schubert, *Chem. Revs.*, **22**, 444 (1938); I. Pascal and D. S. Tarbell, *J. Am. Chem. Soc.*, **79**, 6015 (1957); J. J. Bohning and K. Weiss, *ibid.*, **82**, 4724 (1960).