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The Synthesis of the Enantiometric Quinacrine Mustards and Atabrines and Their Interaction with Human and Rabbit Lymphocytes

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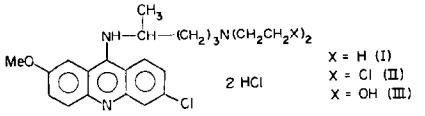
The optical antipodes of both atabrine and quinacrine mustard have been prepared. In both cases one enantiomer exhibited greater fluorescence intensity than the other when allowed to interact with human and rabbit lymphocytes.

The use of atabrine (I) and quinacrine mustard (QM, II) as specific chromosomal strains has been one of the major recent developments in cytogenics (*1*, *2*). The specificity of quinacrine fluorescence for AT rather than GC was first shown by Weisblum and



de Haseth (*3*). The chemical basis for localized cytologic fluorescence appears to be due to enhancement of quinacrine fluorescence by interaction between AT base-pairs and quenching by GC (*4*, *5*).

It has been suggested that the characteristic chromosome fluorescence striations are the result of a binding in those regions where the DNA chains become sufficiently dispersed to prevent bridging (of the chain) by the dye molecules. Detailed hypotheses for this binding have been proposed (*6*, *7*). Recently, the binding sites, the dependence of fluorescence intensity on ionic strength, pH and other parameters have been studied (*8–11*). Although atabrine (I) has been resolved (*12*), no one seems to have considered the possible diastereomeric differences in the binding between D- or L-fluorochromes with the chiral DNA substrate. It seemed of interest to us to resolve atabrine (I) and QM (II) and measure whether the enantiomers when bound to chromosomes or to lymphocytes showed differences in fluorescence intensity. Atabrine (I) was resolved as reported (*12*) (see Table 1).

After considerable experimentation we found that the precursor of QM (II), namely the diol III, could be resolved using the ammonium salt of 3-bromo-(D-camphor)-7-

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TABLE 1

Compound	$[\alpha]_{578}^{23}$	mp (°C)
D-Atabrine dihydrochloride[a] (I)	+350° (0.3 g/100 ml water	250 (dec.)
L-Atabrine dihydrochloride[b] (I)	−334° (0.1 g/100 ml water)	250 (dec.)
D-QM dihydrochloride (II)	+302° (0.2 g/100 ml EtOH)	149–151
L-QM dihydrochloride (II)	−315° (0.3 g/100 ml EtOH)	147–148
D-diol III[c]	+112° (0.3 g/100 ml EtOH)	135–137
L-diol III[c]	−109° (0.2 g/100 ml EtOH)	136–137

[a] Lit. (*12*) $[\alpha]_D^{23}$ + 355° (2.0 g/100 ml water).
[b] Lit. (*12*) $[\alpha]_D^{23}$ − 334° (2.0 g/100 ml water).
[c] Free base.

sulphonic acid. The diol III, namely DL-2-methoxy-6-chloro-9-[4-bis(β-hydroxyethyl)-amino-1-methylbutylamino]acridine, mp 135–137°C (lit. 136–137°C); di-HCl salt, mp 217–219°C (lit. 217–218°C), was made according to the method described by Jones, Price, and Sen (*13*). The optically active diols (see Table 1) were converted to QM dihydrochloride (II) using thionyl chloride (*13*). Rotations are summarized in Table 1.

FLUORESCENCE MEASUREMENTS

Preliminary experiments indicate that, when rabbit and human lymphocytes were treated with solutions of each of the enantiomers of both atabrine and quinacrine mustard at 20 ± 0.1°C and pH 5.5 (*14*), differences in the intensity of the fluorescence emitted were observed and measured. Measurements were made with a Zeiss microscope-Photometer MP 01 equipped with an HBO 100 mercury lamp. Excitation wavelengths used were 450 nm with barrierfilters 53, 44, and 500 nm with barrierfilters 53, 47, 44. Of particular interest is the observation that both D-atabrine and D-QM when bound to these lymphocytes show stronger fluorescence intensity than either L-enantiomer or the racemic mixture, in a significant majority of the measurements.

TABLE 2

Compound	D>L;D>DL	L<D;D>DL	D>L;D<DL	D<L;D<DL	D=L;D=DL	Total number of nuclei measured	Number of independent samples
QM, nuclei	2610	450	390	210	120	3780	82
%	69.0	11.9	10.3	5.6	3.2		
Atabine nuclei	480	120	90	—	180	870	17
%	55.2	13.8	10.3	—	20.7		

Thus in the case of QM a total of 3780 measurements were made. The results were as follows: In 2610 cases (69%) D-QM when bound to nuclei showed stronger fluorescence than either L-QM or DL-QM. See Table 2 for a summary of these results.

It appears from these preliminary results that the enantiomeric fluorochromes manifest differential fluorescence in the biological substrates used. Further quantitative work with DNA polymers is needed to confirm this qualitative specificity (*15*).

Starting Materials

2-Methoxy-6-chloro-9-[4-bis(β-hydroxyethyl)amino-1-methylbutylamino]acridine (III) was prepared by the method of Jones et al. (*13*), mp 135–138°C (lit. 136–138°C); di-HCl salt, mp 218–219°C (lit. 218–219°C).

THE RESOLUTION OF 2-METHOXY-6-CHLORO-9-[4-BIS(β-HYDROXYETHYL)AMINO-1-METHYLBUTYLAMINO]ACRIDINE (III)

A solution was made of 8.70 g (20.2 mmole) of III and 14.50 g (44.2 mmole) of the ammonium salt of 3-bromo-(D-camphor)-sulfonic acid-7 in 300 ml of ethanol and 10 ml of water. Removal of the ammonia and the solvents *in vacuo* left a reddish brown material, which was dissolved in 300 ml of hot 95% ethanol. The solution was filtered to remove any insoluble material and then allowed to crystallize in a cold room at least overnight. A partially resolved salt (9.80 g, fraction 1) with a specific rotation of $[\alpha]_{578}^{23} + 72°$ (0.3 g/100 ml in ethanol) was obtained. The 9.80 g of fraction 1 was dissolved in hot ethanol and after standing at 0°C overnight; 3.5 g of material (fraction 2) was obtained with a specific rotation of $[\alpha]_{578}^{23} + 81°$ (0.3 g/100 ml ethanol). Fraction 2 was treated like fraction 1, whence 1.0 g of fraction 3 was obtained, corresponding to a specific rotation of $[\alpha]_{578}^{23} + 108°$ (0.2 g/100 ml ethanol). Investigation showed that it was no longer profitable to crystallize the enriched bromocamphorsulfonate salt further. The free optically active base III was obtained by dissolving the salt in ethanol–water, adding an excess of ammonia, and extracting with chloroform. The organic extracts were dried over potassium carbonate, and after evaporation there was left a highly viscous oil. This oil was dissolved in boiling dry acetone and the filtrate allowed to stand for 2 days, during which time crystallization occurred. Recrystallization of this material from dry acetone afforded 100 mg of D-2-methoxy-6-chloro-9-[4-bis(β-hydroxyethyl)amino-1-methylbutylamino]acridine (III) having a specific rotation of $[\alpha]_{578}^{23} + 112°$ (0.25 g/100 ml ethanol) and mp 135–137°C. Mass spectrum: M^+ 431.

The mother liquor from fraction 1 was evaporated and the residue was dissolved in water. After adding an excess of ammonia the work up was essentially the same as carried out with the dextro isomer. There was obtained 0.90 g of material, mp 133–136°C, that was found to have a specific rotation $[\alpha]_{578}^{23} - 91°$ (0.25 g/100 ml ethanol). After two recrystallizations from acetone the L-isomer (0.50 g) of III melted at 136–137°C, having a specific rotation $[\alpha]_{578}^{23} - 109°$ (0.20 g/100 ml ethanol). Mass spectrum: M^+ 431.

L-QUINACRINE MUSTARD DIHYDROCHLORIDE (II)

To 5 ml thionyl chloride, cooled in ice water, was added 100 mg (0.26 mmole) of L-III, $[\alpha]_{578}^{23}$ −109°. After stirring for 3 hr at 0°C, the mixture was allowed to stand for 72 hr at room temperature. The excess of thionyl chloride was distilled *in vacuo*, and the residue so obtained was dissolved in a little absolute ethanol. Dry acetone was added to turbidity and after standing for 1 week at −15°C 113 mg (89%) yellowish brown crystals were obtained, having a specific rotation $[\alpha]_{578}^{23}$ − 284°. After recrystallization and after keeping at −15°C for 1 week, the yellow crystals were collected. On heating, these softened with slow decomposition at 147–148°C with shrinkage starting at about 110°C. The specific rotation of L-quinacrine mustard dihydrochloride (II) was $[\alpha]_{578}^{23}$ − 315°, $[\alpha]_{546}^{23}$ − 429° (0.30 g in 100 ml ethanol). Ultraviolet spectrum in 95% ethanol: λ_{max} in nm (log ε) 223 (4.40), 271^{sh} (4.70), 2.85 (4.78), 331 (3.56), 345 (3.69), 405^{sh} (3.78), 426 (4.00), 449 (3.99). In the mass spectrum the parent peak appears at m/e 467 corresponding to M^+-2 HCl.

D-QUINACRINE MUSTARD DIHYDROCHLORIDE (II)

Essentially the same procedure as described for L-quinacrine mustard dihydrochloride was carried out for the D-isomer. The yellow compound had a specific rotation $[\alpha]_{578}^{23}$ + 302°, $[\alpha]_{546}^{23}$ + 314° (0.20 g in 100 ml ethanol). On heating the crystals shrank at 140°C and then softened at 149–151°C with slow decomposition. Ultraviolet spectrum in 95% ethanol: λ_{max} in nm (log ε) 223 (4.35), 270^{sh} (4.58), 284 (4.70), 330 (3.51), 334 (3.63), 405^{sh} (3.76). 426 (3.99), 449 (3.97). In the mass spectrum the parent peak appears at m/e 467 corresponding to M^+-2 HCl.

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