1-β-D-**Ribofuranosyl-1,2,4-triazole-3-thiocarboxamide (2).** A mixture of **4** (8.0 g, 22.7 mmol), Et_3N (14.0 ml), and EtOH (200 ml) was stirred at 25° while H_2S gas was passed into the solution for 2 hr. The solvent was removed and the residue was treated with a solution of NaOMe (600 mg) in MeOH (150 ml) for 3 hr at 25°. After the solution was neutralized with Bio-Rad AG 50W-X2(H), it was filtered, and the solvent was removed. Crystallization of the product from aqueous EtOH provided 4.5 g (76%) of **2** with mp 173–175°: nmr (DMSO-d_6) δ 5.86 (d, 1, $J_{1',2'}$ = 3.5 Hz, 1'-H), 8.92 (s, 1, 5-H), 9.50 and 9.95 (s, 2, NH_2); λ_{max} (H_2O) 233 nm (ε 7600), 297 (8650). *Anal.* ($C_8H_{12}N_4O_4S$) C, H, N, S.

1-β-D-**Ribofuranosyl-1,2,4-triazole-3-carboxamidine Hydrochloride (3).** A mixture of **4** (7.04 g, 20.0 mmol), NH_4Cl (1.07 g, 20.0 mmol), and anhydrous NH_3 (150 ml) was heated in a bomb at 85° for 18 hr. After removal of excess NH_3, the residue was crystallized from MeCN–EtOH to provide 5.30 g (95%) of **3** with mp 177–179° dec: nmr (DMSO-d_6) δ 5.97 (d, 1, $J_{1',2'}$ = 3.5 Hz, 1'-H), 9.26 (s, 1, 5-H). *Anal.* ($C_8H_{14}ClN_5O_4$) C, H, Cl, N.

1-β-D-**Ribofuranosyl-1,2,4-triazole-3-carboxamidoxine (8).** A solution of **4** (3.0 g, 8.52 mmol) and excess NH_2OH in EtOH (100 ml) was refluxed with stirring for 2 hr. The solvent was removed and the product was crystallized from aqueous EtOH to provide 2.0 g (91%) of **8** with mp 212–214° dec. *Anal.* ($C_8H_{13}N_5O_5$) C, H, N.

1-β-D-**Ribofuranosyl-1,2,4-triazole-3-carboxamidrazone (9).** A solution of **4** (1.76 g, 5.0 mmol) in EtOH (50 ml) was treated with N_2H_4 (97%, 1.0 ml) and the solution was stirred at room temperature for 48 hr. The solid material was collected and recrystallized from aqueous EtOH to give 1.2 g (93%) of **9** with mp 179–180° dec. *Anal.* ($C_8H_{14}N_6O_4$) C, H, N.

1-(**2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1,2,4-triazole-5-thiocarboxamide (6).** Treatment of **5** with H_2S and Et_3N in EtOH as in the preparation of **2** followed by chromatography of the crude product over silica gel with $CHCl_3$ and crystallization from Et_2O–CH_2Cl_2 afforded **6** with mp 109–111°: nmr (DMSO-d_6) δ 7.23 (d, 1, $J_{1',2'}$ = 1.5 Hz, 1'-H), 8.26 (s, 1, 3-H), 10.15 and 10.55 (s, 2, NH_2). *Anal.* ($C_{14}H_{18}N_4O_7S$) C, H, N, S.

1-β-D-**Ribofuranosyl-1,2,4-triazole-5-thiocarboxamide (7).** Deacetylation of **6** with NaOMe as in the preparation of **2** and crystallization from EtOH provided **7** with mp 168–169°: nmr (DMSO-d_6) δ 6.86 (d, 1, $J_{1',2'}$ = 2.5 Hz, 1'-H), 8.11 (s, 1, 3-H), 10.10 and 10.50 (br s, 2, NH_2); λ_{max} (H_2O) 230 nm (ε 6000), 287 (6800). *Anal.* ($C_8H_{12}N_4O_4S$) C, H, N, S.

Acknowledgments. The authors wish to thank Martin P. Schweizer for helpful suggestions, John H. Huffman for assistance in obtaining the antiviral data, and O. P. Crews, Jr., and his staff for the large-scale preparation of chemical intermediates.

References

(1) J. T. Witkowski, R. K. Robins, R. W. Sidwell, and L. N. Simon, *J. Med. Chem.*, **15**, 1150 (1972).
(2) R. W. Sidwell, J. H. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, and R. K. Robins, *Science*, 177, 705 (1972).
(3) J. T. Witkowski, R. K. Robins, and R. W. Sidwell, Abstracts, 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972, No. MEDI 19.
(4) J. T. Witkowski, F. A. Lehmkuhl, S. R. Naik, R. W. Sidwell, and R. K. Robins, Abstracts, Twelfth Interscience Conference on Antimicrobial Agents and Chemotherapy, Sept 1972, No. 55.
(5) T. Sato, T. Shimidate, and Y. Ishido, *Nippon Kagaku Zasshi*, 81, 1440 (1960).
(6) G. E. Cipens and V. Grinsteins, *Latv. PSR Zinat. Akad. Vestis, Kim. Ser.*, 204 (1965); *Chem. Abstr.*, **63**, 13243*f* (1965).
(7) J. T. Witkowski and R. K. Robins, *J. Org. Chem.*, **35**, 2635 (1970).
(8) F. A. Lehmkuhl, J. T. Witkowski, and R. K. Robins, *J. Heterocycl. Chem.*, **9**, 1195 (1972).
(9) Y. Ishido, T. Matsuda, A. Hosono, K. Fujii, T. Sato, S. Isome, A. Maruyama, and Y. Kikuchi, *Bull. Chem. Soc. Jap.*, **40**, 1007 (1967).
(10) G. P. Kreishman, J. T. Witkowski, R. K. Robins, and M. P. Schweizer, *J. Amer. Chem. Soc.*, **94**, 5894 (1972).
(11) K. Matsuda and L. T. Morin, *J. Org. Chem.*, **26**, 3783 (1961).
(12) F. C. Schaefer and A. P. Kropcho, *ibid.*, **27**, 1255 (1962).
(13) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).
(14) G. Middlebrook, *Proc. Soc. Exp. Biol. Med.*, 80, 105 (1952).

Notes

Structure–Activity Relationships in Reactivators of Organophosphorus-Inhibited Acetylcholinesterase. 6. 2-Hydroxyiminomethylimidazolium Iodides†

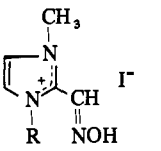
Mario Grifantini, Sante Martelli,

Institute of Pharmaceutical and Organic Chemistry, University of Camerino, Italy

and Maria L. Stein*

Institute of Pharmaceutical and Toxicological Chemistry, University of Naples, Italy. *Received November 10, 1972*

Recently we found[1] that methiodides of *syn*-2-hydroxyiminomethyl-1-methylimidazole (**1**) and of *syn*-2-hydroxyiminomethyl-1-benzylimidazole (**2**) are effective reactivators *in vitro* of acetylcholinesterase (AChE) inhibited by diisopropylphosphorofluoridate (DFP). Compound **2** is about twice as active as compound **1**. The increased reactivating capacity of **2** may be rationalized as due to greater bond strength with the enzyme surface resulting from van der Waals attractions and hydrophobic bonds.



1, R = CH_3
2, R = $CH_2C_6H_5$

We suspected that replacement of the methyl group by bulkier groups might produce agents giving stronger hydrophobic interactions with the enzyme and therefore yielding greater reactivating capacity. Therefore, we have prepared two homologous series of quaternary salts by treating 1-methyl- and 1-benzyl-2-hydroxyiminomethylimidazole with *n*-alkyl iodides, where the alkyl group varies from ethyl to *n*-octyl (Table I).

The reactivating capacity of the new products was determined *in vitro* following the method of Ashani, *et al.*,[2] on bovine erythrocyte AChE inhibited by DFP. Since the ef-

†Presented in part at the 7th International Meeting on Medicinal Chemistry, Lyon, France, Sept 1971. The work was supported by the Italian National Research Council.

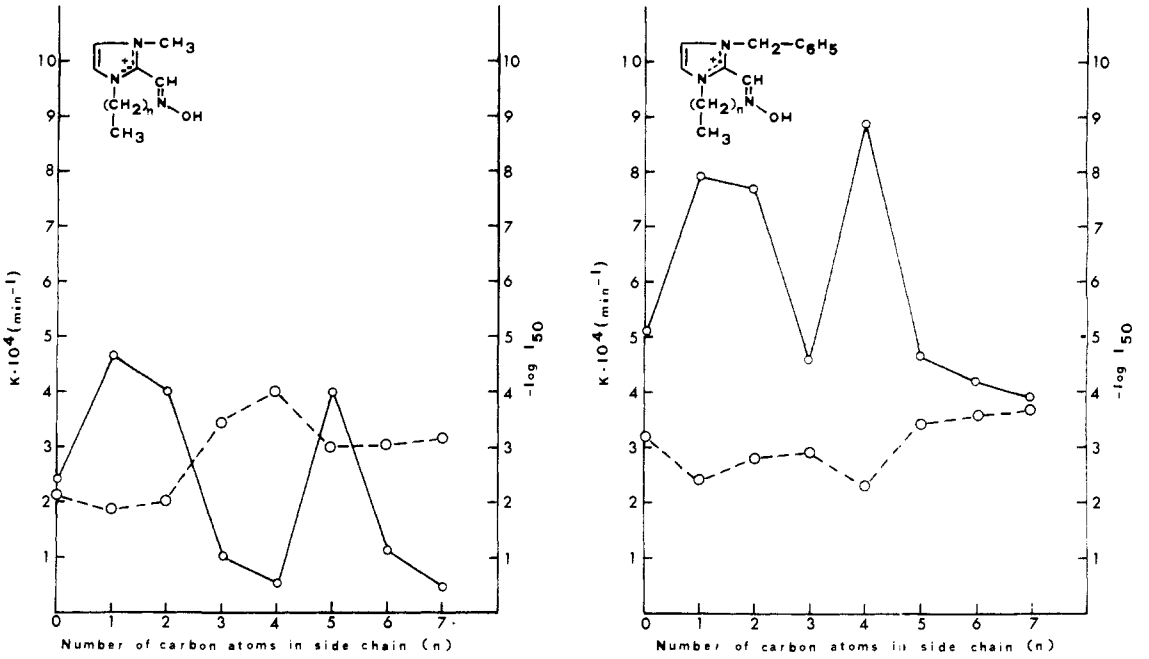


Figure 1. Reactivation rate constants (o—o) of bovine erythrocyte AChE, inhibited by DFP, by means of compounds 1–16 and *anti*-AChE activities as $-\log I_{50}$ (o···o) of the same compounds under identical experimental conditions.

Table I. 1-R_1,3-R_2-2-Hydroxyiminomethylimidazolium Iodides

No.	R_1	R_2	Method of prepn[a]	Mp, °C	Formula[b]
3	CH_3	C_2H_5	A[c]	193–195	$C_7H_{12}IN_3O$
4	CH_3	n-C_3H_7	C[d]	43–45[e]	$C_8H_{14}IN_3O$
5	CH_3	n-C_4H_9	C[d]	86–87	$C_9H_{16}IN_3O$
6	CH_3	n-C_5H_{11}	C[d]	87–89	$C_{10}H_{18}IN_3O$
7	CH_3	n-C_6H_{13}	D[d]	83–85	$C_{11}H_{20}IN_3O$
8	CH_3	n-C_7H_{15}	D[d]	90–92	$C_{12}H_{22}IN_3O$
9	CH_3	n-C_8H_{17}	E[d]	77–79	$C_{13}H_{24}IN_3O$
10	$CH_2C_6H_5$	C_2H_5	B[d]	116–118	$C_{13}H_{16}IN_3O$
11	$CH_2C_6H_5$	n-C_3H_7	C[d]	123–125	$C_{14}H_{18}IN_3O$
12	$CH_2C_6H_5$	n-C_4H_9	C[d]	111–113	$C_{15}H_{20}IN_3O$
13	$CH_2C_6H_5$	n-C_5H_{11}	C[d]	105–107	$C_{16}H_{22}IN_3O$
14	$CH_2C_6H_5$	n-C_6H_{13}	E	Oil	$C_{17}H_{24}IN_3O$
15	$CH_2C_6H_5$	n-C_7H_{15}	E	Oil	$C_{18}H_{26}IN_3O$
16	$CH_2C_6H_5$	n-C_8H_{17}	E	Oil	$C_{19}H_{28}IN_3O$

[a] Yields are low (15–35%); this is due to the instability of these products, especially of those which do not crystallize readily (4, 5, 7, 12, 16). [b] *Anal.* C, H, N. [c] Recrystallized from MeOH–EtOAc. [d] Recrystallized from Me_2CO–EtOAc. [e] Hygroscopic.

fectiveness of a reactivator of this type can be masked by its ability to inhibit AChE, the *in vitro anti*-AChE activity was also measured, under the same experimental conditions, but with noninhibited AChE.

Results and Discussion

The solid lines in Figure 1 show the variation of the reactivation rate constants as a function of the number n of methylene groups in the substituent. The curves are similar for both homologous series up to the butyl derivatives (n = 3). The two series differ starting with the n-pentyl derivatives: here we find a maximum in the series derived from 1-benzyl-2-hydroxyiminomethylimidazole; for the 1-methyl-3-alkylimidazolium salts the maximum occurs with the n-hexyl derivative. All the *N*-benzyl-substituted compounds are stronger reactivators than the corresponding *N*-methyl derivatives.

The *anti*-AChE activities ($-\log I_{50}$) of compounds **1–16** are shown in Figure 1 by the dotted lines; for the series of compounds examined a relationship between the reactivating capacity and the *anti*-AChE activity is apparent. In each series the best reactivator is also the least active inhibitor.

The favorable influence of the benzyl group on the reactivation of phosphoryl-AChE is thus confirmed by our results. A further improvement in this activity is obtained by introduction of certain alkyl groups. The analogous variation in the change of reactivating or inhibiting activities through the two homologous series suggests that both groups of quaternary salts are bonded to the anionic active site with a similar molecular orientation. We do not find it easy to explain the pattern of activity change within each series. In the case of AChE inhibitors, Thomas and Marlow[3] and Belleau[4] have found a nonlinear variation of activity with respect to the alkyl chain length in other quaternary ammonium compounds; Belleau[4,5] attributes this behavior to a different conformational perturbation of the enzyme due to the alkyl chain.

Experimental Section

Melting points of compounds were taken with a Büchi apparatus and are uncorrected. Uv, ir, and nmr spectra were consistent with the assigned structures. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Recrystallization solvents and physical data of the new compounds are given in Table I.

Preparation of the Imidazolium Iodides 3–16. Method A. The n-alkyl iodide (0.015 mol) was added to the oxime (0.01 mol) dissolved in 80 ml of anhydrous Me_2CO; the solution was left to stand for 15 days at room temperature. By evaporating part of the solvent a solid crystallized.

Method B. It is similar to method A, but the solid was obtained by adding EtOAc.

Method C. It is also similar to method A; after evaporation of the solvent, the oily residue was repeatedly washed with EtOAc. By adding a mixture of Me_2CO and EtOAc and by scratching, the oil turned to a solid.

Method D. The n-alkyl iodide (0.015 mol) was added to the oxime (0.01 mol) dissolved in 80 ml of anhydrous Me_2CO and the solution was heated for 10 days at 60° in a sealed container. Removal of the solvent left an oily residue which, after repeated washings with EtOAc, solidified.

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.

References

(1) M. Grifantini, S. Martelli, and M. L. Stein, *J. Pharm. Sci.*, 61, 631 (1972).
(2) Y. Ashani, E. Edery, J. Zahavy, W. Kunberg, and S. Cohen, *Israel J. Chem.*, 3, 133 (1965).
(3) J. Thomas and W. Marlow, *J. Med. Chem.*, 6, 107 (1963).
(4) B. Belleau, *Advan. Drug Res.*, 2, 88 (1965).
(5) B. Belleau in "Physico-chemical Aspects of Drug Action," E. Ariens, Ed., Pergamon Press, Oxford, 1966, p 207.

Some Structural Relationships among Cytotoxic and Antitumor Benzophenanthridine Alkaloid Derivatives

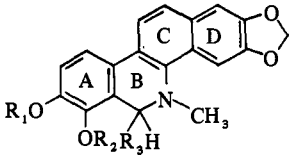
Frank R. Stermitz,*,† Kenneth A. Larson,‡ and Don K. Kim†

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

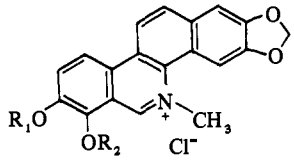
Received February 12, 1973

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**.

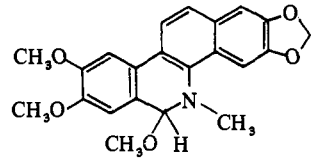
To test this hypothesis we prepared a series of sanguinarine derivatives **5a–f** for extensive cytotoxicity and antileukemia screening. Since nitidine possesses two methoxy groups in ring A, rather than the methylenedioxy grouping of sanguinarine, we also prepared a series of chelerythrine (**6**) derivatives **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.



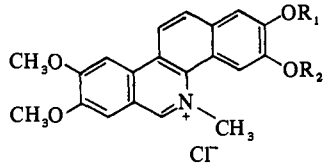
1, R_1, R_2 = $-CH_2-$; R_3 = OH
5a, R_1, R_2 = $-CH_2-$; R_3 = OCH_3
b, R_1, R_2 = $-CH_2-$; R_3 = OCH_2CH_3
c, R_1, R_2 = $-CH_2-$; R_3 = $OCH_2CH_2CH_3$
d, R_1, R_2 = $-CH_2-$; R_3 = $OCH_2CH_2CH_2CH_3$
e, R_1, R_2 = $-CH_2-$; R_3 = $CH_2C(=O)CH_3$
f, R_1, R_2 = $-CH_2-$; R_3 = H
6, R_1 = R_2 = CH_3;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