identical results.) The alcoholic suspension was diluted with 25 ml of water (starting material and other by-products are soluble in the aqueous alkaline medium), and the precipitate was collected by filtration, washed with water, and air dried. The compound (3.1 g, 76.2%) melted at 181–182° and exhibited identical spectral and physical data as the material from Method I.

REFERENCES

(1) L. H. Sternbach and E. Reeder, *J. Org. Chem.*, **26**, 1111(1961).

(2) S. Garattini, E. Mussini, and L. O. Randall, "The Benzodiazepines," Raven, New York, N.Y., 1973.

(3) L. O. Randall, W. Schallek, G. A. Heise, E. F. Keith, and R. E. Bagdon, *J. Pharmacol. Exp. Ther.*, **129**, 163(1960).

(4) G. A. Archer and L. H. Sternbach, *Chem. Rev.*, **68**, 747(1968).

(5) R. Littell and D. Allen, *J. Med. Chem.*, **8**, 722(1965).

(6) H. A. DeWald, I. C. Nordin, Y. J. L'Italien, and R. F. Parcell, *ibid.*, **16**, 1346(1973).

(7) M. Nakanishi, T. Tahara, K. Araki, M. Shiroki, T. Tsumagari, and Y. Takigawa, *ibid.*, **16**, 214(1973).

(8) Farbenfabriken Bayer A.-G., French pat. 1,491,791 (1967); through *Chem. Abstr.*, **69**, P67412d(1968).

(9) H. Horst and U. Bahr, British pat. 1,134,613 (1968); through *Chem. Abstr.*, **70**, P29589e(1969).

(10) H. Wollweber, J. Kurz, and W. Nägele, *Arch. Pharm.*, **304**, 774(1971).

(11) K. Gewald, *Z. Chem.*, **1**, 349(1961).

(12) S. Sunder, M.S. thesis, Auburn University, Auburn, Ala., 1968, p. 52.

(13) W. A. Remers, R. H. Roth, G. J. Gibbs, and M. J. Weiss, *J. Org. Chem.*, **36**, 1232(1971).

(14) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431(1967).

(15) R. I. Geran, H. N. Greenberg, M. M. MacDonald, A. M. Schumacker, and B. J. Abbott, *Cancer Chemother. Rep.*, **3**, 1(1972).

(16) Instruction Booklet 14, "Screening Data Summary Interpretation," Drug Research and Development, Chemotherapy, National Cancer Institute, Bethesda, Md., 1972.

(17) A. Tensho, I. Shimizu, T. Takenawa, H. Kikuchi, and T. Rukujo, *J. Pharm. Soc. Jpn.*, **92**, 879(1972).

(18) E. M. Glenn, B. J. Bowman, W. Kooyers, T. Koslowske, and M. L. Meyers, *J. Pharmacol. Exp. Ther.*, **155**, 157(1967).

(19) L. L. Shaletzky, B. F. Graham, and J. Szmuszkovicz, *J. Med. Chem.*, **12**, 977(1969).

(20) G. C. Gerritsen and W. E. Dulin, *J. Pharmacol. Exp. Ther.*, **150**, 491(1965).

ACKNOWLEDGMENTS AND ADDRESSES

Received July 10, 1975, from the *Department of Medicinal Chemistry, School of Pharmacy, University of Georgia, Athens, GA 30602*

Accepted for publication August 13, 1975.

Adapted in part from a thesis submitted by J. W. Sowell to the University of Georgia in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by Public Health Service Research Grant MH16422 from the National Institutes of Health.

Antimalarial test results were provided through the courtesy of Dr. T. R. Sweeney, Walter Reed Army Institute of Research. Antineoplastic data were made available through the courtesy of Dr. H. B. Wood, Jr., Drug Development Branch, Drug Research and Development, Division of Cancer Treatment, National Cancer Institute. Pharmacological test results from personnel of The Upjohn Co. were provided through the courtesy of Dr. Paul O'Connell, Biological Screening Office, The Upjohn Co., Kalamazoo, Mich. Mass spectral data were provided through the courtesy of the Southeast Environmental Research Laboratory, Athens, Ga.

* Present address: School of Pharmacy, University of South Carolina, Columbia, S.C.

ˣ To whom inquiries should be directed.

Partition Coefficients of Selected Pyridine Carbamates and Comparisons with Their Acetylcholinesterase and Butyrylcholinesterase Inhibitory Potencies

O. ELMO MILLNER, Jr. *, and WILLIAM P. PURCELL ˣ

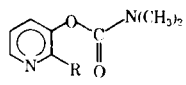
Abstract □ Partition coefficients of a series of 2-substituted 3-(*N,N*-dimethyl)carbamyloxypyridines were determined in an octanol–buffer (pH 7.4) system. The values obtained were compared with the inhibitory potencies of acetylcholinesterase and butyrylcholinesterase. No significant difference was found for the role of hydrophobicity in the two enzyme systems.

Keyphrases □ Partition coefficients—substituted pyridine carbamates, compared with acetylcholinesterase and butyrylcholinesterase inhibitory potencies □ Pyridine carbamates, substituted—partition coefficients compared with acetylcholinesterase and butyrylcholinesterase inhibitory potencies □ Acetylcholinesterase—inhibition by substituted pyridine carbamates compared to their partition coefficients □ Butyrylcholinesterase—inhibition by substituted pyridine carbamates compared to their partition coefficients □ Enzymes—acetylcholinesterase and butyrylcholinesterase, inhibition by substituted pyridine carbamates compared to their partition coefficients

Previously (1), the synthesis and enzymatic evaluation of some pyridine carbamates were reported, and the compounds were used as enzyme inhibitors in a comparative study of the active centers of acetylcholinesterase and butyrylcholinesterase. Fundamental differences in acetylcholinesterase and butyrylcholinesterase regarding their interaction with the selected carbamate inhibitors were presented (1). No results were included, however, on the effect of any chemical or physical properties of the inhibitor molecules on inhibitory activity. The purpose of this paper is to report results of a study on the contribution of relative hydrophobicities to the inhibition of acetylcholinesterase and butyrylcholinesterase by the selected carbamates (1).

Limited literature results comparing the active

Table I—Partition Coefficients (P) and I_{50} Values of Selected Carbamates



Compound	R	$P \pm SD$[a]	Acetylcholinesterase $I_{50} \times 10^5 M$	Butyrylcholinesterase $I_{50} \times 10^5 M$
I	H	—[b]	—[b]	—[b]
II	Cl	11.08 ± 0.61[a]	6.1 (±0.3)[c]	8.75 (±0.44)[c]
III	Br	13.74 ± 0.40[a]	2.15 (±0.11)[c]	6.0 (±0.3)[c]
IV	I	18.26 ± 0.91[a]	0.900 (±0.045)[c]	1.950 (±0.012)[c]
V	NO_2	3.46 ± 0.25[a]	7.9 (±0.4)[c]	19.0 (±1.0)[c]
VI	$CH_2N(CH_3)_2$	0.422 ± 0.068[a]	0.490 (±0.024)[c]	0.144 (±0.007)[c]
VII	$CH_2\overset{+}{N}(CH_3)_3$	—	0.360 (±0.018)[c]	0.135 (±0.007)[c]

[a] Standard deviation ($\sqrt{\Sigma y^2/n}$) of six determinations. [b] Compound underwent immediate autohydrolysis in water. [c] Experimental error determined by repeated measurements for one compound. See Ref. 1.

centers of acetylcholinesterase and butyrylcholinesterase that indicated a difference in the hydrophobic nature of the "nonesteratic" site of acetylcholinesterase and butyrylcholinesterase (2, 3) were based upon qualitative observations; hydrophobicity was assumed to increase with, and increase in, the alkyl nature of organophosphorus inhibitors (3) and pyridine acetate substrates (2). A quantitative assessment of the role of hydrophobic interactions in the inhibition of acetylcholinesterase and butyrylcholinesterase should aid in comparing the hydrophobic nature of the active centers of acetylcholinesterase and butyrylcholinesterase. For this reason, partition coefficients in the octanol–water system (4) were measured as quantitative indexes of hydrophobicity.

EXPERIMENTAL

The synthesis and enzymatic evaluation of the selected compounds were reported previously (1).

Partition Coefficients—Partition coefficients were determined in the octanol–water system (5, 6). Measurements were made using an aqueous phase consisting of 0.1 M phosphate buffer adjusted to pH 7.4. The aqueous phase was shaken vigorously with purified octanol to saturate it with octanol and vice versa so that the phase composition would remain constant during the partition process.

Partitioning was done in 50-ml glass-stoppered centrifuge tubes. A specially designed shaking apparatus was used to ensure vigorous shaking and thorough mixing. The amounts of compound and relative volumes of the two phases were varied. In this manner, any dependence of partitioning on solubility effect (*e.g.*, saturation of one phase with a compound) could be detected. The filled tubes were shaken for 10 min and centifuged until both phases were completely separated. Six determinations were made in this manner for each compound.

Concentrations in the two phases were determined by UV absorption. Absorbance *versus* concentration was determined in both the octanol and the aqueous phases to ensure linearity of absorbance with concentration in both phases in the concentration range used.

Regression Analyses—Linear regressions were performed using a computer program modified from the original program (7).

RESULTS AND DISCUSSION

The results of the partitioning measurements along with the I_{50} values of the respective compounds are given in Table I[1]. Since I undergoes rapid autohydrolysis in aqueous solution, there is no partition coefficient or I_{50} value for this compound.

If one compares the acetylcholinesterase and butyrylcholinesterase inhibitory activities with log P, VI clearly does not fall in line with the other compounds. The tertiary amine substituent of VI is largely protonated at pH 7.4, resulting in its low apparent partition coefficient (8).

The observation that inhibitory activity increases as the van der Waals radii of the halogen in the 2-position increase leads to the speculation that the inhibitory activity of carbamates that are quasisubstrates of cholinesterase, *i.e.*, progressive inhibitors, may be related to the ability of the carbamate to create an anhydrous environment at the catalytic center of the cholinesterase. Since these inhibitors have been shown to carbamylate (1), any condition that facilitates the rate of carbamylation should also increase inhibitory activity. The hydrolysis of carbamates is thought to proceed *via* a mechanism including a nucleophilic attack on the carbonyl carbon similar to the hydrolysis of acetylcholine (9). Weaver and Hutchison (10) showed that, in a nonenzymatic system, nucleophilicity is increased by anhydrous conditions.

The relative hydrophobicities of the halogen should increase with an increase in surface area of the nonpolar group (11, 12). The amount of structured water that is broken down when a hydrophobic moiety leaves the aqueous environment is directly proportional to the gain in the positive entropy for the transfer (11, 13) and, thus, to the strength of the hydrophobic interaction. The hypothesis of Belleau and Lacasse (14) is that the function of the third methyl group of acetylcholine, which has been shown not to contribute to the affinity of acetylcholine for the enzyme, acetylcholinesterase (15), is to expel water from the active site of acetylcholinesterase. The creation of a more anhydrous environment could serve to increase the rate of carbamylation (and thus the inhibitory potencies) by the halogen-substituted pyridine carbamates.

[1] *Note added in proof:* Even though the partition coefficients in Table I are apparent partition coefficients, the true partition coefficients should be the same, since ionization is not present. An exception to this is Compound XI, of course.

REFERENCES

(1) O. E. Millner, Jr., J. W. Stanley, and W. P. Purcell, *J. Med. Chem.*, **17**, 13(1974).

(2) K.-B. Augustinsson, *Biochim. Biophys. Acta*, **128**, 351(1966).

(3) M. I. Kabachnik, A. P. Brestkin, N. N. Godoviko, M. J. Michelson, E. V. Rozengart, and V. I. Rozengart, *Pharmacol. Rev.*, **22**, 355(1970).

(4) A. Leo, C. Hansch, and C. Church, *J. Med. Chem.*, **12**, 766(1969).

(5) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525(1971).

(6) A. Leo and C. Hansch, *J. Org. Chem.*, **36**, 1539(1971).

(7) A. J. Capato, "Single and Multiple Linear Regression Analysis Program," IBM Data Processing Systems, New York, N.Y., 1967.

(8) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solution," Butterworths Scientific Publications, London, England, 1965.

(9) R. D. O'Brien, *Mol. Pharmacol.*, **4**, 121(1968).

(10) W. M. Weaver and J. D. Hutchison, *J. Amer. Chem. Soc.*, **86**, 261(1964).

(11) G. Nemethy, *Angew. Chem. Int. Ed.*, **6**, 195(1967).

(12) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507(1945).

(13) G. Nemethy and H. A. Scheraga, *ibid.*, **36**, 3401(1962).

(14) B. Belleau and C. Lacasse, *J. Chem.*, **7**, 768(1964).

(15) I. B. Wilson and E. Cabib, *J. Amer. Chem. Soc.*, 78, 202(1956).

ACKNOWLEDGMENTS AND ADDRESSES

Received June 23, 1975, from the *Drug Design Division, University of Tennessee, Center for the Health Sciences, Memphis, TN 38163*

Accepted for publication August 4, 1975.

Abstracted in part from a dissertation submitted by O. E. Millner, Jr., to the University of Tennessee in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported by the U.S. Public Health Service through a National Institutes of Health Traineeship (GM-02052) and by the University of Tennessee Cardiovascular Computer Project (HL-09495).

* NIH Trainee (GM-02052). Present address: Research and Development, Norwich Pharmacal Co., Norwich, NY 13815

ˣ To whom inquiries should be directed.

Potential Antitumor Agents: A Cytotoxic Cardenolide from *Coronilla varia* L.

MARTIN WILLIAMS * and JOHN M. CASSADY ˣ

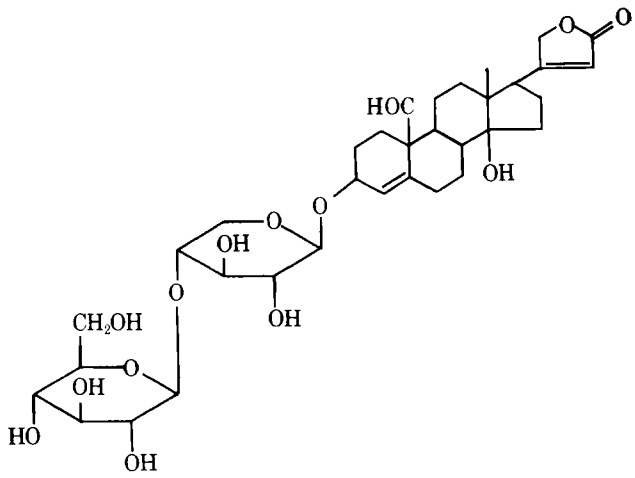
Abstract □ An alcoholic extract of the seeds of *Coronilla varia* L. showed inhibitory activity against KB cells in culture and was fractionated through a series of partitions, column chromatography, and preparative layer chromatography to yield hyrcanoside, daphnoretin, scopoletin, and umbelliferone. Hyrcanoside was also tested in the PS mouse leukemia assay and showed borderline activity.

Keyphrases □ *Coronilla varia*—seeds, alcoholic extract, cardenolide and three coumarins isolated and identified, cytotoxic and antileukemic activity screened □ Cardenolides—hyrcanoside isolated from *Coronilla varia* seeds, cytotoxic and antileukemic activity screened □ Coumarins—isolated from alcoholic extract of *Coronilla varia* seeds, cytotoxic and antileukemic activity screened □ Cytotoxic compounds—alcoholic extract of *Coronilla varia* seeds, cytotoxic and antileukemic activity screened □ Antitumor agents, potential—alcoholic extract of *Coronilla varia* seeds, cardenolide and three coumarins isolated and screened □ Antileukemic agents, potential—alcoholic extract of *Coronilla varia* seeds, cardenolide and three coumarins isolated and screened

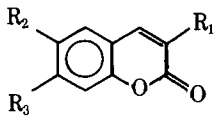
An alcoholic extract of the seeds of *Coronilla varia* L. (var. Penngift) (family Leguminosae) was found to have inhibitory activity against cells derived from human carcinoma of the nasopharynx (KB)[1]. Systematic fractionation of the extract led to the isolation of two cardenolides, one of which was identified as hyrcanoside (I); three coumarins, daphnoretin (II), scopoletin (III), and umbelliferone (IV); and two unidentified compounds. Compounds I–IV have been reported to occur in Russian varieties of *C. varia* (1, 2), and III and IV have also been shown to occur in *C. varia* (var. Penngift) (3). There is considerable interest in varieties of *C. varia* as potential forage crops (4, 5) and in soil erosion control, and recent publications dealing with toxic constituents have appeared (6, 7).

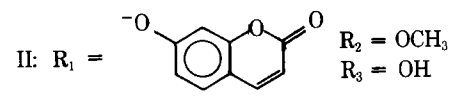
DISCUSSION

An outline of the general fractionation and chromatographic procedures are given in the *Experimental* section, and the biological data



I





III: R_1 = H, R_2 = OCH_3, R_3 = OH
IV: R_1 = H, R_2 = H, R_3 = OH

are presented in Table I. The concentrated alcoholic fraction (C) from percolation of the powdered seed material with ethanol was partitioned between chloroform and water, resulting in concentration of the cytotoxic activity in the aqueous phase (C). Further partitioning of the aqueous phase with mixtures of chloroform and ethanol in various proportions and with 1-butanol brought the activity into the organic phase. Chromatography of Fraction E on silicic acid yielded the coumarins and an unidentified cardenolide with an R_f of 0.56. Chromatography of Fractions G and H on silicic acid, followed by preparative layer chromatography on silica gel, gave hyrcanoside (I) from Fraction G and two unidentified compounds, R_f 0.49 and 0.39, from Fraction H.

Solid 1 (L) obtained from the chloroform fraction (E) was identified as daphnoretin (II) on the basis of a comparison of physical and spectral properties with literature values. Solid 2 (M) and Solid 3 (N) were identified as scopoletin (III) and umbelliferone (IV) by direct comparison of physical and spectral properties with those of authentic

[1] The cell culture and mouse leukemia assays were performed under the auspices of the Division of Cancer Treatment, Drug Research and Development, National Cancer Institute, according to the procedure given in *Cancer Chemother. Rep.*, 25, 22(1962). In KB, a purified substance is considered active if it shows an $ED_{50} \leq 4$ μg/ml. In PS, a compound is considered active if it shows an increase in lifespan (ILS) of ≥25% relative to controls.