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Structure of the Antitumour Agent *m*-AMSA (Amsacrine), $C_{21}H_{19}N_3O_3S$, as the Free Base

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Abstract. $M_r = 393{\cdot}5$, monoclinic, $P2_1/c$, $a = 11{\cdot}176$ (3), $b = 21{\cdot}142$ (4), $c = 8{\cdot}794$ (3) Å, $\beta = 112{\cdot}29$ (2)°, $U = 1922$ Å^3, $Z = 4$, $D_x = 1{\cdot}36$ g cm^{-3} (*cf.* 1·39 g cm^{-3} for HCl salt), Mo $K\alpha$, $\lambda = 0{\cdot}71073$ Å, $\mu = 2{\cdot}01$ cm^{-1}, $F(000) = 824$, $T = 256$ K, $R = 0{\cdot}041$ using 1478 reflections. In the acridine moiety the four outer C atoms are displaced to the same side, with the two central atoms displaced in the opposite direction. The phenyl ring is more nearly planar. The molecular geometry is compared with that of the HCl salt and other derivatives.

Introduction. The antitumour agent 4′-(9-acridinylamino)-3′-methoxymethanesulphonanilide (*m*-AMSA)† has shown dramatic activity against some tumours in clinical trials (Legha *et al.*, 1978; Von Hoff *et al.*, 1978). Its mode of action has been the subject of extensive investigations and it is known to react with DNA (Waring, 1976; Gormley, Sethi & Cysyk, 1978; Deaven, Oka & Tobey, 1978). As a class the 9-anilinoacridines are intercalating agents although their biological activity depends critically on substitution requiring, especially, electron-releasing substituents at the aniline 1′-position. The exact significance of the total substituent requirement is not, however, known. We report the structure of *m*-AMSA itself for comparison with the hydrochlorides of AMSA (which although active lacks the 3′-methoxy group) (Hall, Swann & Waters, 1974) and *m*-AMSA and with the methanesulphonate salt of the 2-methoxy substituent of AMSA (Karle, Cysyk & Karle, 1980).

Experimental. Crystals grown as orange, diamond-shaped plates by slow evaporation from methanol. D_m not measured. CAD-4 diffractometer, Mo $K\alpha$ radiation. 3675 reflections collected, 1478 with $I > 3\sigma(I)$ used; $\omega/2\theta$ mode, hkl range 0–12, 0–24, $\bar{1}0$–10, θ limit 25°; scan range $\theta = (0{\cdot}80 + 0{\cdot}35\tan\theta)°$ was extended by 25% on either side of the peak to record backgrounds; scan speed chosen to return a constant $\sigma(I)$ of $0{\cdot}02I$ with a maximum scan time set at 100 s. Three standard reflections recorded every 3600 s and three orientation-control reflections after every 100 measurements: no significant changes in standards or controls. Standard deviations determined from counting statistics but $\sigma(F_o^2) = [\sigma^2(I) + (fI)^2]^{1/2}/\mathrm{Lp}$ with a modifying factor, $f = 0{\cdot}04$. Weights $1/\sigma^2(F_o)$, *i.e.* $4F_o^2/\sigma^2(F_o^2)$, applied in later least-squares minimization of $\sum w(|F_o| - |F_c|)^2$. Crystal: rectangular plate, developed in {001}, dimensions 0·34 × 0·42 × 0·11 mm. Absorption corrections not applied. Systematic absences $h0l$ (l odd) and $0k0$ (k odd). Structure solved with the phase-determining program *MULTAN* (Germain, Main & Woolfson, 1971). Least-squares refinement [calculations with Enraf–Nonius *SDP* package and with a program derived from *ORFLS* (Busing, Martin & Levy, 1962)] of 253 variables of atomic positions (excluding H atoms) and anisotropic temperature parameters (not H atoms) brought R to 0·041 and R_w to 0·048. Atom scattering factors and anomalous-dispersion coefficients from Cromer & Waber (1974) and Cromer (1974). E.s.d. of an observation of unit weight 1·432; $(\Delta/\sigma)_{max} = 0{\cdot}3$. Final $\Delta\rho$ map showed no residual density. No correction for secondary extinction.

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† The compound is now known as amsacrine but the older nomenclature is retained here because of the comparisons made with other AMSA derivatives.

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Table 1. *Atom coordinates and isotropic thermal parameters*

$$B_{eq} = \tfrac{4}{3}\sum_i\sum_j b_{ij}\mathbf{a}_i.\mathbf{a}_j.$$

	x	*y*	*z*	B_{eq}(Å²)
C(1)	0·9265 (4)	0·4859 (2)	0·1921 (5)	3·4
C(2)	1·0028 (4)	0·5248 (2)	0·3102 (5)	4·0
C(3)	1·1385 (4)	0·5184 (2)	0·3668 (5)	4·1
C(4)	1·1929 (4)	0·4754 (2)	0·3007 (5)	3·7
C(4a)	1·1159 (3)	0·4337 (2)	0·1752 (5)	2·8
C(5)	1·1653 (4)	0·3138 (2)	−0·0862 (6)	4·7
C(5a)	1·1025 (3)	0·3556 (2)	−0·0154 (5)	3·0
C(6)	1·0981 (5)	0·2762 (3)	−0·2105 (6)	6·2
C(7)	0·9620 (5)	0·2761 (3)	−0·2740 (6)	5·8
C(8)	0·8976 (4)	0·3135 (2)	−0·2091 (6)	4·2
C(8a)	0·9646 (4)	0·3559 (2)	−0·0771 (5)	3·0
C(9)	0·9029 (3)	0·3969 (2)	−0·0047 (5)	2·8
C(9a)	0·9788 (3)	0·4381 (2)	0·1211 (5)	2·6
N(10)	1·1770 (3)	0·3931 (2)	0·1092 (4)	3·0
N(11)	0·7682 (3)	0·3986 (2)	−0·0614 (4)	3·7
C(12)	0·6947 (3)	0·3934 (2)	0·0371 (5)	2·8
C(13)	0·5652 (3)	0·4135 (2)	−0·0291 (5)	2·7
C(14)	0·4869 (3)	0·4055 (2)	0·0600 (5)	2·5
C(15)	0·5364 (3)	0·3798 (2)	0·2163 (5)	2·6
C(16)	0·6653 (4)	0·3623 (2)	0·2842 (5)	3·1
C(17)	0·7424 (3)	0·3686 (2)	0·1934 (5)	3·3
N(18)	0·4499 (3)	0·3719 (2)	0·2990 (4)	3·0
S(19)	0·4701 (1)	0·3229 (1)	0·4471 (1)	3·2
O(20)	0·3539 (2)	0·3250 (2)	0·4786 (3)	4·2
O(21)	0·5904 (3)	0·3358 (2)	0·5787 (3)	4·8
C(22)	0·4835 (5)	0·2480 (2)	0·3709 (6)	5·0
O(23)	0·5270 (2)	0·4381 (2)	−0·1813 (3)	4·0
C(24)	0·3924 (4)	0·4507 (2)	−0·2695 (5)	4·4

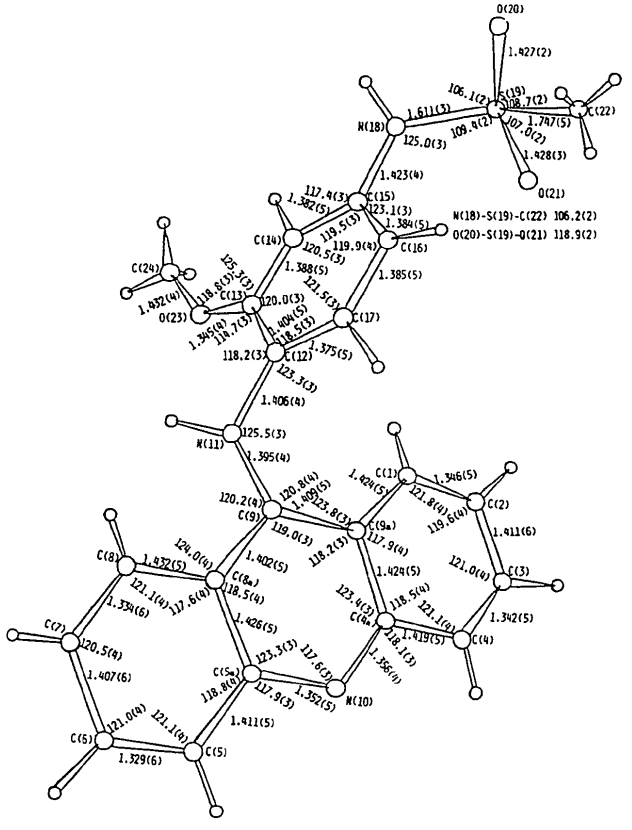

Fig. 1. Numbering scheme, bond lengths (Å) and angles (°).

Discussion. Atomic coordinates are listed in Table 1.* Fig. 1 records the calculated bond lengths and angles and the numbering system.

The overall molecular structure is similar to that already described for *m*-AMSA⁺ (Karle, Cysyk & Karle, 1980) but with the aniline ring disposed with respect to the acridine plane so as to keep the 3′-methoxy substituent away from the heterocycle. The close approaches of O(23) to C(1) and C(9) [C(8) and C(9) of the reference] are not seen, the H atom on C(17) being closest to the ring [H(17)···C(1) = 3·04 Å]. This 'more normal' conformation highlights the interactions found in *m*-AMSA⁺ and adds weight to the suggestion that the acridinium moiety has a considerable electrostatic attraction for O atoms.

It can be seen from the deviation of atoms from planes of best fit (Table 2) that, as in *m*-AMSA.HCl, the acridine group is non-planar, but again differences appear. The cations AMSA⁺, 2-MeO-AMSA⁺ and *m*-AMSA⁺ all show remarkably similar distortions from planarity, the latter notwithstanding the interaction with the 3′-methoxy group already mentioned, with the outer rings twisted to place two atoms of each on one side of the mean plane and two on the other. The 9-aminoacridinium cation is similarly non-planar but to a much smaller degree (Phillips, Ahmed & Barnes, 1960). In *m*-AMSA the four outer atoms C(2), C(3), C(6), C(7) are displaced to the same side, with two central atoms displaced in the opposite direction, to give the group the appearance of having been folded along the line C(9)···N(10). The neutral molecule, 9-aminoacridine, is planar (Talacki, Carrell & Glusker, 1974).

The phenyl ring is more nearly planar than is the acridine, but perhaps shows a slight 'boat' distortion with C(13) and C(16) as bow and stern atoms. The substituent atoms N(11), N(18) and O(23) are out of the mean plane as a consequence.

More detailed comparisons reveal other differences (Table 3). They are clearly electronic in origin and can be seen in changes in bond lengths and angles, particularly in the amino–acridine region. This is perhaps most evident about the bridging atom N(11) where it is found that the C(9)–N(11) bond is appreciably longer in neutral *m*-AMSA, the N(11)–C(12) distance being correspondingly reduced. The angles C(9a)–C(9)–N(11) and C(8a)–C(9)–N(11) are close to 120° [120·8 (4) and 120·2 (4)° respectively] compared with the values found in the cationic species (*e.g.* 117·4 and 123·9° in *m*-AMSA⁺). A comparison of the torsion angles about C(8a),-C(9)–N(11),C(12) and C(9),N(11)–C(12),C(17)

* Lists of structure factors, anisotropic temperature factors and H-atom coordinates have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 39250 (10 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

Table 2. *Deviations* (Å) *of atoms from planes of best fit*

Plane 1: $0{\cdot}2547x + 0{\cdot}6983y - 0{\cdot}6689z + 8{\cdot}4742 = 0$
Plane 2: $-0{\cdot}1246x - 0{\cdot}9148y - 0{\cdot}3841z - 8{\cdot}6861 = 0$

Plane 1				Plane 2			
C(1)	0·124 (4)	C(7)	0·061 (5)	N(11)	0·069 (4)	C(16)*	−0·014 (4)
C(2)	0·174 (4)	C(8)	0·022 (4)	C(12)*	0·012 (4)	C(17)*	0·003 (4)
C(3)	0·109 (4)	C(8a)*	0·007 (4)	C(13)*	−0·016 (4)	N(18)	0·059 (3)
C(4)	0·045 (4)	C(9)*	−0·018 (4)	C(14)*	0·005 (4)	O(23)	−0·028 (3)
C(4a)*	−0·001 (4)	C(9a)*	0·014 (4)	C(15)*	0·010 (4)		
C(5)	0·014 (5)	N(10)*	−0·011 (4)				
C(5a)*	0·007 (4)	N(11)	−0·020 (3)				
C(6)	0·051 (5)						

* Atoms used to define the planes.

Table 3. *Comparison of bond lengths* (Å), *bond angles* (°) *and torsion angles* (°)

	m-AMSA	*m*-AMSA$^+$	AMSA$^+$	2-MeO-AMSA$^+$
C(9)–N(11)	1·395	1·359	1·353	1·338
N(11)–C(12)	1·406	1·431	1·420	1·415
C(9)–N(11)–C(12)	125·5	129·6	127·2	128·3
C(9a)–C(9)–N(11)	120·8	117·4	116·3	119·3
C(8a)–C(9)–N(11)	120·2	123·9	124·1	123·1
C(8a)–C(9)–C(9a)	119·0	118·6	119·3	117·6
C(8a),C(9)–N(11),C(12)	129·0	−160·3	157·1	−149·2
C(9),N(11)–C(12),C(17)	19·1	−136·4	54·6	−139·4
C(4a)–C(9a)	1·424	1·415	1·418	1·392
C(4a)–N(10)	1·356	1·347	1·355	1·373
C(5a)–C(8a)	1·426	1·419	1·429	1·386
C(5a)–N(10)	1·352	1·350	1·365	1·396
C(8a)–C(9)	1·402	1·433	1·424	1·443
C(9)–C(9a)	1·409	1·440	1·441	1·440

shows how the aniline is brought further from the plane of the acridine avoiding the close approaches between atoms of these two rings seen in AMSA$^+$. (The difference in signs between angles for AMSA$^+$ and *m*-AMSA on the one hand and *m*-AMSA$^+$ and 2-MeO-AMSA$^+$ on the other is a result of molecules of opposite chirality being chosen for the respective coordinate lists.) It can be seen that the rotation about C(9)–N(11) is greatest, *i.e.* further from 180°, in *m*-AMSA where this bond is lengthened, whereas that about N(11)–C(12), the shortened bond in *m*-AMSA, is the least. (In this latter set of angles deviations occur from 0 to 180° reflecting the reversed orientation of the anilino methoxy group between *m*-AMSA and *m*-AMSA$^+$ to which attention has already been drawn.) The result is to move the phenyl moiety a little further from the acridine than occurs in the cationic species. Equally in line with the two bond-length changes just mentioned it is found that C(8a)–C(9) and C(9)–C(9a) are shorter than in the cations whereas C(4a)–C(9a) and C(5a)–C(8a) tend to be longer. The overall pattern of longer and shorter bonds is much the same in the acridine moiety of all compounds but the neutral species exhibits shorter bonds in each of the end rings at C(1)–C(2), C(3)–C(4), C(5)–C(6) and C(7)–C(8). The longer end bonds are of much the same length in all molecules.

The 125·0° angle at N(18) is, with those of the other three compounds, greater than 120° and there is, similarly, hydrogen bonding with a donor. Interestingly the H atom on N(18) refined to a position 0·39 Å above the trigonal plane defined by C(15),N(18),S(19), paralleling the finding in AMSA$^+$.

Although there are significant differences in detail between the neutral and cationic species their overall molecule shapes are the same in accord with the conclusion (Karle, Cysyk & Karle, 1980) that, thus far, there is not an obvious one-to-one correspondence between structures in the crystals and their clinical activities. Attention has been drawn to the need for more subtle changes such as the requirement for a non-planar acridine moiety (Hempel, Hall, Dauter, Bogucka-Ledochowska & Konitz, 1979), a feature which, if necessary, is by no means sufficient (Karle, Cysyk & Karle, 1980). The likely effect of the binding of the aniline section to the minor groove of DNA has also been discussed (Baguley, Denny, Atwell & Cain, 1981). Thus the correct combination of charge distribution and overall shape may not only require substituents in the right place but also those with the right spatial and electronic properties as well. Attention has been drawn to the fact that a 1′-substituent would be located close to a backbone phosphate if it lay in the minor groove (Baguley, Denny, Atwell & Cain, 1981) suggesting that this 'end' of the molecule is as vital to activity as the intercalating acridine group.

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