configuration is known. Supporting evidence may be derived from a linear regression treatment of the shift-gradient data.

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Registry No.—**1**, 39707-76-1; **2**, 35784-92-0; **3**, 54797-99-8; **4**, 54307-95-8; **5**, 53438-14-5; **6**, 14133-63-2; **7**, 54307-96-9; **8**, 25018-67-1; Eu(fod)$_3$, 17631-68-4.

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Syntheses of 2-Substituted 1,N^6-Ethenoadenosines[1]

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The syntheses of some 2-substituted (–SH, –SC_2H_5, –SO_3^-, –OH, –$NHNH_2$, –N_3, and –NH_2) 1,N^6-ethenoadenosines are described. The fluorescent properties of the derivatives were affected by the substituents. While substituents –SH, –SC_2H_5, –NH_2, –$NHNH_2$, and –N_3 quenched the fluorescence, substituents –OH and –SO_3^- enhanced the fluorescence.

1,N^6-Ethenoadenosine[1] (ε-adenosine, **1**) is a fluorescent analog of adenosine. The phosphate derivatives of ε-adenosine have been found to be useful substrates in numerous enzyme reactions.[2] However, the fluorescence emission maximum of these derivatives is 410 nm and they are not suitable for cytochemical investigation where either tissues or cells possess autofluorescence in this range. The synthesis of a new fluorescent adenosine analog, 2-aza-ε-adenosine (**3**), that could be useful for such purposes was therefore undertaken in our laboratory. The synthesis of this new compound and its properties have recently been reported.[3] In several instances, the phosphate derivatives of this new fluorescent adenosine analog have been found to be better substrates than the corresponding ε analogs.[4,5] Furthermore, compound **3** was found to be cytotoxic against a mammary tumor cell line.[6] Since the synthetic objectives in our laboratory are to provide, first, fluorescence nucleosides and nucleotides that could be useful probes for protein–oligonucleotide interaction,[7,8] second, fluorescent nucleoside substrates that can be used as histochemical or cytochemical substrates for localizing enzymes at cellular level,[9,10] and third, potential chemotherapeutic agents, the preparation of other 2-substituted ethenoadenosines is therefore of interest. The present paper reports the synthesis and some properties of these new nucleoside derivatives.

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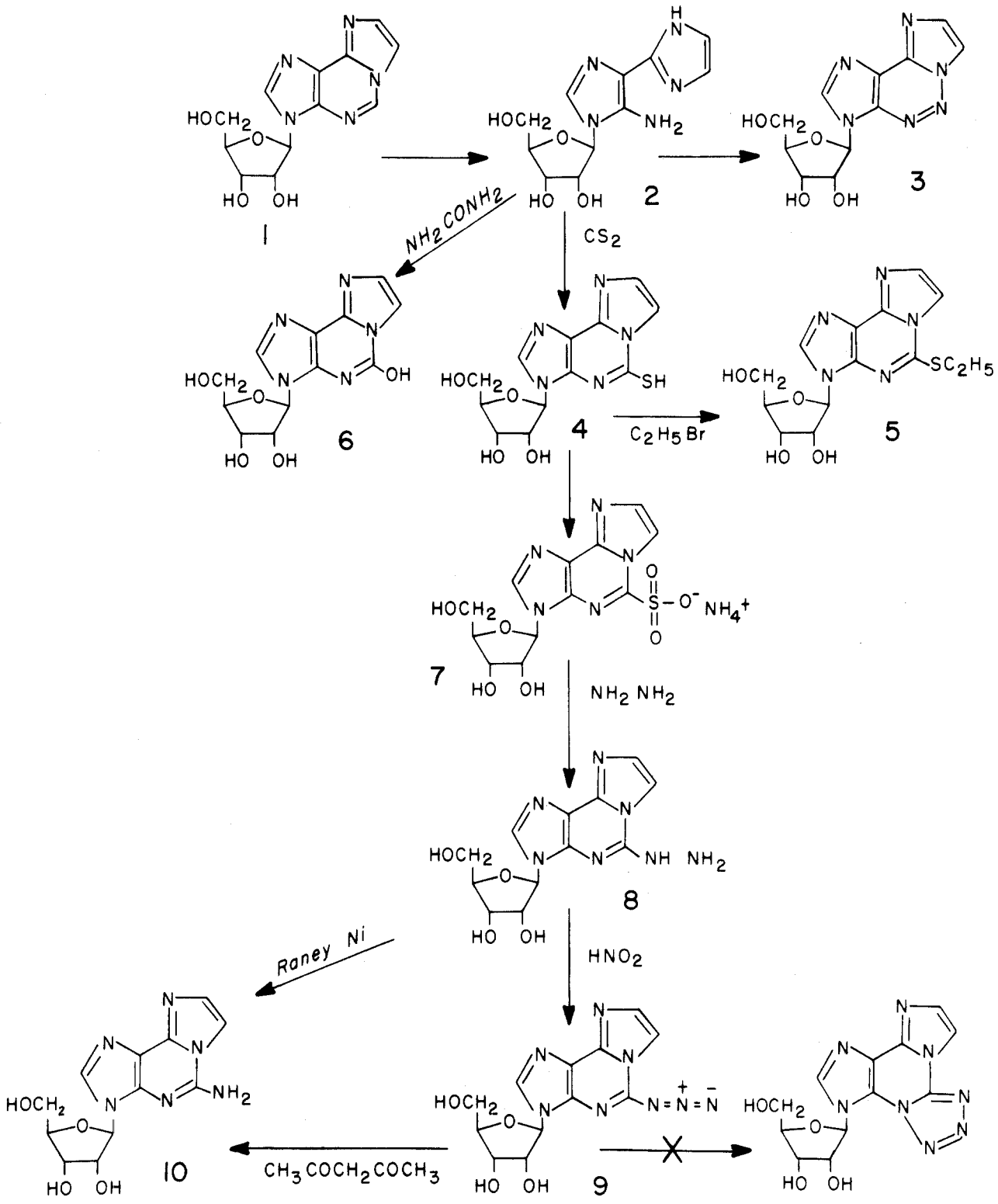
The preparation of **2** was described in our recent paper[6] (Scheme I). Conversion of **2** to 1,N^6-etheno-2-mercaptoadenosine (2-mercapto-ε-A, **4**) was accomplished by carbon disulfide in pyridine. The ultraviolet absorption spectrum of the nucleoside **4** showed a maximum (pH 7) at 317 nm, but when alkylated as in **5**, the uv spectrum (Table I) showed a hypsochromic shift of 35 nm to 282 nm (pH 7). Thus, it is likely that **4** exists predominantly in the thiono form in neutral solution, as in the case of 6-mercaptopurine riboside[11] and 2-mercaptoinosine.[12] When **2** was heated with urea at 150° under nitrogen, 1,N^6-etheno-2-hydroxyadenosine (2-hydroxy-εA, **6**) was isolated as the major product. This compound, however, does exist in the enol form, as its ir spectrum shows no carbonyl absorption. Compound **6** is also a good fluorescent compound with emission maximum at 430 nm and an excitation maximum at 315 nm (Table II). While the mercapto derivative **4** is nonfluorescent, the quantum yield of **6** was 0.68 at pH 5.5. The fluorescence of this compound is unique among all other fluorescent ε-adenosine derivatives. These compounds are usually quenched in acidic solution because of the protonation of the imidazole ring,[1b] but the fluorescence of **6** was quenched at alkaline pH as well as acidic pH (Figure 1). Two pK_a values of 2.40 and 6.75 were found from the titration curve. The low pK_a was due to the protonation of the imidazole ring and the higher pK_a corresponded to the ionization of the phenolic acidic proton, as shown in Scheme

Scheme I



II. Therefore, only the neutral form of **6** fluoresces, and this property should serve as a useful indicator in cytochemical experiments in biological systems.

Compound **5** was prepared by ethylation of **4**. Similar to other 2-alkylthiopurine ribosides, this compound was found to resist nucleophilic substitution reaction.[13] It did not undergo amination reaction and was stable in alkaline solution. In order to facilitate the nucleophilic substitution on the ethylthio group, the conversion of **5** into 2-ethylsulfonyl-ϵ-A was attempted with oxidizing agents, including hydrogen peroxide and *N*-chlorosuccinimide, without success. An alternate route to obtain other 2-substituted compounds from **4** was therefore investigated. It was found that compound **4** was rapidly oxidized with *N*-chlorosuccinimide to yield the ammonium salt of ϵ-adenosine-2-sulfonic acid (ϵ-A-2-sulfonate), **7**, in good yield. The structure of **7** was confirmed by its ir spectrum with an absorption band at 1210 cm^{-1} for the sulfonic acid group. Compound **7** was also found to have a fluorescence emission maximum at 460 nm when excited at 335 nm, and formed an insoluble salt when silver ion was added to a solution of less than 10^{-4} *M*. The fluorescence characteristic as well as the low

Table I
Some Analytical Data on 2-Substituted 1,N^6-Ethenoadenosine

Compd	Name	Mp, °C	R_f value TLC$_1$ S_1	R_f value TLC$_2$ S_2	Uv max, nm (ε) pH 1	pH 7	pH 13	Empirical formula	Calcd/Found C	H	N
4	2-Mercapto-ε-A	225–227 dec		0.55	312 (18,000)	317 (15,200)	318 (14,400)	$C_{12}H_{13}N_5O_4S \cdot H_2O$	42.22 42.29	4.42 4.26	
5	2-Ethylthio-ε-A	186–187	0.50		292 (17,100)	282 (8800)	292 (9350)	$C_{14}H_{17}N_5O_4S$	47.86 47.91	4.88 4.80	19.94 20.01
6	2-Hydroxy-ε-A	194 dec	0.15	0.65	294 (14,200)	292 (13,000)	281 (15,300)	$C_{12}H_{13}N_5O_5 \cdot H_2O$	44.31 43.80	4.54 4.53	21.53 21.86
7	ε-A-2-Sulfonate	223–225		0.70	280[a] (9030)	312[b] (4200)	264[c] (9240)	$C_{12}H_{12}N_5O_7S \cdot NH_4$	37.12 37.12	4.15 4.35	21.65 21.68
8	2-Hydrazino-ε-A	146–148	0.22	0.54	282 (11,100)	280 (11,100)	291 (11,700)	$C_{12}H_{15}N_7O_4 \cdot 1^1/_2H_2O$	41.37 41.19	5.20 5.14	28.14 27.83
9	2-Azido-ε-A	162–164 dec	0.67	0.75	278 (15,400)	283 (7160)	273 (8330)	$C_{12}H_{12}N_8O_4$	43.37 43.28	3.64 3.81	33.73 33.73
10	2-Amino-ε-A	169–171	0.45	0.70	278 (11,500)	283 (10,900)	276 (12,400)	$C_{12}H_{14}N_6O_4 \cdot {}^1/_2H_2O$	45.71 45.77	4.79 5.09	26.66 26.57

[a] Also 233 nm (ε 19,500). [b] Also 270 nm (ε 5250) and 235 (21,000). [c] Also 235 nm (ε 22,000).

Table II
Fluorescence Properties of 2-Substituted 1,N^6-Ethenoadenosines

Compd[a, b]	Excitation max, nm	Emission max, nm	Buffer (pH)	Quantum yield
1	300	415	Phosphate (7.0)	0.56[b]
3	358	495	Citrate (7.0)	0.16
6	315	430	Citrate (5.5)	0.68
7	335	460	Citrate (7.0)	0.69

[a] Compounds 2, 4, 5, 8, 9, and 10 are nonfluorescent. [b] See ref 2.

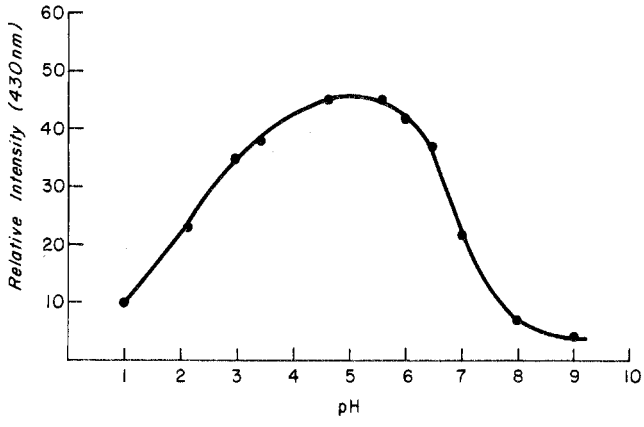


Figure 1. Variation in fluorescent intensity of 6 with pH. Potassium citrate (0.1 *M*) was used as buffer (excitation at 315 nm).

solubility of the silver salt should make 7 a useful probe for our electron cytochemical study. ε-A-2-sulfonate was stable under acidic conditions, but lost fluorescence irreversibly at alkaline pH. The sulfonic acid group was resistant to amination at 50° after treatment with ammonia for 2 days, but when treated with hydrazine, readily converted at room temperature to 1,N^6-etheno-2-hydrazinoadenosine (2-hydrazino-ε-A, 8), in good yield. Compound 8 was nonfluorescent and stable at neutral and acidic pH, but could be oxidized easily in alkaline solution.

When 2-hydrazino-ε-A was treated with nitrous acid, the azide derivative 9 was obtained. Its structure was confirmed by the characteristic ir absorption of the azide group.[14,15] The NMR spectrum of 9 in DMSO revealed only one set of protons, which ruled out any possibility of the tetrazole form (Scheme II). Thus, it is obvious that while other 2-azido purines do rearrange to the tetrazole form, the 2-azido-ε-adenosine exists exclusively in the azido form. 2-Azido-ε-A is only slightly soluble in water and is sensitive to light. The use of this compound for photolabeling is currently under study in our laboratory. As expected, compound 9, when treated with triphenylphosphine, yielded a yellow triphenylphosphine imine. When 9 was allowed to react with acetylacetone in an alcohol solution in the presence of triethylamine,[16] 1,N^6-etheno-2-aminoadenosine (2-amino-ε-A, 10) was obtained in good yield. This novel method of preparing 10 is preferred over the alternate route via Raney nickel reduction of 8. The structure of 10 was confirmed by both NMR and mass spectra.

It is well known that the substituents in the 2 position of purine nucleosides are less reactive than substituents in the

Scheme II

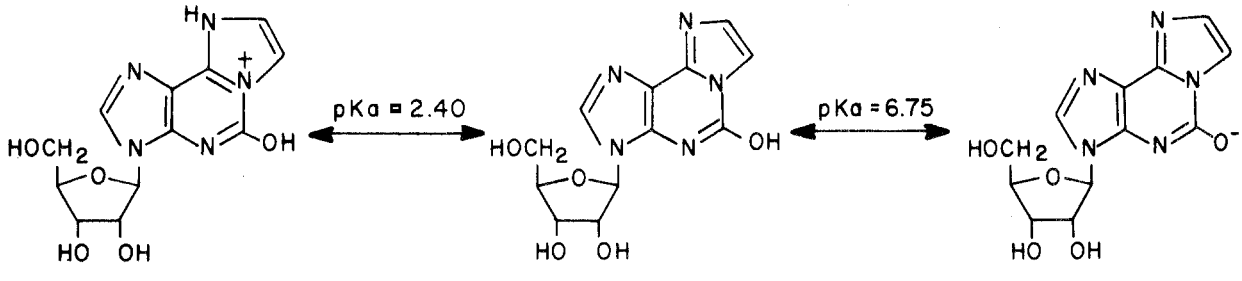
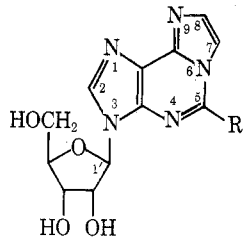


Table III
NMR Chemical Shifts and Coupling Constants of 2-Substituted 1,N^6-Ethenoadenosine[a]



Compd	R	δH_7 ($J_{H_7-H_8}$)	δH_8	δH_2	δH_5	$\delta H_{1'}$ ($J_{H_{1'}-H_{2'}}$)
1	H	8.61 (2.0)	8.20	9.01	9.80	6.18 (5.5)
3	$C^5-R = N$	8.85 (1.8)	8.01	9.13		6.36 (4.5)
4	SH	8.40 (2.0)	7.83	8.43		6.04 (5.0)
5[b]	SC_2H_5	7.83 (1.3)	7.66	8.50		6.10 (5.5)
6	OH	7.75 (2.0)	7.52	8.09		5.87 (5.0)
7	$-SO_3^-NH_4^+$	8.47 (1.5)	7.66	8.70		6.17 (5.5)
8	$-NHNH_2$	7.98 (1.5)	7.44	8.18		6.02 (5.5)
9	$-N_3$	7.71 (1.8)	7.55	8.50		6.00 (5.5)
10	$-NH_2$	7.99 (1.5)	7.53	8.18		5.96 (5.5)

[a] The spectra were measured in a 10% solution of DMSO-d_6 at 38°. [b] Also δ 1.33 (t, J = 7.5 Hz), 3.05 (q, J = 7.5 Hz).

6 position. The present study has shown that the substituent in the 5 position (equivalent to the 2 position of purine) of the 1,N^6-ethenoadenosine is even less reactive than substituents in the 2 position of purine nucleosides. The 2-mercapto, 2-ethylthio, and even ϵ-A-2-sulfonate were resistant to hydrolysis and amination. Only ϵ-A-2-sulfonate reacted with hydrazine successfully. Thus, the etheno ring influence on the 2 position bears no resemblance to the 9,10 position of the phenanthrene ring.

All NMR spectra of the ϵ-adenosine derivative show one singlet and two doublets in the aromatic region for the base protons. The assignment of these signals is listed in Table III. Interestingly, all fluorescent derivatives have a lower field absorption than the nonfluorescent derivatives. The biochemical properties, as well as the chemotherapeutic aspect of these compounds, will be reported later.

Experimental Section

Uv absorption spectra were measured on a Beckman Model DB-G recording spectrophotometer. Fluorescence spectra were determined with an Aminco-Bowman spectrofluorometer. NMR spectra were measured with a Varian NMR spectrometer, Model A-60. Chemical shifts are given in parts per million on a δ scale; coupling constants are expressed in Hz; Me_4Si was used as internal standard. Thin layer chromatography was carried out by the ascending method with Eastman Chromagram Sheets 6065 (cellulose with fluorescent indicators, TLC_1) and 6060 (silica gel with fluorescent indicators, TLC_2). Solvent systems are: S_1, ethanol–ammonium acetate, 1 M (7:3, v/v); S_2, methanol–chloroform (1:2, v/v). Elemental analysis (C, H, N) were performed by Micro-Analysis, Wilmington, Del. Melting points were determined on a Mel-Temp capillary melting point apparatus and are uncorrected.

1,N^6-Etheno-2-ethylthioadenosine (5). To a suspension of 2-mercapto-ϵ-A[6] (650 mg, 2 mmol) and anhydrous potassium carbonate (276 mg, 2 mmol) in DMF (7 ml) was added 150 μl of ethyl bromide (2.2 mmol). The mixture was heated at 55° (oil bath) for 1 hr. Judging by TLC_2 (S_2), all 2-mercapto-ϵ-A (R_f 0.10) had reacted to give a single spot with R_f 0.50. The solvent was removed by evaporation after removal of the inorganic insoluble material by filtration. The residue was crystallized with absolute ethanol to give pure **5** (580 mg, 83%).

1,N^6-Etheno-2-hydroxyadenosine (6). A mixture of 2[6] (1 g, 3.5 mmol) and urea (2 g, 3.3 mmol) was heated in an oil bath (150–160°) under a slow stream of nitrogen for 8 hr. Judging by TLC_2 (S_2), most of **2** had reacted. The reaction mixture was cooled to room temperature and the residue was taken up by 20 ml of water and evaporated to dryness. The residue was washed with ethanol and acetone and finally recrystallized in 20 ml of ethanol to give 0.34 g (31%) of **6**. A small amount of unreacted urea in the product was removed by a silica gel column. The column was first washed with 20% methanol in chloroform to elute the urea and the product was recovered by 40% methanol in chloroform.

Ammonium 1,N^6-Ethenoadenosine-2-sulfonate (7). To a suspension of 2-mercapto-ϵ-A (1 g, 3.1 mmol) in 30 ml of water, N-chlorosuccinimide (1.25 g, 9.3 mmol) was added. The mixture was allowed to stand at 40° for 2 hr. After 1 hr, a clear solution was obtained. The reaction mixture was then neutralized carefully with concentrated ammonium hydroxide. The solid was then washed with 20 ml of absolute ethanol to remove the succinimide. The insoluble residue was again washed with 5 ml of cold water to remove the inorganic salt, and recrystallized in hot water after treatment with charcoal to give 0.6 g of **7** (53%) as a creamy white solid.

1,N^6-Etheno-2-hydrazinoadenosine (8). Compound **7** (1 g, 2.6 mmol) was added slowly to 10 ml of 98% hydrazine with stirring. After the completion of this addition, the brown solution was allowed to stand at room temperature for 15 min. Ice water (15 ml) was then added to this solution and the solution was concentrated with a rotary evaporator. The residue was washed with 5 ml of water to remove the inorganic salt and the product was then dissolved in 60 ml of hot 50% ethanol. Upon standing at 0° overnight, pure **8** crystallized and was filtered and dried to give 0.58 g (69%) as white crystals. It is stable in the solid state, but it can easily be oxidized in alkali or on TLC plates by air to give a blue-colored compound: mass spectrum m/e 174 (B + 1 − NH).

1,N^6-Etheno-2-azidoadenosine (9). To a solution of **8** (200 mg, 0.63 mmol) in 5 ml of acetic acid and 1 ml of water at 0°, sodium nitrite (50 mg, 0.65 mmol) in 1 ml of water was added dropwise. The mixture was allowed to stand at 0° for 15 min and then concentrated to remove the solvents. The last traces of acetic acid were removed by coevaporation with two 5-ml portions of water. The residue was crystallized with 20 ml of 50% ethanol after treatment with charcoal to give pure **9** as needle-like crystals (180 mg, 87%).

1,N^6-Etheno-2-aminoadenosine (10). A. Conversion from 8 with Raney Nickel. A suspension of **8** (640 mg, 2 mmol) in 20 ml of water was treated with 1 ml of Raney nickel (Alfa Inorganics, Inc.). The mixture was heated in a water bath (90–95°) for 3 hr. Judging by TLC_2 (S_2), all of the 2-hydrazine-ϵ-A had reacted. The catalyst was removed by filtration and washed well with hot water. The filtrate was evaporated to dryness and the residue was crystallized in 80% ethanol after being treated with charcoal. Compound **10**, 260 mg (42%), was recovered as a white solid: mass spectrum m/e 306 (parent ion), 174 (B + 1).

B. Conversion from 9 with Acetylacetone. Compound **9** (500 mg, 1.5 mmol) was mixed with 4 ml of acetylacetone, 3 ml of triethylamine, and 4 ml of ethanol. The solid dissolved slowly under stirring. The solution was allowed to stand at room temperature for 30 min. The product precipitated as a white solid. The solvent was re-

moved by rotary evaporator, and the residue was recrystallized with 50% ethanol to give 420 mg (91%) of product that has the same R_f values and uv spectra as **10**.

Registry No.—**1**, 39007-51-7; **2**, 50663-83-7; **3**, 50663-82-6; **4**, 54277-40-6; **5**, 54277-41-7; **6**, 54277-42-8; **7**, 54277-43-9; **8**, 54277-44-0; **9**, 54277-45-1; **10**, 54277-46-2; urea, 57-13-6; *N*-chlorosuccinimide, 128-09-6; hydrazine, 302-01-2; acetylacetone, 123-54-6.

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Reactions of Grignard Reagents with Nitrosamines[1a]

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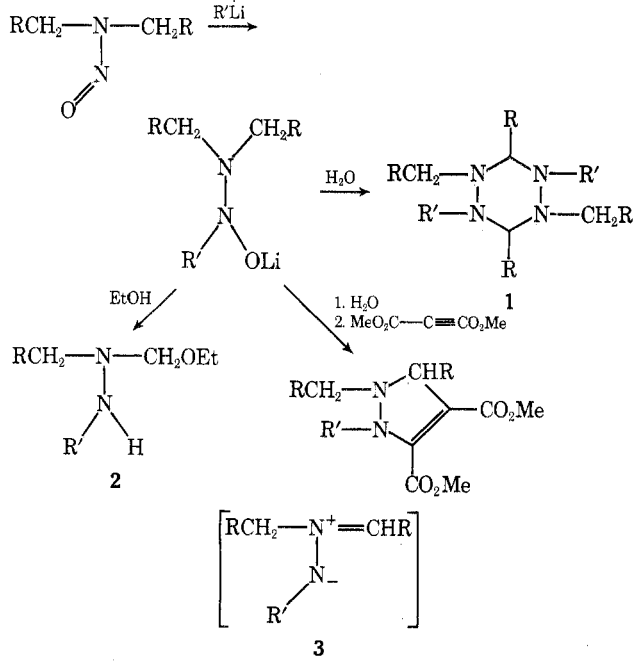
Reaction of aliphatic and alicyclic nitrosamines with excess phenyl-, cyclohexyl-, or *tert*-butylmagnesium halide gave trisubstituted hydrazines resulting from α-carbon and nitroso nitrogen alkylation. Benzylmagnesium chloride and *N*-nitrosodimethylamine gave hydrazones.

In previous reports[2,3] we described the reactions of some nitrosamines[4] with phenyl- and *tert*-butyllithium. This study (Scheme I) demonstrated that nucleophilic attack on the nitroso moiety gave *sym*-hexahydrotetrazines **1**, ethoxymethylhydrazines **2**, and other products, all presumably derived from a dipolar intermediate **3** generated after addition of either water or ethanol to the reaction mixture. The intermediate **3** was readily trapped with dimethyl acetylenedicarboxylate.

Scheme I
Reactions of Nitrosamines with Organolithium Reagents



We have extended our investigation to include reactions of Grignard reagents which serve to complement previous work and offer an expanded view of the reactions of nitrosamines with organometallics.

The earliest studies on the reaction of nitrosamines with Grignard reagents were reported over 60 years ago.[5,6] Wieland and Fressel[5] examined possible routes to the synthesis of hydroxyhydrazines, nitrogen homologs of hydroxylamines, by the condensation of nitrosamines with Grignard reagents. They found, however, that the reaction of ethylmagnesium iodide with diethylnitrosamine (DENA) gave the diethylhydrazone of acetaldehyde. Phenylmagnesium bromide and DENA gave 1,1-diethyl-2-phenylhydrazine and 1-ethyl-1-(α-phenylethyl)-2-phenylhydrazine. It was postulated that the latter product was formed via a diaziridine intermediate, which opened to add an additional mole of Grignard reagent to an α carbon.

Formation of Trisubstituted α-Carbon Substituted Hydrazines. In the present study dimethylnitrosamine (DMNA), diethylnitrosamine (DENA), *N*-nitrosopiperidine (PipNO), and *N*-nitrosopyrrolidine (PyrNO) were treated with cyclohexyl-, *tert*-butyl-, phenyl-, and benzylmagnesium halides. All reactions were run in ether solvent at 0° in an inert atmosphere with reaction times of 1–3 hr.

The addition of an excess of organomagnesium reagent to the nitrosamine gave, after work-up, a trisubstituted hydrazine which had incorporated 2 mol of Grignard reagent, one at a nitroso nitrogen and one at an α carbon of the aliphatic nitrosamine (Table I). Structure assignments were based on NMR and ir analyses. For example, the NMR spectrum of hydrazine **4** displayed singlets at 2.20 (3 H, *N*-methyl) and 3.55 ppm (2 H, *N*-benzyl), an exchangeable proton (NH) at 4.10 ppm, and aromatic multiplets between 6.5 and 7.3 ppm. The ir spectrum displayed an NH stretch at 3.07 μ.

The NMR spectrum of 1-ethyl-1-(α-cyclohexylethyl)-2-cyclohexylhydrazine (**8**) from the reaction between DENA