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*Acta Cryst.* (1993). B**49**, 342–347

# Structures of Quinone Imine Metabolites Related to the Anti-Cancer Drug Amsacrine

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(*Received* 7 *April* 1992; *accepted* 26 *August* 1992)

## Abstract

(5): *N*-(9-Acridinyl)-3-methoxy-1,4-benzoquinone monoimine, $C_{20}H_{14}N_2O_2$, $M_r = 314.3$, monoclinic, $P2_1/c$, $a = 13.451$ (8), $b = 7.007$ (4), $c = 17.864$ (12) Å, $\beta = 117.26$ (4)°, $V = 1497$ (2) Å$^3$, $Z = 4$, $D_m = 1.36$ (1), $D_x = 1.395$ g cm$^{-3}$, Mo $K\alpha$, $\lambda = 0.71069$ Å, $\mu = 0.99$ cm$^{-1}$, $F(000) = 656$, $T = 138$ (5) K, $R = 0.049$ for 1556 reflections. (8): *N*-(9-Acridinyl)-2-methoxy-1,4-benzoquinone monoimine, $C_{20}H_{14}N_2O_2$, $M_r = 314.3$, triclinic, $P\bar{1}$, $a = 9.365$ (1), $b = 13.318$ (2), $c = 6.918$ (3) Å, $\alpha = 96.45$ (3), $\beta =$

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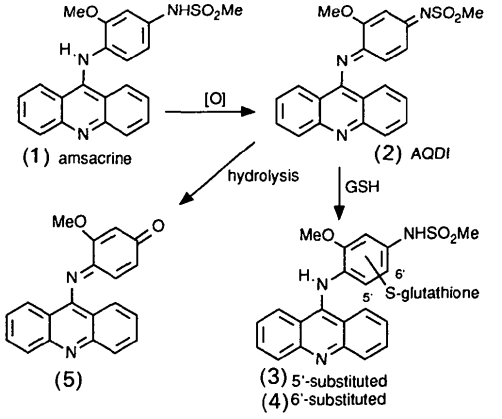
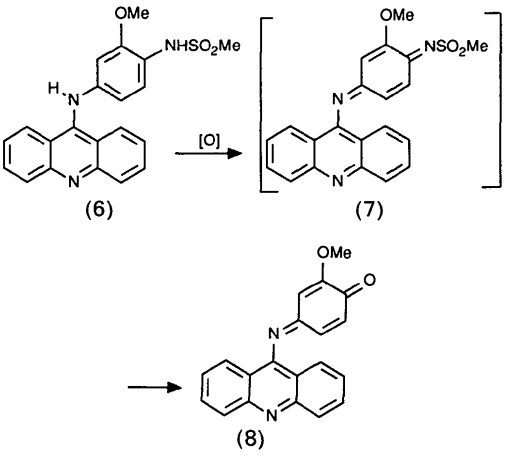
105.30 (2), $\gamma = 110.11$ (1)°, $V = 761.6$ (4) Å$^3$, $Z = 2$, $D_m = 1.35$ (1), $D_x = 1.371$ g cm$^{-3}$, Mo $K\alpha$, $\lambda = 0.71069$ Å, $\mu = 0.98$ cm$^{-1}$, $F(000) = 328$, $T = 292$ (1) K, $R = 0.075$ for 1009 reflections. (13): *N*-(9-Acridinyl)-5-dimethylamino-2-methoxy-1,4-benzoquinone monoimine, $C_{22}H_{19}N_3O_2$, $M_r = 357.4$, triclinic, $P\bar{1}$, $a = 8.091$ (7), $b = 10.078$ (2), $c = 11.716$ (3) Å, $\alpha = 108.39$ (2), $\beta = 99.63$ (4), $\gamma = 95.87$ (3)°, $V = 881.4$ (9) Å$^3$, $Z = 2$, $D_m = 1.33$ (1), $D_x = 1.347$ g cm$^{-3}$, Mo $K\alpha$, $\lambda = 0.71069$ Å, $\mu = 0.95$ cm$^{-1}$, $F(000) = 376$, $T = 173$ (5) K, $R = 0.034$ for 1460 reflections. The molecular geometries are described and discussed.

## Introduction

The major low-molecular-weight metabolites of the clinical anti-leukemia drug amsacrine [(1), *m*-AMSA] are the conjugates (3) and (4), resulting from nucleophilic 1,4-addition of glutathione (the most ubiquitous cellular thiol) to the initially formed quinone diimine [(2), AQDI] (Shoemaker, Cysyk, Padmanhaban, Bhat & Malspeis, 1982; Gaudich & Prybylski, 1983; Robbie, Palmer, Denny & Wilson, 1990). Although the putative intermediate AQDI has




not been detected in cells (Robbie, Palmer, Denny & Wilson, 1990; Robbie, Baguley, Denny, Gavin & Wilson, 1988), it is readily prepared synthetically by mild oxidation of amsacrine (Shoemaker, Cysyk, Padmanhaban, Bhat & Malspeis, 1982). While relatively stable to hydrolysis [its hydrolysis product (5) has been detected as a metabolite], AQDI is very reactive towards nucleophiles (Lee, Palmer & Denny, 1988), and in intact cells is rapidly converted to a combination of low-molecular-weight conjugates [mainly (3) and (4)] and macromolecular adducts (Robbie, Palmer, Denny & Wilson, 1990). In contrast, the corresponding quinone diimine (7) of the biologically inactive isomer *o*-AMSA (6) is very unstable, with mild oxidation of (6), even under rigorously dry conditions, yielding only (8), the hydrolysis product of (7) (Lee, Palmer & Denny, 1988).




The intermediacy of (2) has been suggested as important in the biological activity of amsacrine (Shoemaker, Cysyk, Gormley, DeSouza & Malspeis, 1984). While this view has been contested (Robbie, Palmer, Denny & Wilson, 1990), the difference in stability of the two quinone diimine primary metabolites [(2) and (7)] of the corresponding isomeric 9-anilinoacridines amsacrine (1) and *o*-AMSA (6), where the only difference is placement of the methoxy group, is nevertheless of interest. Previous crystallographic studies on the conformations of the isomeric parent compounds (1) and (6) have shown that the methoxy group exerts a significant steric effect (Buckleton & Waters, 1984; Abraham, Cutbush, Kuroda, Neidle, Acheson & Taylor, 1985; Karle, Cysyk & Karle, 1980; Buckleton & Clark, 1992). We report here comparative crystallographic studies on the two corresponding quinone imines (5) and (8) (being the closest available compounds to the quinone diimines of interest), and on the product (13) of nucleophilic substitution of (8) with dimethylamine (Lee, Palmer & Denny, 1988).

## Experimental

Crystals were obtained as dark-brown tablets by slow evaporation from methanol. Crystal sizes: (5) 0.42 × 0.13 × 0.18 mm, (8) 0.16 × 0.23 × 0.18 mm, (13) 0.20 × 0.24 × 0.14 mm. Densities were measured by KCl/KBr gradient column. Diffractometer: (5) Nicolet *R*3, (8) and (13) Nonius CAD-4. Mo $K\alpha$, graphite monochromator, $\lambda = 0.71069$ Å. $2\theta/\omega$ scans, variable scan speeds and widths. Lattice parameters refined using 25 reflections in the ranges: (5) $17.0 \le 2\theta \le 24.4°$, (8) $18.4 \le 2\theta \le 24.2°$, (13) $20.3 \le 2\theta \le 30.0°$. $[(\sin\theta)/\lambda]_{max}$: (5) 0.6388, (8) 0.5723, (13) 0.5947 Å$^{-1}$. Range of *hkl*: (5) $hk \pm l$, (8)

$\pm h \pm k - l$, (13) $h \pm k \pm l$. For (5) 2938 independent reflections measured ($0 \le h \le 17$, $0 \le k \le 9$, $-23 \le l \le 23$), 1561 observed [$I > 3\sigma(I)$]. For (8) 2384 independent reflections measured ($-12 \le h \le 12$, $-17 \le k \le 17$, $-9 \le l \le 0$), 1119 observed. For (13) 2350 independent reflections measured ($0 \le h \le 12$, $-14 \le k \le 14$, $-16 \le l \le 16$), 1520 observed. Three standard reflections checked every 100 reflections, (5) 600, 040, 006, (8) $16\bar{2}$, $\bar{4}02$, $3\bar{6}0$, (13) 251, $\bar{2}2\bar{6}$, $3\bar{3}4$. No significant variations for (5), maximum variations for (8) 13.6, (13) 13.0%. Corrections for Lorentz and polarization factors and crystal decay for (8) and (13). Absorption corrections not required. $R_{int}$ = (5) 0.023, (8) 0.042, (13) 0.018.

Structure solution by direct methods using *SHELXS*86 (Sheldrick, 1986). Refinement was by full-matrix least squares on $F$ using *SHELX*76 (Sheldrick, 1976). Atomic scattering factors were taken from *International Tables for X-ray Crystallography* (1974, Vol. IV). H atoms were located and refined isotropically for (5) and (13), and placed in calculated positions assuming a riding model for (8). Parameters in least-squares refinement: (5) 273, (8) 222, (13) 396. Weights $w = [\sigma^2(F) + gF^2]^{-1}$, $g$ for (5) $1.43 \times 10^{-3}$, (8) $9.67 \times 10^{-4}$, (13) $1.58 \times 10^{-2}$. Final residuals $R$ and $wR$: (5) 0.049, 0.053, (8) 0.075, 0.076, (13) 0.034, 0.041. $(\Delta/\sigma)_{max}$ for non-H-atom positions in final refinement cycle: (5) 0.13 $x$ of C(2′), (8) −0.18 $z$ of C(2″), (13) 0.09 $z$ of C(6′). Maximum and minimum heights in final difference Fourier map: (5) 0.26, −0.25, (8) 0.27, −0.38, (13) 0.12, −0.19 e Å$^{-3}$. Final $S$ values (5) 1.38, (8) 2.81, (11) 0.16. The final atomic positions and equivalent isotropic temperature factors are in Tables 1, 3 and 5 for compounds (5), (8) and (13) respectively. Similarly, bond lengths and angles are in Tables 2, 4 and 6.*

Table 1. *Atomic positions and equivalent isotropic temperature factors for* $C_{20}H_{14}N_2O_2$ *(meta isomer)* (5)

$B_{eq} = (8\pi^2/3)\sum_i \sum_j U_{ij} a_i^* \mathbf{a}_i . \mathbf{a}_j$.

| | $x$ | $y$ | $z$ | $B_{eq}$ (Å$^2$) |
|---|---|---|---|---|
| N(9′) | 0.7483 (2) | 0.2063 (4) | 0.58690 (18) | 1.9 (1) |
| N(10) | 1.0326 (2) | 0.1346 (4) | 0.82376 (17) | 1.8 (1) |
| O(1″) | 0.5638 (2) | −0.2573 (4) | 0.31902 (17) | 3.1 (2) |
| O(3″) | 0.56654 (19) | 0.3208 (4) | 0.45707 (16) | 2.6 (1) |
| C(1) | 0.9739 (3) | 0.1536 (5) | 0.5993 (2) | 2.0 (2) |
| C(1a) | 0.9511 (3) | 0.1520 (5) | 0.6689 (2) | 1.6 (2) |
| C(1′) | 0.6012 (3) | −0.1554 (6) | 0.3820 (2) | 2.2 (2) |
| C(2) | 1.0813 (3) | 0.1437 (5) | 0.6100 (3) | 2.3 (2) |
| C(2′) | 0.5576 (3) | 0.0342 (5) | 0.3816 (2) | 2.0 (1) |
| C(3) | 1.1719 (3) | 0.1309 (5) | 0.6917 (2) | 2.3 (2) |
| C(3′) | 0.6023 (3) | 0.1463 (5) | 0.4492 (2) | 1.9 (1) |
| C(3″) | 0.4617 (3) | 0.3838 (7) | 0.3887 (3) | 2.7 (2) |
| C(4) | 1.1542 (3) | 0.1278 (5) | 0.7602 (2) | 2.0 (1) |
| C(4a) | 1.0435 (3) | 0.1388 (5) | 0.7522 (2) | 1.7 (2) |
| C(4′) | 0.7026 (3) | 0.0832 (5) | 0.5269 (2) | 1.6 (2) |
| C(5) | 0.9141 (3) | 0.1324 (5) | 0.8900 (2) | 2.2 (2) |
| C(5a) | 0.9280 (3) | 0.1401 (5) | 0.8155 (2) | 1.7 (2) |
| C(5′) | 0.7408 (3) | −0.1127 (5) | 0.5292 (2) | 1.9 (1) |
| C(6) | 0.8104 (3) | 0.1363 (5) | 0.8856 (3) | 2.4 (2) |
| C(6′) | 0.6929 (3) | −0.2259 (6) | 0.4621 (2) | 2.4 (1) |
| C(7) | 0.7141 (3) | 0.1515 (5) | 0.8070 (3) | 2.4 (1) |
| C(8) | 0.7227 (3) | 0.1616 (5) | 0.7347 (2) | 2.2 (1) |
| C(8a) | 0.8301 (3) | 0.1563 (5) | 0.7364 (2) | 1.7 (1) |
| C(9) | 0.8434 (3) | 0.1635 (5) | 0.6632 (2) | 1.7 (2) |

Table 2. *Bond distances* (Å) *and bond angles* (°) *for* $C_{20}H_{14}N_2O_2$ *(meta isomer)* (5)

| | | | |
|---|---|---|---|
| C(4′)—N(9′) | 1.291 (5) | C(3′)—C(2′) | 1.332 (5) |
| C(9)—N(9′) | 1.410 (5) | C(4)—C(3) | 1.349 (6) |
| C(4a)—N(10) | 1.353 (5) | C(4′)—C(3′) | 1.493 (5) |
| C(5a)—N(10) | 1.346 (5) | C(4a)—C(4) | 1.431 (5) |
| C(1′)—O(1″) | 1.229 (5) | C(5′)—C(4′) | 1.460 (5) |
| C(3′)—O(3″) | 1.344 (5) | C(5a)—C(5) | 1.428 (5) |
| C(3″)—O(3″) | 1.448 (5) | C(6)—C(5) | 1.360 (6) |
| C(1a)—C(1) | 1.410 (5) | C(8a)—C(5a) | 1.428 (5) |
| C(2)—C(1) | 1.369 (6) | C(6′)—C(5′) | 1.332 (6) |
| C(4a)—C(1a) | 1.439 (5) | C(7)—C(6) | 1.412 (6) |
| C(9)—C(1a) | 1.407 (5) | C(8)—C(7) | 1.349 (6) |
| C(2′)—C(1′) | 1.450 (6) | C(8a)—C(8) | 1.432 (5) |
| C(6′)—C(1′) | 1.482 (6) | C(9)—C(8a) | 1.399 (5) |
| C(3)—C(2) | 1.412 (6) | | |

| | | | |
|---|---|---|---|
| C(9)—N(9′)—C(4′) | 122.4 (3) | C(4)—C(4a)—C(1a) | 118.3 (3) |
| C(5a)—N(10)—C(4a) | 117.2 (3) | C(3′)—C(4′)—N(9′) | 117.2 (3) |
| C(3″)—O(3″)—C(3′) | 116.5 (3) | C(5′)—C(4′)—N(9′) | 125.3 (3) |
| C(2)—C(1)—C(1a) | 121.2 (4) | C(5′)—C(4′)—C(3′) | 117.5 (3) |
| C(4a)—C(1a)—C(1) | 118.5 (3) | C(6)—C(5)—C(5a) | 120.9 (4) |
| C(9)—C(1a)—C(1) | 124.6 (3) | C(5)—C(5a)—N(10) | 118.3 (3) |
| C(9)—C(1a)—C(4a) | 116.9 (3) | C(8a)—C(5a)—N(10) | 123.7 (3) |
| C(2′)—C(1′)—O(1″) | 122.2 (4) | C(8a)—C(5a)—C(5) | 118.0 (3) |
| C(6′)—C(1′)—O(1″) | 120.0 (4) | C(6′)—C(5′)—C(4′) | 121.1 (4) |
| C(6′)—C(1′)—C(2′) | 117.8 (4) | C(7)—C(6)—C(5) | 120.6 (4) |
| C(3)—C(2)—C(1) | 120.3 (4) | C(5′)—C(6′)—C(1′) | 121.0 (4) |
| C(3′)—C(2′)—C(1′) | 121.5 (4) | C(8)—C(7)—C(6) | 120.9 (4) |
| C(4)—C(3)—C(2) | 120.7 (4) | C(8a)—C(8)—C(7) | 120.4 (4) |
| C(2′)—C(3′)—O(3″) | 126.7 (4) | C(8)—C(8a)—C(5a) | 119.2 (3) |
| C(4′)—C(3′)—O(3″) | 112.7 (3) | C(9)—C(8a)—C(5a) | 118.2 (3) |
| C(4′)—C(3′)—C(2′) | 120.6 (4) | C(9)—C(8a)—C(8) | 122.6 (3) |
| C(4a)—C(4)—C(3) | 121.1 (4) | C(1a)—C(9)—N(9′) | 122.0 (3) |
| C(1a)—C(4a)—N(10) | 124.2 (3) | C(8a)—C(9)—N(9′) | 117.6 (3) |
| C(4)—C(4a)—N(10) | 117.6 (3) | C(8a)—C(9)—C(1a) | 119.9 (3) |

## Description of the crystal structures

The crystals consist of monomers of neutral molecules. The molecular geometries of (5), (8) and (13) are shown in Figs. 1, 2 and 3 respectively. Atoms are depicted as 50% probability surfaces. The two basic structural features of the molecules are the acridine fused-ring system and the substituted quinone imine ring, the two being joined by the single bond C(9)—N(9′).

The acridine rings in the three compounds are essentially identical with respect to bond lengths and angles. Each exhibits the same pattern of short–long–short *etc.* bonds as one proceeds outwards from N(10), consistent with the pattern expected on the basis of the merging of the four possible Kekulé structures of acridine. The only noteworthy difference between the three rings is their respective planarities. (5) and (8) are essentially planar, whereas (13) is partially distorted towards a butterfly geometry by a small fold along the C(9)—N(10) vector. The deviation from planarity is intermediate between that of *m*-AMSA (1) and *m*-AMSA.H$^+$ (Buckleton

* Lists of anisotropic thermal parameters, H-atom positions, observed and calculated structure factors, and least-squares planes have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 55555 (21 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England. [CIF reference: HU0417]

Table 3. *Atomic positions and equivalent isotropic temperature factors for* $C_{20}H_{14}N_2O_2$ *(ortho isomer)* (8)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$

| | $x$ | $y$ | $z$ | $B_{eq}$ (Å$^2$) |
|---|---|---|---|---|
| O(1'') | 0.7801 (8) | 0.4421 (5) | −0.3544 (10) | 5.5 (4) |
| O(2'') | 0.7373 (7) | 0.5126 (5) | −0.0088 (11) | 5.5 (5) |
| N(9') | 0.8987 (8) | 0.2445 (5) | 0.2695 (11) | 4.1 (4) |
| N(10) | 1.0645 (8) | −0.0157 (6) | 0.2536 (10) | 3.3 (3) |
| C(1) | 1.2333 (12) | 0.2873 (7) | 0.3167 (15) | 4.6 (6) |
| C(1a) | 1.1154 (11) | 0.1807 (6) | 0.2822 (13) | 3.3 (5) |
| C(1') | 0.7979 (9) | 0.3934 (7) | −0.2168 (15) | 3.6 (5) |
| C(2) | 1.3877 (12) | 0.3051 (7) | 0.3419 (15) | 5.2 (5) |
| C(2') | 0.7824 (10) | 0.4273 (7) | −0.0160 (16) | 4.1 (6) |
| C(2'') | 0.7170 (12) | 0.5512 (8) | 0.1790 (19) | 6.1 (6) |
| C(3) | 1.4368 (11) | 0.2158 (8) | 0.3372 (15) | 5.0 (6) |
| C(3') | 0.8167 (9) | 0.3771 (6) | 0.1420 (14) | 3.6 (5) |
| C(4) | 1.3274 (10) | 0.1115 (7) | 0.3052 (14) | 4.2 (5) |
| C(4a) | 1.1648 (10) | 0.0893 (7) | 0.2806 (13) | 3.4 (4) |
| C(4') | 0.8671 (9) | 0.2858 (6) | 0.1116 (14) | 2.9 (3) |
| C(5) | 0.8059 (11) | −0.1437 (7) | 0.2129 (13) | 3.9 (5) |
| C(5a) | 0.9113 (10) | −0.0326 (6) | 0.2368 (12) | 3.2 (5) |
| C(5') | 0.8738 (10) | 0.2466 (7) | −0.0901 (14) | 4.0 (5) |
| C(6) | 0.6506 (12) | −0.1690 (7) | 0.1950 (14) | 4.7 (5) |
| C(6') | 0.8417 (9) | 0.2979 (6) | −0.2436 (14) | 3.6 (4) |
| C(7) | 0.5875 (11) | −0.0862 (8) | 0.1973 (15) | 5.1 (6) |
| C(8) | 0.6833 (11) | 0.0201 (8) | 0.2182 (14) | 4.1 (5) |
| C(8a) | 0.8480 (11) | 0.0501 (7) | 0.2377 (12) | 3.3 (5) |
| C(9) | 0.9530 (11) | 0.1578 (7) | 0.2572 (14) | 3.7 (5) |

Table 4. *Bond distances* (Å) *and bond angles* (°) *for* $C_{20}H_{14}N_2O_2$ *(ortho isomer)* (8)

| | | | |
|---|---|---|---|
| C(1')—O(1'') | 1.217 (10) | C(3')—C(2') | 1.368 (11) |
| C(2')—O(2'') | 1.342 (9) | C(4)—C(3) | 1.364 (11) |
| C(2'')—O(2'') | 1.426 (11) | C(4')—C(3') | 1.462 (10) |
| C(4')—N(9') | 1.288 (10) | C(4a)—C(4) | 1.406 (11) |
| C(9)—N(9') | 1.415 (10) | C(5')—C(4') | 1.458 (11) |
| C(4a)—N(10) | 1.348 (10) | C(5a)—C(5) | 1.430 (11) |
| C(5a)—N(10) | 1.344 (10) | C(6)—C(5) | 1.342 (11) |
| C(1a)—C(1) | 1.413 (11) | C(8a)—C(5a) | 1.419 (10) |
| C(2)—C(1) | 1.342 (12) | C(6')—C(5') | 1.349 (11) |
| C(4a)—C(1a) | 1.443 (10) | C(7)—C(6) | 1.420 (12) |
| C(9)—C(1a) | 1.402 (12) | C(8)—C(7) | 1.357 (12) |
| C(2')—C(1') | 1.472 (12) | C(8a)—C(8) | 1.417 (11) |
| C(6')—C(1') | 1.472 (10) | C(9)—C(8a) | 1.400 (12) |
| C(3)—C(2) | 1.414 (12) | | |

| | | | |
|---|---|---|---|
| C(2'')—O(2'')—C(2') | 116.6 (8) | C(4)—C(4a)—C(1a) | 117.7 (9) |
| C(9)—N(9')—C(4') | 119.7 (8) | C(3')—C(4')—N(9') | 115.4 (8) |
| C(5a)—N(10)—C(4a) | 116.2 (7) | C(5')—C(4')—N(9') | 125.3 (7) |
| C(2)—C(1)—C(1a) | 121.9 (9) | C(5')—C(4')—C(3') | 119.3 (8) |
| C(4a)—C(1a)—C(1) | 118.5 (8) | C(6)—C(5)—C(5a) | 120.6 (9) |
| C(9)—C(1a)—C(1) | 124.2 (8) | C(5)—C(5a)—N(10) | 116.1 (8) |
| C(9)—C(1a)—C(4a) | 117.3 (8) | C(8a)—C(5a)—N(10) | 125.2 (8) |
| C(2')—C(1')—O(1'') | 123.0 (8) | C(8a)—C(5a)—C(5) | 118.7 (8) |
| C(6')—C(1')—O(1'') | 120.4 (9) | C(6')—C(5')—C(4') | 120.2 (8) |
| C(6')—C(1')—C(2') | 116.5 (8) | C(7)—C(6)—C(5) | 120.6 (9) |
| C(3)—C(2)—C(1) | 119.9 (9) | C(5')—C(6')—C(1') | 122.3 (9) |
| C(1')—C(2')—O(2'') | 112.1 (8) | C(8)—C(7)—C(6) | 120.5 (10) |
| C(3')—C(2')—O(2'') | 126.1 (10) | C(8a)—C(8)—C(7) | 120.5 (9) |
| C(3')—C(2')—C(1') | 121.8 (8) | C(8)—C(8a)—C(5a) | 119.0 (8) |
| C(4)—C(3)—C(2) | 120.3 (9) | C(9)—C(8a)—C(5a) | 117.6 (8) |
| C(4')—C(3')—C(2') | 119.8 (9) | C(9)—C(8a)—C(8) | 123.5 (8) |
| C(4a)—C(4)—C(3) | 121.6 (8) | C(1a)—C(9)—N(9') | 120.0 (8) |
| C(1a)—C(4a)—N(10) | 124.1 (8) | C(8a)—C(9)—N(9') | 120.1 (8) |
| C(4)—C(4a)—N(10) | 118.2 (8) | C(8a)—C(9)—C(1a) | 119.7 (8) |

& Waters, 1984; Abraham, Cutbush, Kuroda, Neidle, Acheson & Taylor, 1985; Karle, Cysyk & Karle, 1980). The quinone imine rings in (5) and (8) are very similar, but the additional substituent in (13) causes bond length changes within the ring to minimize the close interaction between the —$NMe_2$ C(5'') atom and N(9'), and the ring is partially buckled away from planarity towards a flattened boat conformation. The acridine rings in (8) and (13) are partially stacked in the crystals in the typical

Table 5. *Atomic positions and equivalent isotropic temperature factors for* $C_{22}H_{19}N_3O_2$ (13)

$B_{eq} = (8\pi^2/3)\sum_i\sum_j U_{ij} a_i^* a_j^* \mathbf{a}_i.\mathbf{a}_j.$

| | $x$ | $y$ | $z$ | $B_{eq}$ (Å$^2$) |
|---|---|---|---|---|
| N(5'') | 0.8640 (3) | 0.3467 (2) | 0.16864 (19) | 2.6 (1) |
| N(9') | 0.7556 (3) | 0.2271 (2) | 0.33363 (17) | 2.3 (1) |
| N(10) | 0.6875 (3) | 0.0459 (2) | 0.60767 (17) | 2.2 (1) |
| O(1'') | 0.2792 (3) | 0.2710 (2) | −0.01558 (17) | 3.7 (1) |
| O(2'') | 0.2084 (2) | 0.05399 (18) | 0.05804 (14) | 2.7 (1) |
| C(1) | 0.8211 (3) | −0.0602 (3) | 0.3062 (2) | 2.6 (1) |
| C(1a) | 0.7610 (3) | 0.0263 (3) | 0.4086 (2) | 2.1 (1) |
| C(1') | 0.3996 (4) | 0.2418 (3) | 0.0476 (2) | 2.6 (1) |
| C(2) | 0.8629 (4) | −0.1876 (3) | 0.3040 (2) | 3.0 (1) |
| C(2') | 0.3661 (3) | 0.1298 (3) | 0.1034 (2) | 2.3 (1) |
| C(2'') | 0.1604 (3) | −0.0549 (3) | 0.1079 (2) | 2.6 (1) |
| C(3) | 0.8458 (4) | −0.2390 (3) | 0.4011 (2) | 3.1 (2) |
| C(3') | 0.4844 (3) | 0.1174 (3) | 0.1937 (2) | 2.1 (1) |
| C(4) | 0.7861 (3) | −0.1624 (3) | 0.4979 (2) | 2.7 (1) |
| C(4a) | 0.7428 (3) | −0.0263 (3) | 0.5070 (2) | 2.2 (1) |
| C(4') | 0.6501 (3) | 0.2062 (2) | 0.2320 (2) | 2.0 (1) |
| C(5) | 0.6021 (3) | 0.2578 (3) | 0.7251 (2) | 2.5 (2) |
| C(5a) | 0.6566 (3) | 0.1783 (3) | 0.6181 (2) | 2.1 (1) |
| C(5') | 0.6999 (3) | 0.2885 (2) | 0.1521 (2) | 2.2 (1) |
| C(5'') | 0.9055 (4) | 0.4435 (3) | 0.1041 (3) | 3.9 (2) |
| C(5''') | 1.0115 (4) | 0.2992 (3) | 0.2258 (3) | 3.4 (2) |
| C(6) | 0.5748 (4) | 0.3921 (3) | 0.7428 (2) | 2.9 (2) |
| C(6') | 0.5712 (4) | 0.3085 (3) | 0.0701 (2) | 2.5 (2) |
| C(7) | 0.5982 (4) | 0.4573 (3) | 0.6555 (2) | 2.9 (2) |
| C(8) | 0.6467 (3) | 0.3854 (3) | 0.5505 (2) | 2.5 (1) |
| C(8a) | 0.6766 (3) | 0.2433 (3) | 0.5273 (2) | 2.0 (1) |
| C(9) | 0.7241 (3) | 0.1626 (3) | 0.4202 (2) | 2.0 (1) |

Table 6. *Bond distances* (Å) *and bond angles* (°) *for* $C_{22}H_{19}N_3O_2$ (13)

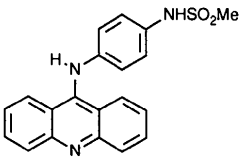
| | | | |
|---|---|---|---|
| C(5')—N(5'') | 1.351 (3) | C(6')—C(1') | 1.422 (4) |
| C(5'')—N(5''') | 1.454 (4) | C(3)—C(2) | 1.409 (4) |
| C(5''')—N(5'') | 1.467 (4) | C(3')—C(2') | 1.348 (4) |
| C(4')—N(9') | 1.286 (3) | C(4)—C(3) | 1.348 (4) |
| C(9)—N(9') | 1.406 (4) | C(4')—C(3') | 1.449 (3) |
| C(4a)—N(10) | 1.347 (3) | C(4a)—C(4) | 1.424 (4) |
| C(5a)—N(10) | 1.352 (3) | C(5')—C(4') | 1.505 (4) |
| C(1')—O(1'') | 1.240 (4) | C(5a)—C(5) | 1.423 (3) |
| C(2')—O(2'') | 1.346 (3) | C(6)—C(5) | 1.348 (4) |
| C(2'')—O(2'') | 1.443 (4) | C(8a)—C(5a) | 1.434 (4) |
| C(1a)—C(1) | 1.432 (3) | C(6')—C(5') | 1.370 (4) |
| C(2)—C(1) | 1.353 (4) | C(7)—C(6) | 1.403 (4) |
| C(4a)—C(1a) | 1.433 (4) | C(8)—C(7) | 1.358 (4) |
| C(9)—C(1a) | 1.404 (4) | C(8a)—C(8) | 1.423 (4) |
| C(2')—C(1') | 1.493 (4) | C(9)—C(8a) | 1.400 (3) |

| | | | |
|---|---|---|---|
| C(5'')—N(5'')—C(5') | 119.0 (2) | C(4)—C(4a)—C(1a) | 118.0 (2) |
| C(5''')—N(5'')—C(5') | 125.4 (2) | C(3')—C(4')—N(9') | 124.9 (3) |
| C(5'')—N(5'')—C(5''') | 114.4 (2) | C(5')—C(4')—N(9') | 116.7 (2) |
| C(9)—N(9')—C(4') | 123.4 (2) | C(5')—C(4')—C(3') | 118.3 (2) |
| C(5a)—N(10)—C(4a) | 117.4 (2) | C(6)—C(5)—C(5a) | 121.2 (3) |
| C(2'')—O(2'')—C(2') | 117.4 (2) | C(5)—C(5a)—N(10) | 118.4 (3) |
| C(2)—C(1)—C(1a) | 120.5 (3) | C(8a)—C(5a)—N(10) | 123.5 (2) |
| C(4a)—C(1a)—C(1) | 118.7 (2) | C(8a)—C(5a)—C(5) | 118.1 (2) |
| C(9)—C(1a)—C(1) | 123.6 (3) | C(4')—C(5')—N(5'') | 119.9 (2) |
| C(9)—C(1a)—C(4a) | 117.7 (2) | C(6')—C(5')—N(5'') | 122.9 (3) |
| C(2')—C(1')—O(1'') | 119.3 (2) | C(6')—C(5')—C(4') | 117.0 (2) |
| C(6')—C(1')—O(1'') | 123.7 (3) | C(7)—C(6)—C(5) | 120.8 (2) |
| C(6')—C(1')—C(2') | 117.0 (2) | C(5')—C(6')—C(1') | 123.2 (3) |
| C(3)—C(2)—C(1) | 120.9 (3) | C(8)—C(7)—C(6) | 120.5 (3) |
| C(1')—C(2')—O(2'') | 112.6 (2) | C(8a)—C(8)—C(7) | 120.8 (3) |
| C(3')—C(2')—O(2'') | 126.8 (3) | C(8)—C(8a)—C(5a) | 118.5 (2) |
| C(3')—C(2')—C(1') | 120.5 (2) | C(9)—C(8a)—C(5a) | 117.8 (2) |
| C(4)—C(3)—C(2) | 120.6 (3) | C(9)—C(8a)—C(8) | 123.6 (3) |
| C(4')—C(3')—C(2') | 120.4 (3) | C(1a)—C(9)—N(9') | 121.0 (2) |
| C(4a)—C(4)—C(3) | 121.4 (3) | C(8a)—C(9)—N(9') | 118.8 (2) |
| C(1a)—C(4a)—N(10) | 123.7 (2) | C(8a)—C(9)—C(1a) | 119.6 (2) |
| C(4)—C(4a)—N(10) | 118.3 (3) | | |

self-intercalative fashion but no such stacking occurs in (5). The torsion angles defining the relative orientation of acridine and quinone imine rings are C(1a)—C(9)—N(9)—C(4') 75.5, 81.6, 85.1°; C(9)—N(9')—C(4')—C(3') [C(5') in (13)] 179.9, 178.2, 177.4° in (5), (8) and (13) respectively.

## Discussion

A comparison of crystallographic and NMR studies carried out on the parent compounds (1) and (6) provides information about the steric effects of the methoxy group in the two different positions. 9-Anilinoacridines can exist in two possible conformations [rotamers around the N(9)—C(4′) bond]. These cannot be distinguished for unsubstituted compounds such as AMSA (9), but are different for asymmetrically substituted derivatives such as (1) and (6). The likely lowest-energy conformation(s) of (9) are shown by the crystal structure of AMSA (Hall, Swann & Waters, 1974), but NMR studies of this compound show it to be in free rotation in solution at 293 K (Lee, Palmer & Denny, 1988), suggesting the energy barrier between the rotamers is small for compounds with no 3′- or 5′-substituents.

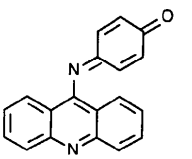



(9)

The 3′-OMe derivative amsacrine (1) would be expected, on steric grounds, to take up a conformation where the OMe group is distal from the acridine ring, and this is confirmed by a crystal structure of the free base (Buckleton & Waters, 1984; Abraham, Cutbush, Kuroda, Neidle, Acheson & Taylor, 1985). However, a crystal structure determination of the cationic form shows it to be in the alternative proximal conformation (Karle, Cysyk & Karle, 1980) where extra stability is gained by electrostatic attraction between the 3′-OMe O atom and the protonated acridine ring. Energy calculations show that a small number of interconvertible low-energy conformations are possible (Abraham, Cutbush, Kuroda, Neidle, Acheson & Taylor, 1985).

The crystal structure of the 2′-OMe derivative (6) in its free-base form shows it to be in the conformation in which the OMe group is again distal from the acridine (Buckleton & Clark, 1992).

In the quinone imines the barrier to free rotation around the N(9)—C(4′) bond to form the two different rotamers is much higher, and NMR studies of the unsubstituted derivatives (10) (Lee, Palmer & Denny, 1988) show that there is indeed restricted rotation in solution at 293 K. Our crystal structure determination shows that the 3′-methoxy compound (5) exists in the same conformation as the parent free base, with the methoxy group in the expected position, distal from the acridine ring.




(10)

The structure which the 2′-methoxyquinone imine (8) would be predicted to adopt is less obvious. Previous studies of NMR chemical shift positions suggested that the 3′-proton was in the deshielding region closest to the acridine ring and on the basis of this the proximal conformation has been proposed (Lee, Palmer & Denny, 1988). However, the crystal structure shows it quite clearly to be in the alternative conformation, with the OMe (and therefore also the 3′-H) distal from the acridine. It is possible that the different solution and solid-state conformations are due to crystal packing forces.

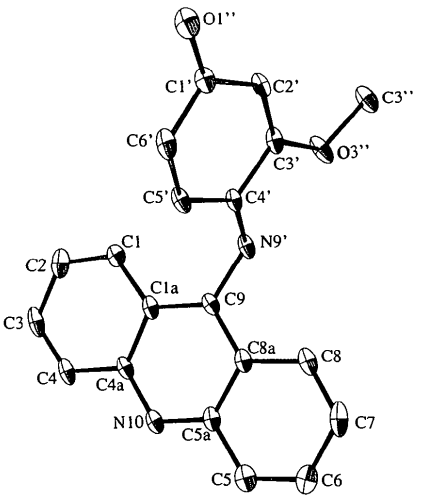



Fig. 1. Molecular geometry and atomic numbering for $C_{20}H_{14}N_2O_2$ (*meta* isomer) (5).

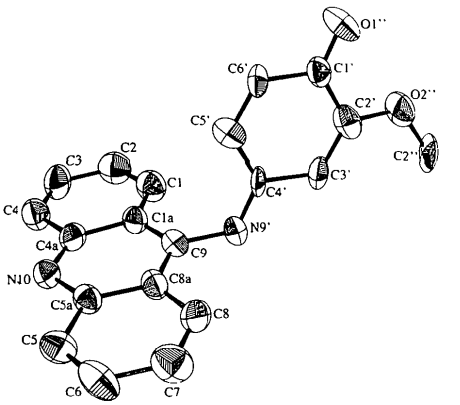
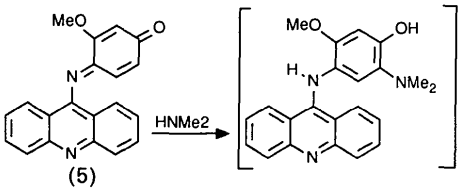
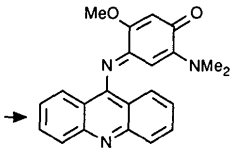



Fig. 2. Molecular geometry and atomic numbering for $C_{20}H_{14}N_2O_2$ (*ortho* isomer) (8).

The quinones (5) and (8) undergo ready substitution with a variety of nucleophiles, primarily *via* 1,4-addition with respect to the imine (Lee, Palmer & Denny, 1988). Thus, reaction of (5) with dimethylamine gave a 96% yield of (11), the product of addition to the (sterically available) 6′-position followed by facile reoxidation. In contrast, (8) gave a

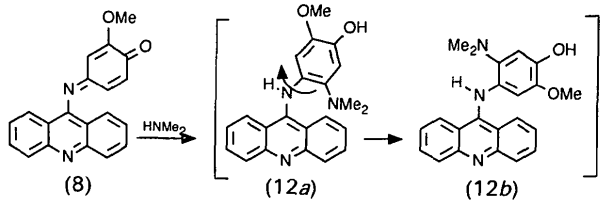



(5)

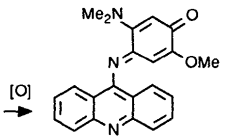



(11)

low (16%) yield of (13). The sluggish nature of this reaction may reflect the fact that the first step is addition at the sterically hindered 5′-position of (8) to form the phenol (12*a*). This would rapidly undergo rotation about the N(9)—C(4′) bond to give the more stable phenol rotamer (12*b*), before this underwent facile oxidation to give (13). The conformation of (13) (—OMe proximal, —$NMe_2$ distal to the acridine ring) is confirmed by the crystal structure analysis.




(8) (12*a*) (12*b*)




(13)

Thus an understanding of the conformational preferences in this series of 9-anilinoacridines and their related quinone imine oxidation products sheds light on their subsequent reactions.

Although the present study of the quinone imine metabolites does not directly explain the differences in biological activity between *m*- and *o*-AMSA themselves, it does add to the body of structural information available on the crystal structures of both active

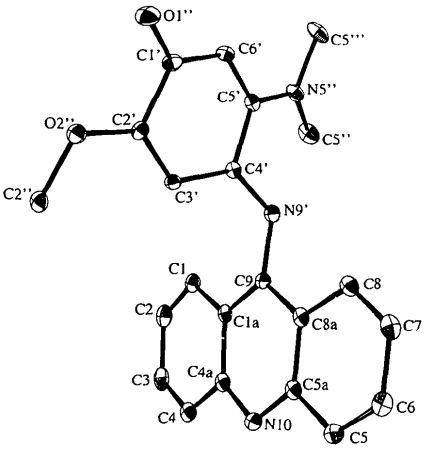



Fig. 3. Molecular geometry and atomic numbering for $C_{22}H_{19}N_3O_2$ (13).

and inactive acridine-based DNA intercalators. An interesting trend has emerged from the crystal structures of 9-anilinoacridines in which there is no further substituent on the acridine ring. All those which exhibit anti-tumour activity (including the *m*-AMSA metabolite) do not incorporate acridine stacking in the solid state, whereas all those which are inactive or only marginally active (including the *o*-AMSA metabolite) do. It will be of interest to see if this trend continues as further related structures are reported, although there have been no reports of significant self-stacking of 9-anilinoacridines in solution.

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