d, 1 H, 1'-OH), 3.74, 3.76 (each s, 6 H), 4.09 (d, 1 H, J = 10.0 Hz, H-1'), 4.14 (t, 2 H, J = 6.0 Hz, H-3), 4.12, 4.41 (AB q, 2 H, J = 7.0 Hz, H-3'), 4.35, 4.72, 4.20, 4.80 (each AB q, J = 14 Hz), 5.20, 5.75 (each s, 2 H, $=CH_2$), 6.06 (s, 1 H, 6-OH), 6.74 (d, 4 H, J = 8.0 Hz), 7.0-7.6 (m, 7 H), 7.9-8.05 (m, 2 H); ^{13}C NMR δ 21.8 (q), 29.7 (t, C-4), 45.4, 46.9 (each t), 55.2 (q), 63.3 (t), 77.1 (s, C-2'), 80.5 (d, C-1'), 81.6 (t, C-3'), 86.5, 96.5 (each s, C-5 and C-8), 117.8 (t), 143.0 (s, C-5), 113.6, 114.2, 128.1, 128.4, 129.5, 132.9 (each d), 128.8, 158.7, 159.0 (each s), 166.0, 166.4, 166.8 (each s). Anal. Calcd for $C_{35}H_{38}N_2O_{10}$: C, 65.00; H, 5.92; N, 4.33. Found: C, 65.23; H, 6.05; N, 4.28.

Acknowledgment. We are grateful to Fujisawa Pharmaceutical Co. for a kind gift of bicyclomycin.

Registry No. **1**, 38129-37-2; (±)-**2**, 94807-54-2; (+)-**2**, 117249-39-5; (-)-**2**, 95341-59-6; **3**, 79243-92-8; **4**, 95237-55-1; **5**, 117305-43-8; **6**, 117305-44-9; **7**, 117305-45-0; **8**, 63777-16-2; **9**, 95237-57-3; **10**, 117183-89-8; **11**, 117183-90-1; **12**, 117183-91-2; **13**, 117183-92-3; **14**, 117249-34-0; (±)-**14**, 117249-40-8; **15**, 117183-93-4; **16**, 117249-35-1; **19**, 117249-36-2; **20**, 117249-37-3; **21**, 117249-38-4; **24**, 117201-61-3; **25**, 95694-70-5; **26**, 117183-94-5; **28**, 95237-56-2; **29**, 117183-95-6; **30**, 117183-96-7; **33**, 117183-97-8; **34**, 117183-98-9; (*S*)-(-)-MTPA, 17257-71-5; CH_2=C(OMe)CH_3, 116-11-0; ClSi(*t*-Bu)Me_2, 18162-48-6; 4-$MeOC_6H_4CH_2Br$, 2746-25-0; PhCOCl, 98-88-4.

Reactivity of Quinone Imine and Quinone Diimine Metabolites of the Antitumor Drug Amsacrine and Related Compounds to Nucleophiles

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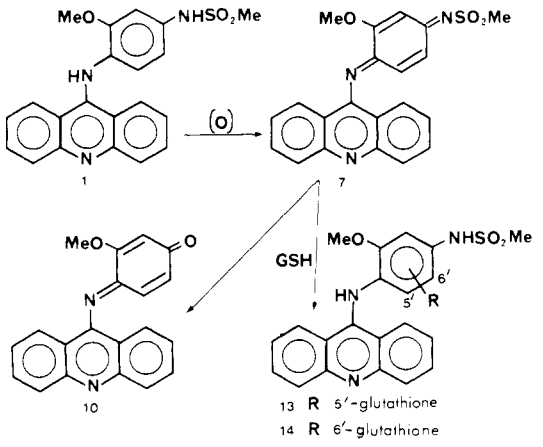
The quinone diimine AQDI (**7**) and the quinone imine AQI (**10**) (products of oxidative metabolism of the clinical antileukemia drug amsacrine (**1**)) and related compounds were prepared, and their reactions with a variety of nucleophiles were studied. Reaction of both quinone diimines and quinone imines with methanethiol gave reduced products resulting from 1,4-addition, while reaction with methylamine and dimethylamine gave almost exclusively quinonoid products, resulting from 1,4-addition followed by reoxidation. These results clarify the mechanism by which certain metabolites of amsacrine are probably formed. Detailed NMR studies of the quinonoid products show that the presence of a bulky group in the 3'-position results in the anilino ring being restricted to one conformer, where the group is distal from the acridine ring.

Recent studies[1,2] on the metabolism of the clinical antileukemic drug amsacrine (**1**) in rats have identified the glutathione 5'-conjugate **13** as the main biliary metabolite. This is postulated to be formed by nucleophilic 1,4-addition to an intermediary quinone diimine **7** (AQDI) (Scheme I). Other metabolites detected include the quinone imine **10**, presumably formed by competing hydrolysis of **7**. Chemical reaction of AQDI with glutathione has been shown to be rapid,[3] resulting in a 60:40 mixture of the 5'-adduct **13** and the isomeric 6'-adduct **14**. Oxidation of amsacrine to AQDI is facile, with an oxidation potential for the reversible two-electron oxidation of 280 mV,[4] and can be accomplished readily by liver microsomes[5] or by reaction with MnO_2.[1] We have also observed the reaction to occur spontaneously under neutral conditions; dilute solutions of amsacrine free base in aqueous methanol slowly produce AQDI, while solutions of the hydrochloride salt do not.

The role of this facile oxidation in the biological activity of amsacrine is not well understood, but is clearly important. Some studies have reported that AQDI is more cytotoxic than amsacrine itself,[5] while rapid cleavage of DNA is observed in the presence of amsacrine, oxygen, and

Scheme I



copper salts, suggesting the possibility of redox cycling of the drug.[6] It is also possible that the quinone diimine could act as an alkylating agent toward biological macromolecules, since the well-studied quinone imines *N*-acetyl-*p*-benzoquinone imine (**19**) and *N*-(4-ethoxyphenyl)-*p*-benzoquinone imine (**20**) (the major oxidative metabolites of the drugs acetaminophen and phenacetin respectively[7,8]) are known to exert their cytotoxic effects via arylation of both glutathione and protein thiols.[9,10]

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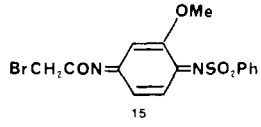

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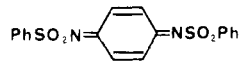

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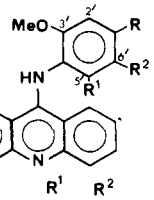
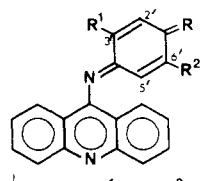
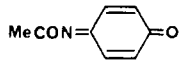


	R	R^1	R^2
17a	OH	H	SMe
17b	$NHSO_2Me$	H	SMe
17c	$NHSO_2Me$	SMe	H
17d	NMe_2	H	H



	R	R^1	R^2
18a	O	OMe	SMe
18b	O	H	NMe_2
18c	O	NMe_2	NMe_2
18d	O	OMe	NMe_2
18e	O	NMe_2	OMe
18f	O	OMe	NHMe
18g	NMe	OH	NHMe



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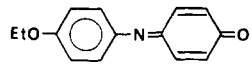

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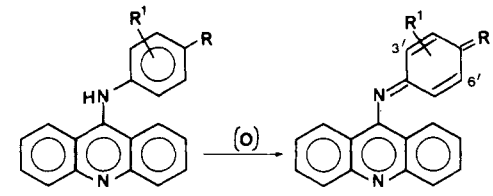
A study of the reactivity of AQDI and related compounds with nucleophiles of the type likely to be found in biological systems is therefore of interest. Additionally, AQDI belongs to the relatively small group of *N*-sulfonylbenzoquinone diimines (another recent example[11] is the protein cleavage reagent **15**). Although detailed studies of symmetrical N^1,N^4-disulfonylbenzoquinone diimines (especially the diphenyl derivative **16**) indicate a complex and varied chemistry,[12-14] there have been few investigations of the reactions of unsymmetrical sulfonylquinone diimines.

We report here the preparation of the quinone diimines **7–9** from amsacrine (*m*-AMSA) (**1**), the biologically inactive isomer *o*-AMSA (**2**), and the unsubstituted derivative AMSA (**3**), respectively, and the reaction of these compounds with a variety of nucleophiles. The corresponding quinone imines **10–12** were also prepared from the phenols **4–6** (Scheme II) and studied.

Results and Discussion

Preparation of Quinone Diimines 7–9 and Quinone Imines 10–12. These compounds were prepared by MnO_2 oxidation of the corresponding amsacrine analogues as reported,[1] although the 3′-methoxy derivative **7** was the only one of the three that could be obtained completely pure. The unsubstituted compound **9** was so susceptible to hydrolysis of the sulfonamide group that even under anhydrous conditions it was contaminated with 10–20% of the quinone imine hydrolysis product **12**. The 6′-methoxy analogue **8** could not be obtained at all, with only the corresponding hydrolysis product **11** being produced under all conditions. While N-unsubstituted and *N*-alkylbenzoquinone diimines are known to be very unstable to hydrolysis, undergoing a formal 1,2-addition of water

Scheme II



	R	R^1		R	R^1
1	$NHSO_2Me$	3′-OMe	7	NSO_2Me	3′-OMe
2	$NHSO_2Me$	2′-OMe	8	NSO_2Me	6′-OMe
3	$NHSO_2Me$	H	9	NSO_2Me	H
4	OH	3′-OMe	10	O	3′-OMe
5	OH	2′-OMe	11	O	6′-OMe
6	OH	H	12	O	H

followed by loss of amine to give the corresponding benzoquinone imines,[13] *N*-acyl and *N*-sulfonyl analogues (e.g., compounds **15** and **16**) are much more stable.[12,13] Thus the relative instability of the parent compound **9** in the present series is somewhat surprising. Within the series, reactivity to hydrolysis is related to the electronic properties of the 3′-substituent. The 3′-OMe derivative **7** is stable, as is the 3′-NMe_2 compound.[4] In contrast, the 3′-F and 3′-Cl analogues were relatively unstable and could not be prepared free of the corresponding quinone imines.[15] The high instability of the 6′-OMe derivative **8** may be due to steric and inductive effects.

For structural identification of these compounds and their reaction products, the anilino ring proton resonances in the ^1H NMR spectra were particularly useful. In both the reduced and oxidized species, the position of substitution in the anilino ring could be easily determined by the coupling pattern. For the unsubstituted compounds **3** and **6**, the observation of only two doublets (J = 9 Hz) at δ 7.20 and 6.74 shows that there is free rotation of the anilino ring about the aniline N–C bond, making H-2′ and H-6′ equivalent, as also are H-3′ and H-5′. The 2′-OMe derivatives **2** and **5** show three proton resonances with a characteristic splitting pattern allowing their individual assignments, with the sharpness of this pattern also suggesting free rotation of the anilino ring. The 3′-OMe compounds **1** and **4** seem more likely to take up the conformation that has the OMe group held away (distal) from the acridine system.[16] The clean NMR spectra of these compounds again suggest only one conformation, but cannot establish which it is.

However, the corresponding quinonoid compounds do show more complex spectra, due to restricted rotation about the aniline N=C bond. Thus the unsubstituted quinone diimine **9** shows four doublets due to the aniline protons, with the previously equivalent 2′,6′ and 3′,5′ pairs now being resolved by residing in different spatial environments (close to and remote from the acridine system). Model building suggests that the 5′ and 6′ protons (the ones proximal to the acridine system as the structures are depicted) will be shielded by its ring currents. This is particularly so for the 5′ proton, to which the signal at δ 7.21 is assigned, with the signal at δ 7.48 being attributed to the 3′ proton. Similar reasoning lies behind resonance assignments for the unsubstituted quinone imine **12**. The 3′-OMe compounds are assumed to have the structure shown in the diagrams, with the OMe lying distal to the

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acridine system and the 5′ proton proximal to it. For the quinone diimine **7**, the doublet at δ 6.60 (J = 8.8 Hz) must be from the 5′-proton, and it can be seen to be coupled to an upfield signal, thus assigning the resonance at δ 6.32 to the 6′-proton.

Although the quinone diimine derived from the 2′-OMe methanesulfonamide **2** was too unstable to be isolated, the NMR spectrum of the corresponding quinone imine (**11**) (derived from the 2′-OMe phenol **5**) indicates that it takes up the conformation shown, with the OMe group lying proximal to the acridine system, in the 6′-position. The doublet at δ 6.77 (J = 9.6 Hz) is unequivocally assigned to the proton adjacent to the quinone carbonyl (2′ as the structure is written) and is coupled to a downfield signal, which allows assignment of the resonance at δ 7.72 to the adjacent (3′) proton. This leaves the high-field signal at δ 5.74 to be assigned to the proton adjacent to the OMe. By analogy with data from the other compounds in the series, this high-field signal is from the proton proximal to the acridine system (and in its shielding region), indicating that the conformation of compound **11** is as written. For clarity it is therefore referred to as a 6′-OMe derivative.

The quinone imines (**10–12**) were similarly prepared from the corresponding phenols (**4–6**), and all were stable. As with the quinone diimines, anilino ring substitution was assigned largely by examination of the proton coupling patterns. Although sharp spectra were obtained from the starting phenols, the corresponding quinone imines **10–12** do not give highly resolved spectra, and some individual proton assignments are more tentative.

Reactions with Methanethiol. In view of the previous work done on the reaction of amsacrine quinone diimine (**7**) and amsacrine quinone imine (**10**) with glutathione,[1-3] studies were carried out using methanethiol as the simplest sulfur nucleophile.

Reaction of the quinone imine **10** gave a mixture of reduced starting material (**4**) and one product, which was identified as the 6′-regioisomer (**17a**) by virtue of the two sharp singlets seen at δ 6.56 and 6.54 and assigned to H-2′ and H-5′. The identification was made by NMR analysis of the crude reaction product, since **17a** was relatively unstable. On chromatography the only product recovered (apart from reduced starting material **4**) was a small amount of what appeared to be a reoxidation product (**18a**). The result is in agreement with previous work[3] on the reaction of **10** with glutathione, where a single product with singlet resonances at δ 6.75 and 6.64 was obtained, although the NMR assignments were reversed by these authors.

As shown previously with glutathione,[3] reaction of the quinone diimine **7** with methanethiol was extremely rapid and gave two regioisomeric products (**17b** and **17c**) together with a small amount (16%) of reduced starting material (**1**). The less polar isomer (21%) showed two sharp singlets in the NMR spectrum at δ 7.28 and 6.60, resulting from the uncoupled protons at 2′ and 5′ respectively, and was assigned structure **17b**. The major (36%) and more polar 5′-regioisomer (**17c**) showed two single-proton doublets (J = 2.1 Hz) at δ 6.76 and 6.68, from the meta-coupled 2′ and 6′ protons. This agrees well with previous work[3] on the reaction of **7** with glutathione, where the less polar regioisomer (**14**) showed two singlets at δ 6.99 and 6.91 Hz, while the more polar isomer (**13**) had two doublets (J = 1.8 Hz) centered at 6.91 and 6.71 ppm. During the separation, it was apparent that the 6′-thiomethyl regioisomer (**17b**) was appreciably more unstable than the 5′-isomer (**17c**), undergoing decomposition to baseline material. In one study of the in vivo metabolism of amsacrine, only the 5′-glutathione conjugate was isolated.[2] Later authors[3] have suggested that this was in fact an unresolved mixture of the 5′- and 6′-conjugates, but it seems possible, considering that the isolation procedure included preparative TLC, that the 6′-conjugate was selectively lost by decomposition.

These results are consistent with the known reactivity of quinone diimines toward thiol nucleophiles.[12,17] For the monoimine **10**, the sole product results from 1,4-addition to the imine, whereas the diimine **7** gives products resulting from 1,4-addition to both imine moieties. The lack of reaction adjacent to the electron-donating 3′-OMe is consistent with the known electronic effects on quinone imine reactivity[14] and also may have a steric component.

Reactions with Dimethylamine. No previous work has been carried out on the reaction of these compounds with amine nucleophiles, and all three available quinone imines (**10–12**) were studied.

The unsubstituted quinone imine **12** gave two isolable, quinonoid products, but there was a low overall recovery of material (ca. 30%). The major product was the 6′-substituted derivative **18b**, resulting from 1,4-addition followed by reoxidation.[12] The structure was assigned by following similar reasoning to that for compound **11**. A sharp six-proton singlet at δ 2.81 showed the presence of the NMe_2 group, while a one-proton doublet (J = 8.5 Hz) was assigned to the proton (2′) adjacent to the quinone carbonyl. This was downfield coupled, allowing assignment of the signal at δ 7.30 to H-3′, leaving the most upfield resonance at δ 4.50 to be assigned to H-5′, on the side proximal to the acridine system. A small amount (8%) of a more polar 3′,6′-disubstituted product (**18c**) was also obtained from a further addition to **18b** followed by reoxidation. This compound showed two sharp, uncoupled one-proton singlets at δ 5.65 and 4.59, assigned to H-2′ and H-5′ respectively. When the favored 6′-position was blocked as in quinone imine **11**, reaction was very slow and the major product isolated was unchanged starting material (67%), together with a small amount (16%) of the 3′-substitution product (**18e**). The isomeric 3′-methoxyquinone imine **10** gave solely the 6′-addition product **18d** in high yield (60%), together with 30% of reduced starting material (**4**).

Reaction of the corresponding quinone diimine **7** with dimethylamine gave a single product in 95% yield. Its NMR spectrum showed peaks assigned to H-2′, H-5′, and H-6′, with a coupling pattern identical with that for amsacrine (**1**), together with loss of the methanesulfonamide protons typically at 3.1–3.3 ppm and appearance of a six-proton singlet at δ 2.94. The compound was thus assigned structure **17d** and results from 1,2-addition of dimethylamine with subsequent displacement of methanesulfonamide. This is analogous to the major product found in the reaction of **16** with dimethylamine.[18]

Reactions with Methylamine. Reaction of the quinone imine **10** for a short time (10 s) gave the 6′-substituted product **18f** in 22% yield, together with a 22% yield of the corresponding quinone diimine demethylated compound **18g**, but the major product (52%) was reduced starting material (**4**). If the reaction time was extended to 5 min, only trace amounts of **18f** could be detected, while the yield of **18g** increased to 45%, suggesting that it is formed from the initial addition product (**18f**). Elemental analysis of **18g** was consistent with a formula of $C_{21}H_{18}N_4O$, and the structure was confirmed by NMR. The two signals at δ

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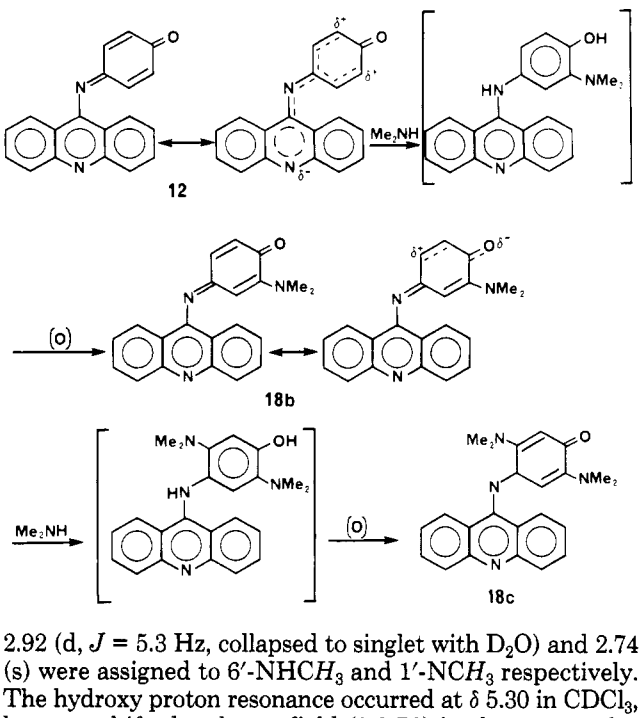
Scheme III



2.92 (d, J = 5.3 Hz, collapsed to singlet with D_2O) and 2.74 (s) were assigned to 6′-NHCH_3 and 1′-NCH_3 respectively. The hydroxy proton resonance occurred at δ 5.30 in $CDCl_3$, but was shifted to lower field (δ 9.76) in the more polar solvent DMSO-d_6, showing its acidic character.

Reaction of the corresponding quinone diimine **7** with methylamine gave one major unstable product. Purification was attempted, but its rapid decomposition precluded identification.

Conclusions

Reaction of both the quinone imine **10** and quinone diimine **7** with methanethiol gave the products of 1,4-addition to the imine moieties: the unstable compound **17a** from **10** and the two possible products **17b** and **17c** from **7**. This agrees with previous work[3] using glutathione as the S-nucleophile. The fact that all the initially formed products were in the reduced form is presumably due to the presence of excess thiol acting as reducing agent. Reaction with the alkylamines methylamine and dimethylamine gave almost exclusively quinonoid products, resulting from 1,4-addition to the imine followed by reoxidation. This is not surprising, given the facile oxidation of the starting materials and the fact that substitution with alkylamines renders the compounds even more susceptible to adventitious oxidation.[4] The reaction of the unsubstituted quinone imine **12** with dimethylamine is outlined in Scheme III. Initial reaction at the 6′-position followed by reoxidation gives the first product (**18b**), which although less reactive undergoes further addition at the 3′-position followed by further reoxidation to give **18c**. Similar considerations can be used to explain the pattern of products from reaction of the other quinone imines (**10** and **11**) with the alkylamines.

However, reaction of the quinone diimine **7** with dimethylamine gave a virtually quantitative yield of **17d**, the product of 1,2-addition to the N-sulfonylimine followed by elimination of methanesulfonamide. Previous work with symmetrical bis(N-sulfonylimines) such as **16** has shown that this is a common reaction mode with alkylamines.[18]

In terms of the chemistry of quinone imines, this work on the reactivity of the unsymmetrical quinone imine **10** and quinone diimine **7** has shown that their chemistry is essentially predictable from the rules obtained from previous studies of (largely) the symmetrical compound **16**. In terms of the metabolic fate of amsacrine (**1**) and other compounds which can form benzoquinone imine species in vivo, this work suggests that reactions with amine nucleophiles (both small molecular weight and protein-bound) could also play a role in their metabolism. Previous studies[2] of the metabolism of amsacrine (**1**) have shown the presence of small amounts of 9-aminoacridine, which presumably arises via oxidative formation of the quinone diimine **7** followed by 1,2-addition/elimination at the 4′-nitrogen.

The detailed NMR studies carried out in the course of this work to identify the reaction products have revealed some general rules useful for structure determination. The chemical shift value (δ) of proton H-3′ was always larger than that of H-5′, while that of H-2′ was similarly always larger than that of H-6′. Of the anilino protons, H-2′ always showed the best resolution.

The work has also provided insights into the conformation of the quinonoid compounds. In the quinone imine series, the presence of a bulky group such as dimethylamino at the 3′-position results in the anilino ring being restricted to one conformer with the bulky group distal from the acridine ring. This is indicated by the H-5′ proton resonances occurring at higher field and by sharp signals from the anilino protons in general.

Experimental Section

Where analyses are indicated only by symbols for the elements, results obtained were within ±0.4% of the theoretical values. Melting points were determined on an Electrothermal apparatus by using the supplied, stem-corrected thermometer and are as read. ^{1}H NMR spectra were recorded at 400 MHz. Flash chromatography was carried out by using Merck silica gel 60 (230–400 mesh).

Preparation of Quinone Imines. N(4′)-(9-Acridinyl)-3′-methoxy-1′,4′-benzoquinone imine (10) was prepared as reported[4] from the phenol (**4**) as a black solid: mp 256–259 °C; ^{1}H NMR (DMSO-d_6) δ 8.18–7.52 (m, 8 H, acridine protons), 6.35 (br s, 1 H, H-6′), 6.24 (s, 1 H, H-2′), 4.00 (br s, 1 H, H-5′), 3.35 (s, 3 H, OCH_3). In comparison, ^{1}H NMR data for **4** (in DMSO-d_6) is as follows: δ 7.75–6.90 (m, 8 H, acridine protons), 7.10 (d, 1 H, J = 8.2 Hz, H-5′), 6.60 (d, 1 H, J = 2.3 Hz, H-2′), 6.53 (dd, 1 H, J = 8.2, 2.3 Hz, H-6′), 3.54 (s, 3 H, OMe).

N(4′)-(9-Acridinyl)-6′-methoxy-1′,4′-benzoquinone Imine (11). A solution of 2-methoxy-4-nitrophenol (3.1 g, 18.3 mmol) in MeOH (100 mL) was hydrogenated over 5% Pd/C (0.1 g) until uptake ceased. Since partial precipitation of the air-sensitive aminophenol had occurred, the suspension was immediately added to a solution of 9-chloroacridine (3.9 g, 18.2 mmol) in MeOH (40 mL). A drop of concentrated HCl was added, and the mixture was heated to reflux for 2 min and then filtered to remove the hydrogenation catalyst. The MeOH was then allowed to boil off, and EtOAc was periodically added until crystallization of the product began. The cooled mixture was then filtered to recover **4′-(9-acridinylamino)-2′-methoxyphenol (5)** as the hydrochloride (6.1 g, 94%). A sample was crystallized from MeOH/EtOAc as orange plates, mp 279–281 °C. ^{1}H NMR of free base ($CDCl_3$): δ 8.5–7.0 (m, 8 H, acridine protons), 6.70 (d, 1 H, J = 8.0 Hz, H-6′), 6.42 (br s, 1 H, H-3′), 6.16 (br d, 1 H, J = 8.0, H-5′), 3.67 (s, 3 H, OCH_3). Anal. Calcd for $C_{20}H_{16}N_2O_2$·HCl: C, 68.80; H, 4.86; N, 7.94; Cl, 10.05. Found: C, 68.91; H, 4.94; N, 7.88; Cl, 10.11.

The free base of the above phenol (3.4 g, 93 mmol) was suspended in EtOAc/Me_2CO (1:1) (600 mL) and treated with commercial activated MnO_2 (12 g) for 2 h with rapid stirring. The dark mixture was filtered, and solvent was removed under vacuum to give **11** as a black solid, which was filtered through alumina in CH_2Cl_2 and recrystallized from EtOAc/Et_2O (vapor diffusion) as black crystals (3.0 g, 89%): mp 258–260 °C; ^{1}H NMR (DMSO-d_6) δ 8.56–7.53 (m, 8 H, acridine protons), 7.22 (br s, 1 H, H-3′), 6.77 (d, 1 H, J = 9.6 Hz, H-2′), 5.74 (br s, 1 H, H-5′), 3.57 (s, 3 H, OCH_3). Anal. Calcd for $C_{20}H_{14}N_2O_2$: C, 76.41; H,

4.49; N, 8.91. Found: C, 76.25; H, 4.5; N, 8.65.

***N*(4′)-(9-Acridinyl)-1′,4′-benzoquinone imine (12)** was prepared as reported[4] as a black, crystalline solid: mp 215–218 °C; ^{1}H NMR (DMSO-d_6) δ 8.18–7.54 (m, 8 H, acridine protons), 7.77 (br s, 1 H, H-3′), 6.95 (br s, 1 H, H-5′), 6.47 (br s, 2 H, H-2′ and H-6′).

Preparation of Quinone Diimines. *N*(1′)-(Methylsulfonyl)-*N*(4′)-(9-acridinyl)-3′-methoxy-2′,5′-cyclohexadiene-1′,4′-diimine (7) was prepared by oxidation of amsacrine (1) with MnO_2 as described.[1] Crystallization from EtOAc/Et_2O gave black needles: mp 218–220 °C; ^{1}H NMR (DMSO-d_6) δ 8.20–7.55 (m, 8 H, acridine protons), 7.01 (br s, 1 H, H-2′), 6.60 (d, 1 H, J = 8.8 Hz, H-6′), 6.32 (br, 1 H, H-5′), 4.05 (b s, 3 H, OCH_3), 3.30 (s, 3 H, SO_2CH_3). In comparison, ^{1}H NMR data for amsacrine (1) (DMSO-d_6) is as follows: δ 8.05–7.05 (m, 8 H, acridine protons), 7.00 (d, 1 H, J = 2.1 Hz, H-2′), 6.85 (dd, 1 H, J = 8.1, 2.1 Hz, H-6′), 6.75 (d, 1 H, J = 8.1 Hz, H-5′), 3.53 (s, 3 H, OMe), 3.00 (s, 3 H, SO_2Me).

***N*(1′)-(Methylsulfonyl)-*N*(4′)-(9-acridinyl)-2′,5′-cyclohexadiene-1′,4′-diimine (9).** 4′-(9-Acridinylamino)methanesulfonanilide (3)[19] (2.5 g, 6.35 mmol) was dried at 60 °C under vacuum for 12 h and suspended in dry EtOAc (500 mL). Activated MnO_2 (10 g, dried at 100 °C for 12 h) was added, and the mixture was stirred for 4 h at 20 °C and filtered through a predried Celite pad. The filtrate was evaporated under reduced pressure to give 9 as a black solid (2.5 g, 100%), contaminated with 10–15% of the corresponding quinone imine hydrolysis product 12. The ^{1}H NMR spectrum of the crude product in DMSO-d_6 gave the following signals assinged to 9: δ 7.48 (d, 1 H, J = 9.0 Hz, H-3′), 7.21 (d, 1 H, J = 9.3 Hz, H-5′), 6.77 (d, 1 H, J = 9.0 Hz, H-2′), 6.42 (d, 1 H, J = 9.3 Hz, H-6′), 3.35 (s, 3 H, SO_2CH_3).

Similar oxidation of 4′-(9-acridinylamino)methanesulfon-*o*-anisidide (*o*-AMSA) (2),[20] even under scrupulously anhydrous conditions, gave only the quinone imine 11, the product of oxidation followed by hydrolysis of the resulting desired but unstable quinone diimine 8.

Reactions of Methanethiol with Quinone Imines. A. Quinone Imine 10. A solution of 10 (500 mg, 1.59 mmol) in dry CH_3CN (50 mL) was treated with a slow stream of gaseous MeSH until the black color was discharged. Excess reagent was immediately removed at 20 °C under vacuum, and the solvent was similarly removed at 40 °C. The resulting crude solid (570 mg) contained two main products, one of them unstable, in approximately equal amounts. ^{1}H NMR spectral analysis of the mixture identified the unstable compound as **17a**: ^{1}H NMR (DMSO-d_6) δ 6.56 (s, H-2′), 6.54 (s, H-5′), 3.53 (s, OCH_3), 2.26 (s, SCH_3). This compound decomposed during column chromatography of the mixture on silica, and only a small amount (40 mg) of the reoxidation product **18a** was isolated: mp (EtOAc) 215–217 °C; ^{1}H NMR (acetone-d_6) δ 8.20–7.50 (m, 8 H, acridine protons), 6.22 (s, 1 H, H-2′), 5.88 (br s, 1 H, H-5′), 4.05 (s, 3 H, OCH_3), 1.72 (s, 3 H, SCH_3). Anal. Calcd for $C_{21}H_{16}N_2O_2S$: C, 69.99; H, 4.47; N, 7.77; S, 8.89. Found: C, 69.46; H, 4.45; N, 7.71; S, 8.52. Later eluates gave reduced starting material 4, identified by TLC, ^{1}H NMR spectra, and mixed melting point.

B. Quinone Diimine 7. A solution of 7 (1.4 g, 3.57 mmol) was treated similarly, and the resulting product was chromatographed on silica. Elution with EtOAc/petroleum ether (1:1) gave a fraction, which was rechromatographed on silica in the same solvent system to give ***N*-[4′-(9-acridinylamino)-3′-methoxy-6′-(methylthio)phenyl]methanesulfonamide (17b)** (300 mg, 21%), which was recrystallized from CH_2Cl_2 as a brown-orange solid: mp 211–212 °C; ^{1}H NMR ($CDCl_3$) δ 8.20–7.30 (m, 8 H, acridine protons), 7.28 (s, 1 H, H-2′), 6.60 (s, 1 H, H-5′), 4.03 (s, 3 H, OCH_3), 2.97 (s, 3 H, SO_2CH_3), 2.05 (s, 3 H, SCH_3). Anal. Calcd for $C_{22}H_{21}N_3O_3S_2$: C, 60.11; H, 4.82; N, 9.56; S, 14.59. Found: C, 59.65; H, 4.9; N, 9.6; S, 14.4.

Further elution of the original column with EtOAc/petroleum ether (1:1) gave ***N*-[4′-(9-acridinylamino)-3′-methoxy-5′-(methylthio)phenyl]methanesulfonamide (17c)** (570 mg, 36%) as an orange solid, which was recrystallized from CH_2Cl_2: mp 135 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.44–6.97 (m, 8 H, acridine protons), 6.76 (d, 1 H, J = 2.1 Hz, H-2′), 6.68 (d, 1 H, J = 2.1 Hz, H-6′), 3.51 (s, 3 H, OCH_3), 3.03 (s, 3 H, SO_2CH_3), 2.41 (s, 3 H, SCH_3). Anal. Calcd for $C_{22}H_{21}N_3O_3S_2$: C, 60.11; H, 4.82; N, 9.56; S, 14.59. Found: C, 60.00; H, 4.63; N, 9.55; S, 14.6.

Continued elution with the same solvent mixture gave the reduced starting material 1 (220 mg, 16%).

Reactions of Dimethylamine with Quinone Imines. A. Quinone Imine 12. A suspension of 12 (600 mg, 2.11 mmol) in dry CH_3CN (20 mL) was treated with excess dry gaseous dimethylamine. The reaction was complete within 2 min. Excess reagent was removed by degassing under reduced pressure at 20 °C, and the solvent was then removed at 40 °C. The residue was chromatographed on silica. EtOAc/petroleum ether (1:1) first eluted ***N*(4′)-(9-acridinyl)-6′-(dimethylamino)-1′,4′-benzoquinone imine (18b)** (170 mg, 25%), which was crystallized from EtOAc/petroleum ether as black needles: mp 176–177 °C; ^{1}H NMR ($CDCl_3$) δ 8.23–7.41 (m, 8 H, acridine protons), 7.30 (br s, 1 H, H-3′), 6.62 (d, 1 H, J = 8.5 Hz, H-2′), 4.50 (br s, 1 H, H-5′), 2.81 (s, 6 H, NMe_2). Anal. Calcd for $C_{21}H_{17}N_3O$: C, 77.03; H, 5.23; N, 12.64. Found: C, 76.65; H, 5.3; N, 12.65.

Later eluates gave ***N*(4′)-(9-acridinyl)-3′,6′-bis(dimethylamino)-1′,4′-benzoquinone imine (18c)** (30 mg, 8%), which crystallized from CH_2Cl_2/EtOAc as black needles: mp 190–192 °C; ^{1}H NMR ($CDCl_3$) δ 8.82–7.40 (m, 8 H, acridine protons), 5.65 (s, 1 H, H-2′), 4.59 (s, 1 H, H-5′), 3.43 (s, 6 H, NMe_2), 2.74 (s, 6 H, NMe_2). Anal. Calcd for $C_{23}H_{22}N_4O\cdot0.5H_2O$: C, 72.79; H, 6.07; N, 14.77. Found: C, 72.5; H, 5.75; N, 14.4.

B. Quinone Imine 10. Similar reaction of 10 (650 mg, 2.07 mmol) with gaseous dimethylamine gave a product, which was chromatographed on silica. EtOAc eluted ***N*(4′)-(9-acridinyl)-6′-(dimethylamino)-3′-methoxy-1′,4′-benzoquinone imine (18d)** (440 mg, 60%), which crystallized from EtOAc as scarlet-black needles: mp 204–205 °C; ^{1}H NMR ($CDCl_3$) δ 8.20–7.40 (m, 8 H, acridine protons), 5.92 (s, 1 H, H-2′), 4.69 (s, 1 H, H-5′), 4.03 (s, 3 H, OCH_3), 2.75 (s, 6 H, NMe_2). Anal. Calcd for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.75. Found: C, 73.8; H, 5.3; N, 11.7. EtOAc/MeOH (10:1) eluted the reduced form of the starting material 4 (200 mg, 30%), identified by TLC with an authentic sample: ^{1}H NMR (DMSO-d_6) δ 7.75–6.90 (m, 8 H, acridine protons), 6.60 (d, 1 H, J = 8.2 Hz, H-5′), 6.48 (d, 1 H, J = 2.3 Hz, H-2′), 6.38 (dd, 1 H, J = 8.2 and 2.3 Hz, H-6′), 3.54 (s, 3 H, OCH_3).

C. Quinone Imine 11. Similar reaction of 11 (700 mg, 2.23 mmol) with gaseous dimethylamine was slower. Workup of the reaction after 5 min followed by chromatography on silica in EtOAc gave considerable amounts of starting material (470 mg, 67%), followed by ***N*(4′)-(9-acridinyl)-3′-(dimethylamino)-6′-methoxy-1′,4′-benzoquinone imine (18e)** (130 mg, 16%), which was crystallized from CH_2Cl_2/EtOAc as black needles: mp 217–219 °C; ^{1}H NMR ($CDCl_3$) δ 8.24–7.48 (m, 8 H, acridine protons), 5.82 (s, 1 H, H-2′), 5.09 (s, 1 H, H-5′), 3.45 (s, 6 H, NMe_2), 3.21 (s, 3 H, OCH_3). Anal. Calcd for $C_{22}H_{19}N_3O_2$: C, 73.92; H, 5.36; N, 11.75. Found: C, 73.4; H, 5.2; N, 11.6.

D. Quinone Diimine 7. Similar reaction of 7 (450 mg, 1.15 mmol) gave a residue, which was chromatographed on silica. Elution with EtOAc/MeOH (20:1) gave **4′-(9-acridinylamino)-*N*,*N*-dimethyl-3′-methoxyaniline (17d)** (370 mg, 95%), which crystallized from CH_2Cl_2/EtOAc as brown crystals: mp 181–182 °C; ^{1}H NMR ($CDCl_3$) δ 8.12–7.26 (m, 8 H, acridine protons), 6.67 (d, 1 H, J = 8.7 Hz, H-5′), 6.43 (d, 1 H, J = 2.6 Hz, H-2′), 6.19 (dd, 1 H, J = 8.7, 2.6 Hz, H-6′), 3.93 (s, 3 H, OCH_3), 2.94 (6 H, NMe_2). Anal. Calcd for $C_{22}H_{21}N_3O\cdot H_2O$: C, 74.97; H, 6.29; N, 11.93. Found: C, 74.6; H, 6.1; N, 11.8.

Reactions of Methylamine with Quinone Imines. A. Quinone Imine 10. A suspension of 10 (210 mg, 0.67 mmol) in CH_3CN was treated with excess dry gaseous methylamine at 20 °C for 10 s. The excess reagent was removed by degassing under reduced pressure at 25 °C, followed by removal of solvent. Chromatography of the residue on silica and elution with EtOAc/petroleum ether (1:1) gave, firstly, ***N*(1′)-methyl-*N*(4′)-(9-acridinyl)-3′-hydroxy-6′-(methylamino)-2′,5′-cyclohexadiene-1′,4′-diimine (18g)** (50 mg, 22%), which crystallized from CH_2Cl_2/MeOH as an orange-brown solid: mp 254–256 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.76 (s, 1 H, exchangeable with D_2O, O*H*), 7.30–6.55 (m, 8 H, acridine protons), 7.08 (d, 1 H, J = 5.2

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Hz, exchangeable with D_2O, NH), 5.47 (s, 1 H, H-2′), 5.32 (s, 1 H, H-5′), 2.78 (d, 3 H, J = 5.2 Hz, collapses to singlet with D_2O, $NHCH_3$), 2.65 (s, 3 H, $=NCH_3$); ^1H NMR ($CDCl_3$) δ 7.30–6.70 (m, 8 H, acridine protons), 6.25 (d, 1 H, J = 5.3 Hz, exchangeable with D_2O, NH), 5.63 (s, 1 H, H-2′), 5.35 (s, 1 H, H-5′), 5.30 (s, 1 H, H-5′), 5.30 (s, 1 H, exchangeable with D_2O, OH), 2.92 (d, 3 H, J = 5.3 Hz, collapses to singlet with D_2O, $NHCH_3$), 2.74 (s, 3 H, $=NCH_3$). Anal. Calcd for $C_{21}H_{18}N_4O$: C, 73.65; H, 5.30; N, 16.36. Found: C, 73.20; H, 4.96; N, 15.98.

Later eluates gave **N(4′)-(9-acridinyl)-6′-(methylamino)-3′-methoxy-1′,4′-benzoquinone imine (18f)** (50 mg, 22%) as a black solid: mp 193–194 °C (from EtOAc); ^1H NMR ($CDCl_3$) δ 8.20–7.40 (m, 8 H, acridine protons), 6.00 (s, 1 H, H-2′), 5.63 (d, 1 H, J = 5.0 Hz, exchangeable with D_2O, NH), 4.55 (s, 1 H, H-5′), 4.05 (s, 3 H, OCH_3), 2.35 (d, 3 H, J = 5.0 Hz, collapses to singlet with D_2O, $NHCH_3$). Anal. Calcd for $C_{21}H_{17}N_3O_2$: C, 73.5; H, 5.00; N, 12.2. Found: C, 73.21; H, 4.72; N, 12.11. Further elution with EtOAc/MeOH (10:1) gave reduced starting material **4** (110 mg, 52%), identified by TLC and mixed melting point.

With longer reaction time (5 min), under similar conditions, the following product distribution was obtained: **18g** (45% yield); **18f** (trace amount); and reduced starting material **4** (50%).

B. Quinone Diimine 7. Similar treatment of **7** (300 mg, 0.77 mmol) gave a major product (TLC), which was unstable and decomposed when purification by chromatography was attempted.

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Thermolysis of 7-(Acylamino)-7-azabenzonorbornadienes and 1-(Acylamino)aziridines. Generation and Trapping of Monosubstituted Azamines[1]

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The thermolysis of 7-[[[(9-fluorenylmethyl)oxy]carbonyl]amino]- and 7-(benzoylamino)-7-azabenzonorbornadienes (**2a** and **2b**) in various solvents has been studied. In the absence of an olefinic trapping agent the major products other than naphthalene are the corresponding hydrazides **6a,b**. In cyclohexene as solvent, the aziridines **7a,b** are formed, suggesting that the azamine **3** is ejected and captured by the olefin. For the olefin *cis*-4-methyl-2-pentene, the reaction occurs with greater than 95% stereoselectivity in further agreement with a labile azamine intermediate. This represents the first demonstration that a monosubstituted azamine has independent existence and reacts with olefin faster than it undergoes 1,2-hydrogen shift. Synthesis of the related 7-phthalimido-7-azabenzonorbornadiene (**17**) was achieved via rearrangement of the corresponding isophthalimide derivative **18**, which could be obtained by reaction of phthaloyl chloride with hydrazine **1**. Thermolysis of **17** caused fragmentation to naphthalene and phthaloylazamine **13** as shown by trapping of the latter. This reaction represents a new thermal source of transient species **13**. For synthetic purposes more practical intermediates for the generation of **3** are the aziridines **21** and **22**. The cis analogues (**23**) of **21** proved to be relatively stable thermally. A new route is presented for the synthesis of 1-amino-*cis*-2,3-diphenylaziridine.

In a previous paper, the use of 7-[[[(9-fluorenylmethyl)oxy]carbonyl]amino]-7-azabenzonorbornadiene (**2a**) as a storage form of the thermally sensitive hydrazine **1** was reported.[2] Although **2a** could be obtained as a crystalline solid and was far easier to handle ($t_{1/2}$ 70 min, $CDCl_3$, 37 °C) than oily **1** ($t_{1/2}$ 15 min, $CDCl_3$, 37 °C), it decomposed at its melting point (84 °C) or upon standing in solution at room temperature for several hours. Since the thermal decomposition of **2a** was accompanied by the formation of naphthalene (85%), it became of interest to determine whether the initial reaction involved fragmentation to the azamine **3**. Along with naphthalene, hydrazide **6a** was isolated from all such decompositions carried out in a variety of neutral solvents. Formation of **6a** can be rationalized as arising from capture of **3** by initial reactant **2** via adduct **4**, extrusion of **5**, and subsequent loss of nitrogen (Scheme I). Analogy for such extrusion reactions is available in the fragmentation of related ylides[3] and amine oxides.[4,5]

More direct evidence for the finite existence of **3** was sought by olefin trapping reactions. Indeed, thermolysis of **2a** in cyclohexene at 55 °C led to the isolation in 44% yield of aziridine **7a**, the structure of which was established by its alternate synthesis by reaction of authentic **9**[6] with

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