

## The Structure of Hycanthonone Methanesulfonate, $[C_{20}H_{24}N_2O_2S][CH_3SO_3H]$ : An Antischistosomal Agent\*

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The structure of the methanesulfonate of hycanthonone, 1-[2-(diethylamino)ethylamino]-4-(hydroxymethyl)-thioxanthone-9-one, has been determined by X-ray diffraction with three-dimensional counter data. The compound crystallizes with four formula units in a monoclinic unit cell of symmetry  $P2_1/a$ , with parameters  $a = 26.194$  (1),  $b = 8.7997$  (4),  $c = 10.6373$  (5) Å, and  $\beta = 116.420$  (7)°. The structure was solved by the heavy-atom technique and refined to a reliability index,  $R(F)$ , of 0.033, based on 3538 independent observed data corrected for absorption and for isotropic extinction. All H atoms were located and refined isotropically. The crystallographic evidence indicates that approximately 88% of the molecules in the specimen used are the alcohol species with a  $-CH_2OH$  group at the 4 position, while the remaining 12% are the corresponding aldehyde species. The average estimated standard deviation for bond distances between nonhydrogen atoms is 0.002 Å, and for bond angles it is 0.2°. The molecular parameters are all within the range of expected values. The two outer rings are planar to within 0.02 Å, and these planes intersect along the central  $S \cdots C$  line to form a slightly bent configuration with a dihedral angle of 167.7°. There is an intramolecular hydrogen bond between the proximal N and the carbonyl O. The hycanthonone base is protonated at the terminal N, and this proton and the hydroxyl H are hydrogen bonded to different O atoms of two neighboring methanesulfonate anions, thus forming infinite stacks of hydrogen-bonded molecules around the crystallographic twofold screw axes. The methanesulfonate anion, which furnishes two of its three O atoms as acceptors for interionic hydrogen bonds with two neighboring hycanthonone cations, has the expected configuration with approximately  $C_{3v}$  symmetry. Its charge is delocalized, as evidenced by the fact that all S–O bonds are equal to within 0.01 Å.

### Introduction

Hycanthonone, a major active metabolite of lucanthonone (Rosi, Peruzzotti, Dennis, Berberian, Freele, Tullar & Archer, 1967), was developed by Sterling–Winthrop Research Institute and has been used in the treatment of schistosomiasis, estimated to affect more than 200 million people in the tropics (Hubert, Bueding & Hartman, 1974). While a possible genetic hazard from the use of hycanthonone has been reported on the basis of a microbial test (Hartman, Levine, Hartman & Berger, 1971), Russell (1975) concluded, from results of the most extensive investigation carried out so far with mice, that the expected mutation rate in mammals at the therapeutic dose is unlikely to be more than a small fraction of the naturally occurring, spontaneous rate. A large number of biological and physiological experiments (in systems ranging from *in vivo* to subcellular, and from mammalian to bacterial) have been carried out on hycanthonone and lucanthonone in relation to their adverse mutagenic, teratogenic, and carcinogenic activities, as well as their well-established anti-schistosomidal activities (for reviews, see Weinstein &

Hirschberg, 1971; Hirschberg, 1975). The accumulated evidence now seems to suggest: (i) hycanthonone binds DNA to form an intercalative complex in a manner similar to that for actinomycin (Jain & Sobell, 1972; Sobell & Jain, 1972) and ethidium bromide (Tsai, Jain & Sobell, 1975); (ii) the three-ring heterocyclic system in a planar configuration provides strong hydrophobic interaction with the DNA base pairs; (iii) the proximal N of the molecule has an intramolecular hydrogen bond; and (iv) the terminal N contributes to hycanthonone activity by interacting with a phosphate group at the periphery of the DNA helix, thereby stabilizing the drug–DNA complex.

Recently, the structure of lucanthonone (as free base), the metabolic precursor of hycanthonone, has been reported in an X-ray crystallographic study (Neidle, 1976). Our X-ray study on hycanthonone methanesulfonate was undertaken to confirm the overall molecular conformation and to provide additional detailed stereochemical information for structure–activity relation studies.

### Experimental

Orange crystals of hycanthonone methanesulfonate were grown at 4°C from an ethanol–water (1:1) solution of

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Table 1. *Crystal data*

[C <sub>20</sub> H <sub>24</sub> N <sub>2</sub> O <sub>2</sub> S][CH <sub>3</sub> SO <sub>3</sub> H]	$\beta = 116.420 (7)^\circ$
FW 452.6	$V = 2195.8 \text{ \AA}^3$
Monoclinic	$Z = 4$
Space group $P2_1/a$	$D_{\text{calc}} = 1.368 \text{ g cm}^{-3}$
$a = 26.1935 (13) \text{ \AA}$	$D_{\text{obs}} = 1.40 (2)$
$b = 8.7997 (4)$	$F(000) = 960$
$c = 10.6373 (5)$	$\mu(\text{Cu } K\alpha) = 24.23 \text{ cm}^{-1}$
	$t = 24^\circ\text{C}$

Etrenol (product of Sterling Drug Inc.), kindly furnished to us by Dr J. F. de Serres of NIEHS, Research Triangle, NC. The space group and approximate unit-cell parameters were determined from Weissenberg and precession photographs. 12 strong reflections in the  $2\theta$  range of  $130$  to  $135^\circ$  were centered with an Oak Ridge computer-controlled diffractometer (Busing, Ellison, Levy, King & Roseberry, 1968) by use of Ni-filtered Cu  $K\alpha_1$  ( $\lambda = 1.54051 \text{ \AA}$ ) radiation. Cell parameters were refined by the least-squares method. Density was determined by flotation in mixtures of 1,2,3-trichloropropane and 1-chloropropane. Crystal data are summarized in Table 1.

A crystal with the shape of an irregular hexagonal prism of dimensions  $0.18 \times 0.32 \times 0.70 \text{ mm}$  was glued on the tip of a thin glass fiber and was exposed to air during X-ray intensity data collection. The longest dimension, corresponding to the  $b$  axis (prism axis), was nearly parallel to the  $\phi$  axis of the diffractometer. The mosaic ranges of the crystal about three reciprocal directions were estimated to be approximately  $0.2^\circ$ . The total estimated exposure time at 40 kV and 16 mA was 150 h. The intensities of 3973 nonzero independent reflections with  $2\theta \leq 137^\circ$  (equivalent to a minimum spacing of  $0.828 \text{ \AA}$ ) were measured with the  $\theta$ - $2\theta$  scan technique with Ni-filtered Cu  $K\alpha$  radiation.\* Of these, 3538 reflections had corrected intensities greater than three times the corresponding standard deviations, and they were subsequently utilized throughout the structural analysis. Absorption corrections calculated by the method of Busing & Levy (1957), and isotropic extinction corrections (Coppens & Hamilton, 1970) incorporated in the revised least-squares refinement programs *ORXFLS3* (Busing, Martin & Levy, 1962), were applied.

Variances  $\sigma^2(F_o^2)$  for use in least-squares weighting were estimated according to the empirical expression:

$$\sigma^2(F_o^2) = A^2 s^2 \{ [G + (t_G/t_B)^2 B] + 0.0016 [G - (t_G/t_B) B]^2 \} / (Lp)^2,$$

where  $A$  = correction factor on the intensity for absorption,  $s$  = scale factor on the intensity,  $G$  = gross count,  $B$  = background count,  $t_G/t_B$  = ratio of the counting times of gross intensity and background and  $Lp$  = Lorentz-polarization factor.

### Solution and refinement of the structure

Trial positional parameters for the two independent S atoms were obtained from inspection of a three-dimensional Patterson map (program *FORDAPER* of A. Zalkin, modified by G. Brunton). The C, N, and O atoms were located on the basis of normal chemical criteria in two successive Fourier syntheses. This starting model consisting of 30 nonhydrogen atoms was refined with the full-matrix least-squares program *ORXFLS3*, the function minimized being  $\sum w|F_o^2 - sF_c^2|^2$ , where the weights  $w$  are the reciprocals of the variances  $\sigma^2(F_o^2)$ . Four cycles of refinement with all 30 atoms treated isotropically resulted in an unweighted reliability index,  $R(F^2)$ , of 0.278. The refinement was continued with all atoms assigned anisotropic temperature factors. After four cycles  $R(F^2)$  was 0.173. Bond-length calculations carried out at this stage with the program *ORFFE* (Busing, Martin & Levy, 1964) revealed the overall correctness of the structure.

A three-dimensional difference synthesis, phased on all nonhydrogen atoms, was calculated next, and 28 peaks were interpreted as H atoms. The refinement was carried out further with nonhydrogen atoms assigned anisotropic and H atoms assigned isotropic temperature factors. After four cycles, the  $R(F^2)$  value had dropped to 0.079. All parameters settled down except for those of the hydroxyl hydrogen, H( $a$ ), which had an unrealistically high temperature factor of  $25 \text{ \AA}^2$ . Examination of another difference Fourier map phased on all atoms except H( $a$ ) and of the previous difference Fourier map revealed the persistence of a perplexing large ( $1.0 \text{ e \AA}^{-3}$ ) peak,  $1.27 \text{ \AA}$  from the C and  $1.08 \text{ \AA}$  from the O of the hydroxymethyl moiety. A partially disordered model for the  $-\text{CH}_2\text{OH}$  moiety in which two alternative conformations exist was ruled out because of stereochemical considerations, which were later confirmed by the results of the successful refinement. Instead, it was assumed that the crystal specimen was (or had become) a mixed crystal, with the  $-\text{CH}_2\text{OH}$  species and the corresponding oxidized  $-\text{CHO}$  species coexisting but having different occupancies. The otherwise uninterpretable strong peak then represented the O site of the  $-\text{CHO}$  moiety. The H site of the  $-\text{CHO}$  moiety was assumed to be effectively the same as that of H( $14a$ ) of the  $-\text{CH}_2\text{OH}$  moiety (Fig. 1). Every other atom in the molecule was considered to occupy a single site.

\* The  $2\theta$  step width was  $0.05^\circ$ , and backgrounds were measured at the beginning and end of each scan. Scan widths to which the width of the  $\alpha_1$ - $\alpha_2$  doublet separation was added, counting times for each point in a scan, and  $t_B$ , total background counting times, were as follows: For  $2\theta < 90^\circ$ , width =  $1.4^\circ$ , time per step = 2 s,  $t_B = 20$  s; for  $2\theta = 90$  to  $137^\circ$ , width =  $1.6^\circ$ , time per step = 3 s,  $t_B = 60$  s. For  $2\theta = 125$  to  $137^\circ$ , no filter was used. Reflections for which overlap was calculated to be possible (J. R. Einstein, unpublished work) were remeasured with the filter.

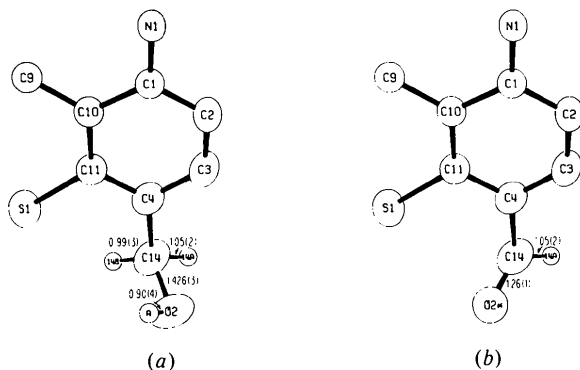


Fig. 1. Configurations of (a) alcohol and (b) aldehyde moieties.

This and all subsequent figures (except Fig. 6) were prepared with the program ORTEP-II (Johnson, 1976). The final bond angles ( $^{\circ}$ ) are: C(4)–C(14)–O(2) 112.2 (2), C(4)–C(14)–H(14a) 113 (1), C(4)–C(14)–H(14b) 107 (2), O(2)–C(14)–H(14a) 97 (1), O(2)–C(14)–H(14b) 116 (2), H(14a)–C(14)–H(14b) 110 (2), C(14)–O(2)–H(a) 108 (1), C(4)–C(14)–O(2\*) 116.0 (7) and O(2\*)–C(14)–H(14a) 126 (2).

Table 2. Positional parameters for nonhydrogen atoms

The e.s.d.'s of the last significant figures are given in parentheses in all tables and in the text.

	$x (\times 10^5)$	$y (\times 10^4)$	$z (\times 10^4)$
S(1)	1338 (2)	1108.7 (6)	2082.6 (5)
S(2)	20976 (2)	2176.0 (4)	1893.1 (4)
C(1)	14413 (6)	2031 (2)	5952 (2)
C(2)	15314 (7)	3563 (2)	5734 (2)
C(3)	12045 (8)	4248 (2)	4472 (2)
C(4)	7805 (7)	3494 (2)	3338 (2)
C(5)	–4641 (8)	–1446 (2)	1780 (2)
C(6)	–6035 (8)	–2774 (2)	2205 (3)
C(7)	–2824 (8)	–3309 (3)	3558 (2)
C(8)	1846 (8)	–2491 (2)	4481 (2)
C(9)	8540 (7)	–337 (2)	5091 (2)
C(10)	9864 (6)	1215 (2)	4846 (2)
C(11)	6724 (6)	1983 (2)	3552 (2)
C(12)	0 (7)	–586 (2)	2719 (2)
C(13)	3330 (6)	–1120 (2)	4074 (2)
C(14)	4825 (9)	4328 (3)	1955 (2)
C(15)	22368 (7)	2074 (2)	8362 (2)
C(16)	27663 (7)	2045 (2)	8115 (2)
C(17)	37414 (8)	3021 (2)	8793 (2)
C(18)	36262 (12)	3374 (4)	7316 (3)
C(19)	33714 (7)	2960 (2)	10584 (2)
C(20)	36074 (10)	1423 (3)	11194 (2)
C(21)	24202 (17)	2586 (5)	3690 (3)
N(1)	17682 (6)	1344 (2)	7195 (1)
N(2)	32220 (5)	3113 (2)	9046 (1)
O(1)	11674 (6)	–1054 (2)	6164 (1)
O(2)*	8409 (11)	4519 (3)	1277 (3)
O(3)	15008 (6)	1901 (2)	1496 (2)
O(4)	22047 (8)	3469 (2)	1223 (2)
O(5)	23726 (6)	801 (1)	1732 (2)

\* Oxygen atom of alcohol species with an occupancy factor of 0.881 (7) (see text).

The refinement was continued with anisotropic temperature factors for 29 'full' atoms and one 'partial' atom [alcohol O(2), occupancy  $m$ ], and with isotropic temperature factors for 27 full H atoms and three partial atoms [aldehyde O(2\*), occupancy  $1 - m$ ; and alcohol H(14b) and H(a), occupancies  $m$ ]. The position of H(14a), one of the 27 full H atoms, represented the average site for both alcohol and aldehyde moieties. Only the occupancy factor of O(2) was adjusted with the other parameters in the refinement, the occupancy factors of the other partial atoms being constrained properly. At the end of the refinement, the values of  $R(F^2)$  and  $R_w(F^2)$  [the latter defined as  $(\sum w|F_o^2 - sF_c^2|^2/wF_o^4)^{1/2}$ ] stood at 0.048 and 0.097 respectively. The value of  $R(F)$  was 0.033. Parameter shifts for the nonhydrogen atoms in the last cycle of the refinement were all less than 2% of the corresponding estimated standard deviations (e.s.d.'s) except for O(2\*) (14%), whereas the maximum shift in the H parameters was 16% of the corresponding e.s.d. for H(14b). The value of  $m$  was 0.881 (7). A final difference map was essentially flat [maximum residual density was 0.3 e

Table 3. Positional and isotropic thermal parameters for hydrogen atoms and the oxygen atom of the aldehyde species

	$x (\times 10^4)$	$y (\times 10^3)$	$z (\times 10^3)$	$B (\text{Å}^2)$
H(2)	1813 (8)	415 (3)	650 (2)	4.1 (4)
H(3)	1281 (10)	526 (3)	435 (3)	5.5 (5)
H(5)	–677 (10)	–107 (3)	82 (3)	5.2 (5)
H(6)	–914 (12)	–331 (3)	153 (3)	7.0 (7)
H(7)	–400 (11)	–419 (3)	382 (3)	6.4 (6)
H(8)	442 (10)	–286 (3)	545 (3)	5.5 (5)
H(14a)	419 (10)	549 (3)	208 (2)	5.2 (5)
H(14b)†	113 (12)	381 (4)	140 (3)	6.0 (7)
H(15a)	2130 (7)	315 (2)	851 (2)	3.2 (4)
H(15b)	2301 (8)	149 (2)	918 (2)	4.0 (4)
H(16a)	2676 (7)	232 (2)	721 (2)	2.9 (3)
H(16b)	2912 (9)	108 (3)	823 (2)	4.0 (4)
H(17a)	3894 (9)	204 (3)	907 (2)	4.3 (5)
H(17b)	4002 (10)	367 (3)	942 (3)	4.9 (5)
H(18a)	3418 (16)	424 (5)	699 (4)	9.8 (10)
H(18b)	3403 (14)	250 (4)	671 (3)	7.8 (7)
H(18c)	4002 (13)	337 (4)	735 (3)	7.5 (7)
H(19a)	3029 (8)	321 (2)	1067 (2)	3.6 (4)
H(19b)	3636 (8)	375 (2)	1103 (2)	3.4 (4)
H(20a)	3646 (12)	138 (3)	1213 (3)	6.9 (6)
H(20b)	3352 (11)	58 (3)	1074 (3)	6.0 (6)
H(20c)	3966 (13)	120 (4)	1120 (3)	7.5 (7)
H(21a)	2760 (14)	282 (4)	391 (3)	7.4 (8)
H(21b)	2335 (13)	167 (4)	415 (4)	8.2 (8)
H(21c)	2238 (15)	346 (5)	388 (4)	9.9 (11)
H(a)†	1010 (17)	362 (5)	131 (4)	9.1 (11)
H(b)	1689 (9)	39 (3)	726 (2)	4.5 (5)
H(c)	3075 (8)	398 (2)	881 (2)	3.3 (4)
O(2*)‡	527 (7)	374 (2)	93 (2)	5.6 (4)

† Hydrogen atoms of alcohol species each with an occupancy factor of 0.881 (7) (see text).

‡ Oxygen atom of aldehyde species with an occupancy factor of 0.119 (see text).

$\text{\AA}^{-3}$  in the vicinity of S(1)]. The scattering factors used were those of: Dawson (1960) for S; Berghuis, Haanappel, Potters, Loopstra, MacGillavry & Veenendaal (1955) for C, N, and O; Stewart, Davidson & Simpson (1965) for H. The Hönl corrections,  $\Delta f' = 0.319$  and  $\Delta f'' = 0.557$ , were employed for the S atoms (Cromer, 1974).

Final values of the atomic parameters and their e.s.d.'s are given in Table 2 for nonhydrogen atoms (positional parameters) and in Table 3 for H atoms and the partial aldehyde O atom (positional and isotropic thermal parameters).\*

## Discussion

### Molecular configuration

The crystal structure of hycanthonone methanesulfonate consists of hycanthonone  $[\text{C}_{20}\text{H}_{25}\text{N}_2\text{O}_2\text{S}]^+$  cations and methanesulfonate  $[\text{CH}_3\text{SO}_3]^-$  anions linked together by hydrogen bonds (see below). Fig. 2 is a stereoscopic view of the hycanthonone cation. Bond distances and angles for the cations are given in Fig. 3.

\* Lists of structure factors and anisotropic thermal parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 32931 (5 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 13 White Friars, Chester CH1 1NZ, England.

Although not shown for the sake of clarity, all H atoms are in reasonable positions (Fig. 2) with C—H, N—H, O—H distances ranging from 1.05 (2)  $\text{\AA}$  [for C(14)—H(14a)] to 0.84 (2)  $\text{\AA}$  [for N(2)—H(c) and C(21)—H(21a)]. The average value of 28 non-hydrogen—hydrogen bond distances is 0.95  $\text{\AA}$ , which is in agreement with other values obtained by X-ray diffraction methods.

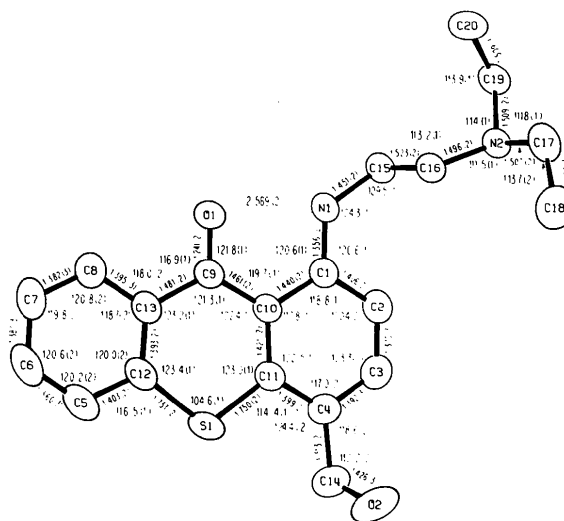


Fig. 3. Bond lengths ( $\text{\AA}$ ) and angles ( $^\circ$ ) for nonhydrogen atoms with their e.s.d.'s.

Table 4. Distances and angles in the hydrogen-bond systems

Symmetry code: (i)  $0.5 - x, 0.5 + y, 1 - z$ .

$A-H \cdots B$	Distances ( $\text{\AA}$ )			Angles ( $^\circ$ )	
	$A \cdots B$	$A-H$	$H \cdots B$	$\angle A-H \cdots B$	$\angle H-A \cdots B$
$N(1)-H(b) \cdots O(1)$	2.569 (2)	0.88 (2)	1.85 (2)	138 (2)	29 (2)
$O(2)-H(a) \cdots O(3)$	2.827 (2)	0.90 (4)	1.93 (4)	169 (4)	7 (4)
$N(2)-H(c) \cdots O(5)^i$	2.749 (2)	0.84 (2)	1.92 (2)	170 (2)	7 (2)

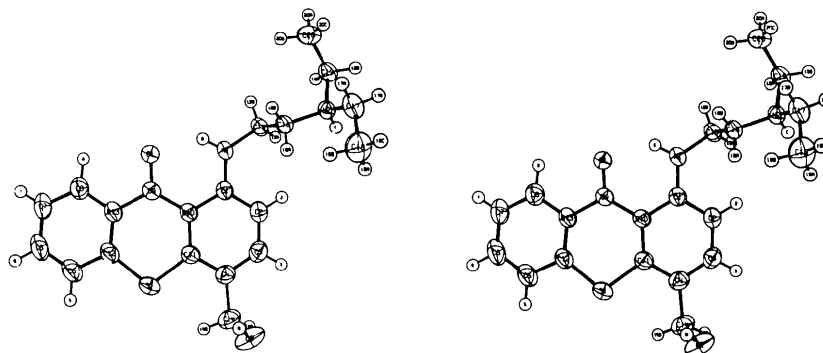


Fig. 2. Stereoscopic view of the hycanthonone cation.

Table 5. Equations of best molecular planes, and perpendicular distances (Å) of atoms from these planes

Calculations were performed with the program written by Smith (1962).  $X, Y, Z$  are orthogonal coordinates expressed in Å and are related to the monoclinic-cell coordinates by the transformation  $X_k = ax_k + cz_k \cos \beta$ ,  $Y_k = by_k$ , and  $Z_k = cz_k \sin \beta$ .

- (a)  $0.8971X - 0.2948Y - 0.3291Z = -1.5553$   
 (b)  $0.8973X - 0.3115Y - 0.3127Z = -1.5263$   
 (c)  $0.8132X - 0.4920Y - 0.3110Z = -1.5857$   
 (d)  $0.8068X - 0.4917Y - 0.3274Z = -1.6205$   
 (e)\*  $0.8547X - 0.4094Y - 0.3192Z = -1.7882$

	(a)	(b)	(c)	(d)	
C(1)	0.021	0.056	C(5)	0.011	0.031
C(2)	-0.003	0.005	C(6)	-0.001	0.015
C(3)	-0.017	-0.039	C(7)	-0.007	-0.013
C(4)	0.019	-0.009	C(8)	0.005	-0.020
C(11)	-0.019	-0.003	C(13)	-0.013	-0.018
C(10)	-0.001	0.010	C(12)	0.005	-0.012
C(9)	-0.109†	-0.053	C(9)	0.083†	0.039
S(1)	0.045†	0.032	S(1)	-0.028†	-0.021
O(1)	0.022†	0.105†	O(1)	0.330†	0.268†

Dihedral angle between (a) and (c)  $167.7^\circ$

Dihedral angle between (b) and (d)  $169.4^\circ$

\* Deviations of atoms are not given for this plane, which was calculated for all 14 atoms of the three-ring system.

† Values for atoms not included as members of the plane.

There is an intramolecular hydrogen bond, the parameters of which are given in Table 4, between the proximal N(1) and the carbonyl O(1). The existence of this hydrogen bond has been considered to be essential for optimum interaction of the drug with DNA *in vivo* (Hirschberg, 1975).

All C atoms of the two outer rings are essentially coplanar, as shown in Table 5 planes (a) and (c), whereas the middle-ring members, C(9) and S(1), are further from these planes. When all 14 atoms of the three rings are included in the plane calculation [Table 5 plane (e)], the mean deviation from the least-squares plane is  $0.116 \text{ \AA}$ , a value which can be compared with  $0.05 \text{ \AA}$  reported for lucanthone (Neidle, 1976). The thioxanthene fragment may be considered to be formed by the fusion of two nearly planar  $C_6H_4SC$  and  $C_6H_2SC$  moieties [Table 5 planes (b) and (d)] along the common  $S \cdots C$  line. This type of configuration has previously been reported in the structures of thioxanthene-9-ol 10-oxide (Ternay, Chasar & Sax, 1967), *cis*-9-methylthioxanthene 10-oxide (Jacobs & Sundaralingam, 1969) and  $\alpha$ - and  $\beta$ -flupenthixol (Post, Kennard & Horn, 1975). The thioxanthene fragment in each of the above-mentioned structures is far from coplanar, the dihedral angles between the two outer rings being  $132.4$ ,  $127.2$ ,  $152$  and  $143^\circ$  respectively. In contrast to these folded configurations, here the dihedral angle between the least-squares planes (a) and (c) (Table 5) is  $167.7^\circ$ , while that between planes (b) and (d) is

$169.4^\circ$ . The structures of two indazole derivatives of hycanthone, in which the proximal N atoms are parts of fourth rings leading to a substituent on the 9 position, have recently been determined in our laboratory (C. H. Wei, unpublished results). Thus, 8-chloro-2-[2-(diethylamino)ethyl]-5-hydroxymethyl-2H-

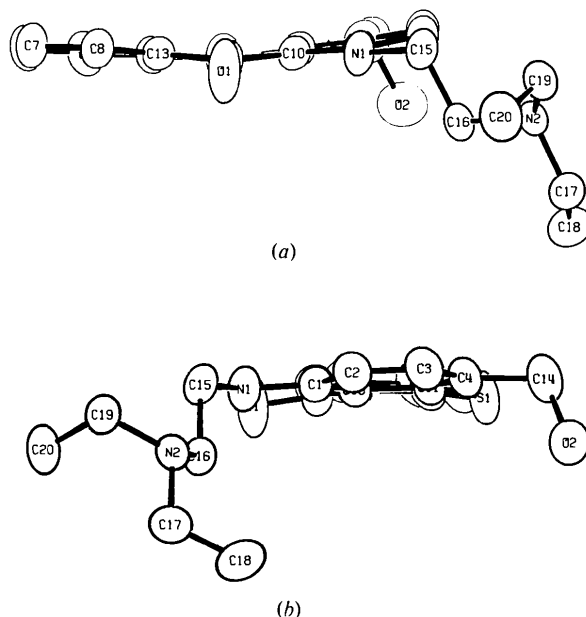


Fig. 4. Two views of the hycanthone cation, showing the conformation of the side chain.

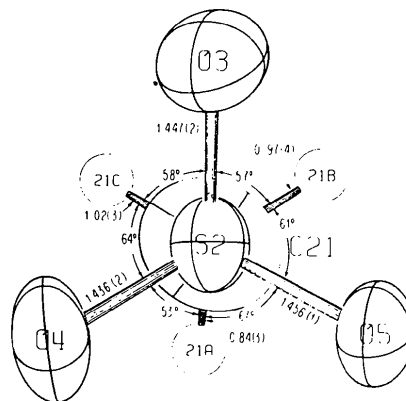


Fig. 5. View of the methanesulfonate anion down the  $S(2)-C(21)$  bond direction. Bond lengths (Å) and conformational angles ( $^\circ$ ) are shown. The hidden bond  $S(2)-C(21)$  has a length of  $1.749(3) \text{ \AA}$ . The bond angles involving nonhydrogen atoms are:  $O(3)-S(2)-C(21)$   $106.7(2)$ ,  $O(4)-S(2)-C(21)$   $105.8(1)$ ,  $O(5)-S(2)-C(21)$   $106.0(2)$ ,  $O(3)-S(2)-O(4)$   $114.3(1)$ ,  $O(4)-S(2)-O(5)$   $112.6(1)$  and  $O(5)-S(2)-O(3)$   $110.7(1)$ . Other pertinent angles are:  $O(3)-O(4)-O(5)$   $59.3(1)$ ,  $O(4)-O(5)-O(3)$   $60.7(1)$  and  $O(5)-O(3)-O(4)$   $60.0(1)$ .

[1]benzothiopyrano[4,3,2-*cd*]indazole (IA-4 MES) and dechloro IA-4 base (Bueding, Fisher & Bruce, 1973; Bueding, 1974) have corresponding dihedral angles of  $177.1$  and  $177.5^\circ$  respectively. These values indicate

that the indazole derivatives have thioxanthene ring systems much closer to the proposed coplanarity (Weinstein & Hirschberg, 1971; Hirschberg, 1975) than that for hycanthonc itself.

The existence of the intramolecular hydrogen bond (see above) between the proximal N atom and the carbonyl O atom necessarily results in a small torsion angle of  $-2.0(2)^\circ$  for atoms C(15)–N(1)–C(1)–C(2). This value is in agreement with  $1^\circ$  observed for lucanthonc. As an aid in visualizing the side-chain conformation, two views are shown in Fig. 4.

In this structure and those of the derivatives, IA-4 MES and dechloro IA-4 base, the terminal N atoms invariably act as donors in intermolecular hydrogen bonds with neighboring O atoms. It is to be expected that the flexibility of the side chain contributes to the ability of the terminal N atom to interact in this way with DNA phosphate residues, so as to stabilize the complexes formed by this family of drugs.

The methanesulfonate anion has the expected configuration with approximately  $C_{3v}$  symmetry. A view down the S(2)–C(21) bond is shown in Fig. 5. The three O atoms are in an almost perfectly staggered arrangement with respect to the three H atoms attached to C(21). Despite the fact that only two O atoms, O(3) and O(5), are involved in interionic hydrogen bonding (see below), all three S–O bonds are equal to within  $\pm 0.01 \text{ \AA}$ , suggesting that the three bonds are chemically equivalent.

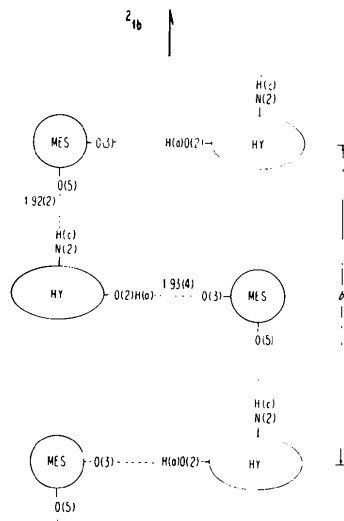


Fig. 6. Schematic illustration of the interionic hydrogen bonding around a twofold screw axis at  $a/4$  and  $c/2$ . HY and MES represent hycanthonc and methanesulfonate ions respectively. Ions at the same height in the figure belong to the same asymmetric unit. The figure shows repeats along a full  $b$  range.

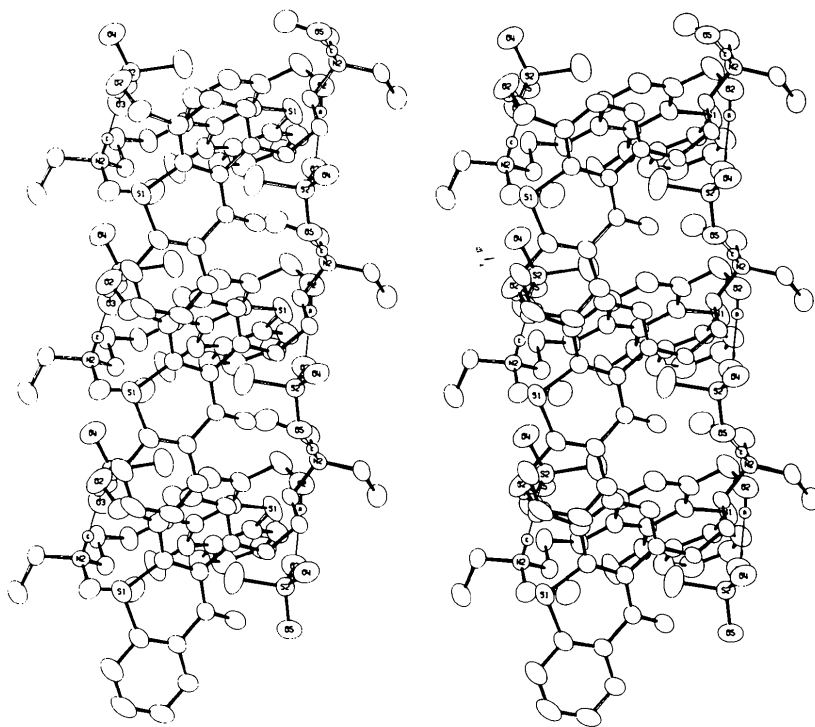


Fig. 7. Stereoscopic view of hydrogen-bonding scheme from a direction perpendicular to  $b$ .

### Hydrogen bonding and packing

The molecular packing is governed by two types of interactions: (i) electrostatic interaction and hydrogen bonding involving two O atoms [O(3) and O(5)] of the methanesulfonate anion, the terminal N(2), and the hydroxyl O(2); (ii) stacking of ring systems of hycanthonium cations. As shown in Fig. 6, the hydroxyl O(2) of the hycanthonium cations is hydrogen-bonded to O(3) of the neighboring methanesulfonate anion in the same asymmetric unit *via* the O(2)—H(*a*)···O(3) bond. The hycanthonium base is protonated at the terminal N(2), which donates H(*c*) to make a hydrogen bond with O(5) of another methanesulfonate anion related to the basic one (*x, y, z*) by twofold screw axis symmetry ( $\frac{1}{2} - x, \frac{1}{2} + y, 1 - z$ ). Each methanesulfonate anion in turn furnishes two O atoms as acceptors in the hydrogen bonding with two neighboring cations. These two interionic hydrogen bonds, the parameters of which are included in Table 4, result in infinite stacks of the molecules around the twofold screw axes, as elucidated in a stereoscopic view perpendicular to **b** (Fig. 7).

Another point of interest is that the thioxanthene ring systems stack across centers of symmetry. The distance calculated between the basic ring system [the least-squares plane of which is given in Table 5(*e*)] and the adjacent ring system [related to the basic one by a center of symmetry at  $(0, 0, \frac{1}{2})$ ] is 3.51 Å.

The [010] projection of the unit cell is given in Fig. 8, which shows the packing. The shortest intermolecular contacts, other than those involved in the hydrogen bonding, are 3.22 Å for C(16)···O(4) (between non-

hydrogen atoms) and 2.49 Å for O(4)···H(19*a*) (between nonhydrogen and H atoms).

### Comments on mixed structure

The assumption of a mixed crystal containing both the hydroxymethyl and the corresponding aldehyde species led to results which are convincing from a crystallographic point of view. The refinement converged well and resulted in reasonable atomic parameters for all atoms (Tables 2 and 3). The difference map became essentially flat. The final distances and angles for both —CH<sub>2</sub>OH and —CHO moieties are stereochemically reasonable within experimental errors (Fig. 1). The hypothesis, involving an increase of five in the number of varied parameters, brought about a decrease in  $R_w(F^2)$  from 0.1095 to 0.0974 (ratio 1.124), for 3145 degrees of freedom. Interpolation (Pawley, 1970) in the Hamilton tables (Hamilton, 1965) for  $\alpha = 0.001$  gives an  $\chi^2_{5,3145,0.001}$  value of 1.003. Hence, the hypothesis of the mixed-structure model cannot be rejected at the 0.1% significance level.

In the preparation of hycanthonium from its parent, lucanthonium, by microbiological conversion in fermentation media of *Aspergillus schlerotiorum*, the formation of the aldehyde species along with the alcohol species has been observed (Rosi *et al.*, 1967). However, the drug as obtained from the manufacturer was considered to be 99.9% pure (F. C. Nachod, private communication). The NMR spectra taken in D<sub>2</sub>O

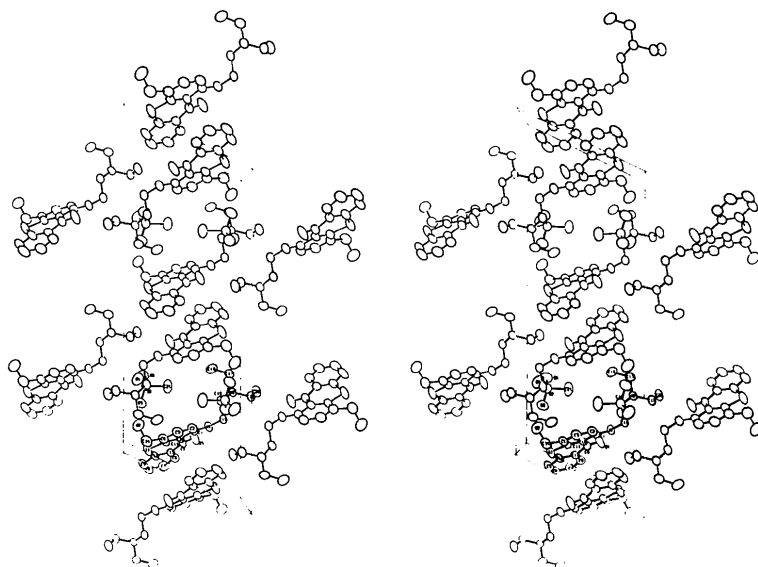


Fig. 8. Stereoscopic pair showing the unit-cell packing down **b** for the alcohol species. The axial system is right-handed. For the sake of clarity, hydrogen atoms are not included, and only the reference molecule is labeled. The origin of the unit cell is in the lower left, rear corner.

solutions for the original drug powder and for the powder exposed to unfiltered X-rays for 5 d show no detectable signals in the chemical shift range of 10–11 p.p.m., which would have indicated the aldehyde species to be present. Comparison of the IR spectrum for the crystalline sample with that for the original drug powder also failed to show a detectable difference in the absorption region of 1695–1665  $\text{cm}^{-1}$ , characteristic for aromatic aldehyde (Pouchert, 1970). Oxidation might conceivably have taken place during the crystallization process from solution, which took two weeks, or during a one-year period of storage of the crystalline sample prior to the collection of X-ray data. However, not enough crystalline material (of the same batch) was available for a more definitive spectroscopic study. Therefore, confirmation of the presence of the aldehyde species by noncrystallographic techniques remains to be accomplished.

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