Synthetic Spectroscopic Models Related to Coenzymes and Base Pairs. VI. The Synthesis of (ω-Arylalkyl)-6,7-dimethylisoalloxazines, Spectroscopic Model Compounds Related to Flavin–Adenine Dinucleotide[1]

NELSON J. LEONARD AND RONALD F. LAMBERT

Department of Chemistry and Chemical Engineering, University of Illinois, Urbana, Illinois 61801

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A general method has been employed for the synthesis of 9-(ω-arylalkyl)-6,7-dimethylisoalloxazines (**1**) in which the flavin and arene nuclei are linked by n = 2, 3, and 6 methylene groups. These compounds serve as spectroscopic models for the study of interactions between the terminal rings as a function of separation and conformation. The following have been prepared: 9-[2-(aden-9-yl)ethyl]-6,7-dimethylisoalloxazine, Fl-C_2-Ad (**1a**, n = 2), 9-[3-(aden-9-yl)propyl]-6,7-dimethylisoalloxazine, Fl-C_3-Ad (**1a**, n = 3), and 9-[6-(aden-9-yl)-hexyl]-6,7-dimethylisoalloxazine, Fl-C_6-Ad (**1a**, n = 6); 6,7-dimethyl-9-[2-(3-indolyl)ethyl]isoalloxazine, Fl-C_2-Ind (**1b**, n = 2), and 6,7-dimethyl-9-[3-(3-indolyl)propyl]isoalloxazine, Fl-C_3-Ind (**1b**, n = 3); trimethylenebis-9,9′-(6,7-dimethylisoalloxazine), Fl-C_3-Fl (**1c**, n = 3), and hexamethylenebis-9,9′-(6,7-dimethylisoalloxazine, Fl-C_6-Fl (**1c**, n = 6); 6,7-dimethyl-9-(3,4,5-trimethoxybenzyl)isoalloxazine, Fl-C_1-TMB (**1d**, n = 1), and 6,7-dimethyl-9-(3,4,5-trimethoxyphenethyl)isoalloxazine, Fl-C_2-TMB (**1d**, n = 2).

In order to study the intramolecular interactions characteristic of flavin–adenine,[2-11] flavin–indole,[5,12-15] flavin–flavin,[5,10] and flavin–trimethoxybenzene pairs, a series of 9-(ω-arylalkyl)-6,7-dimethylisoalloxazine derivatives (**1**)[16] has been prepared, with the flavin and arene nuclei linked by n = 2, 3, and 6 methylene groups. These compounds provide models in which the spectroscopic properties can be directly related to 1:1 interactions between the terminal rings in solutions that are sufficiently dilute.[1] It was considered that observation of the electronic and fluorescence spectra would aid in the understanding of the interactions of flavin and arenes in general[17-20] and of the isoalloxazine[21] and adenine portions of flavin–adenine dinucleotide (FAD)[16] in particular. The concept of linking arene nuclei in proximity for examination of actual 1:1 interaction, *i.e.*, intramolecular association, without undue electronic and steric distortion, has been utilized in other situations to assess the geometric constraints placed on electronic excitation energy transfer.[22-28] Moreover, a single trimethylene bridge has been found sufficient to provide the molecular configuration conducive to eximer formation in 1,3-diphenylpropane,[29] to intramolecular association of pyridinyl diradicals,[30] and to maximum base–base interaction, in aqueous solution, in analogs of NAD^+[1,31a] and dinucleotides.[1,31b] The trimethylene bridge permits the terminal rings to lie in parallel or near-parallel planes. In the models **1a–d** (n = 3) the ring positions of attachment of the trimethylene bridge automatically limit any plane-parallel conformations to *syn* varieties. Thus, by comparing the spectroscopic behavior of Fl-C_3-Ad (**1a**, n = 3) with that of the related natural compound, *e.g.*, FAD, it may be possible either to accept or to exclude certain conformations of the latter. As examples, synthetic Fl-C_3-Ad (**1a**, n = 3) can assume the folded FAD conformation favored by Sarma, Dannies, and Kaplan,[10] but not those favored by Miles and Urry,[11] by Tsibris, McCormick, and Wright,[7] or by Song.[32] Moreover, synthetic Fl-C_3-Fl (**1c**, n = 3) is prevented from existing in the *anti* conformation, with the two isoalloxazine moieties inverted, which was suggested as the likely arrangement for intermolecular complexing of these moieties with increasing concentration of FAD and FMN.[10] Also synthesized were compounds of type **1a–d** having n = 2, where the shorter (ethylene) chain does not permit the flavin and arene nuclei to lie in parallel planes, and n = 6, where the tendency for intramolecular ring interaction may be balanced, or overbalanced, by the increased flexibility of the longer chain.

Before the desired spectroscopic models **1a–d** were synthesized efficiently, certain exploratory reactions

(1) For earlier papers in this series, see (a) N. J. Leonard, T. G. Scott, and P. C. Huang, *J. Amer. Chem. Soc.*, **89**, 7137 (1967); (b) D. T. Browne, J. Eisinger, and N. J. Leonard, *ibid.*, **90**, 7302 (1968); (c) N. J. Leonard, K. Golankiewicz, R. S. McCredie, S. M. Johnson, and I. C. Paul, *ibid.*, in press; (d) N. J. Leonard, H. Iwamura and J. Eisinger, *Proc. Nat. Acad. Sci. U. S.*, in press; T. G. Scott, R. D. Spencer, N. J. Leonard, and G. Weber, *J. Amer. Chem. Soc.*, in press.

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(9) G. Tollin, *ibid.*, **7**, 1720 (1968).

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(11) D. W. Miles and D. W. Urry, *ibid.*, **7**, 2791 (1968).

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(14) V. Massey and H. Ganther, *Biochemistry*, **4**, 1161 (1965).

(15) I. Isenberg, A. Szent-Györgyi, and S. L. Baird, Jr., *Proc. Nat. Acad. Sci. U. S.*, **46**, 1307 (1960).

(16) Abbreviations: Fl-C_n-Ad (**1a**), Fl-C_n-Ind (**1b**), Fl-C_n-Fl (**1c**), Fl-C_n-TMB (**1d**), FAD (flavin–adenine dinucleotide), NAD^+ (nicotinamide–adenine dinucleotide).

(17) K. Yagi, T. Ozawa, and K. Okuda, *Biochim. Biophys. Acta*, **35**, 102 (1959).

(18) K. Yagi, T. Ozawa, and T. Nagatzu, *Nature*, **184**, 982 (1959).

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(30) M. Itoh and E. M. Kosower, *J. Amer. Chem. Soc.*, **89**, 3655 (1967).

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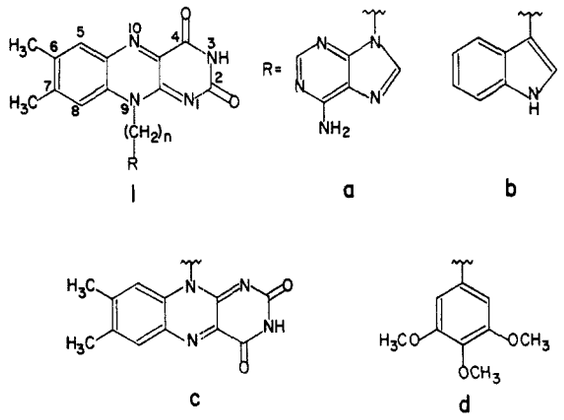
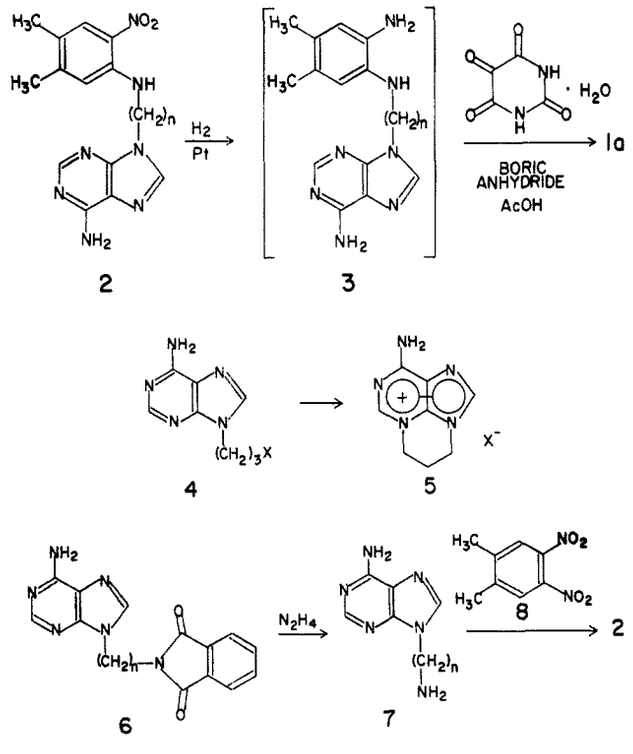


were run and then either discarded or utilized depending upon the individual results. Intially, attention was focused on preparing the FAD analog **1a** (n = 3) *via* an intermediate such as **2** (n = 3), which could then be reduced to **3** (n = 3), and converted into 9-[3-(aden-9-yl)-propyl]-6,7-dimethylisoalloxazine, Fl-C_3-Ad (**1a**, n = 3), by the method of Kuhn, Weygand, and Möller.[33] An approach to **2** (n = 3) *via* the appropriate 9-haloalkyladenine, obtainable by the treatment of adenine in dimethylformamide with sodium hydride and the alkylene dihalide,[1a,31a,34,35] proceeded as far as 9-(3-chloropropyl)adenine[36] and 9-(3-bromopropyl)adenine (**4**), but failed when facile intramolecular cyclization to the 3,9-cyclotrimethyleneadenine halide (**5**)[1b,31a,37] supervened over attempted alkylation of 6-nitro-N-(*p*-tolylsulfonyl)-3,4-xylidine[38] using **4**. Alkylation of 3,4-xylidine and of 3,5-dimethoxyaniline with 9-(3-chloropropyl)adenine in the solid melt proceeded to give 9-[3-(3,4-dimethylanilino)propyl]adenine and 9-[3-(3,5-dimethoxyanilino)propyl]adenine, respectively, but these intermediates failed in reaction with violuric acid in an attempted isoalloxazine synthesis following the method of Hemmerich, Prijs, and Erlenmeyer.[39]



Finally, synthetic difficulties were circumvented by utilizing the 9-(ω-aminoalkyl)adenines (**7**), which were obtainable by treatment of the sodium salt of adenine with N-(ω-bromoalkyl)phthalimides to give the corresponding 9-(ω-phthalimidoalkyl)adenines (**6**), followed by removal of the phthaloyl group with hydrazine in the usual manner. In the employment of a slight modification of published procedure,[40-42] 9-(3-aminopropyl)adenine (**7**, n = 3) readily displaced a nitro group in *o*-dinitrobenzene. 9-[3-(2-Nitroanilino)propyl]adenine precipitated directly from the reaction mixture when isopropyl alcohol was used as the solvent. Controls were run on the successive steps leading from an intermediate of type **2** through **3** to **1a**. Thus, refluxing alloxan monohydrate and 9-benzyladenine in ethanol or acetic acid during 24 hr and subjection of 9-benzyladenine to hydrogenation in ethanol–acetic acid in the presence of platinum oxide at 3 atm for 24 hr resulted in no change in the adenine moiety. Hydrogenation of N-benzyl-2-nitroaniline in ethanol with platinum oxide, followed by condensation of the intermediate with alloxan gave the expected 9-benzylisoalloxazine. Under identical conditions, the intermediate 9-[3-(2-nitroanilino)propyl]adenine was converted into the dedimethyl FAD analog 9-[3-(2-(aden-9-yl)propyl]isoalloxazine, the spectral properties of which were consistent with the expected structure. The method was then applied to combinations of **7** with **8**, 4,5-dimethyl-1,2-dinitrobenzene, which was obtained by oxidation of commercially available 6-nitro-3,4-xylidine with 30% hydrogen peroxide in acetic acid.[43,44]

In outline, the general synthetic method (**2** → **3** → **1a**) was used in preparing 9-[2-(aden-9-yl)ethyl]-6,7-dimethylisoalloxazine, Fl-C_2-Ad (**1a**, n = 2), 9-[3-(aden-9-yl)propyl]-6,7-dimethylisoalloxazine, Fl-C_3-Ad (**1a**, n = 3), and 9-[6-(aden-9-yl)hexyl]-6,7-dimethylisoalloxazine, Fl-C_6-Ad (**1a**, n = 6). Starting with tryptamine and homotryptamine, the corresponding indolyl compounds, 6,7-dimethyl-9-[2-(3-indolyl)ethyl]-isoalloxazine, Fl-C_2-Ind (**1b**, n = 2), and 6,7-dimethyl-9-[3-(3-indolyl)propyl]isoalloxazine, Fl-C_3-Ind (**1b**, n = 3), were prepared, and from 3,4,5-trimethoxybenzylamine and 3,4,5-trimethoxyphenethylamine, 6,7-dimethyl-9-(3,4,5-trimethoxybenzyl)isoalloxazine, Fl-C_1-TMB (**1d**, n = 1), and 6,7-dimethyl-9-(3,4,5-trimethoxyphenethyl)isoalloxazine, Fl-C_2-TMB (**1d**, n = 2), respectively.

(33) R. Kuhn, F. Weygand, and E. F. Möller, *Chem. Ber.* **76**, 1044 (1943).
(34) K. L. Carraway, Ph.D. Thesis, University of Illinois, 1966.
(35) N. J. Leonard, K. L. Carraway, and J. P. Helgeson, *J. Heterocycl. Chem.*, **2**, 291 (1965).
(36) J. H. Lister and G. M. Timmis, *J. Chem. Soc.*, 327 (1960).
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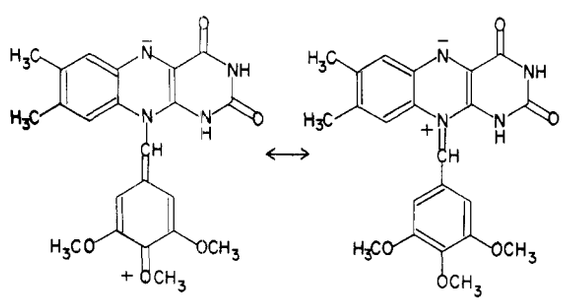
The quantitative electronic absorption data for the flavin precursors of general structure **2** and related compounds were taken in 95% ethanol and are tabulated in the Experimental Section. In no case was a new transition, not characteristic of either aromatic moiety alone, detected.

An interesting observation was made during the final step of the synthesis of the Fl-C_1-TMB (**1d**, $n = 1$) and Fl-C_2-Ad (**1a**, $n = 2$) derivatives. In addition to the desired product a minor amount of lumichrome (6,7-dimethylalloxazine) was also obtained. The formation of lumichrome was probably either the result of a photolytic cleavage of the product or a hydrogenolytic dealkylation of the intermediate in synthesis (*e.g.*, **2**, $n = 2$). During subsequent handling an increased photolability relative to 6,7-dimethyl-9-propylisoalloxazine (**1**, R=CH_3, $n = 2$) was observed for the Fl-C_1-TMB. Since this cannot be ascribed to an ineffective intramolecular quenching, one possible explanation might lie in the formation of the reactive tautomer of Fl-C_1-TMB, *e.g.*, **9** (only two canonical resonance structures shown) which would be expected to display an increased propensity toward hydrolytic cleavage.



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The modified Kuhn and Weygand[33,40,42] isoalloxazine synthesis also proved useful for the preparation of the bisflavin derivatives. Reaction of 2 equiv of 4,5-dimethyl-1,2-dinitrobenzene (**8**) with trimethylenediamine and with hexamethylenediamine yielded the precursors, trimethylenebis-N,N′-(4,5-dimethyl-2-nitroaniline) (**10**, $n = 3$) and hexamethylenebis-N,N′-(4,5-dimethyl-2-nitroaniline) (**10**, $n = 6$), respectively, which were characterized satisfactorily and converted *via* catalytic hydrogenation in methanol–acetic acid into intermediates **11** ($n = 3, 6$) and thence by alloxan condensation into trimethylenebis-9,9′-(6,7-dimethylisoalloxazine), Fl-C_3-Fl (**1c**, $n = 3$), and hexamethylenebis-9,9′-(6,7-dimethylisoalloxazine), Fl-C_6-Fl (**1c**, $n = 6$). Analytically pure material was obtained by eluting the bisflavin compounds (**1c**) from a silica gel column with anhydrous formic acid–chloroform. A modification of the same chromatographic procedure was utilized in the final purification of all of the flavin derivatives in order to render them adequate specimens for mass spectrographic analysis. The reluctance of these materials to accept solvent precluded reliance on thin layer and paper chromatography as sufficient purity criteria, since a controlled spectrum of R_f values could not be achieved. Difficulty in obtaining satisfactory elemental microanalyses for flavin derivatives is a well-known problem.[33,45,46] Nevertheless, once techniques

(45) P. Hemmerich, B. Prijs, and H. Erlenmeyer, *Helv. Chim. Acta*, **42**, 2164 (1959).

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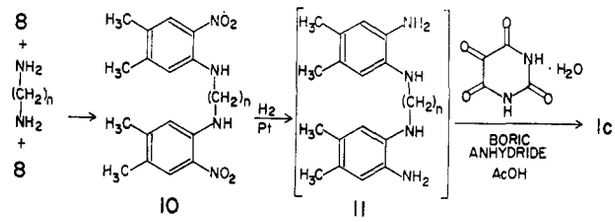

10 **11**

were established for obtaining the materials free of traces of "bound water," satisfactory elemental analyses were obtained.[47] Mass spectrometry was used to confirm the final structures (**1a–d**) and to indicate the absence of any contamination. The electronic spectroscopy (*e.g.*, hypochromism) and the fluorescence emission and quenching behavior of these compounds will be considered in detail in a separate publication.[48,49] Of particular interest is the comparison of the relative quantum yields with the relative excited state lifetimes for compounds **1a–d** *vs.* their composite flavin and arene moieties, all in propylene glycol solution. This comparison has provided information concerning dynamic quenching as opposed to quenching by intramolecular complex formation. In general, the extent of intramolecular interaction has been shown to depend upon flavin–arene proximity.

Experimental Section[50]

Optical Measurements.—For quantitative measurements in propylene glycol a specified amount of material weighed to the nearest 0.01 mg in an aluminum boat was placed inside a 100-ml "low actinic" volumetric flask. Then 20 ml of propylene glycol (purified) was added and the mixture heated on a steam bath with constant agitation until a homogeneous solution resulted. To the solution at room temperature was added additional glycol to give the desired 3.00×10^{-5} M concentration. The small deviations between the specified and actual material weight were always positive in order to allow the small volume correction to be made by addition of solvent to the flask. The electronic absorption spectrum at this concentration was determined in 5-mm NIR quartz cells. A portion of this solution was diluted 1:1 by transfer to a 50-ml low actinic volumetric flask containing exactly 25 ml of propylene glycol. The electronic absorption spectrum of this solution, 1.5×10^{-5} M, recorded in 10-mm silica cells was shown to be identical with that previously recorded. This solution was also shown to obey Beer's law in 20- and 50-mm cells on appropriate dilution.

Nmr Spectra.—All nmr spectra reported herein were determined (R. F. Lambert) on a Varian Associates A-60 spectrometer using TMS as an internal standard. Routine spectra for the most part were determined in standard solvents. Solubility problems,

(47) The authors wish to express their appreciation to Mr. Josef Nemeth and his staff for the special care exercised in both the handling and drying of these samples for microanalysis, for weighing samples for quantitative measurements, and for the osmometric molecular weight determinations.

(48) The results were presented in preliminary form at the Seventh International Congress of Biochemistry, Tokyo, Aug 19–25, 1967 [R. F. Lambert, G. Weber, and N. J. Leonard, paper F-8., no. 7].

(49) R. F. Lambert, Ph.D. Thesis, University of Illinois, 1967.

(50) Melting points are corrected. Electronic absorption spectra were recorded on a Cary Model 15 spectrophotometer. Nuclear magnetic resonance spectra were determined on a Varian A-60 spectrometer. Infrared spectra were determined on a Perkin-Elmer Model 521 spectrophotometer. Molecular weights are determined by mass spectrometry unless otherwise indicated. Mass spectra were determined using an Atlas CH-4 low resolution spectrometer. The authors wish to thank J. Wrona for running the mass spectra and Ester Ming-See Yang for graphing the results.

Normal laboratory lighting was filtered through Rohm and Haas red transparent Plexiglas sheets, 3 mm thick, opaque below 550 nm, during treatment of isoalloxazine derivatives, unless otherwise indicated. Standard laboratory glassware was purchased from Corning Glass (low actinic). Special equipment and chromatography columns were rendered impermeable to uv light by dyeing the Pyrex glass with an inorganic amber stain (no. 2747) generously supplied by B. F. Drakefeld and Co., Inc., 45–47 Park Place, New York 7, N. Y.

TABLE I

QUANTITATIVE ELECTRONIC ABSORPTION DATA IN 95% ETHANOL[a]

	Neutral				0.1 *N* HCl				0.1 *N* NaOH			
Compound	λ_{max}	ϵ	λ_{min}	ϵ	λ_{max}	ϵ	λ_{min}	ϵ	λ_{max}	ϵ	λ_{min}	ϵ
Ad-C_3-CN	2620	14,200	2260	1,400	2600	14,300	2300	2,500	2600	14,600	2378	5,300
Ad-C_6-NH_2	2620	14,000	2270	4,199	2590	14,600	2335	4,700	2620	14,600	2370	5,200
Ad-C_3-Phth	2950 sh	3,400			2950 sh	3,400			2950 sh	1,786		
	2620	15,800	2470	11,500	2600	15,900	2470	11,600	2600	17,300	2400	11,300
	2340	16,200	2380	14,900	2430	16,500	2390	15,200				
	2320	18,500			2320	19,800						
Ad-C_3-NAn	4250	6,200	3250	2,900	4220	5,900	3250	2,900	4250	6,200	3250	5,900
	2595	18,200	2470	16,550	2575	18,200	2470	16,800	2575	18,500	2470	17,600
	2350	21,400	2270	14,300	2350	22,600	2270	18,200	2350 sh			
DMNAn-C_3	4430	6,300	3430	300	4430	6,400	3430	400	4430	6,600	3430	600
	2935	6,100	2725	3,000	2935	6,600	2725	3,200	2935	7,000	2725	4,800
	2370	24,200	2120	5,400	2370	24,600	2120	5,400	2350 sh	27,000		
DMNAn-C_2-Ad	4300	5,700	3400	300	4300	5,800	3400	600	4300	5,800	3400	600
	2900 sh	5,700			2900 sh	6,700			2950 sh	6,400		
	2600	18,600	2520	16,000	2575	20,000	2520	19,000	2600	19,500	2520	18,700
	2380	23,000	2225	14,000	2370	25,400	2225	16,600	2350 sh	26,900		
DMNAn-C_3-Ad	4350	6,360	3450	510	4350	6,000	3450	500	4380	6,900	3450	1,000
	2930 sh	6,400			2930 sh	6,700			2900 sh	7,400		
	2600	19,400	2510	17,000	2580	14,400	2510	17,000	2590	21,000	2520	20,300
	2380	26,800	2230	16,500	2380	27,100	2240	17,900	2350 sh	29,900		
DMNAn-C_6-Ad	4900	6,400	3450	380	4400	6,400	3450	380	4400	6,400	3450	750
	2925 sh	6,400			2950 sh	6,400			2925 sh	7,000		
	2570	19,700	2530	19,000	2560	19,900	2530	19,000	2570	20,500	2530	19,900
	2380	28,300	2240	15,900	2380	28,300	2250	19,700	2350 sh	30,700		
NAn-C_3-NAn	4250	12,200	3250	6,100	4250	15,900	3250	1,500	4250	12,500	3250	1,500
	2800	9,200	2675	7,300	2800	11,600	2660	8,900	2800	10,700	2660	8,900
	2320	42,500	2000		2320	46,200			2350 sh	47,000		
DMNAn-C_2-DMNAn	4350	10,000	3450	1,200	4350	10,300	3450	1,500	4400	10,300	3450	2,300
	2920	11,200	2700	7,600	2930	11,800	2730	7,700	2930	12,400	2740	8,800
	2370	36,300	2100	11,800	2380	37,500	2120	11,900	2350 sh	40,500		
DMNAn-C_3-DMNAn	4350	11,800	3400	400	4400	11,400	3950	800	4400	10,800	3450	1,700
	2920	11,400	2730	6,100	2930	11,900	2730	6,300	2930	12,500	2740	7,050
	2370	44,900	2120	9,700	2370	44,000	2120	7,800	2350	47,400		
DMNAn-C_6-DMNAn	4420	13,600	3470	200	4420	13,500	3430	200	4450	13,100	3450	300
	2940	14,800	2730	8,600	2925	14,100	2740	7,800	2950	14,400	2730	8,800
	2365	49,200	2130	7,000	2370	49,600	2140	5,700	2350 sh	24,200		
DMNAn-C_1-TMB	4350	7,200	3400	1,400	4350	7,900	3400	1,900	4350	8,200	3400	2,100
	2920	7,900	2730	6,300	2900	8,400	2730	6,700	2950	2,100	2730	7,500
	2370	34,900	2220	25,000	2350	38,300	2200	28,700	2350 sh	40,300		
DMNAn-C_6-TMB	4400	5,400	3450	600	4430	5,400	3450	600	4430	5,800	3450	1,200
	2920	5,600	2750	4,000	2950	5,800	2750	4,000	2930	6,600	2750	5,200
	2370	27,900	2200	17,900	2370	28,000	2200	17,000				
DMNAn-C_2-Ind	4450	6,500	3450	700	4450	6,500	3450	700	4450	6,800	3450	1,300
	2910	11,600	2870	10,900	2910	11,900	2870	11,000	2910	13,100	2870	11,900
	2830	11,500	2650	9,700	2830	11,600	2650	9,800	2370 sh	33,200	2625	5,800
	2370 sh	28,700			2370 sh	27,200						
	2240?	42,900	2100	23,000	2250?	43,100	2100	23,500				
DMNAn-C_3-Ind	4425	6,900	3400	600	4425	6,900	3400	600	4450	7,400	3400	130
	2920	11,900	2870	10,600	2920	11,900	2870	10,600	2950	12,500	2870	11,900
	2840	9,900	2625	7,800	2840	9,990	2625	7,800	2890	6,900	2625	5,800
	2370 sh	27,300			2370 sh	27,300			2370 sh	16,000		
	2240 ?	46,200	2100	23,800	2240 ?	46,200	2100	23,800				

[a] Abbreviations: λ, wavelength in Å; ε, molar extinction coefficient; Ad-C_n, ω-(aden-9-yl)alkyl-; An, anilino-; NAn, 2-nitroanilino-; DMNAn, 4,5-dimethyl-2-nitroanilino-, TMB, 3,4,5-trimethoxybenzene; Ind, indolyl-; Phth, N-phthalimido.

however, often dictated utilization of somewhat more imaginative conditions. It was found that a mixture of $CDCl_3$ and TFA was superior to either solvent alone and permitted the ready identification of N–H protons in addition to improving the spectral resolution. For 9-substituted adenine derivatives, dichlorotetrafluoroacetone deuterate proved most satisfactory.[51] Unfortunately, the limited solubility of many flavin–arene pairs precluded determination of their nmr spectra.

Chromatography.—Homogeneity of the flavin–arene preparations were established in the following manner: ascending paper chromatography in butanol–formic acid–water (6:2:2), in butanol–pyridine–ammonium hydroxide (6:2:2), and in butanol–formic acid–chloroform (4:3:3); thin layer chromatography on silica gel (Eastman chromagram sheets) in anhydrous formic acid–chloroform media (1:4), in glacial acetic acid–methanol media (3:7), and in ethanol–chloroform–anhydrous formic acid media (5:4:1).

Ultraviolet Absorption Spectra of Intermediates.—Spectral evidence was used to follow progress in the synthetic procedures outlined below and to obtain confirmation that each reaction had proceeded satisfactorily. The ultraviolet maxima and minima of the intermediates are assembled in Table I.

9-Benzylisoalloxazine.—A stirred ethanolic solution of 1.68 g (0.01 mol) of *o*-dinitrobenzene and 2.14 g (0.02 mol) of benzyl-

(51) We wish to thank Dr. William Middleton, Central Research Department, Experimental Station, E. I. du Pont de Nemours and Co., for a generous gift of dichlorotetrafluoroacetone.

amine was heated under reflux for 1 hr under nitrogen. On cooling, N-benzyl-2-nitroaniline separated as orange crystals, which were purified further either by recrystallization from isopropyl alcohol or by chromatography on alumina, yield 1.58 g (69%), mp 74–75°. The simplified nmr spectrum ($CDCl_3$) showed signals at τ 1.88, 2.02 (2 H, doublets superimposed on a broad resonance, ArC_5H, NH); 2.79 (5 H, singlet, C_6H_5); 2.87, 3.24, 3.38, 3.49, 3.62 (major signals, total area 3, ArH); 3.55 (2 H, doublet, CH_2). A mixture of 458 mg (2 mmol) of pure N-benzyl-2-nitroaniline and 5 mg of platinum oxide in 50 ml of 95% ethanol was hydrogenated at 3 atm at 23° for 1 hr in a Parr apparatus. The colorless solution was filtered free of catalyst in an inert atmosphere, and the solvent was evaporated under nitrogen to an oil. Then 50 ml of glacial acetic acid, 336 mg (2.1 mmol) of alloxan monohydrate and 248 mg (3.56 mmol) of boric anhydride were introduced, and the slurry was warmed at 60° for 10 min with constant agitation on a steam bath. The solvent was evaporated *in vacuo*, the solids were treated with methanol, which was then removed on the rotary evaporator. This step was repeated to assure removal of the boric acid. The crude solid was then recrystallized from a 5% acetic acid solution in portions and finally from an acetic acid–methanol–water solution (1:2:7) as a bright yellow-green solid, mp 326° dec, homogeneous on tlc, yield 410 mg (67%). The qualitative electronic absorption spectrum showed $\lambda_{max}^{5\%\ AcOH}$ 437, 337, 268 nm (reported[52] for 9-methylisoalloxazine: 440, 335, 268 nm). The nmr spectrum (DMSO) showed signals at τ −1.85 (1 H, br, CONHCO); 1.35–2.52 (major signals at 1.48, 1.85, 2.25, total area 9, ArH); 3.66 (2 H, s, CH_2).

Anal. Calcd for $C_{17}H_{12}N_4O_2$: C, 67.10; H, 3.97; N, 18.41. Found: C, 66.58; H, 4.04; N, 18.48.

4,5-Dimethyl-1,2-dinitrobenzene (8).[44]—To 8.31 g (0.05 mol) of 4,5-dimethyl-2-nitroaniline was added 300 ml of glacial acetic acid, 90 ml of 30% hydrogen peroxide, and 6.0 ml of sulfuric acid. The mixture was stirred for 5 hr at 65–70°, 150 ml of acetic acid and 45 ml of peroxide were added, and heating was continued overnight.[43] The solution was diluted with water, and the precipitated dimethyldinitrobenzene was filtered, washed with water, and recrystallized from isopropyl alcohol–water as colorless needles, mp 118–119° (lit.[53] 118°), 4.32 g. Further dilution of the cooled filtrate afforded a second crop, recrystallized as above: 1.98 g (in all 64%); nmr ($CDCl_3$) τ 2.30 (2 H, ArH); 7.58 (6 H, CH_3). Purity was important at this stage because purification became more difficult at later stages.

Anal. Calcd for $C_8H_8N_2O_4$: C, 48.98; H, 4.11; N, 14.28. Found: C, 48.87; H, 4.22; N, 14.22.

4,5-Dimethyl-2-nitro-N-propylaniline.—To a magnetically stirred solution of 0.56 g (29 mmol) of 4,5-dimethyl-1,2-dinitrobenzene in 50 ml of isopropyl alcohol was added 1.93 g (31 mmol) of *n*-propylamine, and the solution was heated at reflux for 8 hr under nitrogen. The orange solid which separated was washed with cold isopropyl alcohol and recrystallized from isopropyl alcohol as orange leaflets: mp 66–66.5°; 585 mg (98%); nmr ($CDCl_3$) τ 2.14 (2 H, broad base, s, C_3H superimposed on NH); 3.36 (1 H, s, C_6H); 6.75 (2 H, q, J = 7.5 ± 0.5 cps, NCH_2); 7.76 (3 H, s, C_5CH_3); 7.84 (3 H, s, C_4CH_3); 8.34 (2 H, p, J = 7.5 ± 0.5 cps, NCH_2CH_2); 8.93 (3 H, uneven, CH_2CH_3).

Anal. Calcd for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.52; H, 7.87, N, 13.52.

6,7-Dimethyl-9-propylisoalloxazine (1, R = CH_3, n = 2).—A solution of 1.196 g (5.65 mmol) of 4,5-dimethyl-2-nitro-N-propylaniline in 80 ml of 95% ethanol was hydrogenated at 3 atm and 20° in the presence of 25 mg of platinum oxide during 2 hr. The colorless solution was filtered free of catalyst, under nitrogen, into an amber-stained round-bottomed flask and the solvent was evaporated under reduced pressure. Then 50 ml of glacial acetic acid, 910 mg (5.65 mmol) of alloxan monohydrate, and 785 mg (11.3 mmol) of boric anhydride were introduced and the slurry was warmed at 60° for 15 min under nitrogen, with constant agitation. An orange solid separated on cooling. The reaction mixture was diluted with 100 ml of water and heated under reflux until a clear brown solution resulted. The orange needles which deposited on standing were recrystallized from 30% acetic acid: yellow flakes, homogeneous by tlc (silica gel) and paper chromatography; mp 332–334°; yield 1.17 g (73%); λ_{max}^{MeOH} (0.218 *N* AcOH) 443 nm (ϵ 12,200), 350 (8,350), 267 (34,400); λ_{min} 385 (4,450), 300 (1,940); $\lambda_{max}^{pH\ 7}$ (0.05 *M* phosphate buffer) 445 (16,700), 368 (13,850), 266 (38,000); λ_{min} 395 (10,000), 303 (4,300), 242 (11,200); nmr ($CDCl_3$, TFA) τ 1.85, 2.25 (2 H, s, s, $C_{5,8}H$); 5.21 (2 H, uneven t, NCH_2); 7.34, 7.48 (6 H, s, s, $FlCH_3$); 8.0 (2 H, br, CH_2CH_3); 9.03 (uneven t, 3 H, CH_2CH_3). The mass spectrum showed the following major fragments: *m/e* 284 (M^+), 269 ($FlCH_2CH_2^+$), 242 (base peak, FlH^+).

Anal. Calcd for $C_{15}H_{16}N_4O_2$ (284.3): C, 63.37; H, 5.67; N, 19.71. Found: C, 63.25; H, 5.69; N, 19.99.

9-(3-Bromopropyl)adenine.—To a slurry of 25 g (0.19 mol) of adenine in 350 ml of dry dimethylformamide stirred under nitrogen was added in 0.5-g portions a total of 4.56 g (0.19 mol) of pentane-washed sodium hydride.[34,35] The solution was stirred at room temperature for 1 hr and was then transferred under nitrogen to a 500-ml-pressure equalized dropping funnel. The sodium salt suspension was added slowly to a large excess of trimethylene dibromide with vigorous stirring. After an additional 4 hr, the mixture was poured into 3 l. of ether. After 8 hr the precipitate was filtered and washed with ether and then with water to remove the 9,3-cyclized salt.[31a] The crude solid was recrystallized from water and dried *in vacuo*, yield 4.1 g (8%) of compound **4**, X = Br, homogeneous on tlc (silica gel) with uv and nmr spectra identical with those of a sample of 9-(3-chloropropyl)adenine prepared by Huang.[31a] The compound underwent conversion to the cyclized derivative **5**, X = Br, above 120°, with decomposition above 330° without melting.

9-(3-Cyanopropyl)adenine (4, X = CN).—To a stirred suspension of sodium adenine prepared from 25 g (0.19 mol) of adenine and 4.56 g (0.19 mol) of pentane-washed sodium hydride in 350 ml of anhydrous DMF was added 27.5 g (0.186 mol) of freshly distilled γ-bromobutyronitrile and stirring was continued for 3 hr. The resulting slightly yellow solution was treated with Norit, filtered through Celite, the solvent was concentrated *in vacuo*, and the crude product was triturated with ether. Recrystallization in portions from methanol–ethanol gave 10 g (27%) of analytically pure crystals, mp 198–200°, ν_{max}^{KBr} 2242 cm^{-1} (C≡N). The filtrates afforded a second crop, 10.3 g, mp 192–193°, which was not further purified. A third crop, 2.6 g, was recovered, the uv spectrum of which suggested the presence of a 3-alkylated derivative. The nmr spectrum of the major product (TFA) showed signals at τ 1.75, 2.25 (4 H, s, s, superimposed on a broader resonance, aromatic H and NH_2); 5.2 (2 H, unsymmetrical t, NCH_2); 7.35 (4 H, br, CH_2CH_2CN).

Anal. Calcd for $C_9H_{10}N_5$: C, 53.45; H, 4.98; N, 41.56. Found: C, 53.65; H, 4.84; N, 41.34.

9-[3-(3,4-Dimethylanilino)propyl]adenine.—To a magnetically stirred solution of 11.5 g (0.075 mol) of freshly distilled 3,4-dimethylaniline under nitrogen at 50° was slowly added, in portions, 2.21 g (1.03 mmol) of 9-(3-chloropropyl)adenine. After the melt was stirred at 70° for 3 hr an exothermic reaction ensued and the temperature rose to 96°, and after 8 hr additional time the reaction mixture solidified. The cooled solids were extracted repeatedly with ether, and the resulting material was filtered and taken up in 50 ml of water (pH 3.7). The pH was adjusted with sodium bicarbonate to 7.2, and the suspension was extracted with ether. The aqueous layer was filtered, and the crude material was recrystallized from isopropyl alcohol: mp 192–193°; yield 1.8 g (15%); $\lambda_{max}^{95\%\ EtOH}$ (0.1 *N* HCl) 298 nm (sh) (ϵ 1,800), 258 (16,300), 243 (13,400), λ_{min} 246 (11,000), 239 (11,900); $\lambda_{max}^{95\%\ EtOH}$ 298 (sh) (1,800), 254 (21,200), λ_{min} 227 (7,500); $\lambda_{max}^{95\%\ EtOH}$ (0.1 *N* NaOH) 295 (sh) (3,300), 252 (25,700), λ_{min} 236 (20,800); nmr (TFA) τ 0.82, 1.25 (5 H, s, s, superimposed on a broader resonance, $AdC_{2,8}H$, NH's), 2.76 (3 H, br s, AnH's), 5.22 (2 H, br, CH_2N^+), 6.21 (2 H, br, $AdCH_2$), 7.36 (2 H, br, center CH_2), 7.62 (6 H, s, $AnCH_3$).

Anal. Calcd for $C_{16}H_{20}N_6$: C, 64.83; H, 6.80; N, 28.36. Found: C, 64.58; H, 6.77; N, 28.74.

9-[3-(3,5-Dimethoxyanilino)propyl]adenine.—This compound was made in a similar manner from 9-(3-chloropropyl)adenine and 3,5-dimethoxyaniline, though in low yield (9%) *via* this route: mp 280–281°; $\lambda_{max}^{95\%\ EtOH}$ (0.1 *N* HCl) 258 nm (ϵ 16,300), λ_{min} 239 (8,450); $\lambda_{max}^{95\%\ EtOH}$ 255 (22,000), λ_{min} 234 (12,000); $\lambda_{max}^{95\%\ EtOH}$ (0.1 *N* NaOH) 254 (24,200), λ_{min} 237 (16,300).

Anal. Calcd for $C_{16}H_{20}N_6O_2$: C, 58.52; H, 6.14; N, 25.59. Found: C, 58.35; 6.28; N, 25.63.

9-Propyladenine (4, X = H).—To a stirred slurry of 13.51 g (0.1 mol) of adenine in 500 ml of dry dimethylformamide was added 4.8 g (0.1 mol) of sodium hydride as a 50% dispersion in mineral oil. After 2 hr, 13.1 g (0.106 mol) of 1-bromopropane was introduced and stirring was continued for 18 hr. The solu-

(52) R. Kuhn and F. Weygand, *Chem. Ber.*, **70**, 1302 (1937).
(53) R. Kuhn and F. Weygand, *ibid.*, **68**, 1282 (1935).

tion was evaporated under reduced pressure, and the colorless semisolid was extracted with chloroform. Removal of the chloroform under reduced pressure and recrystallization of the residue from isopropyl alcohol gave analytically pure needles: mp 178–179° (lit.[54] 175°); 5.53 g (32%); λ_{max}^{MeOH} (0.218 *M* AcOH) 262 nm (ε 14,700), λ_{min} 249 (12,100); $\lambda_{max}^{pH\ 7}$ (0.05 *M* phosphate buffer) 262 nm (15,300), λ_{min} 238 (2,330); nmr [$ClF_2CC(OD)_2CF_2Cl$] τ 1.00, 2.00 (2 H, s, s, $AdC_{2,8}H$); 5.72 (2 H, br, $AdCH_2$); 8.05 (2 H, m, CH_2CH_3); 9.00 (3 H, uneven t, CH_3). The mass spectrogram indicated the following major fragments: *m/e* 177 (M^+); 148 ($AdCH_2^+$); 135 (base peak, Ad^+).

Anal. Calcd for $C_8H_{11}N_5$ (177.2): C, 54.22; H, 6.26; N, 39.52. Found: C, 54.28; H, 6.17; N, 39.77.

9-(3-Phthalimidopropyl)adenine (**6**, *n* = 3).—A suspension of sodium adenide in 700 ml of dry DMF, prepared from 25.0 g (0.185 mol) of adenine and 8.65 g (0.18 mol) of sodium hydride, was stirred with 49.6 g (0.185 mol) of N-(3-bromopropyl)-phthalimide for 3 days. The precipitate was filtered and the filtrate was evaporated to a semisolid *in vacuo*. The semisolid was swirled with ether and filtered. The combined solids were washed successively with water, ethanol, and ether. Recrystallization in portions from ethanol afforded 44.8 g (77%) of product homogeneous on tlc (silica gel): mp 272–274°; nmr (TFA) τ 0.05, 0.92 (4 H, two signals superimposed on a br resonance, NH_2, $AdC_{2,8}H$), 1.70 (4 H, s, PhH), 5.04 (2 H, diffuse peak, $AdCH_2$), 5.84 (2 H, diffuse peak, CH_2Ph), 7.25 (2 H, diffuse peak, center CH_2). The ultraviolet spectra for this and other intermediates are given in Table I.

Anal. Calcd for $C_{16}H_{14}N_6O_2$: C, 59.61; H, 4.38; N, 26.09. Found: C, 59,47; H, 4.47; N, 25.99.

9-(3-Aminopropyl)adenine (**7**, *n* = 3).—To a stirred solution of 12.88 g (0.04 mol) of 9-(3-phthalimidopropyl)adenine in 700 ml of refluxing ethanol, under nitrogen, was added 5.0 g (0.1 mol) of 98% hydrazine hydrate. The solution was heated under reflux overnight, and the solvent was removed under reduced pressure. To the residue maintained in an inert atmosphere was added 100 ml of 2 *N* hydrochloric acid. The precipitated phthalhydrazide was filtered, washed with water, and the filtrates were evaporated to dryness *in vacuo*. The residue when recrystallized from methanol gave the desired amine dihydrochloride as the hydrate, 10.66 g. The material was dissolved in water and stirred for 6 hr with 180 g of dry Dowex 1-X-8 (OH^-) ion-exchange resin. The resin was filtered, and the filtrate was passed through a column containing 150 g of fresh Dowex hydroxide. The resin beds were washed several times with water and then with methanol, and the combined filtrates were evaporated to dryness under reduced pressure. Fractional crystallization from isopropyl alcohol gave a total of 3.7 g (48%) of material homogeneous on tlc (silica gel): mp 182–183°; nmr (TFA) τ 0.01, 0.93 (4 H, two signals superimposed on a br resonance, $AdC_{2,8}H$, $AdNH_2$), 2.08 (3 H, s, NH_3^+), 4.93 (2 H, diffuse peak, $AdCH_2$), 6.3 (2 H, diffuse peak, CH_2N), 7.08 (2 H, diffuse peak, center CH_2).

Anal. Calcd for $C_8H_{12}N_6$: C, 49.99; H, 6.29; N, 43.73. Found: C, 50.17; H, 6.65; N, 42.38.

Anal. Calcd for the **dihydrochloride hemihydrate**, $C_8H_{14}Cl_2N_6 \cdot {}^1/_2 H_2O$: C, 35.02; H, 5.52; N, 30.65; Cl, 25.86. Found: C, 35.14; H, 5.47; N, 30.71; Cl (titrametric), 24.9.

9-[3-(2-Nitroanilino)propyl]adenine.—A stirred solution of 2.6 g (8 mmol) of 1,2-dinitrobenzene with a slight excess of 9-(3-aminopropyl)adenine in 30 ml of isopropyl alcohol was heated at reflux overnight under nitrogen and the orange precipitate which separated was filtered, washed with cold isopropyl alcohol, and recrystallized from dilute acetic acid as an orange powder: mp 255–256°; yield 1.33 g (53%); nmr (TFA) τ 1.00–2.35 (9 H, br, with major peaks at 1.00, 1.50, 2.33, $AdC_{2,8}H$, AdC_6NH_2, C_6H_4NH), 5.25 (2 H, br, $AdCH_2$), 6.16 (2 H, br, $ArNCH_2$), 7.24 (2 H, br, center CH_2).

Anal. Calcd for $C_{11}H_{15}N_7O_2$: C, 53.67; H, 4.83; N, 31.38. Found: C, 53.72; H, 4.87; N, 31.33.

9-[3-(Aden-9-yl)propyl]isoalloxazine.—A slurry of 0.85 g (2.71 mmol) of 9-[3-(2-nitroanilino)propyl]adenine and 30 mg of platinum oxide in 70 ml ethanol, 5 ml water, 20 ml methanol, and 5 ml acetic acid at 40° was hydrogenated at 3 atm for 6 hr in a Parr apparatus. The colorless solution was filtered free of catalyst in an inert atmosphere, and the solvents were evaporated *in vacuo*. Then 50 ml of glacial acetic acid, 450 mg (2.8 mmol) of alloxan monohydrate and 334 mg (4.8 mmol) of boric anhydride were introduced and the slurry was warmed to 60° for 15 min on a steam bath under nitrogen with constant agitation. The solvent was evaporated *in vacuo*, the residue treated with methanol and evaporated to dryness. The crude solids were recrystallized twice from dilute acetic acid to a yellow-orange powder, mp 347° dec, yield 0.35 g (33%).

Anal. Calcd for $C_{18}H_{15}N_9O_2 \cdot H_2O$: C, 53.07; H, 4.21; N, 30.96. Found: C, 52.95; H, 4.46; N, 31.17.

9-[3-(4,5-Dimethyl-2-nitroanilino)propyl]adenine (**2**, *n* = 3).—A stirred solution of 4.12 g (0.0215 mol) of 9-(3-aminopropyl)adenine and 3.92 g (0.02 mol) of 4,5-dimethyl-1,2-dinitrobenzene (**8**) in 50 ml of isopropyl alcohol was refluxed for 8 hr under nitrogen. The orange precipitate which separated was filtered from the hot solution and washed with hot isopropyl alcohol. Further concentration of the filtrate and fractional crystallization of the combined solids from 30% acetic acid–methanol gave 2.686 g (39%) of analytically pure material: mp 243–244°; nmr (TFA) τ 0.90, 1.40, 1.70, 2.40 (br signals, total area 7, $AdC_{2,8}H$, $AdNH_2$, ArNH, $ArC_{3,6}H$), 5.17 (2 H, br, $AdCH_2$), 6.02 (2 H, br, $ArNHCH_2$), 7.58 (8 H, br, $ArCH_3$, center CH_2).

Anal. Calcd for $C_{16}H_{19}N_7O_2$: C, 56.29; H, 5.61; N, 28.72. Found: C, 56.17; H, 5.67; N, 28.75.

9-[3-(Aden-9-yl)propyl]-6,7-dimethylisoalloxazine (**1a**, *n* = 3) (**Fl-C_3-Ad**).—A slurry of 1.19 g (3.5 mmol) of [3-(4,5-dimethyl-2-nitroanilino)propyl]adenine and 92 mg of platinum oxide in 100 ml of ethanol, 5 ml of water, 10 ml of methanol, and 20 ml of acetic acid was hydrogenated overnight at 3 atm at 35–45°. The colorless solution was filtered free of catalyst, under nitrogen, into an amber-stained flask, and the solvent was evaporated under reduced pressure. Then 50 ml of glacial acetic acid, 0.565 g (3.5 mmol) of alloxan monohydrate, and 0.485 g (7 mmol) of boric anhydride were introduced, and the stirred slurry was warmed at 60° for 30 min under nitrogen. The solvent was evaporated under reduced pressure, and the residue was treated with methanol and reevaporated to ensure the removal of boric acid. The residue was recrystallized several times in small portions from 30% acetic acid: yellow powder; mp 258° dec; homogeneous on tlc (silica gel) and paper chromatography; yield 724 mg (48%); λ_{max}^{MeOH} (0.218 *N* AcOH) 443 nm (ε 10,600), 350 (7,350), 267 (42,200), λ_{min} 385 (4,100), 300 (1,350); $\lambda_{max}^{pH\ 7}$ (0.05 *M* phosphate buffer) 450 (9,350) 368 (8,670), 265 (34,700), λ_{min} 403 (6,000), 308 (3,660), 238 (16,500). The mass spectrogram indicated the following major fragments: *m/e* 417 (M^+), 282 ($FlC_3H_5^+$), 256 ($FlH{=}CH_2^+$ or $FlCH_3^+$), 242 (base peak, FlH^+).[55] A sample dried at room temperature *in vacuo* had the correct analysis for a monohydrate.

Anal. Calcd for $C_{20}H_{19}N_9O_2 \cdot H_2O$ (435.2): C, 55.15; H, 4.87; N, 28.96. Found: C, 55.20; H, 4.87; N, 30.12.

A sample treated as hygroscopic was dried at 100° for 10 hr in vacuum.

Anal. Calcd for $C_{20}H_{19}N_9O_2$ (417.4): C, 57.54; H, 4.59; N, 30.21. Found: C, 57.76; H, 5.07; N, 30.45.

9-(6-Phthalimidohexyl)adenine (**6**, *n* = 6).—Conditions similar to those used for **6**, *n* = 3, were employed for this compound, which was made from 13.5 g (0.1 mol) of adenine, 4.85 g of sodium hydride, and 33.4 g (0.018 mol) of N-(6-bromohexyl)-phthalimide. Recrystallization from methanol and recovery of additional product by chromatography of the mother liquors on silica gel and elution with methanol gave a total yield of 24.36 g (67%) of **6**, *n* = 6: homogeneous on tlc (silica gel); mp 187–180°; nmr (TFA) τ 0.70, 1.25 (4 H, s, s superimposed on a broader resonance, $AdC_{2,8}H$, $AdNH_2$), 2.15 (4 H, s, PhH), 5.42 (2 H, br, $AdCH_2$), 6.16 (2 H, br, $PhCH_2$), 8.36 [8 H, m, $(CH_2)_4$].

9-(6-Aminohexyl)adenine (**7**, *n* = 6).—This compound was made from the phthalimido derivative by the procedure described for **7**, *n* = 3, in 65% yield of analytically pure material recrystallized from isopropyl alcohol–methanol: mp 164–165°; nmr (TFA) τ 0.82, 1.30 (4 H, s, s superimposed on a broader resonance, $AdC_{2,8}H$, NH_2), 2.15 (3 H, br, NH_3^+), 5.44 (2 H, br, $AdCH_2$), 6.80 (2 H, br, $CH_2NH_3^+$), 8.52 [8 H, br, $(CH_2)_4$].

Anal. Calcd for $C_{11}H_{18}N_6$: C, 56.39; H, 7.74; N, 35.87. Found: C, 56.38; H, 7.79; N, 36.12.

(54) C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 866 (1962).

(55) Bar graphs of *m/e vs.* total abundance representing the mass spectra of all of the models **1** are provided in ref 49.

9-[6-(4,5-Dimethyl-2-nitroanilino)hexyl]adenine (2, n = 6).—This homolog of 2, n = 3, was made by the same method, but 18-hr reflux time was allowed: mp 159–163°; yield 77%; nmr ($CDCl_3$) τ 1.65 (2 H, s, AdH), 2.08, 2.15 (2 H, two signals, $AnC_{3,6}H$), 3.42 (1 H, s, AnNH), 3.61 (2 H, s, $AdNH_2$), 5.85 (2 H, t, $AdCH_2$), 6.58, 6.78, 6.86, 7.01 (major peaks of m, area 4, $NHCH_2CH_2$), 7.82, 7.92 (6 H, s, s, $AnCH_3$), 8.58 [6 H, br m, $(CH_2)_3$].

Anal. Calcd for $C_{19}H_{25}N_7O_2$: C, 59.52; H, 6.58; N, 25.58. Found: C, 59.24; H, 6.55; N, 25.85.

9-[6-(Aden-9-yl)hexyl]-6,7-dimethylisoalloxazine (1a, n = 6) (**Fl-C_6-Ad**).—The method of preparation was that used for 1a, n = 3. The method of purification was by chromatography in chloroform–formic acid solution through a column of Davidson silica gel (no. 923). Elution with formic acid–chloroform (1:4) followed by evaporation gave a bright yellow powder, 647 mg, homogeneous on tlc and paper chromatography. A sample was prepared for analysis by recrystallization from methanol–chloroform–acetic acid and was shown by mass spectrometry to be uncontaminated. The mass spectrogram indicated the following major fragments: m/e 459 (M^+); m/e 284 ($FlHCH_2$-$CH_2CH_2^+$); 242 (base peak FlH^+). In propylene glycol λ_{max} 445 nm (ϵ 11,300), 355 (7,700), 268 (44,600), λ_{min} 387 (4,700), 302 (1,650), 239 (15,300) values were obtained.

Anal. Calcd for $C_{23}H_{25}N_9O_2$ (459.50): C, 60.12; H, 5.49; N, 27.44. Found: C, 60.21; H, 5.62; N, 27.99.

9-(2-Phthalimidoethyl)adenine (6, n = 2).—The compound was made in a 24-hr period from adenine, sodium hydride, and N-(2-bromoethyl)phthalimide following the directions for 6, n = 3: plates from methanol, mp 256–260°; yield 18%; nmr (TFA) τ 0.65 (3 H, s superimposed on a broader signal, AdH, NH_2), 1.43 (1 H, s, AdH), 2.00 (4 H, s, phth H's), 5.02 (2 H, br, $AdCH_2$), 5.86 (2 H, br, $PhCH_2$).

Anal. Calcd for $C_{15}H_{12}N_6O_2$: C, 58.43; H, 3.92; N, 27.27. Found: C, 58.29; H, 3.96; N, 27.17.

9-(2-Aminoethyl)adenine (7, n = 2).—Compound 7 was prepared by hydrazinolysis of 6, n = 2: yield 34%, mp 225–227° (lit.[36] 219–221°).

Anal. Calcd for $C_7H_{10}N_6$: C, 47.18; H, 5.67; N, 47.14. Found: C, 47.06; H, 5.57; N, 47.15.

9-[2-(4,5-Dimethyl-2-nitroanilino)ethyl]adenine (2, n = 2).—Prepared as was 2, n = 3, from equimolar quantities of 4,5-dimethyl-1,2-dinitrobenzene (8) and 9-(2-aminoethyl)adenine (7, n = 2) using a reflux period of 18 hr, crystallized from methanol–chloroform and recrystallized from isopropyl alcohol: mp 275–276°; yield 48%; homogeneous on tlc; nmr ($CDCl_3$, TFA) τ 0.65–1.60 (br m with major peaks at 0.7, 1.2, 1.4, area 5.3, AdH, NH_2, ArNH), 2.03 (1 H, s, ArC_3H), 2.40 (1 H, s, ArC_6H), 5.16 (2 H, br, $AdCH_2$), 6.00 (2 H, br, NCH_2), 7.66 (6 H, br, $ArCH_3$).

Anal. Calcd for $C_{15}H_{17}N_7O_2$: C, 55.03; H, 5.24; N, 29.96. Found: C, 54.90; H, 5.16; N, 29.63.

9-[3-(Aden-9-yl)ethyl]-6,7-dimethylisoalloxazine (1a, n = 2) (**Fl-C_2-Ad**).—This compound was prepared as in the case of 1a, n = 3. The crude material was dissolved in a minimum volume of formic acid–chloroform (1:1) and chromatographed on a column of Davidson silica gel (no. 923). The first fraction eluted from the column with chloroform–formic acid (8:1), after evaporation and recrystallization from methanol, gave 25 mg of lumichrome (6,7-dimethylalloxazine) identical with an authentic sample by absorption and fluorescence spectroscopy and by its behavior on tlc. The desired product was eluted in subsequent fractions as a bright yellow band, collected in 20-ml fractions. The fractions were eluted with ethanol and allowed to stand overnight at 4°. The tlc-homogeneous material derived from the column was shown by mass spectrometry to be uncontaminated. The mass spectrogram indicated the following major fragments: m/e 403 (M^+); 270 ($FlHCH_2CH_2^+$); 242 (base peak, FlH^+. In propylene glycol λ_{max} 445 nm (ϵ 10,300), 355 (6,700), 268 (36,300), λ_{min} 390 (4,800), 302 (2,000), 240 (14,000) values were obtained.

Anal. Calcd for $C_{19}H_{17}N_9O_2$ (403.3): C, 56.56; H, 4.25; N, 31.26. Found: C, 55.40; H, 4.58; N, 31.38.

4,5-Dimethyl-N-(3-indolylpropyl)-2-nitroaniline.—The directions for 2, n = 3, were applied to the synthesis of this compound from 8, homotryptamine hydrochloride, and triethylamine. The crude solid product was digested with ether. The ether was concentrated and chromatographed on 300 g of Woelm alumina (neutral, activity I), Evaporation of the ether eluents gave 745 mg of unreacted 4,5-dimethyl-1,2-dinitrobenzene, mp 118–118.5°. Elution of the sharp red band with ether–tetrahydrofuran (1:1) gave, on evaporation and recrystallization from isopropyl alcohol–methanol, 790 mg (25%) of analytically pure, deep red needles: mp 164–165°; nmr ($CDCl_3$) τ 2.07 (1 H, singlet, ArC_3H), 2.54, 2.80, 3.00, 3.12 (complex m, total area 6, ArC_6H and indole), 6.55 (2 H, t, NCH_2), 7.08 (2 H, t, $IndCH_2$), 7.78 (8 H, br s, $ArCH_3$, center CH_2).

Anal. Calcd for $C_{19}H_{21}N_3O_2$: C, 70.57; H, 6. 55; N, 12.99. Found: C, 70.45; H, 6.42; N, 12.91.

6,7-Dimethyl-9-[3-(3-indolyl)propyl]isoalloxazine (1b, n = 3) (**Fl-C_3-Ind**).—Prepared from 4,5-dimethyl-N-(3-indolylpropyl)-2-nitroaniline by the general method described for 1a, n = 3. The crude product was taken up in 100 ml of methanol–acetic acid (3:1), and water was added just to turbidity. The solution was allowed to stand overnight at room temperature. The red solid which precipitated was filtered, dissolved in chloroform–acetic acid (1:1), and passed onto a column containing 120 g of silica gel. Elution with a 10% solution of acetic acid in chloroform gave, on evaporation and recrystallization from methanol–chloroform (3:1), 421 mg of analytically pure material: mp 315° dec; yield 50%; nmr ($CDCl_3$, TFA) τ 2.00 (1 H, s, FlC_5H), 2.70, 2.81, 2.84, 2.95, 3.02 (complex pattern, area 6, FlC_8H and indole H's), 5.25 (2 H, br, $FlCH_2$), 6.80 (2 H, br, $IndCH_2$), 7.56 (s superimposed on a br signal, area 5, FlC_7CH_3, center CH_2), 7.92 (3 H, s, FlC_6CH_3). The mass spectrum indicated the following major fragments: m/e 399 (M^+); 256 ($FlH{=}CH_2^+$ or $FlCH_3^+$); 157 ($IndC_3H_5^+$); 143 ($IndC_2H_3^+$); 130 (base peak, $IndCH_2^+$).

Anal. Calcd for $C_{23}H_{21}N_5O_2$ (399.5): C, 69.16; H, 5.30; N, 17.53. Found: C, 69.01; H, 5.31; N, 17.24.

4,5-Dimethyl-N-(3-indolylethyl)-2-nitroaniline.—The directions for 2, n = 3, were applied to the synthesis of this compound from 8, tryptamine hydrochloride, and triethylamine, and purification was effected as in the case of its homolog (see above): deep red prisms from methanol; mp 150–151°; yield 49%; homogeneous on tlc; nmr ($CDCl_3$, TFA) τ 2.00 (1 H, s, IndNH), 2.21; 2.28, 2.34, 2.41, 2.48, 2.56, 2.64, 2.87 (complex pattern, area 5, trypt H's), 316 (1 H, s, AnNH), 6.22, 6.32, 6.43, 6.52, 6.68, 6.79, 6.91 (degenerate A_2B_2, area 4, CH_2CH_2), 7.75, 7.82 (6 H, two broad based s, $AnCH_3$).

Anal. Calcd for $C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.58. Found: C, 69.66; H, 6.23; N, 13.84.

6,7-Dimethyl-9-[3-(2-indolyl)ethyl]isoalloxazine (1b, n = 2) (**Fl-C_2-Ind**).—This compound was prepared in the usual manner from the intermediate described above and was obtained as a pink powder on recrystallization from methanol–chloroform–formic acid and then from chloroform–formic acid: homogeneous on tlc and paper chromatography; yield 62%; in propylene glycol λ_{max} 445 nm (ϵ 11,200), 360 (7,650), 271 (33,300); λ_{min} 387 (5,650), 312 (2,550), 242 (13,000).

The mass spectrum indicated major fragments at m/e 385 (M^+, Fl-Ind); 387 (FlH_2-Ind); 269 ($FlCH_2CH_2^+$); 271 (FlH_2-$CH_2CH_2^+$); 257 ($FlH_2{=}CH_2^+$); 242 (FlH^+); 143 (base peak, $IndCH{=}CH_2^+$); 130 ($IndCH_2^+$); 115 [$Ind(-H_2)^+$].

Anal. Calcd for $C_{22}H_{19}N_5O_2$ (385.4): C, 68.56; H, 4.97; N, 18.17. Found: C, 68.78; H, 5.21; N, 17.96.

4,5-Dimethyl-2-nitro-N-(3,4,5-trimethoxyphenethyl)aniline.—This compound was prepared from 8, 3,4,5-trimethoxyphenethylamine hydrochloride (mescaline hydrochloride), and triethylamine according to the general directions for 2, n = 3: orange needles from methanol; mp 139–139.5°; yield58 %; homogeneous on tlc; nmr ($CDCl_3$) τ 1.98 (2 H, broad based s, $AnC_{3,6}H$), 3.28 (1 H, s, NH), 3.38 (2 H, s, ArH's), 6.06 (6 H, s, m-OCH_3), 6.11 (3 H, s, p-OCH_3), 6.48 (2 H, m, $ArCH_2$), 7.05 (2 H, m, NCH_2), 7.73, 7.87 (6 H, s, s, $AnCH_3$).

Anal. Calcd for $C_{19}H_{24}N_2O_5$: C, 63.32; H, 6.71; N, 7.77. Found: C, 63.61; H, 6.64; N, 8.00.

6,7-Dimethyl-9-(3,4,5-trimethoxyphenethyl)isoalloxazine (1d, n = 2) (**Fl-C_2-TMB**).—Prepared from 4,5-dimethyl-2-nitro-N-(3,4,5-trimethoxyphenethyl)aniline by the general method (see 1a, n = 3), the product was recrystallized from acetic acid–chloroform: mp 198–200° dec; nmr ($CDCl_3$, TFA) τ 1.90 (1 H, br s, CONHCO), 2.75 (center of two signals, area 2.3, contains $CHCl_3$ signal, $FlC_{5,8}H$), 3.66 (2 H, s, $ArC_{2,6}H$), 5.04 (2 H, br, NCH_2), 6.25 (3 H, s, p-OCH_3), 6.30 (6 H, s, m-OCH_3), 6.94 (2 H, br, $ArCH_2$), 7.52, 7.55 (6 H, s, $FlCH_3$). The mass spectrum indicated major fragments at m/e 436 (M^+, Fl-TMB); 438 (FlH_2-TMB); 256 ($FlH{=}CH_2^+$); 242 (base peak, FlH^+); 194 ($TMBCH{=}CH_2^+$); 181 ($TMBCH_2^+$); 167 (TMB^+).

Anal. Calcd for $C_{23}H_{24}N_4O_5$ (436.5): C, 63.29; H, 5.54; N, 12.84. Found: C, 63.05; H, 5.55; N, 12.88.

4,5-Dimethyl-2-nitro-N-(3,4,5-trimethoxybenzyl)aniline.—Prepared by the general method (see **2**, n = 3) from **8**, 3,4,5-trimethoxybenzylamine hydrochloride, and triethylamine, this compound was obtained as orange needles from cyclohexane: mp 140–140.5°; yield 24%; nmr ($CDCl_3$) τ 1.50–1.90 (1 H, br, ArNH), 2.11 (1 H, s, ArC_3H), 3.38, 3.42 (3 H, two signals, ArH's), 5.53, 5.60 (2 H, two signals, $ArCH_2$), 6.16 (9 H, s, $ArOCH_3$), 7.78, 7.83 (6 H, s, s, $ArC_{4,5}CH_3$, respectively).

Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.42; H, 6.40; N, 8.09. Found: C, 62.49; H, 6.40; N, 8.09.

6,7-Dimethyl-9-(3,4,5-trimethoxybenzyl)isoalloxazine (**1d**, n = 1) (**Fl-C_1-TMB**).—Prepared by the general method (see **1a**, n = 3) from 4,5-dimethyl-2-nitro-N-(3,4,5-trimethoxybenzyl)-aniline, the product was obtained pure by chromatography in chloroform–acetic acid on silica gel and rechromatography in chloroform followed by elution with 5% methanol in chloroform: mp 292–293° dec; nmr ($CDCl_3$, TFA) τ 1.95 (1 H, s, FlC_5H), 2.35 (1 H, s, FlC_8H), 3.50 (2 H, s, $ArC_{2,6}H$), 4.05 (2 H, s, $ArCH_2$), 6.13, 6.19, 6.25 (9 H, s, s, s, $ArOCH_3$), 7.47, 7.52 (6 H, s, s, $FlC_{7,8}CH_3$, respectively. The mass spectrogram indicated the following major fragments at m/e 422 (M^+); 421 $(M - 1)^+$; 242 (FlH^+); 182 (base peak, $TMBCH_3^+$); 167 (TMB^+).

Anal. Calcd for $C_{22}H_{22}N_4O_5$ (422.4): C, 62.55; H, 5.25; N, 13.26. Found: C, 62.67; H, 5.23; N, 13.69.

Trimethylenebis-N,N′-(4,5-dimethyl-2-nitroaniline) (**10**, n = 3).—To a solution of 4.0 g (20.4 mmol) of 4,5-dimethyl-1,2-dinitrobenzene (**8**) in 50 ml of isopropyl alcohol was added 0.74 g (10 mmol) of trimethylenediamine. The stirred solution was refluxed overnight under nitrogen, and the orange precipitate which formed was filtered from the hot solution and washed with isopropyl alcohol. The filtrate was shown by tlc to contain mainly starting material, which was recovered by crystallization from a mixture of acetic acid and methanol. Recrystallization from 2:1 methanol–chloroform gave material of mp 207–208°, which was further purified by recrystallization from methanol–acetic acid–water (6:3:1) to give analytically pure material, mp 208–210°, yield 700 mg (19%). The nmr spectrum was consistent with the expected structure.

Anal. Calcd for $C_{19}H_{24}N_4O_4$: C, 61.28; H, 6.50; N, 15.04. Found: C, 61.02; H, 6.66; N, 15.34.

Trimethylenebis-9,9′-(6,7-dimethylisoalloxazine) (**1c**, n = 3) (**Fl-C_3-Fl**).—A mixture of 1.08 g (2.91 mmol) of trimethylenebis-N,N′-(4,5-dimethyl-2-nitroaniline) and 0.25 g of platinum oxide in 135 ml of methanol and 15 ml of acetic acid was hydrogenated at 3 atm and ambient temperature during 6 hr. The colorless solution was removed from the hydrogenator under a positive pressure of hydrogen, filtered free of catalyst in an inert atmosphere and evaporated to an oil under reduced pressure. Then 50 ml of glacial acetic acid, 940 mg (5.9 mmol) of alloxan monohydrate, and 800 mg (11.9 mmol) of boric anhydride were introduced under nitrogen, and the stirred slurry was warmed at 60° for 30 min on a steam bath. The deeply colored solution was evaporated to dryness *in vacuo*. The residue was treated with methanol, the slurry was reevaporated, and the crude solid was washed with water and dried. The material was dissolved in a minimum volume of 1:1 formic acid–chloroform and passed through a column of Davidson silica gel. The product was eluted with chloroform–formic acid (8:1) as a bright yellow band, collected in 50-ml fractions. The fractions were diluted with 150 ml of methanol and allowed to stand overnight at 4° in a refrigerator. The tlc-homogeneous material derived from the first three fractions was shown to be analytically pure. The material derived from subsequent fractions was collected and rechromatographed on a fresh column. In this manner, 384 mg (25%) of a bright yellow powder was collected and shown by mass spectrometry to be uncontaminated: nmr ($DCCl_3$, TFA) τ 1.84 (4 H, br, aromatic H's), 4.50 (4 H, br, $FlCH_2$), 7.30, 7.34 (*ca.* 15 H, s, s, superimposed on another resonance, Fl-CH_3, center CH_2); in propylene glycol λ_{max} 443 nm (ϵ 20,200), 355 (15,000), 268 (56,600), λ_{min} 390 (8,800), 302 (3,330), 242 (18,300). The mass spectrum indicated the following major fragments: m/e 524 (M^+); 284 ($FlHCH_2CH_2CH_2^+$); 242 (base peak, FlH^+).

Anal. Calcd for $C_{27}H_{24}N_8O_4$ (524.5): C, 61.82; H, 4.61; N, 21.37. Found: C, 61.79; H, 4.80; N, 21.28.

Hexamethylenebis-N,N′-(4,5-dimethyl-2-nitroaniline) (**10**, n = 6).—To a refluxing solution of 3.50 g (17.8 mmol) of 4,5-dimethyl-1,2-dinitrobenzene (**8**) in 80 ml of isopropyl alcohol was added dropwise over a 3 hr period a solution of 1.03 g (8.9 mmol) of hexamethylenediamine. The solution was heated at reflux for 3 days (the reaction was followed by tlc). The red-orange precipitate which separated was filtered and washed with isopropyl alcohol. Recrystallization from a mixture of methanol and chloroform and then from a mixture of methanol, chloroform and acetic acid gave 718 mg of material, homogeneous on tlc, mp 180–181°. The combined filtrates were evaporated under reduced pressure, and the solids were digested in ether. The ether was concentrated and chromatographed on 100 g of silica gel. Evaporation of the ether eluents gave 2.06 g of unreacted 4,5-dimethyl-1,2-dinitrobenzene. The ether-insoluble residues were taken up in chloroform and poured onto the column. Elution with ether–chloroform (1:1) gave an additional 85 mg of analytically pure hexamethylenebis-N,N′-(4,5-dimethyl-2-nitroaniline), bringing the total yield to 22%: nmr ($CDCl_3$) τ 2.11 (2 H, s, 2 ArC_3H), 3.40 (2 H, s, 2 ArC_6H), 6.68 (center m, with major peaks at 6.53, 6.64, 6.74, 6.83, total area 4, 2 NCH_2), 7.74, 7.83 (two br's, total area 12, $ArCH_3$'s), 8.42 [8 H, br, $(CH_2)_4$].

Anal. Calcd for $C_{22}H_{30}N_4O_4$: C, 63.75; H, 7.18; N, 13.52. Found: C, 63.32; H, 7.18; N, 13.65.

Hexamethylenebis-9,9′-(6,7-dimethylisoalloxazine) (**1c**, n = 6) (**Fl-C_6-Fl**).—A mixture of 795 mg (1.92 mmol) of hexamethylenebis-N,N′-(4,5-dimethyl-2-nitroaniline) and 0.15 g of platinum oxide in 50 ml of ethanol and 70 ml of acetic acid at 78° was hydrogenated at 3 atm for 4 days. After 2 days the reduction was interrupted in order to add 100 mg additional of fresh catalyst. The mixture was removed from the hydrogenator under a positive pressure of hydrogen and filtered free of catalyst in an inert atmosphere. The colorless solution was evaporated under reduced pressure to an oil. Then 50 ml of glacial acetic acid, 615 mg (3.85 mmol) of alloxan monohydrate, and 534 mg of boric anhydride (7.68 mmol) were introduced, under nitrogen, and the stirred slurry was warmed at 60° for 30 min. The deep red solution was evaporated to dryness *in vacuo*. The residue was treated with methanol and evaporated to dryness *in vacuo*. This step was repeated to assure removal of boric acid. The solid was taken up in a mixture of chloroform and formic acid and passed onto 210 g of silica gel. Elution with 10% acetic acid–chloroform gave, on evaporation and recrystallization from methanol–acetic acid–chloroform, a bright yellow powder homogeneous on tlc and paper chromatography. An analytical sample was prepared by recrystallization from methanol–acetic acid. The mass spectrum showed that the material was free of contaminating substances: m/e 566 (M^+); 242 (base peak, FlH^+).

Anal. Calcd for $C_{30}H_{30}N_8O_4$ (566.6): C, 63.59; H, 5.33; N, 19.78. Found: C, 63.45; H, 5.48; N, 20.54.

Ethylenebis-N,N′-(4,5-dimethyl-2-nitroaniline) (**10**, n = 2).—This compound was made in the same way as the two homologs **10**, n = 3 or 6, and purified by chromatography in tetrahydrofuran on silica gel, elution of the column with ether–tetrahydrofuran (1:1), and recrystallization of the residue of these fractions from methanol: mp 271–273° dec; nmr (TFA) τ 1.90 (2 H, s, ArC_3H), 2.70 (2 H, s, ArC_6H), 5.91 (4 H, s, CH_2CH_2), 7.55, 7.63 (12 H, s, s, $ArCH_3$).

Anal. Calcd for $C_{18}H_{22}N_4O_4$: C, 60.32; H, 6.19; N, 15.65. Found: C, 60.23; H, 6.00; N, 15.52.

N,N′-Trimethylenebis(2-nitroaniline).—A pyridine solution of 16.55 g (0.105 mol) of *o*-chloronitrobenzene and 3.4 g (0.05 mol) of trimethylenediamine was heated at reflux under nitrogen for 21 hr. The pyridine was evaporated *in vacuo*, and the crude solid was extracted with hot petroleum ether to remove unreacted *o*-chloronitrobenzene. The yellow-orange precipitate was recrystallized from methanol–chloroform: mp 148–149°; yield 6.3 g (39%); nmr ($CDCl_3$, TFA) τ 1.65, 1.80, 2.17, 2.32, 2.44, 2.68, 2.85, 3.00, 3.10 (major signals, total area 8, ArH), 6.43 (4 H, uneven q, 2 × CH_2N), 7.66 (2 H, uneven p, center CH_2).

Anal. Calcd for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.10; N, 17.71. Found: C, 56.84; H, 4.98; N, 17.67.

Registry No.—**1**, R = CH_3, n = 2, 21708-13-4; **1a**, n = 2, 21708-14-5; **1a**, n = 3, 31708-15-6; **1a**, n = 6, 21708-16-7; **1b**, n = 2, 21708-17-8; **1b**, n = 3, 21708-18-9; **1c**, n = 3, 21708-19-0; **1c**, n = 6, 21708-20-3; **1d**, n = 1, 21708-21-4; **1d**, n = 2, 21708-22-5; **2**, n = 2, 21708-23-6; **2**, n = 3, 21708-24-7; **2**, n = 6, 21708-

25-8; **4**, x = H, 707-98-2; **4**, x = CN, 21708-27-0; **6**, n = 2, 21708-28-1; **6**, n = 3, 21708-29-2; **6**, n = 6, 21708-30-5; **7**, n = 3, 21708-31-6; **7**, n = 6, 21708-32-7; **10**, n = 2, 21708-33-8; **10**, n = 3, 21708-34-9; **10**, n = 6, 21708-35-0; Ad-C_3-NAn, 21708-36-1; DMNAn-C_3, 21708-37-2; NAn-C_3-NAn, 21708-38-3; DMNAn-C_1-TMB, 21708-39-4; DMNAn-C_2-TMB, 21708-40-7; DMNAn-C_2-Ind, 21708-41-8; DMNAn-C_3-Ind, 21708-42-9; 9-benzylisoalloxazine, 21708-43-0; 9-(3-bromopropyl)adenine, 21708-44-1; 9-[3-(3,4-dimethylanilino)propyl]adenine, 21766-51-8; 9-[3-(3,5-dimethoxyanilino)propyl]adenine, 21708-45-2; 9-[3-(aden-9-yl)-propyl]isoalloxazine, 21708-46-3.

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Reaction of Hydroxylamine with 3,3-Disubstituted 2,4-Pentanediones. Formation of Novel Isoxazole Derivatives

DAVID T. MANNING AND HAROLD A. COLEMAN

Research and Development Department, Union Carbide Corporation, Chemicals and Plastics, South Charleston, West Virginia

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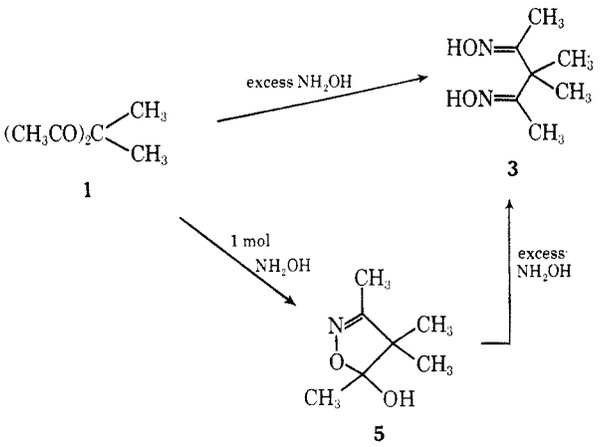
3,3-Disubstituted 2,4-pentanediones reacted with hydroxylamine to give good yields of novel 2-isoxazolin-5-ols. 3,4,4,5-Tetramethyl-2-isoxazolin-5-ol (**5**), prepared in this manner, underwent ring opening on treatment with excess hydroxylamine, forming 3,3-dimethyl-2,4-pentanedione dioxime (**3**). By contrast, all efforts to isolate a dioxime by hydroxylamine treatment of the 4,4-dipropargyl analog (**7**) of **5** failed. 3,5-Dimethyl-4,4-di(2-propynyl)-2-isoxazolin-5-ol (**7**) reacted with alcohols, in the presence of acids, to form the corresponding 5-alkoxy derivatives. Dehydration of **7** by treatment with thionyl chloride and pyridine gave a 5-methylene-2-isoxazoline (**15**) whose high stability contrasts markedly with analogous *exo*-methylene enamines of the related pyrazole series. Upon heating to 145°, **15** underwent exothermic isomerization to an allene, 5-(2,3-butadienyl)-3-methyl-4-(2-propynyl)isoxazole (**16**); a novel Claisen–Cope-type rearrangement is evidently involved. Attempted purification of **16** by alumina column chromatography caused an unexpected isomerization to a new, highly cross-conjugated allene, 5-(1,3-butadienyl)-3-methyl-4-(1,2-propadienyl)isoxazole (**18**), in near-quantitative yield.

Our interest in simple derivatives of 3,3-disubstituted 2,4-pentanediones led to a study of their reaction products with hydroxylamine. Gnichtel and Schönherr have reported[1] that 3,3-dimethyl-2,4-pentanedione (**1**) and 3,3-diethyl-2,4-pentanedione (**2**) react with hydroxylamine to give the expected dioximes **3** and **4**. This contrasted with our observation of cyclic (pyrazole) products resulting from reaction of 3,3-disubstituted 2,4-pentanediones with methylhydrazine, an isostere of hydroxylamine.[2] At the same time, we employed approximately equimolar amounts of diketones and methylhydrazine whereas Gnichtel and Schönherr used a substantial excess of hydroxylamine. Some other standard carbonyl derivatives of 3,3-disubstituted 2,4-pentanediones have been reported[3–8] although it is not evident, in all cases, whether cyclic or acyclic derivatives were obtained.

Results

Reaction of a 1:1 mol ratio of hydroxylamine with 3,3-dimethyl-2,4-pentanedione (**1**) afforded the novel 3,4,4,5-tetramethyl-2-isoxazolin-5-ol (**5**) in 61% yield. Treatment of **5** with excess hydroxylamine then gave Gnichtel and Schönherr's dioxime **3** in a yield of 48%

SCHEME I



(Scheme I). The reaction of 3,3-di(2-propynyl)-2,4-pentanedione (**6**) with hydroxylamine was then studied under various conditions. Treatment of **6** with a 4:1 mol ratio of hydroxylamine in refluxing aqueous ethanol produced only the 2-isoxazolin-5-ol (**7**) in yields as high as 85%. Examination of **7** showed it to be an acid, soluble in aqueous NaOH, and recoverable as an insoluble precipitate on adjustment to pH 6. Use of Gnichtel and Schönherr's conditions (2.88 mol of hydroxylamine hydrochloride in ethanol–pyridine) gave **7** (29%) along with its ethoxy derivative **8** (32%) (Scheme II) but not the dioxime. On the other hand, reaction of **6** with a high (10:1) excess of hydroxylamine gave **7** along with a minor unidentified impurity appearing, in the nmr spectrum, as a singlet at δ 1.8 ppm. Such a resonance is about what would be expected for a methyl group of the unknown dioxime of **6**.

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