Circular Dichroism and Carbon-13 Nuclear Magnetic Resonance Spectra of (S)-5-Alkyl-5-(2'-pentyl)-2-thiobarbituric Acids

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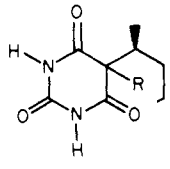
The circular dichroism (CD) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra of (S)-5-(2'-pentyl)-2-thiobarbituric acid and several (S)-5-alkyl-5-(2'-pentyl)-2-thiobarbituric acids were obtained. The results were compared to CD and ^{13}C NMR spectral data obtained from (S)-5-(2'-pentyl)barbituric acid and (S)-5-alkyl-5-(2'-pentyl)barbituric acids.

It has been generally accepted that the duration of action of barbiturates and thiobarbiturates is determined solely by the degree of ionization, lipid solubility (partition coefficient), and protein binding. However, the large differences in the duration of action of optical isomers of some barbiturates and thiobarbiturates in animals[1] and humans[2] show that other factors must also be involved. Since the molecular basis of the mode of action of barbiturates is still not clearly understood, it is difficult to explain the difference in activity of the stereochemical isomers. It has been speculated that the difference in activity results from either different rates of biotransformation or differences in stereochemical fit at the site of absorption into the central nervous system or at the intracellular receptor.[3] Regardless of what the difference is due to, it is clear that the absolute stereochemical and conformational features of both the barbituric or thiobarbituric acid ring and/or the C-5 substituents are of potential importance for elucidating the mechanism of pharmacological action. In order to gain structural information, we have investigated the chiroptical and spectral properties of these compounds. In an earlier study we reported[4] the circular dichroism (CD) and ^{13}C NMR properties of (S)-5-(2'-pentyl)barbituric acid (1, R = H)[5] and several (S)-5-alkyl-5-(2'-pentyl)barbituric acids (1, R = alkyl).[5] In this paper we present studies on some 5-alkyl-5-(2'-pentyl)-2-thiobarbituric acids (2)[5] and compare the results to those obtained from 1.



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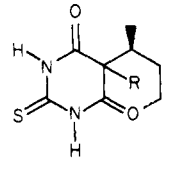

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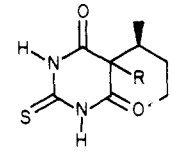
Results

The uv and CD curves of 5-ethyl-5-(2'-pentyl)-2-thiobarbituric acid (2, R = C_2H_5), 5-allyl-5-(2'-pentyl)-2-thiobarbituric acid (2, R = C_3H_5), and 5-(2'-pentyl)-2-thiobarbituric acid (2, R = H), measured from 220 to 320 nm in methanol–0.1 N HCl (1:1), are presented in Figure 1. The uv spectra of all three compounds are quite similar, each showing two λ_{max} at ~288 and ~238 nm with the longer wavelength maximum having approximately 2–3 times the molecular extinction coefficient as the lower wavelength maximum. These data are consistent with a common skeletal structure for all three derivatives in the acidic aqueous methanol solution.

The CD spectra of both 2 (R = C_2H_5) and 2 (R = C_3H_5) show a negative CE at 289 nm and two positive CE's at 266 and 234–235 nm, respectively (Table I). All three CE's were of similar magnitude. The unsubstituted analog 2 (R = H) showed a positive CE at 288 nm and three much smaller CE's at 256, 250, and 242 nm, respectively. In the case of the (S)-5-alkyl-5-(2'-pentyl)barbituric acids we established that the long wavelength CE was due to an $n-\pi^*$ transition.[4] Due to an unfavorable elipticity to absorption ratio in the case of the (S)-5-alkyl-5-(2'-pentyl)-2-thiobarbituric acids, we were unable to conduct the CD solvent studies needed to establish the transition responsible for this CE. However, we did study the effect of solvent on the uv spectra. Table II lists the uv maxima of 2 (R = C_2H_5), 2 (R = C_3H_5), and 2 (R = H) in various solvents. The longest wavelength maximum (~289 mn) is shifted to lower wavelength on going from polar to less

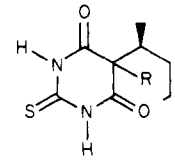
Table I. Observed Maxima and Molar Ellipticities of (S)-5-Alkyl-5-(2'-pentyl)-2-thiobarbituric Acids in 50% v/v Methanol–0.1 N HCl



R	λ, nm	$[\theta] \times 10^{-3}$	λ, nm	$[\theta] \times 10^{-3}$	λ, nm	$[\theta] \times 10^{-3}$
H[a]	288	+3.3				
C_2H_5	289	−4.4	266	+3.5	234	+6.1
C_3H_5	289	−3.2	266	+2.5	236	+4.0

[a] This compound also shows the following very weak CE's: $[\theta]_{256}$ +746, $[\theta]_{250}$ +658, and $[\theta]_{242}$ +439.

Table II. Observed Maxima and Molar Extinction Coefficient of (S)-5-Alkyl-5-(2'-pentyl)-2-thiobarbituric Acids



Solvent	R	λ, nm	$[\epsilon] \times 10^{-3}$
Acetonitrile	H	284	20.3
Dichloroethane	H	286	21.2
Dioxane	H	287	22.4
50% v/v methanol–0.1 N HCl	H	287.5	27.4
Acetonitrile	C_2H_5	284	26.0
Dichloroethane	C_2H_5	284	23.9
Dioxane	C_2H_5	287	23.4
50% v/v methanol–0.1 N HCl	C_2H_5	288	21.2
Acetonitrile	C_3H_5	285.5	27.8
Dichloroethane	C_3H_5	284	23.1
Dioxane	C_3H_5	288	24.8
50% v/v methanol–0.1 N HCl	C_3H_5	287	24.6

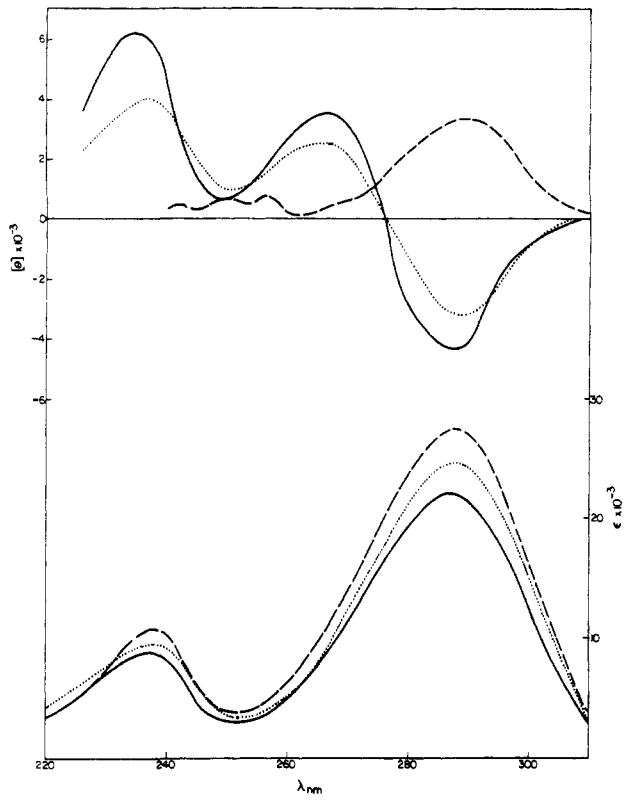


Figure 1. CD (upper curves) and uv (lower curves) spectra of 2, R = C_2H_5 (———); 2, R = C_3H_5 (· · · · ·); and 2, R = H (- - - - -) in 50% v/v methanol–0.1 N hydrochloric acid.

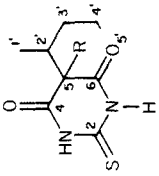
polar solvents. These studies suggest that this band is due to a $\pi-\pi^*$ transition. Since the λ_{max} of this band is identical with the λ_{max} of the CE observed in the CD spectra, this CE might also be due to a $\pi-\pi^*$ transition. However, it is also possible that the long wavelength CE is due to a weaker transition which is obscured by the larger $\pi-\pi^*$ transition.

The ^{13}C NMR chemical shifts relative to tetramethylsilane for 5-(2'-pentyl)-2-thiobarbituric acid and four 5-alkyl-5-(2'-pentyl)-2-thiobarbituric acids in Me_2SO-d_6 are listed in Table III. The chemical shift assignments were made by direct comparison to the 2-oxo analogues[6] and by single-frequency, off-resonance decoupling experiments. The chemical shift of the carbons of the 5-(2'-pentyl) side chain and the 5-alkyl group of **2** is essentially identical with those found in the corresponding 2-oxo analogue **1**. However, as expected, the chemical shift of the barbituric acid rings is different in the two cases. The C-2 thiocarbonyl appears at quite lower field than the C-2 of the 2-oxo analogue. The C-4 and C-6 carbonyls appear at slightly higher field and the C-5 at slightly lower field in the thio analogues.

Discussion

Recently we reported[4] that the circular dichroism (CD) curves of (*S*)-5-(2'-pentyl)barbituric acid (1, R = H)[5] and several (*S*)-5-alkyl-5-(2'-pentyl)barbituric acids (1, R = alkyl)[5] show three Cotton effects (CE's) in the 200–250-nm range. In the case of 1 (R = alkyl), the two longer wavelength CE's (~260 and ~250 nm) were negative, and the shorter wavelength CE (~212 nm) was positive. In contrast, the longer wavelength CE's were positive and the shorter wavelength CE negative with 1 (R = H). In a separate report[6] we presented the ^{13}C NMR chemical shifts of some 5-alkyl-5-(2'-pentyl)barbituric acids (1) in dimethyl sulfoxide-d_6 solution (Me_2SO-d_6). The spectra of 1 showed that the compounds existed exclusively in the triketo form

Table III. Carbon-13 Chemical Shifts (in ppm) of 5-Alkyl-5-(2'-pentyl)-2-thiobarbituric Acids[a]



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Chemical shifts, ppm[b,c]

R	^{1}'CH_3,^{2}'CH_2,^{3}'CH_2,^{4}'CH_2,^{5}'CH_3					R				Ring	Carbonyl[d]			
	C-1' (q)	C-2' (d)	C-3' (t)	C-4' (t)	C-5' (q)	C-1*[e]	C-2*[f]	C-3*	C-4*	C-5*	C-5 (s)	C-2 (s)	C-4 (s)	C-6 (s)	$\delta_{C-4} - \delta_{C-6}$
H[g]	16.69	36.54	35.70[h]	20.05	14.01[h]						52.92[i]	179.99	168.97	168.19	0.78
	(18.54)	(28.10)		(20.78)							(98.85)	(172.68)	(159.51)	(159.51)	
CH_3	14.06[j]	42.05	33.07	20.34	14.06[j]	17.08					54.53	179.26	171.41	171.27	0.14
^{2}*CH_3'*CH_2[k]	14.16	41.90	33.46	20.20	13.91	27.56	9.52				59.94	178.82	171.07	170.73	0.34
^{5}*CH_3'^{4}*CH_2'^{3}*CH_2'^{2}*CH_2'*CH_2	14.12	42.19	33.47	20.24	13.78	34.49	21.76	31.32	24.51	13.92	59.19	178.83	171.14	170.79	0.35
^{1}*CH_2=^{2}*CH'*CH_2	14.10	41.85	33.36	20.20	13.91	38.42	131.91	120.14			59.06	178.63	170.49	170.14	0.35

[a] Numbering of positions is shown in 2. [b] Shifts are in parts per million relative to tetramethylsilane. [c] Signal multiplicity obtained from single-frequency off-resonance experiments is given in parentheses. [d] The designation C-4 and C-6 is arbitrary. [e] The 5-ethyl, 5-pentyl, and 5-allyl derivatives showed a triplet; the 5-methyl showed a quartet. [f] The 5-ethyl showed a quartet; 5-pentyl showed a triplet; 5-allyl showed a doublet. [g] The values in parentheses are due to the tautomeric forms B and C. [h] This resonance was twice as large as the other resonances and thus has the same value for both tautomer A as well as B and C. [i] This peak is a doublet in SFORS. [j] The terminal methyl of the pentyl group and C-1' methyl appeared as one peak with intensity approximately twice that of the C-5' methyl. [k] A. Fratiello, M. Mardviassian, and E. Chavey, *J. Magn. Reson.*, 12, 221 (1973), reported the ^{13}C NMR spectra of this compound in dioxane, methylene chloride, and water. Our assignments in Me_2SO-d_6 are in agreement with these authors.

Table IV. ^{13}C Chemical Shifts (in ppm) of 5-(2'-Pentyl)-2-thiobarbituric Acid in Several Solvents[a]

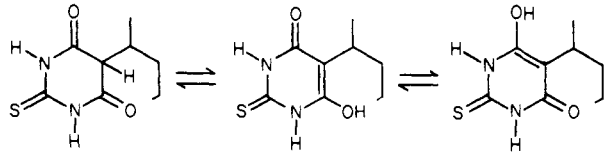
	Chemical shifts, ppm[b,c]									
	$^{1'}CH_3{}^{2'}CH^{3'}CH_2{}^{4'}CH_2{}^{5'}CH_3$					Ring	Carbonyl[d]			
Solvent	C-1' (q)	C-2' (d)	C-3' (t)	C-4' (t)	C-5' (q)	C-5 (d)	C-2 (s)	C-4 (s)	C-6 (s)	$\delta_{C-4} - \delta_{C-6}$
Dioxane	16.67	38.03	36.52	20.82	14.04	53.59	180.03	169.06	168.18	0.88
Acetonitrile-d_3	17.07	38.61	36.76	21.16	14.19	54.17	180.02	169.10	168.32	0.78
Acetone-d_6	16.41	37.50	36.15	20.47	13.59	53.07	179.73	168.56	167.69	0.87
Methanol[e]	17.01	39.00	37.16[f]	21.35	14.18	54.07	181.00	170.42	169.45	0.97
	(18.92)	(29.99)		(22.19)	(14.43)	(100.45)		(162.08)	(162.08)	
Methanol-d_4[e]	17.01	38.96	37.11[f]	21.36	14.18	54.03[g]	181.00	170.42	169.44	0.98
	(18.92)	(29.94)		(22.19)	(14.43)	(100.35)		(161.84)	(161.84)	

[a-d] See corresponding footnotes in Table III. [e] The values in parentheses are due to the tautomeric forms B and C. [f] This resonance is twice as large as the other resonances and thus has the same value for tautomer A as well as B and C. [g] Superimposed on this peak was a triplet, $J_{C-5} = 7.3$ Hz, due to exchange of the C-5 hydrogen for a deuterium in tautomer A.

and that C-4 and C-6 carbonyls were nonequivalent. The degree of nonequivalence of carbonyl carbons 4 and 6 changed as the 5-alkyl group was varied. More importantly, it was pointed out that the chemical shift difference, $\delta_{C-4} - \delta_{C-6}$, of the biologically inactive 5-monoalkyl derivative (R = H) was quite different from the biologically active 5,5-dialkyl derivatives. Our results also showed that the chemical shift, δ_{C-2}, for the carbonyl carbon at the 2 position on the ring of 1 (R = H) was ~1 ppm lower than that of the shifts for the same carbon in the 5-alkyl compounds (1, R = alkyl). Thus, even though the C-2 carbonyl is far removed from the 5-alkyl substituents in 1, the resonance in the case of 1 (R = H) has been shifted downfield relative to 1 (R = alkyl). These ^{13}C NMR results together with our reported CD results indicate a possible difference in steric relationships between the latter compounds and the former. Recent theoretical studies support this hypothesis.[7]

The reversal in sign of the longer wavelength CE in going from the (S)-5-alkyl-5-(2'-pentyl)-2-thiobarbituric acids to the unsubstituted analogue is similar to the results observed with the (S)-5-alkyl-5-(2'-pentyl)barbituric acids (1)[4] and indicates a similar difference in steric relationship between the substituted and unsubstituted 2'-pentyl-2-thiobarbituric acids in methanol–1 N HCl (1:1) solution.

The ^{13}C NMR spectra of 5-alkyl-5-(2'-pentyl)-2-thiobarbituric acids (2, R = alkyl) show that these compounds, like the oxo derivatives 1, exist exclusively in the triketo forms. In contrast, the ^{13}C NMR spectrum of 5-(2'-pentyl)-2-thiobarbituric acid (2, R = H) in Me_2SO-d_6 shows, in addition to the resonances due to tautomer A, several resonances which can be attributed to tautomers B and C. The effect of solvent on this tautomerization is summarized in Table IV. In dioxane, acetonitrile-d_3, and acetone-d_6, 2 (R = H) exists exclusively in form A. In methanol and methanol-d_4 resonances due to both tautomer A and tautomers B and C are present. In methanol-d_4 the C-5 hydrogen of 2 (R = H) is partially exchanged by deuterium.



A B C

A comparison of the difference $\delta_{C-4} - \delta_{C-6}$ of 2 to those of 1 shows that the same general trends exist in both series. That is, with 1 (R = H) the difference $\delta_{C-4} - \delta_{C-6}$ is larger, and the chemical shift of the C-2 thiocarbonyl is downfield relative to the 5,5-dialkyl derivatives (1, R = alkyl).

Biologically inactive 5-monosubstituted barbituric and 2-thiobarbituric acids possess larger pK_a values and usually lower lipid solubility relative to the biologically active, 5,5-dialkylbarbituric and 2-thiobarbituric acids.[8] In addition, our CD and ^{13}C NMR studies show that for barbiturates and 2-thiobarbiturates which possess a 5-(2'-pentyl) substituent, the steric relationship between the barbituric acid and probably the 2-thiobarbituric acid ring and the C-5 substituent(s) is different in the 5-(2'-pentyl)-monosubstituted and 5-alkyl-5-(2'-pentyl)-disubstituted cases.

Experimental Section

General. Melting points were determined on a Kofler hot-stage microscope using a calibrated thermometer. Ir spectra were measured with a Perkin-Elmer 221 spectrophotometer. All observed rotations at the sodium D line were determined with a Perkin-Elmer Model 141 polarimeter (1-dm cell). Mass spectra were determined on an AE1-MS 902 spectrometer. Microanalyses were carried out by Micro-Tech Laboratories, Skokie, Ill.

The ir, uv, NMR, and mass spectra of all the (S)-5-alkyl-5-(2'-pentyl)-2-thiobarbituric acids were in agreement with the assigned structures. The purity of the compounds was checked by GLC analysis using a Varian Aerograph Model 2100-2 gas chromatograph equipped with a flame ionization detector. Glass columns (6 ft × 1/8 in.) packed with 3.8% SE-30 or 3.8% OV-17 on 100–120 mesh Chromosorb W (AWS) were used.

Solvents. Distilled water, spectroquality dioxane, dichloroethane, and acetonitrile from Matheson Coleman and Bell and spectrograde methanol from Fisher Scientific Co. were used without further purification. The deuterated NMR solvents were obtained from Stohler Isotope Chemicals.

Circular dichroism measurements were made at ambient temperatures (~25°) with a Durrum-Jasco Model-20 ORD–CD spectropolarimeter calibrated with d-10-camphorsulfonic acid (0.313° ellipticity for a 1 mg/ml solution in water using a 1.0-cm cell at 290.5 nm). The cell compartment was continually purged with dry purified nitrogen. Time constants of 16 and a low scanning speed (~100 nm/h) were used. Measurements were made at a path length of 0.01 cm and for a concentration of 2.0 mg/ml. Special precautions were taken to assure that the CD bands in regions of strong absorption and weak ellipticity were real.

Several CD spectra were recorded at a sensitivity setting of 1.0 mdeg/cm for every compound studied. The CD spectra are expressed in terms of molar ellipticity $[\theta]$ in deg l./(mol cm), defined by

$$[\theta] = \psi M/10lc$$

where ψ is the measured ellipticity in degrees, l is the path length in centimeters, c is the concentration in grams per milliliter, and M is the molecular weight. The results obtained are summarized in Figure 1 and Table I.

Ultraviolet absorption spectra were obtained on a Cary Model 14 spectrophotometer using silica cells of 0.1- and 1.0-cm path length.

The ^{13}C NMR spectra were determined at 25.03 MHz on a

modified JEOL JNM-PS-100 FT-NMR interfaced with a Nicolet 1085 Fourier transform computer system. Spectra were obtained in either 5- or 10-mm tubes. The spectra were recorded at ambient temperature by using the deuterium resonance of the solvent as the internal lock signal.[9] All proton lines were decoupled by a broad band (~2500 Hz) irradiation from an incoherent 99.538-MHz source. Interferograms were stored in 8K of computer memory (4K output data points in the transformed phase corrected real spectrum), and chemical shifts were measured on 5000-Hz sweep-width spectra. Typical pulse widths were 12.5 μs (45° flip angle), and the delay time between pulses was fixed at 1.0 sec. Normally 1012 (twice as many for single-frequency off-resonance experiments) data accumulations were obtained on a 100 mg/2 ml of solvent sample. The chemical shifts reported are believed accurate to within ±0.05 ppm.

5-Alkyl-5-(2'-pentyl)-2-thiobarbituric Acids (2).[5] The title compounds were prepared by a procedure previously reported for similar 5-alkyl-5-(2'-pentyl)-2-thiobarbituric acids.[10] The following new compounds were prepared. 5-Methyl-5-(2'-pentyl)-2-thiobarbituric acid (2, R = CH_3) was recrystallized from EtOAc–hexane and had mp 85–86°. Anal. ($C_{10}H_{16}N_2O_2S$) C, H, N, S. 5-*n*-Pentyl-5-(2'-pentyl)-2-thiobarbituric acid (2, R = C_5H_{11}) was recrystallized from EtOAc–hexane and had mp 97–100°. Anal. ($C_{14}H_{24}N_2O_2S$) C, H, N, S. (*S*)-5-(2'-Pentyl)-2-thiobarbituric acid (2, R = H) was recrystallized from EtOH–H_2O and had mp 140–141°: $[\alpha]^{24}D$ +2.45° (*c* 0.204, CH_3OH). Anal. ($C_9H_{14}N_2O_2S$) C, H, N, S.

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References and Notes

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Syntheses of Heterocyclic Fused Thiazole Acetic Acids. 2

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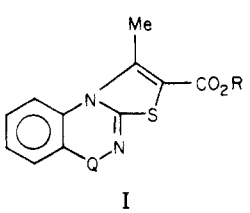
A number of tricyclic and bicyclic fused thiazole-2-acetic acid derivatives were prepared and the chemistry and biological properties of these compounds are discussed. Many of the esters exhibited antitubercular activity. The bicyclic thiazole-2-acetic acids had antidepressant activity. Interesting antimetastatic activity against Lewis lung tumor in mice was found with several compounds, in particular, the thiazolo[3,2-*a*]benzimidazole-2-acetic acid derivative XI.

We recently described the syntheses of fused heterocyclic thiazole carboxylic acids I as potential pharmacological agents.[1] The reports of thiazole acetic acid derivatives II (Myalex)[2] and III[3] as potential antiinflammatory agents prompted us to expand our series of compounds from fused heterocyclic thiazolocarboxylic acid to acetic acid derivatives such as IV and V.



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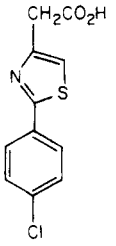

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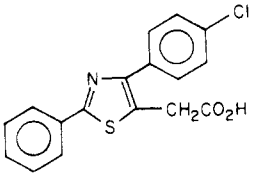

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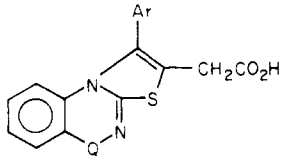
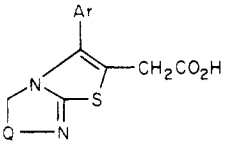


IV



V

In order to prepare compounds of type IV and V, appropriate 2-mercapto heterocycles were allowed to react with 3-aroyl-3-bromopropionic acids and esters. The nature of the products was quite dependent on the reaction conditions and the type of intermediates employed. In all cases the products were identified by spectral data, particularly infrared, as well as analytical data. The compounds were screened in a variety of tests for biological activities, and these results are discussed.

Chemistry. When, for example, 2-mercaptobenzimidazole VI was allowed to react with ethyl 3-bromo-3-*p*-chlorobenzoylpropionate VII in boiling methanol (Scheme I, procedure A) the resultant product was sulfide VIII, isolated as the HBr salt. Upon stirring compound VIII in water or dilute $NaHCO_3$ (procedure B), ring closure took place to form the fused hydroxythiazolo[2,3-*a*]-benzimidazoleacetic acid ethyl ester IX.

When the starting 3-bromo acid X, rather than its ester VII, was allowed to react with VI in a solvent such as hot dimethoxyethane or acetic acid, the fused hydroxythiazoloacetic acid XI HBr (procedure C) was obtained directly in one step. Neutralization of this salt formed the free acid XI. Compound XI was also prepared from IX by alkaline hydrolysis of the ester group (procedure C-1).

Treatment of XI with aqueous hydrochloric acid resulted in dehydration (procedure D) to the thiazolo-[3,2-*a*]benzimidazoleacetic acid XII. The reaction of XI with acetic anhydride produced a rearranged thiazinone XIII, apparently arising from ring opening of the thiazole ring of XI followed by ring closure of the carboxy group onto the nitrogen.[4]

The infrared absorption spectra (KBr) of IX and XI showed one carbonyl peak (carboxy group) at 5.78 and 5.88 μ, respectively, indicating that the compounds exist in the