Metal-Binding Abilities of Antibacterial Heterocyclic Thiones

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Abstract □ Metal-binding stability constants for a series of imidazole, pyrimidine, and related heterocyclic thiones, and a few nonsulfur-containing heterocycles, with Cu (II), Al (III), and Fe (III) ions were determined. Relatively high avidities for the metal ions were found. The magnitudes of the constants, in comparison with those of both cyclic and open-chain compounds of known chelating ability, indicated that four-membered chelate rings involving nitrogen and sulfur atoms were formed. Compounds having the higher metal-binding strengths showed some antimicrobial activity.

Keyphrases □ Thiones, heterocyclic—metal-binding constants, correlated to antibacterial activity □ Heterocyclic thiones—metal-binding constants, correlated to antibacterial activity □ Metal binding to cupric, aluminum, and ferric ions—heterocyclic thiones, binding constants □ Antibacterial activity—correlated to metal-binding ability, heterocyclic thiones □ Structure–activity relationships—heterocyclic thione metal-binding ability, correlated to antibacterial activity

Some relatively simple heterocyclic sulfur compounds have been reported to possess bacteriostatic or bactericidal activity (1, 2). For these compounds (*e.g.*, 2-mercaptoimidazole, 2-mercaptobenzothiazole, and 2-mercaptouracil) no apparent explanation for this activity exists. One possibility is that these compounds may act as metal-binding agents, since other relatively simple structures, notably 8-hydroxyquinoline and related compounds (3), have shown a correlation between metal-binding ability and antibacterial activity. There is no indication in the literature that such compounds have appreciable metal-binding strengths. It was, therefore, of interest to measure metal-binding stability constants for these and some related compounds and to compare these values with their antibacterial activities. It was also desirable to learn the magnitudes of any metal-binding abilities of such sulfur-containing heterocycles which are capable of nitrogen-to-sulfur enolization.

Table I—Ionization Constants (25°)

	Compound	pKa_1	pKa_2
1	2-Mercaptoimidazole	10.79	—
2	1-Methyl-2-mercaptoimidazole	11.22	—
3	2-Mercaptobenzimidazole	9.80	2.60
4	2-Mercaptobenzothiazole	7.83	3.08
5	2-Mercaptouracil	7.73	>2.30
6	6-Methyl-2-mercaptouracil	8.15	2.60
7	Ethyl 2-mercapto-4-hydroxy-pyrimidine-5-carboxylate	10.44	6.70
8	2-Mercapto-4-hydroxypyrimidine-5-carboxylic acid	8.33	3.90
9	2-Methyl-3-hydroxy-4-pyrone	8.53	—
10	1,2,4-Dithiazolidine-5-imino-3-thione	9.10	>2.40
11	3-Acetyltetramic acid	10.50	2.90
12	3-Acetyl-5-methyltetramic acid	10.36	3.72
13	2-Cyano-3,3-dimercaptoacrylonitrile dipotassium salt, monohydrate	10.81	4.31
14	α-Mercaptocinnamic acid	10.22	4.72
15	Salicylic acid[a]	13.82	3.00

[a] W. O. Foye and J. Pecci, *J. Amer. Pharm. Ass., Sci. Ed.*, **49**, 411 (1960).

METHODS

Materials—Analytical reagent grade $CuCl_2 \cdot 2H_2O$, $AlCl_3 \cdot 6H_2O$, and $Fe(NO_3)_3 \cdot 9H_2O$ were used. Carbonate-free 0.01 *M* potassium hydroxide was prepared according to Armstrong (4). Solutions were made in boiled distilled water and stored in polyethylene bottles under nitrogen; they were diluted quantitatively with carbon dioxide-free water just prior to use. Normalities were checked against potassium biphthalate.

The organic compounds were obtained from a commercial source[1], with the exception of the following which were synthesized.

Ethyl 2-Mercapto-4-hydroxypyrimidine-5-carboxylate—The procedure of Ballard and Johnson (5) was used. The product was recrystallized from water and dried at 130° for 12 hr., giving an 82% yield, m.p. 245–248° [lit. (5) m.p. 245°].

2-Mercapto-4-hydroxypyrimidine-5-carboxylic Acid—The procedure of McOmie and White (6) was used. The product was twice recrystallized (water), m.p. 292–295° [lit. (7) m.p. 288–289°].

3-Acetyltetramic Acid—The method of Lacey (8) was employed, giving a 54% yield after repeated extractions with ether and recrystallization from ethyl acetate, m.p. 154–155° [lit. (8) m.p. 155°].

3-Acetyl-5-methyltetramic Acid—The method of Lacey (8) was employed, giving a 46% yield after recrystallization from ethyl acetate–petroleum ether, m.p. 115–116° [lit. (8) m.p. 115–116°].

2-Cyano-3,3-dimercaptoacrylonitrile, Dipotassium Salt, Monohydrate—The procedure of Brown (9) was used, giving an 83% yield, m.p. 313–316° dec. [lit. (10) m.p. 313–316° dec.].

α-Mercaptocinnamic Acid—The procedure of Campaigne and Cline (11) was employed, giving an 87% yield after recrystallization from toluene, m.p. 130–134° [lit. (11) m.p. 133–134°].

The purity of the organic ligands was ascertained by TLC. Chromatogram sheets[2] were spotted with approximately 0.1% solutions of the compounds in either water or ethanol. The developing solvent contained either benzene–methanol (8:2) (Compounds 1–10) or 2-butanone–acetone-formic acid–water (40:2:1:6) (Compounds 11–14). After the plates were removed from the chamber and dried, they were placed in an iodine chamber or sprayed with a solution of bromphenol blue in ethanol (0.06%) containing 1 drop of 10% sodium hydroxide solution. The presence of one spot confirmed the absence of contaminating compounds.

Ionization Constants—The method employed was that of Albert and Serjeant (12), using a research pH meter[3] with glass and calomel electrodes. It consisted of titrations of 0.001 *M* aqueous solutions of the compounds with 0.01 *N* potassium hydroxide in 0.5-ml. portions. The pH was recorded after each addition; each titration thus yielded nine pH values, giving nine values for the pKa which were averaged. Where pH values fell outside the range of 5–9, corrections were made for hydrogen- or hydroxyl-ion concentrations. The pKa values obtained are listed in Table I.

Stability Constants—Potentiometric titrations were carried out under nitrogen in freshly boiled distilled water at 25° using the described equipment. Fifty-milliliter volumes of the 0.001 *M* solutions of the organic ligands were titrated with 0.01 *N* potassium hydroxide solution in 0.5-ml. portions, first in the absence of metal ions and then in the presence of 0.0005 mole of divalent metal salt or 0.00033 mole of trivalent metal salt. Fifty-milliliter volumes of the

[1] Aldrich Chemical Co.
[2] Eastman.
[3] Beckman.

Table II—Stability Constants of Cupric Complexes (25°)

Compound	log K_1	log K_2	log β_2[a]	log β_2[b]
2-Mercaptoimidazole	10.29	9.31	19.60	19.20
1-Methyl-2-mercaptoimidazole	10.71	9.59	20.30	20.26
2-Mercaptobenzimidazole	10.42	9.57	19.99	19.10
2-Mercaptouracil	8.01	6.76	14.77	14.30
6-Methyl-2-mercaptouracil	7.89	6.24	14.13	14.20
Ethyl 2-mercapto-4-hydroxy-pyrimidine-5-carboxylate	7.69	7.22	14.91	12.60
2-Mercapto-4-hydroxypyrimidine-5-carboxylic acid	8.22	5.26	13.48	11.40
2-Methyl-3-hydroxy-4-pyrone	8.11	6.18	14.29	14.32
3-Acetyltetramic acid	10.34	—	—	—
3-Acetyl-5-methyltetramic acid	8.90	5.49	14.39	—
α-Mercaptocinnamic acid	12.32	7.45	19.77	—
Salicylic acid[c]	10.40	7.90	18.30	—

[a] Determined from the sum of the log K values. [b] Determined from: $\log \beta_2 = -2 \log(L^-)$, when $\bar{n} = 1$. [c] J. Volke, *Chem. Listy*, **49**, 1236 (1955).

same quantities of the metal salts were also titrated with 0.01 N potassium hydroxide. After each addition of the titrant, the solution was allowed to mix for 2 min. before the pH reading was made. In the cases of 2-mercaptobenzothiazole and α-mercaptocinnamic acid, 0.001 M solutions were prepared in 50% ethanol.

Calculations were done as previously described (13); values for log K_1, log K_2, log K_3, and either log β_2 or log β_3 were recorded. Values for K_1, K_2, and K_3 were obtained from Eqs. 1–3 according to Flood and Loras (14) and Albert (15):

$$K_1 = \frac{\bar{n}}{(1 - \bar{n})(L^-)} \quad \text{(Eq. 1)}$$

$$K_2 = \frac{(\bar{n} - 1)}{(2 - \bar{n})(L^-)} \quad \text{(Eq. 2)}$$

$$K_3 = \frac{(\bar{n} - 2)}{(3 - \bar{n})(L^-)} \quad \text{(Eq. 3)}$$

Values for log β_2 and log β_3 were obtained from the sum of the log K values. Some values of K_2 or K_3 were not obtained because of precipitation of the metal complexes. Some values of K_1, notably in the case of the ferric complexes, were not uncovered by the method used. (Because of high metal-binding avidity, no values for $\bar{n}$ below 1 were obtained.) Formation curves were plotted ($\bar{n}$ *versus* $-\log [L^-]$) to show whether stepwise complexation may have taken place. No evidence that stepwise complexation was not taking place was given by any of the compounds. The stability constants obtained are listed in Tables II–IV.

Table III—Stability Constants of Aluminum Complexes (25°)

Compound	log K_1	log K_2	log K_3	log β_3[a]
2-Mercaptoimidazole	10.07	9.71	8.98	28.76
1-Methyl-2-mercaptoimidazole	10.42	10.07	9.26	29.75
2-Mercaptobenzimidazole	9.20	8.70	8.06	25.96
2-Mercaptobenzothiazole	7.44	6.90	6.69	21.03
2-Mercaptouracil	7.18	6.70	5.90	19.78
6-Methyl-2-mercaptouracil	7.43	6.98	6.26	20.67
Ethyl 2-mercapto-4-hydroxy-pyrimidine-5-carboxylate	5.80	5.55	5.26	16.61
2-Mercapto-4-hydroxypyrimidine-5-carboxylic acid	8.70	7.00	6.29	21.99
2-Methyl-3-hydroxy-4-pyrone	—	7.95	6.53	—
1,2,4-Dithiazolidine-5-imino-3-thione	8.62	8.16	7.17	23.95
3-Acetyltetramic acid	10.88	8.00	7.88	26.76
3-Acetyl-5-methyltetramic acid	9.44	7.57	7.49	24.50
2-Cyano-3,3-dimercaptoacrylo-nitrile, dipotassium salt, monohydrate	10.77	9.61	8.02	28.40
α-Mercaptocinnamic acid	10.13	9.49	7.39	27.01
Salicylic acid[b]	14.00	10.70	8.60	33.30

[a] Determined from the sum of the log K values. [b] J. Volke, *Chem. Listy*, **49**, 1236(1955).

Table IV—Stability Constants of Ferric Complexes (25°)

Compound	log K_1	log K_2	log K_3	log β_3[a]
2-Mercaptoimidazole	—	11.82	9.96	—
1-Methyl-2-mercaptoimidazole	—	12.12	11.18	—
2-Mercaptobenzimidazole	—	9.88	9.77	—
2-Mercaptobenzothiazole	—	9.60	7.94	—
2-Mercaptouracil	—	8.95	8.25	—
6-Methyl-2-mercaptouracil	—	9.20	8.25	—
Ethyl 2-mercapto-4-hydroxy-pyrimidine-5-carboxylate	—	7.14	6.65	—
2-Mercapto-4-hydroxypyrimi-dine-5-carboxylic acid	9.24	8.16	7.42	24.82
2-Methyl-3-hydroxy-4-pyrone	—	9.43	7.95	—
1,2,4-Dithiazolidine-5-imino-3-thione	—	9.82	8.57	—
3-Acetyltetramic acid	11.37	8.70	8.49	28.56
3-Acetyl-5-methyltetramic acid	10.28	9.00	7.03	26.33
α-Mercaptocinnamic acid	10.32	7.61	6.40	24.33
Salicylic acid[b]	14.70	12.50	11.40	38.60

[a] Determined from the sum of the log K values. [b] J. Volke, *Chem. Listy*, **49**, 1236(1955).

Antibacterial and Antifungal Evaluation—Activities were determined by the use of a modified sensitivity disk method. Three bacteria, one mold, and one yeast were employed. For *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, tryptic soy agar BBL was used; for *Aspergillus niger* and *Candida albicans*, Sabouraud's dextrose agar BBL was used.

Aqueous or acetone solutions of the compounds were prepared, and the concentrations tested were 10 mg./0.1 ml. against the fungi and 1 mg./0.1 ml. against the bacteria. Both 0.1 and 0.01 ml. of the solutions were pipeted onto sterile paper disks (12.7 mm. in diameter) and set overnight in sterile plastic petri dishes. The agar preparations were then smeared with a thin layer of the test organism, the paper disks were placed on the surface, and the Sabouraud's dextrose agar plates were incubated at 30° and the tryptic soy agar plates were incubated at 37° for 72 hr. The radii of the zones of inhibition were then measured. Test results are listed in Table V.

RESULTS AND DISCUSSION

The magnitude of the metal-binding stability constants obtained with the heterocyclic thiones was surprisingly high. With the aluminum and ferric complexes, the avidity for these metal ions approached that of salicylic acid, a relatively strong metal-binding agent, particularly in the case of 2-mercaptoimidazole and its derivatives. With the cupric complexes, the 2-mercaptoimidazoles and α-mercaptocinnamic acid all exceeded salicylic acid in metal-binding strength. It should be considered that in the case of the 2-mercaptoimidazoles and the mercaptopyrimidines, if chelation of the metal is

Table V—Antibacterial and Antifungal Activities

	Zone of Inhibition, Radius, mm.				
Organism: ATCC No.:	*A. niger* 6275	*C. albicans* 10231	*S. aureus* 6538	*E. coli* 15221	*P. aeruginosa* 15442
2-Mercaptoimidazole	—	—	—	20	—
1-Methyl-2-mercapto-imidazole	—	—	—	25	—
2-Mercaptobenzothiazole	18	21	13	—	—
2-Mercaptouracil	—	—	—	22	—
1,2,4-Dithiazolidine-5-imino-3-thione	—	15	15	15	—
3-Acetyl-5-methyltetramic acid	—	15	14	—	—
2-Cyano-3,3-dimercapto-acrylonitrile dipotas-sium salt, monohydrate	21	14	—	23	—
α-Mercaptocinnamic acid	14	24	17	—	—
Salicylic acid	—	20	—	—	—

Table VI—Antibacterial and Antifungal Literature References

Compound	Activity	Reference
2-Mercaptoimidazole	Bacteriostatic	1
1-Methyl-2-mercaptoimidazole	Bacteriostatic	1
2-Mercaptobenzothiazole	Bactericidal	2
2-Mercaptouracil	Bacteriostatic	1
2-Methyl-3-hydroxy-4-pyrone	Weak bacteriostatic	18
1,2,4-Dithiazolidine-5-imino-3-thione	Catalase inhibitor	20
3-Acetyltetramic acid	Bactericidal	19
3-Acetyl-5-methyltetramic acid	Bactericidal	19
Salicylic acid	Fungicidal	21

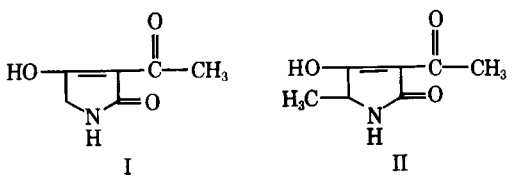
involved, only four-membered rings can be formed; this evidently gives relatively stable complexes where sulfur is one of the atoms of the chelate ring. Four-membered chelate rings of high stability are formed with xanthates, dithiocarbamates, and dithiocarboxylates (16), for example. That chelation is probably involved is indicated by the similarity in metal-binding strengths of the nonsulfur-containing compounds where five- and six-membered chelate rings may be formed. These compounds include the tetramic acid derivatives (I and II), the pyrone, salicylic acid, and most likely the pyrimidine 5-carboxylic acid. In the latter case, bonding to nitrogen and sulfur is also possible.

α-Mercaptocinnamic acid most likely forms a five-membered chelate ring involving the thiol and carboxyl functions. The similarity in binding strengths to metal ions between this compound and the mercaptoimidazoles and the mercaptopyrimidines again argues for chelate structures between nitrogen and sulfur for the latter compounds. Also, the dimercaptoacrylonitrile derivative would be expected to form a chelate ring involving the two sulfurs, and the similarity in metal-binding avidities here suggests chelate ring formation for the mercaptoimidazoles and mercaptopyrimidines. Structure III, therefore, is proposed as an example of a metal complex of the mercaptoimidazoles, mercaptopyrimidines, and other heterocycles observed here where nitrogen–sulfur bonding is possible.

Comparison of the sequences of metal-binding avidities in each of the three series, cupric, aluminum, and ferric, shows the following orders. With cupric ion, the most avid metal-binding compounds in decreasing order of strength in terms of log β_2 are: 1-methyl-2-mercaptoimidazole, 2-mercaptobenzimidazole, α-mercaptocinnamic acid, 2-mercaptoimidazole, salicylic acid, and 3-acetyltetramic acid. Here the strong affinity of cupric ion for sulfur (17) results in higher stability constants than in the case of the nonsulfur chelating systems found with salicylic acid and 3-acetyltetramic acid.

With aluminum ion, the most avid metal-binding compounds in decreasing order of binding strength in terms of log β_3 are: saliylic acid, 1-methyl-2-mercaptoimidazole, 2-mercaptoimidazole, 2-cyano-3,3-dimercaptoacrylonitrile, α-mercaptocinnamic acid, 3-acetyltetramic acid, and 2-mercaptobenzimidazole. Here, several of the nitrogen–sulfur complexing compounds fall between salicylic acid and 3-acetyltetramic acid in metal-binding ability. Since the dimercaptoacrylonitrile derivative and α-mercaptocinnamic acid would be expected to form chelate rings, the mercaptoimidazoles should also form cyclic complexes.

With ferric ion, the sequence in decreasing order of metal-binding avidity was based on the value of log K_2, since many of the log K_1 values were not revealed. The order for the most avid metal-binding compounds is: salicylic acid, 1-methyl-2-mercaptoimidazole, 2-mercaptoimidazole, 2-mercaptobenzimidazole, 1,2,4-dithiazolidine-5-imino-3-thione, 2-mercaptobenzothiazole, 2-methyl-3-hydroxy-4-pyrone, 6-methyl-2-mercaptouracil, and 3-acetyl-5-methyltetramic acid. It appears again from the similarities in binding strengths to the nonsulfur-containing chelating agents that cyclic ferric complexes exist. The fact that α-mercaptocinnamic acid did not fall among the more avid metal-binders in this series is probably due to the smaller size of the ferric ion, which would make a poorer fit in the five-membered chelate ring expected for this compound.



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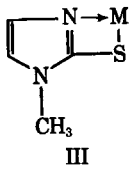
II



III

Some antibacterial or antifungal activity was found for 2-mercaptoimidazole, 1-methyl-2-mercaptoimidazole, 2-mercaptobenzothiazole, and 2-mercaptouracil but not for 2-mercaptobenzimidazole. This agrees with literature reports regarding the activity of these compounds (Table VI). No activity was found for 2-methyl-3-hydroxy-4-pyrone, however, described as weakly bacteriostatic (18). Some activity was found with 3-acetyl-5-methyltetramic acid but not with 3-acetyltetramic acid; both compounds have been reported to be bactericidal (19). In addition, some activity was found for 1,2,4-dithiazolidine-5-imino-3-thione, 2-cyano-3,3-dimercaptoacrylonitrile, and α-mercaptocinnamic acid, which has not been previously reported.

All of these compounds, with the exception of 2-mercaptouracil, have relatively high metal-binding stability constants; in the ferric series, particularly, the compounds with antimicrobial activity constitute the most avid metal-binding compounds. Although α-mercaptocinnamic acid has relatively low stability constants for reaction with ferric ion, it has relatively high constants for cupric and aluminum ions. One notable exception to this correlation between metal-binding stability constants and antimicrobial activity is found with 2-mercaptobenzimidazole; this compound has relatively high constants for all the metal ions measured, particularly for cupric and ferric, but it has shown no antimicrobial activity.

Although the correlation between metal-binding stability constants and antimicrobial activity is not perfect, it does appear that among these relatively simple heterocyclic structures capable of metal binding, those compounds with the greatest avidity for metal ions show antimicrobial effects. Those compounds with lower metal-binding avidities show none of this activity. It appears that the ability to bind metal ions should be considered a possible explanation for the antibacterial or antifungal activity of such structures, in particular for those compounds where nitrogen–sulfur bonding to metal may occur.

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Biopharmaceutical Studies on Guaiacol Glyceryl Ether and Related Compounds V

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Abstract □ Filtration using a crosslinked dextran gel was performed to determine the mechanism by which guaiacol glyceryl ether mononicotinate causes an increase in the solubility of cholesterol. The effects of guaiacol glyceryl ether mononicotinate and its related compounds on the incorporation of radioactive acetate into cholesterol also were investigated to obtain information concerning their hypocholesteremic behavior. Blood levels and urinary metabolites of guaiacol glyceryl ether mononicotinate in man were determined. Although the main purpose of the present study was not to investigate pharmacological effects of guaiacol glyceryl ether mononicotinate, it was noted that this compound displayed some analgesic but no antihistamine or anti-inflammatory activity.

Keyphrases □ Guaiacol glyceryl ether mononicotinate—effect on solubility of cholesterol and incorporation of radioactive acetate, blood levels and urinary metabolites in man □ Hypocholesteremic activity—guaiacol glyceryl ether mononicotinate □ Blood levels—guaiacol glyceryl ether mononicotinate, man □ Urinary metabolites—guaiacol glyceryl ether mononicotinate, man □ Cholesterol, solubility and incorporation of radioactive acetate—effect of guaiacol glyceryl ether mononicotinate

Blood levels of guaiacol glyceryl ether mononicotinate in animals, the effects of it and related compounds on bile in animals, and the hypocholesteremic effect were previously reported[1] (1).

The present study was concerned with guaiacol glyceryl ether mononicotinate; a few of the pharmacological effects, together with the hypocholesteremic mechanism of action of guaiacol glyceryl ether mononicotinate, were investigated. Moreover, blood levels and urinary metabolites of guaiacol glyceryl ether mononicotinate in human test subjects were determined as a preliminary for evaluation of clinical activities of guaiacol glyceryl ether mononicotinate.

EXPERIMENTAL

Effect of Guaiacol Glyceryl Ether Mononicotinate on Squirming and Capillary Permeability—The procedure was performed by the method of Whittle (2) and Naito *et al.* (3). Each group consisted of 10 mice (dd strain, average weight 15 g.).

Tail Withdrawal Reflex in Mice—The method for analgesimetry described in the paper of Ben-Bassat *et al.* (4) was used. Each group consisted of 12 male mice (dd strain, average weight 15 g.).

Anti-Inflammatory Activity—The method for anti-inflammatory testing (involving the use of carrageenin) reported previously (3) was used in this part of the study. Each group consisted of five male rats (Wistar strain, average weight 180 g.).

Antihistamine Activity—Guinea pigs (Hartley strain, average weight 300 g.) were used in the experiment carried out by the procedure of Labelle and Tislow (5). Each group consisted of five male guinea pigs.

Effect of Guaiacol Glyceryl Ether Mononicotinate on Duration of Sleep Induced by Hexobarbital and on Blood Level of Hexobarbital—Each test group consisted of 10 male rats (Wistar strain, average weight 150 g.). Each animal received 100 mg./kg. of hexobarbital intraperitoneally, and guaiacol glyceryl ether mononicotinate was given intraperitoneally 30 min. prior to the hexobarbital injection. The experimental design was the same as that reported by Brodie *et al.* (6).

Column Filtration—The gel[2] washed with distilled water was filled up to a 20-cm. height in a glass tube 15 mm. in diameter. One milliliter of human serum (cholesterol, 530 mg.%) from high cholesterol patients was used as a serum sample. After the addition of a serum sample to the column, 150 ml. of distilled water containing 500 mcg./ml. of either guaiacol glyceryl ether, β-(4-hydroxy-2-methoxyphenoxy)lactic acid, or β-(2-methoxyphenoxy)lactic acid was passed through the column as an eluting solvent and collected in 3-ml. fractions. It was previously ascertained that this volume of the eluant was sufficient to elute the cholesterol. Cholesterol in the eluant was determined by the method of Zurkowski (7).

Incorporation of Acetate into Cholesterol by Rat Liver Slices—Guaiacol glyceryl ether mononicotinate or *meso*-inositol hexanicotinate (inositol niacinate), which is insoluble in water, was passed through a 200-mesh screen to make an homogeneous suspension with water. The other compounds tested were all water soluble.

Male rats (SD strain, average weight 250 g., 7 weeks old) were used for this experiment. After the rats were killed by decapitation, their livers were quickly removed and sliced with a microtome and approximately 500 mg. of slices was placed in 5 ml. of Krebs–Ringer buffer (pH 7.4, a mixture of 41.8 ml. of 0.9% NaCl, 63.8 ml. of 1.15% KCl, 3.0 ml. of 1.22% $CaCl_2$, 1.0 ml. of 2.11% KH_2PO_4, 1.0 ml. of 3.82% $MgSO_4 \cdot 7H_2O$, and 21.0 ml. of 1.30% $NaHCO_3$). The buffer containing sodium acetate-1-^{14}C (2.3 μc./5 μmoles/flask)

[1] Guaiacol glyceryl ether is officially known as glyceryl guaiacolate.

[2] Sephadex G-25 Medium, Pharmacia, Uppsala, Sweden.