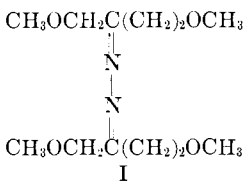
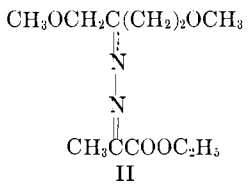
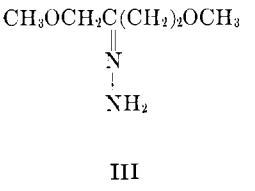
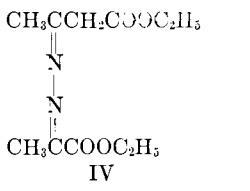
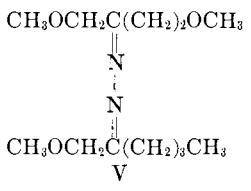

I


II


III


IV


V

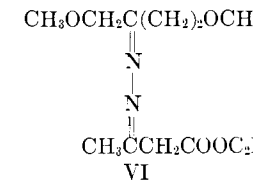

VI

TABLE I

Compounds	Antitumor activity against WA-256: mg/kg	Survivors	Wt. diff., T-C	T/C (%)
I	250	6/6	−27	9
	100	6/6	−14	8
	45	6/6	−9	65
II	200	6/6	−18	18
	100	6/6	−7	72
III	105	6/6	−16	41
	70	6/6	−15	25
	45	6/6	−7	42
	30	6/6	−3	89
1,4-Dimethoxy-4-butanone	400	6/6	−2	60
IV	400	6/6	−3	86

compounds were not reported. Wiley and coworkers[4,5] prepared a series of substituted hydrazones of pyridoxal, indole-3-carboxaldehyde and 2-methoxynaphthaldehyde, but only two of 35 compounds prepared were found to have significant activity against sarcoma 180 in mice.

A survey in the general area of biological activity of azines again revealed little. Some synthetic azine dyes, such as methyl violet and heliotrope, were claimed to have good tuberculostatic effect against human type tubercle bacteria.[6] The molluscacidal activity displayed by azines of certain *p*-benzoquinones[7] was largely attributed to the parent quinones,[7-9] and the well known biological activity of nitrofuran derivatives could possibly be the reason for the antimicrobial activity exhibited by a number of 5-nitro-2-furfural azines.[10,11]

The activity exhibited by compounds I–III is in a way rather unique in that similar activity was not observed by ethyl acetoacetate azine with ethyl pyruvate (IV) nor by 1,4-dimethoxy-2-butanone, the parent compound of I–III. Logically, other azine derivatives of 1,4-dimethoxy-2-butanone, such as compounds V and VI, were prepared for a preliminary structure–activity study. However, the study could not be continued in our laboratory because of the discontinuance of Walker 256 testing system in the general screening.

(4) R. H. Wiley and G. Irick, *J. Med. Pharm. Chem.*, **5**, 49 (1962).
(5) R. H. Wiley and R. L. Clevenger, *ibid.*, **5**, 1367 (1962).
(6) M. G. Good, *Zentr. Bakteriol., Parasitenk. Abt. I. Orig.*, **169**, 99 (1957); *Chem. Abstr.*, **52**, 1357h (1958).
(7) N. Latif and I. Fathy, *J. Org. Chem.*, **25**, 1614 (1960).
(8) A. Halawani and N. Latif, *J. Egypt. Med. Assoc.*, **37**, 957 (1954).
(9) T. von Brand, B. Mehlman, and M. O. Nolan, *J. Parasitol.*, **35**, 475 (1949).
(10) J. D. Johnston, U. S. Patent, 3,099,663 (1963); *Chem. Abstr.*, **60**, 2894g (1964).
(11) J. D. Johnston, U. S. Patent, 3,296,257 (1967); *Chem. Abstr.*, **67**, 3100h (1967).

Experimental Section

1,4-Dimethoxy-2-butanone Azine (I) and 1,4-Dimethoxy-2-butanone Hydrazone (III).—A solution of 32 g (1 mol) of anhydrous N_2H_4 in 250 ml of anhydrous MeOH was stirred in an ice bath while 33 g (0.25 mol) of 1,4-dimethoxy-2-butanone (prepared by the method of Hennion and Kupiecki[12] from 1,4-butyndiol dimethyl ether[13]) in 50 ml of MeOH was added dropwise over a period of 30 min. The reaction mixture was then stirred at room temperature for 16 hr. Removal of MeOH and excess N_2H_4 followed by fractional distillation gave 22 g (60% yield) of the hydrazone (III), bp 91–92° (5.5 mm), $n^{24}D$ 1.4671, and 11.0 g (17% yield) of the azine (I), bp 138–140° (2.6 mm), $n^{25}D$ 1.4664. *Anal.* ($C_6H_{14}N_2O_4$), C, H, N; ($C_{12}H_{24}N_2O_4$), C, H, N.

Ethyl Pyruvate Azine with 1,4-Dimethoxy-2-butanone (II).—A solution of 16.6 g (0.11 mol) of 1,4-dimethoxy-2-butanone hydrazone (III) in 50 m lof anhydrous MeOH was stirred at 0° while 13.2 g (0.11 mol) of ethyl pyruvate in 50 ml of MeOH was added dropwise over a period of 30 min. The mixture was allowed to stir at room temperature overnight and then distilled to give 19.0 g (70% yield) of the azine, bp 119–120° (0.4 mm), $n^{25}D$ 1.4708. *Anal.* ($C_{11}H_{20}N_2O_4$), C, H, N.

Ethyl acetoacetate azine with ethyl pyruvate (IV) was prepared by a procedure analogous to the foregoing. The product, mp 88–89°, was purified by sublimation at 65° (0.1 mm). *Anal.* ($C_{11}H_{18}N_2O_4$), C, H, N.

1-Methoxy-2-hexanone azine with 1,4-dimethoxy-2-butanone (V) was prepared in an analogous fashion, bp 123–126° (2.3 mm), $n^{25}D$ 1.4628. *Anal.* ($C_{13}H_{26}N_2O_3$), C, H, N.

Ethyl acetoacetate azine with 1,4-dimethoxy-2-butanone (VI) had bp 135–138° (0.35 mm), $n^{25}D$ 1.4938. *Anal.* ($C_{12}H_{22}N_2O_4$), C, H, N.

Acknowledgment.—The authors wish to thank Mr. John Gravatt for his assistance in performing analytical measurements.

(12) G. F. Hennion and F. P. Kupiecki, *J. Org. Chem.*, **18**, 1601 (1953).
(13) W. Reppe, *Justus Liebigs Ann. Chem.*, **596**, 38 (1955).

β-Alanyl Thio Esters[1]

GIL CLIFTON[2] AND CHARLES G. SKINNER

Department of Chemistry, North Texas State University, Denton, Texas 76203

Received October 10, 1969

The introduction of chemically active centers in organic molecules which may serve as alkylating agents has been an area of recent interest in the syntheses of

(1) This investigation was supported in part by National Institutes of Health Research Grant No. CA-08102, National Cancer Institute, and by the North Texas State University Faculty Research Fund.
(2) National Defense Education Act Fellow, Title IV.

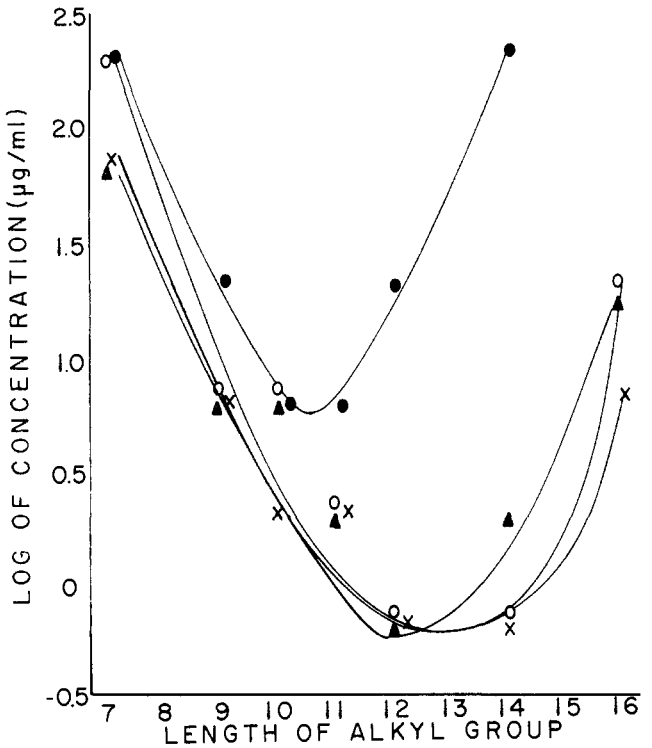


Figure 1.—Effect of chain length on concentration of β-alanyl thioalkyl esters required to completely inhibit growth of microorganisms: (●) *Escherichia coli*, (○) *Lactobacillus arabinosus*, (×) *Lactobacillus casei*, (▲) *Pediococcus cerevisiae*. The legend indicates the number of C atoms in the *n*-alkyl group.

potential chemotherapeutic agents.[3] In addition, long chain alkyl derivatives possessing hydrophilic moieties have detergent characteristics and often possess antibactericidal properties. For example, hexadecyl-, tetradecyl-, and dodecylamine have been reported to inhibit the growth of *Escherichia coli*.[4] In the present study a group of analogs was synthesized possessing a terminal hydrophilic amino group, an internal thio ester linkage which possesses potential alkylating properties, and various hydrophobic long chain hydrocarbon functions. Since several thioesters of amino acids and various other metabolites have been observed to be noncompetitive antimetabolites in several microbial systems,[5,6] it was of interest to examine the physiological effect of introducing a long chain alkyl thio ester grouping into β-alanine which might function as a "carrier moiety."[7]

These compounds were prepared by condensing β-alanyl chloride hydrochloride[8] with the appropriate thiol to produce the corresponding β-alanyl thio ester hydrochlorides (Table I). The general method of synthesis was patterned after Ray and Johnston;[9] all of the compounds were hygroscopic to varying degrees.

The general levels of toxicity to microbial growth for this series of compounds were determined on four

Table I
β-Alanine Thio Ester Hydrochlorides

$$Cl^-NH_3CH_2CH_2C(=O)SR$$

R	Mp, °C	%, Yield	Empirical Formula[a]
n-Bu	110–111	38[b]	$C_7H_{16}ClNOS$
n-Hept	125–127	70	$C_{10}H_{22}ClNOS$
n-Non	117–120[c]	55[b]	$C_{12}H_{26}ClNOS$
n-Decyl	123–125	83[d]	$C_{13}H_{28}ClNOS$
n-Undecyl	126–128	55[b]	$C_{14}H_{30}ClNOS$
n-Dodecyl	121–123	71[e]	$C_{15}H_{32}ClNOS$
n-Tetradecyl	126–126	61[e]	$C_{17}H_{36}ClNOS$
n-Hexadecyl	127–128	71	$C_{19}H_{40}ClNOS$
Benzyl	159–160	75	$C_{10}H_{14}ClNOS$

[a] All compounds possessed the anticipated analysis for C, H, and N. [b] This compound had a broad melting range. [c] Yield based on product after one recrystallization. [d] C, calcd 55.59, found 55.80.

microorganisms as summarized in Figure 1. The concentration levels required for complete growth inhibition ranged from 6 to >200 μg/ml in *E. coli* and from 0.6 to >200 μg/ml in *Lactobacillus arabinosus*, *L. casei*, and *Pediococcus cerevisiae*. It is apparent from Figure 1 that the toxicities of the analogs are dependent upon chain length of the alkyl group. For the lactic acid bacteria the optimum length is between 12 and 14 carbons, and for *E. coli* the chain length inducing the greatest amount of toxicity is between 10 and 11 carbons. In an effort to determine if these toxicities could be reversed by supplements of natural metabolites, the following materials were added concurrently with toxic concentrations of the thio esters: β-alanine, a complete synthetic amino acid mixture, hydrolyzed casein solutions, purine and pyrimidine supplements, a complete vitamin supplement, and high levels of pantothenic acid. However, none of these supplements produced a significant reversal of toxicity in *L. arabinosus*.

The rather consistent relationship between microbial toxicity and chain length as shown in Figure 1 suggested that the inhibitory activity of these derivatives might be a function of the total "amine character" of the molecule rather than to the presence of a thio ester linkage. One might speculate that the lower homologs would be more chemically reactive than the larger molecules and thus possess greater alkylating properties; however, this was not observed. Subsequently, the inhibitory properties (based on total chain length) of an amino thio ester were compared to a corresponding oxygen ester and an analogous long chain amine. The CO–S linkage of the thio ester and the CO–O–CH_2 linkage of the oxygen ester were considered equivalent to a 3-C unit of the amine. Using *L. arabinosus* as the test organism, the *n*-nonyl thio ester and the *n*-decyl ester of β-alanine had identical inhibitory properties: both were toxic to growth at 6 μg/ml. In contrast, *n*-tetradecylamine was toxic to growth at a concentration of 0.6 μg/ml. The relationship between inhibition and total chain length in both the β-alanyl thio ester and *n*-alkyl amine series is demonstrated in Figure 2. A comparison of Figures 1 and 2 suggests that the optimum chain length for growth inhibition is in the range of 18 to 20 C atoms since higher equivalent lengths in the β-alanyl thio ester series become signifi-

(3) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors," John Wiley & Sons, Inc., New York, N. Y., 1967, pp 17–21.
(4) S. Takase, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **74**, 59 (1953).
(5) K. Hayashi, C. G. Skinner, and W. Shive, *Texas Rep. Biol. Med.*, **19**, 277 (1961).
(6) L. C. Smith, K. Hayashi, J. M. Ravel, C. G. Skinner, and W. Shive, *Biochemistry*, **2**, 159 (1963).
(7) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 1954, 2409.
(8) M. Frankel, Y. Lwsekitz, and A. Zilkha, *J. Amer. Chem. Soc.*, **76**, 2814 (1954).
(9) R. A Ray and R. B. Johnston, *J. Med. Chem.*, **8**, 275 (1965).

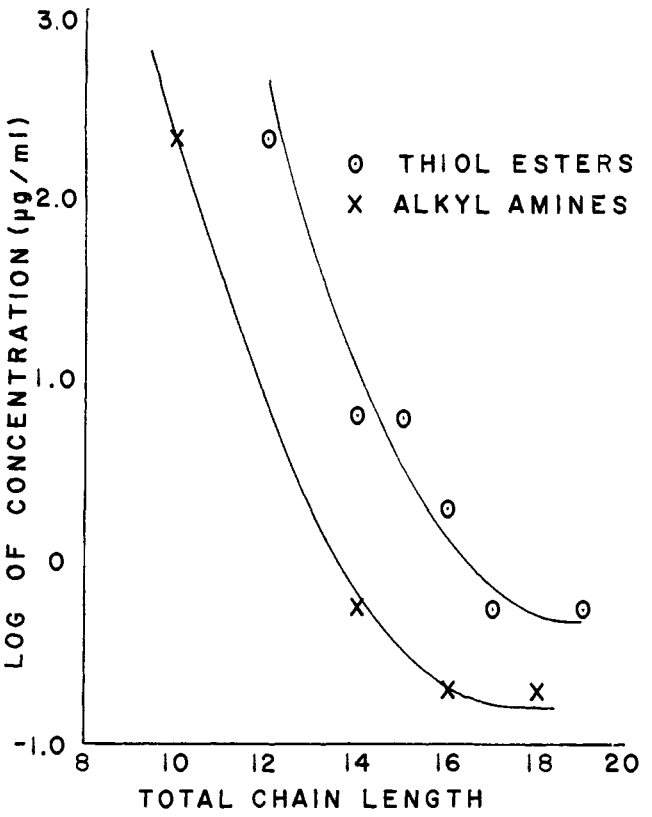


Figure 2.—A comparison of the concentration of β-alanyl thioesters and long chain alkylamines required to completely inhibit the growth of *Lactobacillus arabinosus*. The legend indicates the total number of atoms in each of the chains attached to the amino group.

cantly less toxic. These data suggest that the inhibitory properties of the thio esters are a function of their lipid character rather than of their alkylating ability.

Experimental Section[10]

Biological Testing Procedures.—The organisms used in this study were *E. coli* 9723, *L. arabinosus* 17-5, *L. casei* 7469, and *P. cerevisiae* 8042. An inorganic salts medium was used for *E. coli*,[11] and a previously reported amino acid medium was utilized for *L. arabinosus* and *L. casei*[12] except that the vitamin supplement contained 0.2 μg/ml of calcium pantothenate, and 500 μg of glutamic acid was added per tube for assays with *L. casei*. An acid-hydrolyzed casein medium[13] was used for *P. cerevisiae* with the Salts A concentration increased fourfold. Growth assay times and temperatures were 16 hr at 37° for *E. coli*, 24 hr at 37° for *L. casei*, 20 hr at 30° for *L. arabinosus*, and 21 hr at 30° for *P. cerevisiae*. The amount of growth was determined on a Bausch and Lomb Model 20 spectrometer as per cent transmission at 600 mμ.[14]

β-Alanyl Thio Ester Hydrochlorides.—A 20-ml sample of the appropriate thiol was cooled to appoximately 5°, and 3 g of β-alanyl chloride·HCl[8] was added. The reaction mixture, protected from moisture, was stirred for 15 min at ice bath temperature and then allowed to come to room temperature. Stirring was continued overnight. The mixture was again cooled to 5°, and Et_2O (50 ml) was added to give the desired product which was filtered and washed with dry Et_2O. The resulting solids were recrystallized (MeOH–Et_2O) to yield hygroscopic compounds which were maintained in a desiccator. (See Table I).

***n*-Decyl 3-Aminopropionate Hydrochloride.**—The same procedure was used for this compound as for the thio ester. The solid had mp 84–85° and analyzed satisfactorily for C, H, and N.

(10) Melting points were determined with a Thomas–Hoover Capillary Melting Point Apparatus. The authors are indebted to Mrs. Sarah R. Bryant for assistance with the microbial testing procedures.

(11) E. H. Anderson, *Proc. Nat. Acad. Sci., U.S.*, **32**, 120 (1945).

(12) J. M. Ravel, L. Woods, B. Felsing, and W. Shive, *J. Biol. Chem.*, **206**, 391 (1954).

(13) E. M. Lansford, Jr., W. M. Harding, and W. Shive, *Arch. Biochem. Biophys.*, **73**, 180 (1958).

(14) R. E. Masingale, S. R. Bryant, C. G. Skinner, J. Nash, and P. F. Kruse, Jr., *J. Med. Chem.*, **12**, 152 (1969).

Metal Complexes of 1-Substituted 3-Hydroxyureas

ROBERT E. HARMON, JAMES C. DABROWIAK,[1] DONALD J. BROWN, S. K. GUPTA, MICHAEL HERBERT, AND D. CHITHARANJAN

Department of Chemistry, Western Michigan University, Kalamazoo, Michigan 49001

Received July 28, 1969

A considerable amount of work has been done to indicate the existence of a definite correlation between the metal binding properties of therapeutically active compounds and their activity.[2-4] For instance, therapeutically active analogs of tetracycline form 2:1 complexes with Cu (II), Ni (II), and Zn(II) ions whereas the inactive analogs appear to form only 1:1 complexes.[5] Hydroxyurea (HU)[6] and ethylhydroxyurea (EHU)[7] have been found to be very active against L1210 lymphoid leukemia. When patients with myelogenous leukemia were placed on HU therapy, acetohydroxamic acid was identified in their blood. To explain the mode of action of HU in biological systems, two theories have been proposed. According to Fishbein and Carbone,[8] HU is hydrolyzed to hydroxylamine, which cleaves thio esters such as acetyl-coenzyme A, as shown in eq 1. A second theory[9] suggested that

$$\begin{aligned} &NH_2CONHOH + 2H_2O \longrightarrow NH_2OH + NH_4^+ + HCO_3^- \\ &NH_2OH + CH_3COSCoA \longrightarrow CH_3CONHOH + HS\text{-}CoA \end{aligned} \quad (1)$$

hydroxyurea caused fragmentation of isolated DNA and induced chromosomal abnormalities in mammalian cells. The proposed mechanism is shown in eq 2.

$$NH_2CONHOH \xrightarrow[-H_2O]{\text{oxidation}} 2(HON:) \longrightarrow H_2N_2O_2 \quad (2)$$

Hyponitrous acid ($H_2N_2O_2$) like hydroxylamine and other compounds, causes cleavage of the main chain of cellular DNA. Our results based on the decomposition of Fe(III)–EHU complex suggest that hydroxyureas may be involved in an oxidation–reduction reaction, producing NO. In this paper, we also wish to describe the synthesis, metal-binding properties, and antitumor activity of several 1-substituted 3-hydroxyureas.

The synthesis of 1-substituted 3-hydroxyureas **1a–g** was achieved by a slight modification of the method

(1) J. C. Dabrowiak, M. A. Thesis, Western Michigan University, Kalamazoo, Mich., 1967.

(2) J. T. Doluisio and A. N. Martin, *J. Med. Chem.*, **6**, 20 (1963).

(3) R. C. Warner and I. Weber, *J. Amer. Chem. Soc.*, **75**, 5094 (1953).

(4) K. W. Kohn, *Nature*, **191**, 1156 (1961).

(5) J. T. Doluisio and A. N. Martin, *J. Med. Pharm. Chem.*, **6**, 16 (1963).

(6) B. Stearns, K. Losee, and J. Bernstein, *ibid.*, **6**, (2), 201 (1963).

(7) Private communication, Cancer Chemotherapy National Service Center, Bethesda, Md.

(8) W. Fishbein and P. Carbone, *Science*, **142**, 1069 (1963).

(9) A. Bendich, E. Borenfreund, G. Korngold, and M. Krim, *J. Nat. Cancer Inst.*, **32**, 667 (1964).