Method E. It is similar to method D. The oily residue was chromatographed on a silica gel column, eluting with a mixture of C_6H_6-Me_2CO (60:40). Removal of the solvent from the second fraction of eluate gave an oily residue which crystallized only in the case of **9**.

AChE Reactivation. The reactivation rate constants $K_{obsd} = (1/t) \ln [E/(E - E_t)]$, where E is the total enzyme activity available for reactivation and E_t the activity at time t, were determined following the method of Ashani, *et al.*[2] Each determination was repeated four times.

A solution of 100 U/ml of bovine erythrocyte AChE (Sigma Chemical Co.) in veronal buffer (0.12 M, pH 7.4) was incubated with 5.10^{-6} M DFP at 25°. After 30 min of incubation, 1 vol of 5.10^{-3} M reactivator solution in veronal buffer was added to 2 vol of inhibited enzyme solution. At various times t, 1 ml of sample was diluted with veronal buffer (3 ml) and water (10 ml) and the pH was readjusted to 7.4 by addition of 0.1 N NaOH; 0.1 M ACh perchlorate (1 ml) was added and the rate of ACh hydrolysis was followed with a pH-stat instrument (Copenhagen Radiometer). For the comparison with the activity of 2-pyridylaldoxime (2-PAM), see ref 1.

AChE Inhibition. The *anti*-AChE activity was determined in the same medium and at the same temperature as for the reactivation rate measurement but without DFP. The enzyme preparation was allowed to incubate for 30 min with the quaternary salt and then ACh perchlorate was added; the mixture was maintained at pH 7.4 by the addition of 0.1 N NaOH. The volume was recorded. The determination was repeated using different concentrations of the compound and the I_{50} value was calculated from graphs drawn with ordinates representing concentrations of the inhibitor and abscissas representing the per cent inhibition.

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Some Structural Relationships among Cytotoxic and Antitumor Benzophenanthridine Alkaloid Derivatives

Frank R. Stermitz,*,† Kenneth A. Larson,‡ and Don K. Kim†

Departments of Chemistry and Microbiology, Colorado State University, Fort Collins, Colorado 80521.

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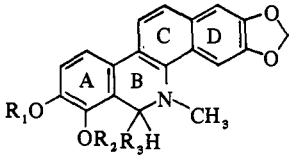
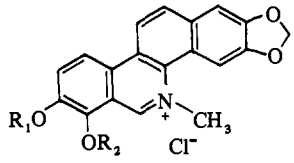
Sanguinarine (**1**) and its acid salt **2** have been known for some time to be cytotoxic at sufficiently low concentrations (<1 μg/ml) to warrant investigation as antitumor agents in more sophisticated systems. However, in spite of extensive tests against many different tumors in mice, **1** and **2** have shown no activity.[1,§] Recently, nitidine chloride (**3**) was reported[2] to be highly cytotoxic and also effective against P388 leukemia in mice while its methoxy derivative **4** showed even greater activity and was also effective against the more resistant L1210 mouse leukemia. The alkoxy derivatives of these alkaloids have solubilities markedly different from the acid salts and it was possible that solubility might have been important in the activity difference between **3** and **4**.

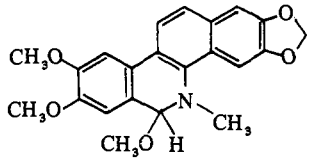
To test this hypothesis we prepared a series of sanguinarine derivatives **5a-f** for extensive cytotoxicity and antileukemia screening. Since nitidine possesses two methoxy groups in ring A, rather than the methylenedioxy grouping of sanguinarine, we also prepared a series of chelerythrine (**6**) derivatives **7a-f** and **8**. These would be closer structural analogs of nitidine than are the sanguinarine derivatives. The synthetic procedures are described in the Experimental Section.



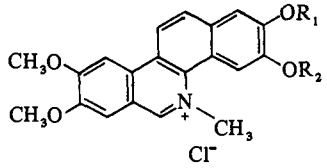
1, R_1, R_2 = $-CH_2-$; R_3 = OH
5a, R_1, R_2 = $-CH_2-$; R_3 = OCH_3
b, R_1, R_2 = $-CH_2-$; R_3 = OCH_2CH_3
c, R_1, R_2 = $-CH_2-$; R_3 = $OCH_2CH_2CH_3$
d, R_1, R_2 = $-CH_2-$; R_3 = $OCH_2CH_2CH_2CH_3$
e, R_1, R_2 = $-CH_2-$; R_3 = $CH_2C(=O)CH_3$
f, R_1, R_2 = $-CH_2-$; R_3 = H
6, R_1 = R_2 = CH_3; R_3 = OH
7a, R_1 = R_2 = CH_3; R_3 = OCH_3
b, R_1 = R_2 = CH_3; R_3 = OCH_2CH_3
c, R_1 = R_2 = CH_3; R_3 = $OCH_2CH_2CH_3$
d, R_1 = R_2 = CH_3; R_3 = $OCH_2CH_2CH_2CH_3$
e, R_1 = R_2 = CH_3; R_3 = $CH_2C(=O)CH_3$
f, R_1 = R_2 = CH_3; R_3 = H



2, R_1,R_2 = $-CH_2-$
8, R_1 = R_2 = CH_3



4



3, R_1,R_2 = $-CH_2-$
9, R_1 = H (or CH_3); R_2 = CH_3 (or H)

Pharmacological Results. The cytotoxicity of **2** was 0.5 μg/ml, while that of the derivatives **5a-f** was in the 4-5 μg/ml range. Cytotoxicity of **8** was 3 μg/ml, while the derivatives **6** and **7a-f** all showed values in the 8-10 μg/ml range. Thus, a decrease in cytotoxicity for the alkoxy derivatives as compared to the acid salts was observed with both sanguinarine and chelerythrine. In addition, chelerythrine and its derivatives, in spite of being closer analogs of nitidine, were less cytotoxic than the corresponding sanguinarines. All compounds **1**, **2**, and **5-8** were tested for antileukemic activity in mice at several dose levels and none were active.

Discussion

First, our work has shown that it is likely that the placement of the functional groups in ring A is of more importance than their constitution. The chelerythrine derivatives (which have two methoxy groups in ring A and, hence, are close analogs of nitidine) thus were all inactive. A simple change of the position of one methoxyl group in ring A has thus converted an inactive compound to one with considerable activity. Nitidine is not an isolated example of activity in this structure series since a very recent report[3] claimed very high activity against P388 leukemia in mice for fagaronine (**9**).

Two possible reasons for these differences can be suggested.

†Department of Chemistry.
‡Department of Microbiology.
§J. L. Hartwell, National Cancer Institute, private communication.

It has been reported[4] that many effective antitumor alkaloids possess two oxygens and one nitrogen, which three functions define a triangle of certain optimum interatomic distances for optimum activity. The O-O bond distances in all the compounds listed here fall within the accepted range for one leg of the triangle. The N-O bond distances in all the compounds are, however, shorter than the minimum value listed[4] for effective structures. However, the closest match does come with **3**, **4**, and **9** where the distances from the ring B nitrogen to the ring A oxygens are 6.2 and 6.3 Å# as compared to the lowest optimum values of about 6.5 and 8.0 Å.

A second more likely possibility is that the important active site of the molecule is the $-C=N^+$ region of ring B in **3** and **9** since the carbon of the iminium function represents an effective alkylation site. This functionality has previously been invoked[5] to account for other biological activities of **2** which disappear upon conversion to the dihydro derivative **5f**. Presence of the peri group of ring A in the sanguinarine and chelerythrine derivatives could sterically block attack at the ring iminium carbon, while this position is open in nitidine and fagaronine. We are actively pursuing structural modifications in these series in order to more closely define structure-activity relationships.

Experimental Section

Synthesis. Commercial "sanguinarine nitrate" (Aldrich Chemical Co. and K and K Laboratories), which is mainly a mixture of sanguinarine, chelerythrine, and protopine nitrates, was used as a source of **2** and **8**. The mixture was converted to a mixture of the free bases and 1 and 6 could be separated and isolated pure by column chromatography on Florisil. Treatment of each with HCl yielded **2** and **8**. The alkoxy derivatives were prepared by recrystallization of 1 and **6** several times from the appropriate solvent (*e.g.*, methanol for **5a** and 7a, ethanol for **5b** and 7b, etc.). The acetonyl derivatives **5e** and 7e were prepared by the literature[6] method for 7e.

Previously known compounds were **1**, **2**, **5a**, **5f**, **6**, **7a**, **7b**, 7e, and 7f. New compounds prepared in this study were **5b** (13,14-dihydro-14-ethoxysanguinarine), mp 200–202° ($C_{22}H_{20}NO_5$); **5c** (13,14-dihydro-14-propoxysanguinarine), mp 191–192° ($C_{23}H_{22}NO_5$); **5d** (13,14-dihydro-14-butoxysanguinarine), mp 172–174° ($C_{24}H_{24}NO_5$); **5e** (13,14-dihydro-14-acetonylsanguinarine), mp 183–184° ($C_{23}H_{20}NO_5$); 7c (12,13-dihydro-13-propoxychelerythrine), mp 202–203° ($C_{24}H_{25}NO_5$); and 7d (12,13-dihydro-13-butoxychelerythrine), mp 192–195° ($C_{25}H_{27}NO_5$). The nmr, mass spectral fragmentations on electron impact, and elemental analyses were consistent with the assigned structures.

Pharmacological Testing. Cytotoxicity was measured *in vitro* against "KB" tumor cells and *in vivo* activity was measured in the P388 and L1210 leukemias in mice.[7] We are indebted to Dr. Jim England and Mr. Noland Dunnan for assistance with these tests. *In vivo* activity of **5a–d**, **5f**, **6**, **7a**, and **7f** was also measured in the P388 and L1210 systems by National Cancer Institute contractors through the cooperation of Dr. Harry B. Wood, Drug Development Branch, National Cancer Institute, U. S. Public Health Service.

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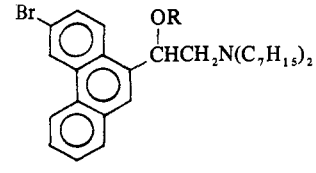
Preparation and Antimalarial Activity of Some Derivatives of 6-Bromo-α-(di-*n*-heptylaminomethyl)-9-phenanthrenemethanol

Robert E. Harmon,* T. S. Lin, and S. K. Gupta

Department of Chemistry, Western Michigan University, Kalamazoo, Michigan 49001. *Received October 18, 1972*

The appearance of drug resistant *falciparum* malaria has stimulated the search for new antimalarials. In their monograph Coatney, *et al.*,[1] discussed the efficacy of many simple 9-phenanthrenemethanols against *Plasmodium gallinaceum* in chicks. The most active compounds in this group were those which contained a Cl or Br atom in positions 3 and 6 and in which R was an unbranched alkyl 4–7 C long. The problem of water insolubility experienced in dialkylaminophenanthrenemethanols can be approached by converting them into ester or salt derivatives with polar ends. This should also improve the transport and absorption of these antimalarials in physiological media. In this paper the preparation and antimalarial activity of several derivatives of 6-bromo-α-(di-*n*-heptylaminomethyl)-9-phenanthrenemethanol (**1a**, R = H) are reported. The synthesis of **1a**† was reported in 1946 by May and Mosettig.[2]

First it was decided to prepare the sulfate and phosphate esters. The crystalline sulfate ester **1b** was prepared in 86% yield by treating a pyridine solution of **1a** · HCl with a pyridine solution of freshly prepared sulfur trioxide–pyridine complex.[3] Several different approaches for the preparation of the dihydrogenphosphate ester **1d** were tried, none of which were successful. As part of the first approach the intermediate diphenyl phosphate ester **1c** was prepared in 91% yield by treating **1a** · HCl with diphenylphosphorochloridate in pyridine.[4] Attempted conversion of diphenyl phosphate ester **1c** to **1d** by catalytic hydrogenation afforded a product whose elemental analysis indicated the absence of phosphorus. Perhaps this product resulted from the cleavage of the O-P bond. Alkaline hydrolysis of **1c** was also unsuccessful.



1a, R = H
b, R = SO_3H
c, R = $PO(OC_6H_5)_2$
d, R = $PO(OH)_2$
e, R = $PO(OC_6H_4NO_2)_2$
f, R = $C(=O)(CH_2)_3CO_2H$

The second approach involved the use of 2-cyanoethyl phosphate in conjunction with dicyclohexyl carbodiimide (DCC).[5-7] Treatment of the amino alcohol **1a** with freshly

#These measurements were performed on Cenco-Petersen molecular models.

†A generous sample of the hydrochloride of 1a was supplied by Walter Reed Army Medical Center, Washington, D. C. 20012.