0.33, was chromatographed on 15 g of alumina (activity 2) by eluting with hexane–EtOAc (95:5) in two 50-ml portions, fractions G'1 and G'2, respectively (0 and 98 mg), and thereafter one 100-ml portion, fraction G'3 (34 mg). Fraction G'3, giving a TLC (hexane–EtOAc, 9:1) R_f 0.24 and 0.33, was chromatographed on 15 g of alumina (activity 2) by eluting with hexane–EtOAc (9:1) in 7 (fraction H'1, 0 mg), 4 (fraction H'2), 2 (fraction H'3), and 24 ml (fraction H'4, 69 mg). Fractions H'2 and H'3 were combined to give, after evaporation of solvent, 3.4 mg of 6'-hydroxyneothiobinupharidine (**2**): TLC (hexane–EtOAc, 9:1) R_f 0.33; uv (acidic 95% EtOH) λ_{max} 275 nm (ϵ 470); ir (CCl_4) 2.85 (wk OH), 3.7 (m, Bohlmann bands), 11.45 μ (st, 3-furyl); ^{1}H NMR (3 mg/0.3 ml $CDCl_3$) δ 7.3 (m, 4 H, 3-furyl α-H), 6.53 and 6.24 (br singlets, 2 H, 3-furyl β-H), 4.34 (br s, ~1 H, OH), 4.08 (br s, 1 H, C-6' H), 3.54 (m, C-4' H), 0.86 (d, 6 H, C-1 CH_3); high-resolution MS, obsd/calcd mass (formula) 510.2935/510.2916 ($C_{30}H_{42}N_2O_3S$), 509.2868/509.2838 ($C_{30}H_{41}N_2O_3S$), 508.2774/508.2759 ($C_{30}H_{40}N_2O_3S$), 492.2755/492.2810 ($C_{30}H_{40}N_2O_2S$), 230.1513/230.1545 ($C_{15}H_{20}NO$), 228.1367/228.1388 ($C_{15}H_{18}NO$), 178.1198/178.1233 ($C_{11}H_{16}NO$), 176.1047/176.1075 ($C_{11}H_{14}NO$); CD (*c* 0.34 mg/ml, neutral 95% EtOH) $[\theta]_{270}$ ±0°, $[\theta]_{253}$ −750°, $[\theta]_{246}$ ±0°, $[\theta]_{239}$ +1690°, $[\theta]_{234}$ ±0°, $[\theta]_{228}$ −4050°, $[\theta]_{227}$ +2850°; CD (*c* 0.34 mg and 1 drop of 0.2 *M* $HClO_4$ in 1 ml of 95% EtOH) $[\theta]_{330}$ ±0°, $[\theta]_{285}$ −1500°, $[\theta]_{280}$ −1650; $[\theta]_{278}$ −1650°, $[\theta]_{270}$ −1500°, $[\theta]_{253}$ −375°, $[\theta]_{231}$ −9150°, $[\theta]_{228}$ −7430°.

Conversion of 6'-Hydroxyneothiobinupharidine to Neothiobinupharidine-6'-d_1 (4). A solution of 1.5 mg of 6'-hydroxyneothiobinupharidine in 5 drops of MeOH was treated with 10 mg of sodium borodeuteride for 1 hr at ambient temperature. Thereafter the solvent was evaporated under a stream of nitrogen and the residue was digested with 5 ml of CH_2Cl_2. The solvent was evaporated from the resulting extract and the residual oil was chromatographed on 2 g of alumina (activity 2) eluted with hexane–Et_2O (8:2), in 10- and 40-ml portions, fractions 1 and 2, and thereafter with 20 ml of benzene which gave fraction 3 comprised of 1.4 mg of neothiobinupharidine-6'-d_1 (**4**): $[\alpha]^{25}D$ −160° (*c* 1.3 mg/ml, 95% EtOH); ir (CCl_4) 3.60 (st, Bohlmann band), 4.90 (wk, C–D), 11.45 μ; ^{1}H NMR (deuteriobenzene) δ 3.21 (d of d, J = 2 and 11.5 Hz, C-6 H eq), 2.60–3.05 (overlapping multiplets, 5 H, C-6' H eq, C-4 and C-4' H, CH_2S), 1.71 (d, J = 11.5 Hz, C-6 H ax), and the 1.55 (d, J = 11.5 Hz, C-6' H ax) observed in the spectrum of neothiobinupharidine was absent; MS *m/e* (rel intensity) 497 (2), 496 (6), (25.6% d_0, 73% d_1, 1.4% d_2), 495 (16.5), 494 (7.5), 360 (7), 231 (14), 230 (26), 179 (17), 178 (100), 136 (8), 94 (22), 81 (11), 79 (10).

Registry No.—**1**, 55869-57-3; **2**, 55869-58-4; **3**, 55869-59-5; **4**, 55869-60-8; **6**, 50478-55-2; **7**, 52002-85-4; neothiobinupharidine, 4850-09-3.

References and Notes

(1) Support of this work by the National Institutes of Health, U.S. Public Health Service (Grant AI 10188), is gratefully acknowledged. The authors are grateful to the National Science Foundation for an equipment grant to the Department of Chemistry, State University of New York, College of Environmental Science and Forestry, toward the purchase of the XL-100-15 spectrometer and the VFT 100L computer used in this study.
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Fluorescent Modification of Guanine. Reaction with Substituted Malondialdehydes

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Received August 29, 1975

Six representative tricyclic 1,N^2-(allylidene)guanine derivatives (**3a–f**), or 10-oxo-9,10-dihydropyrimido[1,2-*a*]purines, which bear a variety of substituents at position 7 (the second carbon of the allylidene bridge), have been prepared by reaction of the corresponding malondialdehydes (**1a–f**) with guanine (**2**) in aqueous 1 *N* HCl at 40°. The substituted malondialdehydes, by pK_a determination, show acidities similar to those of substituted acetic acids and, by their ultraviolet absorption spectra, show amphoteric behavior. The guanine products (**3**), structural analogues of the naturally occurring Y bases, are compared in terms of their NMR, ultraviolet, and fluorescence spectroscopic properties. The three ring protons of the tricyclic 1,N^2-(2-R-allylidene)guanine system show proton magnetic resonance signals at low field in trifluoroacetic acid indicative of aromatic ring current. The ultraviolet spectra of the products (**3**) exhibit long-wavelength absorption in aqueous acidic, neutral, and alkaline solution where guanine does not absorb, and their fluorescence spectra exhibit solvent dependence. In general, 1,N^2-[2-(*p*-methoxyphenyl)allylidene]guanine has the most favorable ultraviolet absorption and fluorescence emission properties, which suggests the potential utility of *p*-methoxyphenylmalondialdehyde in reactions with more complex guanine derivatives.

Recent investigations in our laboratory have been directed toward the preparation of modified tRNA bases[1] or tRNA base analogues[2–4] which are fluorescent. In considering reactions which involve modification of the existing tRNA bases, we have endeavored to devise or elaborate selective reactions which can be carried out under mild aqueous conditions compatible with the stability of nucleosides, nucleotides, coenzymes, and nucleic acids. Using these guidelines, we have turned our attention to the preparation of fluorescent guanine derivatives.

Structural elucidation of the "Y" bases (or "Wye" bases, imidazo[1,2-*a*]purines),[5–9] as naturally occurring tricyclic guanine derivatives found in $tRNA^{Phe}$ from yeast, wheat germ, and other sources, has stimulated the synthesis of nucleosides having related structures.[10,11] The naturally occurring Y bases and the recently prepared synthetic Y-base analogues are fluorescent. Consideration of these findings led us to conclude that reagents capable of cyclization reactions involving the 1-NH and the exocyclic 2-NH_2 substituent of guanine and providing three ring atoms and two double bonds would give convenient access to fluorescent guanine derivatives.[1] Earlier reports have indicated that

Table I
Electronic Absorption Data. Malondialdehydes (1)

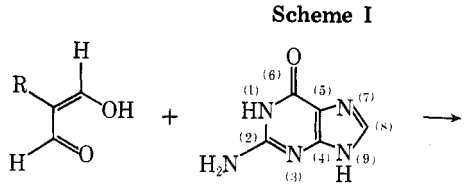
Compd	Acid 90% H_2SO_4 v/v		Acid 0.1 *N* HCl		Neutral[a] pH 6.8 ± 0.1		Alkaline 0.1 *N* NaOH		
	λ_{max}	$\epsilon \times 10^{-4}$	λ_{max}	$\epsilon \times 10^{-4}$	λ_{max}	$\epsilon \times 10^{-4}$	λ_{max}	$\epsilon \times 10^{-4}$	pK_a[b]
1a	270	1.35	250	1.02	275	1.48	275	1.49	4.7
1c	247	1.46	252	1.29	274	2.31	274	2.31	4.1
	305	0.490							
1d	247	2.32	234	1.75	275	2.12	275	2.17	4.3
	292	0.574	265 (sh)	0.895					
	315 (sh)	0.411							
1e	252	2.68	238	1.70	273	2.24	273	2.26	4.0
	300 (sh)	0.355							
1f	243	2.44	250	1.64	272	2.24	272	2.32	3.3
	249	2.48							5.7
	255	2.46							
	260 (sh)	2.05							
	280 (sh)	1.38							

[a] 0.08 *M* KH_2PO_4–Na_2HPO_4 buffer. [b] Solvent H_2O, 25°.

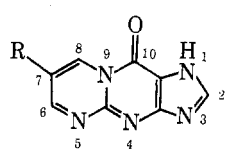
malondialdehyde reacts with DNA,[12] adenine, and guanine[13] to produce fluorescent products of unknown structure which emit at 460 nm upon excitation at 390 nm. In suitable control experiments,[14] it was observed that the highly unstable malondialdehyde alone produces products absorbing at 263 and 345 nm with fluorescence emission at 455 nm and exhibits the same new spots on cellulose TLC as one obtains in the presence of adenosine, cytidine, guanosine, uridine, and ammonium chloride. These results indicated that malondialdehyde offered little promise as a reagent for selective modification of nucleic acid components. In contrast, the substituted malondialdehydes are known to be considerably more stable than the unsubstituted parent molecule and to react with primary and secondary amines to produce a variety of nitrogen-containing heterocycles.[15-17]

Through the work of Arnold[18] and Reichardt[17] variously substituted malondialdehydes are now readily available. Our choice of which substituted malondialdehydes to employ for test reactions with guanine was governed by the requirements that the substituents present enhanced stability, that they be compatible with the display of fluorescence,[19] and that they permit reasonable solubility in aqueous media. We have examined the chemical and spectroscopic properties of six such aldehydes, **1a–f**, and those of their products, **3a–f**, of the respective reactions with guanine (**2**) in acidic aqueous solution.[20]

Scheme I



1a-f **2**



3a-f

R: **a**, CH_3; **b**, CH_3O; **c**, C_6H_5; **d**, *p*-$CH_3OC_6H_4$; **e**, 3,4,5-$(CH_3O)_3C_6H_2$; **f**, *p*-$(CH_3)_2NC_6H_4$

Results and Discussion

Malondialdehydes. The substituted malondialdehydes **1a–e** (Scheme I) were prepared using published procedures.[15,21-23] *p*-Dimethylaminophenylmalondialdehyde (**1f**) had not been described previously. In general, the preparation of these materials involved Vilsmeier formylation of various substrates. Aldehydes **1a** and **1b**, for example, were prepared by formylation of propionaldehyde diethyl acetal[21,24] and methoxyacetaldehyde dimethyl acetal,[22] respectively, while the arylmalondialdehydes **1c–f** were synthesized by formylation of their corresponding arylacetic acids using the general method of Arnold[23] or the modified procedure of Coppola, Hardtmann, and Huegi.[15] Synthesis of **1f** was accomplished by causing *p*-dimethylaminophenylacetic acid[25] to react with a threefold molar excess of Vilsmeier reagent generated by reaction of phosphorus oxychloride with DMF, the formylation solvent. Treatment of the reaction solution with aqueous alkali provided α-(*p*-dimethylaminophenyl)-β-dimethylaminoacrolein as a solid which was easily hydrolyzed in alkaline ethanol–water solution to sodio-*p*-dimethylaminophenylmalondialdehyde. Neutralization of the conjugate base in aqueous acetic acid solution afforded **1f** in good yield.

All malondialdehydes employed in this investigation absorbed strongly in the ultraviolet. Eistert and Haupter[26] presented ultraviolet absorption data for ethoxy- and methoxymalondialdehyde (**1b**), and we include similar data for aldehydes **1a** and **1c–f** in Table I. Under mildly acidic conditions (0.1 *N* HCl), all aldehydes showed absorption maxima in the 230–270-nm region with extinction coefficients of 10000 or higher. In both neutral and alkaline aqueous solution the spectra of all the aldehydes were similar, showing shifts in their maximum absorption to near 275 nm accompanied by significant increases in the values of their extinction coefficients. The spectral changes in the pH range 1–7 are consistent with acidic dissociation of these dialdehydes to their respective anions within these pH extremes.

Osman[27] recently determined a pK_a of 4.46 ± 0.01 for malondialdehyde in water at 25° at an ionic strength of 0.1. A pK_a value of 3.7 for both ethoxy- and methoxymalondialdehyde (**1b**) has been presented by Eistert and Haupter.[26] For comparison, we include pK_a data for **1a** and **1c–f** in Table I as determined in aqueous solution at 25°. The pK_a values in Table I are closely related to the values for representative acetic acids. From the ionization constants presented by Kortüm, Vogel, and Andrussow,[28] the calculated pK_a's in water at 25° are as follows: acetic acid, 4.76; phenylacetic acid, 4.31; and *p*-methoxyphenylacetic acid, 4.36. Hoefnagel and Wepster[29] recently published a pK_a value of 3.75 for the dissociation of the carboxylic acid function in 4-trimethylammoniophenylacetic acid in water at 25°. Consideration of this value and comparison with the assigned dissociation values for the zwitterionic *p*-aminobenzoic acid (COOH, 2.38; NH_3, 4.89)[28] enabled us to assign

Table II
Electronic Absorption Data. 1,N^2-(2-R-allylidene)guanines (3)

Compd name and no.	Formula[a]	Mol wt[b]	Acid 0.1 N HCl λ_{max}	Acid 0.1 N HCl $\epsilon \times 10^{-4}$	Neutral[c] pH 6.8 ± 0.1 λ_{max}	Neutral[c] pH 6.8 ± 0.1 $\epsilon \times 10^{-4}$	Alkaline[d,e] pH 10.1 ± 0.1 λ_{max}	Alkaline[d,e] pH 10.1 ± 0.1 $\epsilon \times 10^{-4}$
1,N^2-(2-Methylallylidene)guanine (3a) or 7-Methyl-10-oxo-9,10-dihydropyrimido[1,2-a]purine	$C_9H_7N_5O$	201.19	218 248 301 (sh) 312 342	2.26 2.44 0.490 0.558 0.490	218 256 309 (sh) 319 355	2.97 2.35 0.359 0.395 0.414	228 250 (sh) 265 315 (sh) 372	3.90 1.59 1.80 0.318 0.340
1,N^2-(2-Methoxyallylidene)guanine (3b) or 7-Methoxy-10-oxo-9,10-dihydropyrimido[1,2-a]purine	$C_9H_7N_5O_2$	217.19	231 259 267 (sh) 287 300 312 (sh) 355	2.16 2.09 1.90 0.428 0.404 0.300 0.381	227 265 (sh) 271 300 (sh) 365	2.81 2.28 2.40 0.285 0.381	270 317 335 379	2.09 0.309 0.271 0.352
1,N^2-(2-Phenylallylidene)guanine (3c) or 7-Phenyl-10-oxo-9,10-dihydropyrimido[1,2-a]purine	$C_{14}H_9N_5O$	263.25	248 289 315 (sh) 360 (sh)	2.26 1.90 0.901 0.305	244 285 320 (sh) 370	2.66 2.17 0.591 0.295	245 287 323 (sh) 375	3.18 2.60 0.566 0.266
1,N^2-(2-p-methoxyphenylallylidene)-guanine (3d) or 7-p-Methoxyphenyl-10-oxo-9,10-dihydropyrimido[1,2-a]purine	$C_{15}H_{11}N_5O_2$	293.28	244 258 306 370 (sh)	1.82 1.84 2.53 0.260	253 256 (sh) 304 370	2.54 2.52 2.59 0.307	254 301 335 380	2.75 2.61 0.614 0.260
1,N^2-[2-(3,4,5-trimethoxyphenyl)-allylidene]guanine (3e) or 7-(3,4,5-Trimethoxyphenyl)-10-oxo-9,10-dihydropyrimido[1,2-a]purine	$C_{17}H_{15}N_5O_4$	353.33	243 307 370 (sh)	2.30 2.14 0.270	253 256 (sh) 304 370	2.47 2.44 2.06 0.285	255 277 295 380	2.62 2.51 2.38 0.285
1,N^2-(2-p-Dimethylaminophenyl-allylidene)guanine (3f) or 7-p-Dimethylaminophenyl-10-oxo-9,10-dihydropyrimido[1,2-a]purine	$C_{16}H_{14}N_6O$	306.32	245 263 (sh) 317 360 (sh)	2.26 2.08 1.14 0.289	222 257 329	2.26 1.98 2.59	231 270 324	2.65 2.01 2.62

[a] Satisfactory analytical data (±0.3% for C, H, N) were reported for all compounds listed in the table. [b] Molecular ions were observed in the mass spectra for all compounds except 3e. [c] 0.08 M KH_2PO_4–Na_2HPO_4 buffer. [d] 0.1 M $NaHCO_3$–Na_2CO_3 buffer. [e] Spectra recorded within 10 min after preparation of alkaline solution.

the two pK_a values for **1f** in order of increasing value to the β-dialdehyde and p-dimethylammonium ionization, respectively.

While these data quantitatively substantiate the acidity of the substituted malondialdehydes, Table I also includes data demonstrating their weak basicity. Eistert and Haupter[26] provided spectroscopic evidence for protonation of ethoxy- and methoxymalondialdehyde (**1b**) in concentrated H_2SO_4. We have included similar data in Table I for aldehydes **1a** and **1c–f**. The ultraviolet spectra of all the aldehydes in 90% H_2SO_4 (v/v) differ significantly from their respective spectra in 0.1 N HCl. The spectrum of **1a** in 90% H_2SO_4 resembled that of its anion indicating that methylmalondialdehyde (**1a**) showed the same "amphoteric halochromism"[30] described for methoxymalondialdehyde (**1b**). Maxima for the arylmalondialdehydes in 90% H_2SO_4 were also shifted with respect to their values in 0.1 N HCl and, in addition, showed marked increases in absorption at longer wavelength. The results are consistent with O-protonation of the enolic β-dialdehyde moiety in strongly acidic solution. In the case of methylmalondialdehyde (**1a**) in particular, we found that this weak basicity could be used to advantage in its purification. Crude methylmalondialdehyde (**1a**) prepared from the sodium enolate salt by the original procedure of Arnold and Šorm,[21] required sublimation, crystallization, and additional sublimation for preparation of analytically pure product. The time required for purification can be shortened as a result of our observation that the neutral aldehyde can be precipitated from ether solution as a crude hydrochloride following addition of excess HCl in diethyl ether. The resulting hygroscopic crystalline solid loses HCl on standing in air. A 1% aqueous solution of this material is strongly acidic to pH paper and shows a positive test for chloride ion with 2% $AgNO_3$ solution. This acid-precipitable material is converted to free methylmalondialdehyde (75% yield) by storing at 1.5 mmHg over solid KOH at 25° for 12 hr.

Synthesis of Tricyclic Products (3). Products of type **3**, substituted 1,N^2-(allylidene)guanine derivatives or 10-oxo-9,10-dihydropyrimido[1,2-a]purines, 1*H* tautomeric form shown (see general formula for *Chemical Abstracts* numbering system)[31] were prepared by treating guanine (**2**) as the hydrochloride with a fivefold molar excess of substituted malondialdehyde (**1**) in 1 N HCl at 40° for 24 hr (Scheme I). These particular conditions proved most effective, although tricyclic products can be obtained under less acidic conditions. Crude hydrochlorides of **3a, 3c, 3d,** and **3e** were precipitated when their reaction solutions were chilled following the reaction incubation. The respective free bases (Table II) were obtained in 40–50% overall yield by acid–base crystallization. Crude product **3f** was recovered as a solid from the neutralized reaction solution and was obtained as a hemihydrate by crystallization from ethanol–water or as the anhydrous material following crystallization of the hemihydrate from absolute methanol. Preparation of the methoxy derivative **3b** required chromatographic purification as the hydrochloride followed by acid–base crystallization. Elemental analyses and mass spectra for the products were completely consistent with the molecular formulas, although the trimethoxyphenyl derivative **3e** failed to show a molecular ion. The molecular ion for all other derivatives appeared as the base peak in the respective spectra. In all cases, the mass spectra recorded at electron beam energies of either 10 or 70 eV were simple and

showed very few fragments, indicating substantial stability in the products—stability indicative of their polycyclic heteroaromatic nature.

The 100-MHz NMR spectra for compounds **3** in trifluoroacetic acid (therefore protonated) were also consistent with structures of a highly aromatic, polycyclic nature. All spectra for the protonated products **3** showed low-field resonances for three aromatic protons in addition to signals assignable to the substituents present in the aldehyde from which the products were derived. The spectrum of the methoxy-substituted compound (**3b**), for example, showed a three-proton singlet at δ 4.17 (downfield from Me_4Si) for the three *O*-methyl protons and coupled doublets at δ 9.10 and 9.30 (J = 3.2 Hz) for the 6 and 8 protons. A one-proton singlet for the 2-H appeared at δ 9.38. The coupling constant for the 6 and 8 protons was of the order of 2 Hz for compounds **3c–f.** In the spectrum of **3a,** a three-proton doublet (J = 1.1 Hz) at δ 2.68 for the 7-methyl group showed coupling with the one-proton quartet (J = 1.1 Hz) at δ 9.49 for the adjacent hydrogen at position 8. Similar splitting through a double bond has been observed for the Y bases synthesized by Kasai et al.[6] Protons 2 and 6 had identical chemical shifts in **3a** and appeared as a two-proton singlet at δ 9.33 in trifluoroacetic acid. The chemical shift for the singlet 2-H resonance appeared between δ 9.3 and 9.5 in the spectra for all products of type **3.** The corresponding 8 proton in the spectrum of protonated guanine (**2**) measured in trifluoroacetic acid appeared at δ 8.92. Clearly, the ring protons in the protonated products **3** are more extensively deshielded than the ring proton of guanine conjugate acid. They are also deshielded with respect to 2,6 and 7 protons observed for the linear tricyclic model, a substituted 10-oxo-9,10-dihydro-1,2,4-triazino[2,3-*a*]purine, in neutral $(CD_3)_2SO$ solution.[11] It would be instructive to inspect the NMR spectrum of the latter type in trifluoroacetic acid. In any case, the extended conjugation and ring current enhancement indicated by the substantial deshielding of all the ring protons coincides with the structure **3** that includes the additional six-membered ring attached linearly to the original guanine.

The linear tricyclic structure **3** is assured over the angular formulation of an N^2,3-(allylidene)guanine, which would have resulted from cyclization involving the exocyclic 2-NH_2 and the 3-NH (alternative, less contributing tautomeric form) of guanine, by analogy with the reaction between a guanine moiety and glyoxal, pyruvaldehyde, or α-keto-β-ethoxybutyraldehyde which leads to 1,N^2 cyclization and linear tricyclic products[32,33] and by the similarities in the ultraviolet spectral characteristics with those of the following linear tricyclic types: Y bases (imidazo[1,2-*a*]purines),[6,8] 1,2,4-triazino[2,3-*a*]purine derivatives,[11] and linear benzopurines (vs. their angular isomers).[2,3] The ultraviolet spectra for the compounds of type **3** (Table II) showed absorption at much longer wavelength, 300–450 nm, than guanine and maximum absorption in the 240–280-nm range at 5–7 times the absorption maxima at long wavelength. The neutral spectra for the aryl-substituted products **3c, 3d,** and **3e** exhibited an additional maximum in the 280–310-nm range, contributed by the benzenoid moiety, with extinction coefficients approaching or exceeding the values for their maxima at shorter wavelength (Figure 1). The neutral spectrum of the *p*-dimethylaminophenyl compound **3f** was exceptional in that its absorption maximum occurred at 329 nm and the spectrum lacked the well-defined maxima or shoulders at longer wavelength characteristic of the spectra for compounds **3a–e.** Ultraviolet spectra of products **3** in 0.1 *N* HCl were shifted with respect to the neutral spectra and exhibited changes consistent with protonation in acidic solution. The spectra in alkaline solution showed bathochromic shifts compared with the respective neutral spectra and general increases in overall absorbance throughout the 200–400-nm region attributable to the loss of a proton from the imidazole portion of the tricyclic system. The choice of pH 10.1 for recording the spectra in alkaline solution was governed by consideration of product stability. The alkaline spectrum of all products **3** changed with time at pH values higher than pH 10.1. The figures reported in Table II were recorded immediately following preparation of the solution and remained unchanged at pH 10.1 for at least 20 min.

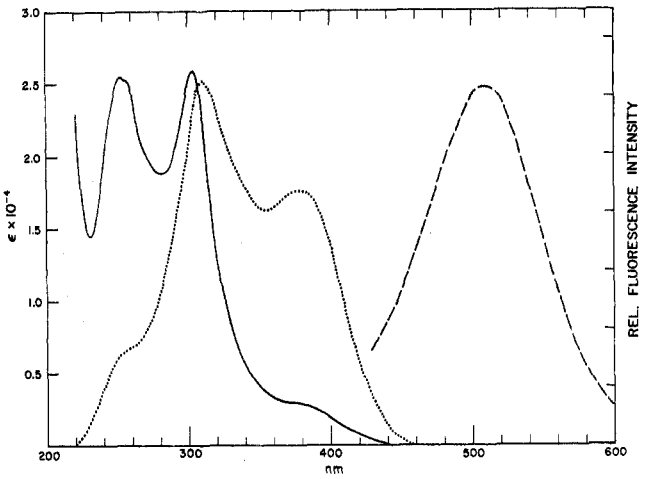


Figure 1. Ultraviolet absorption (—), fluorescence excitation (· · ·), and technical fluorescence emission (- - -) spectra for 1,N^2-[2-(*p*-methoxyphenyl)allylidene]guanine (**3d**) in water, pH 6.8.

Fluorescence Characteristics. Technical fluorescence emission and excitation data for compounds **3** at 25° in neutral aqueous solution and in various solvents at 25° have been summarized in Table III. The fluorescence characteristics of all compounds **3** were generally similar. Compounds **3a–d** exhibited detectable emission at 500 nm (excitation at their respective maxima) at concentrations of the order of 10^{-6}–10^{-5} *M* in water at pH 6.8. The trimethoxyphenyl and *p*-dimethylaminophenyl examples, **3d** and **3e,** however, were very weakly fluorescent in the protic solvents water and ethyl alcohol. Significantly, all compounds **3** showed substantial changes in their excitation and emission characteristics in nonprotic solvents of decreasing dielectric constant. Excitation maxima underwent shifts from 350 nm in water to 397 nm in less polar solvents in agreement with the position of their respective long wavelength ultraviolet absorption. While the wavelength of the emission maxima for compounds **3** did not change significantly with decreasing solvent polarity, the relative quantum yields and fluorescence lifetimes showed substantial increases. The relative quantum yield of **3d** (determined from uncorrected emission spectra), for example, showed an eightfold increase over the solvent series employed accompanied by a fivefold increase in fluorescence lifetime. These changes in emission characteristics were quite analogous to those recently observed in this laboratory for derivatives in the *lin*-benzoadenine series.[4]

After general consideration of all the properties of the six products prepared in this investigation, we concluded that guanine derivatives modified by *p*-methoxyphenylmalondialdehyde (**1d**) showed the greatest promise for future investigations involving fluorescent modification of guanine-containing materials. The long wavelength ultraviolet absorption of products **3d** should make it possible to monitor conveniently the extent of reactions involving **1d** and guanine derivatives using either ultraviolet or fluorescence spectroscopic techniques. Product **3d** can be excited in the

Table III
Fluorescence Emission and Excitation Data

Compd	Solvent	Emission[a] λ_{max}, nm	Excitation[b] λ, nm	τ, nsec	Φ relative[e]
3a	H_2O (pH 6.8)	500	360	0.8[c]	0.004
	EtOH	505	380	1.4[c]	0.010
	DMF	500	397	2.4[d]	0.027
	EtOAc	495	397	2.9[d]	0.038
	Dioxane	500	397	2.5[d]	0.030
3b	H_2O (pH 6.8)	510	375	0.8[c]	0.003
	EtOH	515	397	1.6[c]	0.008
	DMF	515	397	2.5[d]	0.017
	EtOAc	510	397	2.7[c]	0.030
	Dioxane	515	397	2.6[d]	0.026
3c	H_2O (pH 6.8)	505	290, 340, 370	0.7[c]	0.002
	EtOH	510	300, 345, 397	1.5[c]	0.005
	DMF	510	295, 345, 397	2.3[c]	0.008
	EtOAc	495	295, 345, 397	2.9[c]	0.025
	Dioxane	500	300, 345, 397	2.8[d]	0.021
3d	H_2O (pH 6.8)	510	310, 378	0.7[c]	0.004
	EtOH	510	335, 397	1.6[d]	0.006
	DMF	510	340, 397	3.1[d]	0.013
	EtOAc	500	318, 340, 397	3.6[d]	0.031
	Dioxane	500	320, 343, 397	3.7[d]	0.026
3e	H_2O (pH 6.8)	500	320		
	EtOH	500	322, 397	0.8[c]	0.003
	DMF	505	343, 397	1.5[c]	0.008
	EtOAc	498	320, 397	3.3[d]	0.027
	Dioxane	500	327, 397	2.9[d]	0.027
3f	H_2O (pH 6.8)				
	EtOH				
	DMF	535	340	0.8[c]	<0.001
	EtOAc	535	340	1.5[c]	0.001
	Dioxane	535	343	4.1[d]	0.004

[a] Fluorescence emission spectra were measured with excitation at the longest wavelength excitation maximum. [b] Excitation spectra were measured by holding fluorescence emission at 500 nm. [c] Fluorescence lifetime measured by phase only (see ref 38, 39). [d] Fluorescence lifetimes measured by phase and modulation and were identical to within 0.2 nsec (see ref 38, 39). [e] Determined using uncorrected emission spectra by comparison with quinine sulfate (in 0.1 N H_2SO_4) which has a quantum yield of 0.7 (see ref 40).

350–400-nm region, far beyond the usual protein and nucleic acid absorption range. Unlike compounds **3e** and **3f**, compound **3d** fluoresces at 500 nm with readily measurable emission in both protic and nonprotic solvents, thus ensuring the possibility that fluorescence parameters could be measured in aqueous media. Furthermore, of the products **3a–d**, the greatest increase in fluorescence lifetime with changing solvent polarity is observed for **3d**.

The influence of the molecular environment on the fluorescence of **3d** might prove to be very useful if the **3d** fluorophore were incorporated into guanine-containing nucleosides, nucleotides, or nucleic acids. If, for example, a modified and active guanine-containing coenzyme exhibited fluorescence enhancement in the presence of an enzyme specific for the unmodified coenzyme, it should be possible to obtain useful information about the nature of the site at which the enzyme and modified coenzyme interact. Similarly, the **3d** fluorophore in nucleic acids might be capable of monitoring conformational changes in the polymer since the properties of the environment surrounding the modified guanine residue might change as a result of conformational alteration in the larger polymer.[34] In sequels, we will describe the results of our investigations dealing with the preparation, properties, and interactions with enzymes of modified guanine nucleosides and nucleotides.

Finally, in any work employing new reagents for nucleic acid bases, it is advisable to determine whether facile chemical alteration of structure is accompanied by mutagenicity in living organisms. We are indebted to Dr. Graham C. Walker of the University of California, Berkeley, who carefully checked findings that all of the substituted malondialdehydes described here (**1a–f**) were nonmutagenic in Ames' tester strains TA 100 and TA 98 when incorporated into the top agar at a level of 2 mg/plate.[35,36] It may be that metabolic processes compete successfully in faster rates of conversion than the rate of chemical attack on the nucleic acid bases. Or it may be a function of the pH of the medium, since the reaction of the substituted malondialdehydes with guanine derivatives proceeds favorably only below pH 4.5. Chloroacetaldehyde, which has been used for fluorescence labeling of adenine- and cytosine-containing compounds,[1] reverts the *Salmonella* bacterial tester strain TA 100 and thus is mutagenic.[37] Since, in the opinion of Ames and his co-workers,[35–37] there is a high probability that chemicals found to be mutagens in the *Salmonella* test will turn out to be carcinogens, special care should be exercised by those working with reagents that readily (and specifically) alter the nucleic acid bases.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. Nuclear magnetic resonance spectra were recorded on either a Varian A-60 or HA-100 spectrometer using either trifluoroacetic acid or deuteriochloroform as solvent. Tetramethylsilane was used as an internal standard in both solvents. Mass spectra were run on a Varian MAT CH-5 spectrometer coupled with a 620i computer and STATOS recorder. Ultraviolet absorption spectra were obtained on a Beckman ACTA M VI spectrophotometer. For quantitative electronic absorption measurements a specific amount of material was weighed in a volumetric flask and dissolved in 0.01 N HCl. Aliquots of these stock solutions were diluted 1:5 with an appropriate aqueous buffer to arrive at solutions with concentrations of the order of $2–5 \times 10^{-5}$ M in compounds **3** at either pH 6.8 or pH 10.1, or in 0.1 N HCl. The spectra of these solutions were measured against an appropriate solvent blank. Fluorescence emission and excitation spectra were measured on a Hitachi Perkin-Elmer MPF-2A fluorescence spectrophotometer. Fluorescence lifetimes

were determined by phase and modulation as indicated (Table III) using the cross-correlation fluorometer described by Spencer and Weber.[38,39] The exciting light was selected with a monochromator and filtered through a Corning CS-7-54 filter. Emitted light was filtered through a Corning 3-71 filter. Relative quantum yields (Table III) were determined by comparing the area under the uncorrected emission spectra for products 3 with the area beneath the emission spectrum of quinine sulfate (in 0.1 N H_2SO_4), which has a quantum yield of 0.70.[40] All solvents used in fluorescence studies were freshly distilled and checked to ensure absence of emission in the 400–600-nm region when excited at 350 nm. Microanalyses were performed by Mr. Josef Nemeth and staff, who weighed samples for quantitative electronic absorption studies. The pK_a values for the aldehydes were determined potentiometrically for us at the Lilly Research Laboratories, Eli Lilly and Co., Indianapolis, Ind., and we are grateful to Ms. Mary Ann Bogan and Mr. George Maciak for their assistance.

Guanine (ICN Grade) was obtained from ICN Pharmaceuticals, City of Industry, Calif. The hydrochloride was prepared by crystallization from 1 N HCl. **Methylmalondialdehyde (1a)** was prepared through Vilsmeier formylation of propionaldehyde diethyl acetal[24] by the method of Arnold and Šorm[21] and was purified by conversion through its hydrochloride salt. To a vigorously stirred suspension of 5.0 g (0.046 mol) of the sodium salt of methylmalondialdehyde in 100 ml of ether was added 15 ml of 3 N HCl in ether and the suspension was stirred for 10 min. The solution was diluted to 200 ml with ether, stirred for an additional 5 min, and filtered to remove precipitated sodium chloride. The filtrate was acidified by the addition of 45 ml of 3 N HCl in ether and the precipitate was filtered and washed with ether to afford 3.4 g (61%) of presumed methylmalondialdehyde hydrochloride, mp (open tube, uncorrected) partial dec 90°, final dec 100–105°.

A 1% solution of this material in water is acid (pH < 2) to pH paper and shows a positive test for chloride ion when mixed with an equal volume of 2% $AgNO_3$ solution. The acid-precipitable material is converted to free methylmalondialdehyde by storing at 1.5 mmHg, 25° over KOH for 12 hr: yield 1.8 g (75% based on hydrochloride; 45% based on sodium salt); mp 89–89.5° (lit.[21] 88–89.5°); mass spectrum at 10 eV showed peaks at m/e 86 $(M)^+$, 85 $(M - H)^+$, 68 $(M - H_2O)^+$, 57 $(M - CHO)^+$; NMR ($CDCl_3$–Me_4Si) δ 1.74 (s, 3, CH_3), 8.30 (s, 2, CHO), 10.38 (broad, 1, OH). A 1% aqueous solution of this solid is slightly acidic to pH paper (pH 4–5) and shows no turbidity on mixing with an equal volume of 2% $AgNO_3$ solution.

Phenylmalondialdehyde (1c), p-methoxyphenylmalondialdehyde (1d),[15] **and 3,4,5-trimethoxyphenylmalondialdehyde (1e)**[15] were prepared from the corresponding arylacetic acids using the general procedure of Arnold.[23] **Methoxymalondialdehyde (1b)**[26] was obtained through alkaline hydrolysis of 1,3-bis(dimethylamino)-2-methoxytrimethinium perchlorate prepared by Arnold.[22] The hydrolysis procedure employed was that described by Arnold and Šorm[21] for the conversion of α-methyl-β-dimethylaminoacrolein to the sodium salt of methylmalondialdehyde. The recovered sodium salt of methoxymalondialdehyde was neutralized with 1 equiv of HCl in ether, and following filtration of the precipitated sodium chloride, the ether was removed under reduced pressure to afford solid methoxymalondialdehyde (**1b**) which was thoroughly dried and used without further purification. **p-Dimethylaminophenylmalondialdehyde (1f)** was prepared by formylation of p-dimethylaminophenylacetic acid.[25]

α-(p-Dimethylaminophenyl)-β-dimethylaminoacrolein. To 100 ml of chilled dimethylformamide was added 46 g (0.3 mol) of phosphorus oxychloride. The warm solution was allowed to stand for 5 min and 18 g (0.1 mol) of p-dimethylaminophenylacetic acid was added as a solid. The resulting solution was stirred at 75° for 4 hr, cooled to room temperature, and poured over 300 g of ice. Solid sodium hydroxide (36 g, 0.9 mol) was added and the suspension was stirred on ice until all solid had dissolved. The solution was made strongly alkaline by the addition of 200 ml of 10 N NaOH and was chilled to ensure that the temperature did not exceed 40°. After standing for 2 hr, the voluminous solid which precipitated was filtered and resuspended in 500 ml of water with vigorous stirring. The brown, undissolved solid was filtered, washed with water, and dried to afford 11 g (50%) of α-(p-dimethylaminophenyl)-β-dimethylaminoacrolein: mp 155° dec; mass spectrum m/e 218 (M^+); NMR ($CDCl_3$–Me_4Si) δ 2.80 [s, 6, $(CH_3)_2N$–], 2.90 [s, 6, $(CH_3)_2NAr$], 6.80 [s, 1, $(CH_3)_2NCH{=}C<$], 6.85 (m, 4, –Ar–), 9.07 (s, 1, –CHO).

Anal. Calcd for $C_{13}H_{18}N_2O$: C, 71.53; H, 8.31; N, 12.83. Found: C, 71.45; H, 8.16; N, 12.92.

p-Dimethylaminophenylmalondialdehyde (1f). To a solution of 2 g (0.05 mol) of NaOH in 50 ml of 50% aqueous ethanol was added 2.5 g (0.01 mol) of α-(p-dimethylaminophenyl)-β-dimethylaminoacrolein, and the mixture was heated at reflux for 2 hr. The ethanol was removed under reduced pressure and the remaining solution was acidified by the addition of 5 ml of glacial acetic acid. A dark semisolid separated immediately. After brief standing the orange supernatant was decanted and chilled on ice to afford 1.1 g (50%) of p-dimethylaminophenylmalondialdehyde (**1f**): mp 144°; mass spectrum m/e 191 (M^+); NMR ($CDCl_3$–Me_4Si) δ 2.95 [s, 6, $(CH_3)_2N$–], 6.95 (m, 4, –Ar–), 8.53 (s, 2, =CHOH), 11.42 (broad, 1, =CHOH).

Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32. Found: C, 68.90; H, 6.87; N, 7.04.

Reaction of Aldehydes 1a–f with Guanine Hydrochloride. General Procedure. To a solution of 0.18 g (1 mmol) of guanine hydrochloride in 7–10 ml of 1 N HCl was added 5 mmol of aldehyde, and the resulting homogeneous solutions (for reactions involving **1a, 1b,** or **1f**) or suspensions (for reactions with **1c, 1d,** or **1e**) were stirred at 40–45° for 24 hr. The products **3a, 3c, 3d,** and **3e** could be recovered as crude hydrochlorides in 50–60% yield when their respective reaction solutions were chilled to 10° for 1–2 hr following the reaction incubation. After water and ethanol washes of the acid precipitates, the free bases (Table I) were recovered in 40–50% overall yield by cautious neutralization of hot acid solutions of the hydrochlorides. Preparation of **3f** required neutralization of the reaction solution and liberal washing of the crude precipitate by suspension in absolute ethanol and filtration. Following acid–base precipitation from water, the recovered solid was crystallized from 80% aqueous ethanol. Drying under vacuum at room temperature over P_2O_5 for 24 hr afforded an analytical sample of **3f** as the hemihydrate. Recrystallization of the hemihydrate from absolute methanol followed by drying at 110° for 24 hr at 2 mmHg provided an analytical sample of anhydrous **3f** in 25% overall yield. Preparation of **3b** required chromatographic purification. Thus, following neutralization of the acidic reaction solution of **1b** with **2** (threefold scale up), the crude product (300 mg) was dissolved in 5 ml of 1 N HCl and layered on a 4.5 × 50 cm cellulose column. Elution was carried out with isopropyl alcohol–H_2O (7:3). The hydrochloride eluted as a yellow-green band. Following removal of the solvent under reduced pressure the solid residue was twice precipitated from hot acid solution by cautious neutralization to afford 60 mg (10%) of pure **3b.**

Acknowledgment. This work was supported by Research Grant GM-05829 from the National Institutes of Health, U.S. Public Health Service. We are grateful to Professor Gregorio Weber for provision of the equipment used in fluorescence determinations and for his encouragement. Dr. Charles R. Frihart initiated work at the University of Illinois on the fluorescent modification of guanine-containing compounds.

Registry No.—**1a,** 57325-58-3; **1b,** 57325-59-4; **1c,** 4432-64-8; **1d,** 53868-40-9; **1e,** 53868-43-2; **1f,** 57325-60-7; **2** HCl, 635-39-2; **3a,** 57325-61-8; **3b,** 57325-62-9; **3c,** 57325-63-0; **3d,** 57325-64-1; **3e,** 57325-65-2; **3f,** 57325-66-3; α-(p-dimethylaminophenyl)-β-dimethylaminoacrolein, 57325-67-4; dimethylformamide, 68-12-2; p-dimethylaminophenylacetic acid, 17078-28-3.

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Self-Immolative Asymmetric Synthesis. I. Allylic Rearrangement of Optically Active Amine Oxide[1]

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Received July 9, 1975

Transfer of chirality from tetracoordinate nitrogen to trigonal carbon was achieved in the allylic rearrangement of (*R*)-(+)-*N*-*trans*-crotyl-*N*-methyl-*p*-toluidine oxide to (*S*)-(+)-*O*-methylvinylcarbinyl-*p*-tolylhydroxylamine with nearly complete conservation of chirality. The present thermally allowed [2,3]sigmatropic rearrangement proceeds via a transition state conformation such as to meet the orbital symmetry requirements in a doubly suprafacial fashion.

Extensive studies on [2,3]sigmatropic rearrangements have been made during the last few years and the accumulated knowledge[3] suggests that this process proceeds through a five-membered cyclic transition state of a doubly suprafacial migration. The transition state is of the Hückel type, and since six electrons participate, the reaction is expected to be thermally allowed in accordance with the Woodward–Hoffmann orbital symmetry rule.[4]

The [2,3] shifts are the anionic equivalent of the Cope rearrangement, and like the [3,3] changes, are not confined to carbon systems,[5] but also involve many hetero systems. The Wittig,[6] Stevens,[7] and Meisenheimer rearrangements of allylic systems,[8] the Sommelet rearrangement,[9] the rearrangements of allylic sulfonium ylides,[10] sulfenates,[11] phosphinates,[12] amidoammonium salts[13] and other hetero systems[14] can be categorized as [2,3]sigmatropic processes.

It is also known that this process is accompanied by a second pathway of higher activation energy, shown to be a radical-pair mechanism. The mechanistic difference depends on molecular environment and reaction conditions. In cases where the substrate has an allylic group, the concerted [2,3] shift competes favorably with the radical process. The former usually has the lower activation energy, as revealed by the fact that the proportion of the product which is formed by the concerted pathway increases at lower temperature. In contrast, the rearrangement of nonallylic compounds proceeds through a radical dissociation–recombination as demonstrated in the Wittig rearrangement of nonallylic ethers[15] and in the rearrangement of benzylamine oxide.[16]

In a preliminary report,[1] we described the first example of self-immolative asymmetric synthesis, in which the chirality on tetracoordinate nitrogen atom of (+)-*N*-*trans*-crotyl-*N*-ethyl-*p*-toluidine oxide (**2a**) was transferred, upon heating, completely to the trigonal carbon to give (+)-*O*-methylvinylcarbinyl-*N*-ethyl-*p*-tolylhydroxylamine (**3a**). However, since neither the absolute configuration nor the maximum rotation of **2a** was heretofore known, it was impossible to assess the degree of stereoselectivity and to formulate the transition state topology with certainty.

We now present unambiguous stereochemical evidence supporting the concerted nature of the [2,3]sigmatropic rearrangements of allylic amine oxide.

(*R*)-(+)-*N*-*trans*-Crotyl-*N*-methyl-*p*-toluidine oxide[17] (**2b**) was derived from the parent amine (**1**) by oxidation with *O,O*-dibenzoyl-L-pertartaric acid in chilled chloroform. Reflux of (+)-**2b** in 10% aqueous sodium hydroxide for 30 min gave (+)-*O*-methylvinylcarbinyl-*N*-methyl-*p*-tolylhydroxylamine (**3b**, $[\alpha]_D$ 2.42°) in 90% yield. This shows that a sigmatropic [2,3] allylic shift took place in the present system, as was the case with the *N*-ethyl homologue.[1] The absolute configuration of the newly created tetrahedral carbon was correlated by the sequential reduction to (*S*)-2-butanol (**5**) (Scheme I). Catalytic hydrogenation of (+)-**3b** over platinum oxide yielded (+)-*O*-2-butyl-*N*-methyl-*p*-tolylhydroxylamine (**4**, $[\alpha]_D$ 2.38°). Hydrogenol-