The marked decrease in the rate of return of monoamine oxidase activity approximately 48 hr. following inhibition with pargyline and treatment with emetine, may be the result of the partial inhibition of monoamine oxidase synthesis by emetine. According to the mechanism proposed by Grollman (13), protein synthesis is inhibited by emetine at the site of peptide bond formation, a late step in the synthesis sequence. It seemed relevant to use an inhibitor of protein synthesis which acts early in the sequence, in order to possibly predict some properties of the *m*-RNA responsible for the direction of MAO synthesis. Chloramphenicol was used for this purpose, since it is known from the work of Weisberger (14) that chloramphenicol inhibits protein synthesis at the site of the attachment of the *m*-RNA to the ribosomes. The results of this study showed a complete inhibition of monoamine oxidase synthesis after 48 hr. There was a decrease in the rate of MAO synthesis approximately 48 hr. following teatment with either emetine or chloramphenicol suggesting a constant lag time for the inhibition of protein synthesis in the rat liver. Currently, further studies on the synthesis of MAO and other mitochondrial proteins are in progress.

REFERENCES

(1) G. Arnaiz and E. Robertis, *J. Neurochem.*, **9,** 503(1962).

(2) C. Schnaitman, V. G. Erwin, and J. W. Greenawalt, *J. Cellular Biol.*, **32,** 719(1967).

(3) S. H. Barondes, *J. Neurochem.*, **13,** 721(1966).

(4) M. Chessin, B. Dubnick, G. Lieson, and C. C. Scott, *Ann. N.Y. Acad. Sci.*, **80,** 597(1959).

(5) L. Hellerman and V. G. Erwin, *J. Biol. Chem.*, **243,** 5234 (1968).

(6) D. S. Beattie, R. E. Basford, and S. B. Koritz, *ibid.*, **242,** 4584(1967).

(7) W. C. Schneider and G. H. Hogeboom, *ibid.*, **123,** 8(1950).

(8) C. W. Tabor, H. Tabor, and S. M. Rosenthal, *ibid.*, **208,** 645(1954).

(9) V. G. Erwin and L. Hellerman, *ibid.*, **242,** 4230(1967).

(10) H. Weissbach, W. King, A. Sjoersdm, and S. Udenfriend, *ibid.*, **234,** 81(1959).

(11) V. G. Erwin and R. D. Dietrich, *ibid.*, **241,** 3533(1966).

(12) R. D. Dietrich, L. Hellerman, and J. Wein, *ibid.*, **237,** 560(1962).

(13) A. P. Grollman, *Proc. Nat. Acad. Sci.*, **56,** 1867(1966).

(14) A. S. Weisberger and S. Wolfe, *Federation Proc.*, **23,** 976 (1964).

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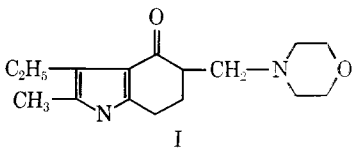
Synthesis of 2-Ethyl-3-methyl-5-morpholinomethyl-4-keto-4,5,6,7-tetrahydrothionaphthene

J. SAM and J. R. MOZINGO, JR.*

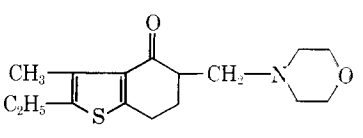
Abstract □ The title compound was prepared and evaluated for central nervous system, cardiovascular, autonomic, endocrine, anti-inflammatory, antiallergic, and metabolic activities. No significant activity was noted.

Keyphrases □ 2-Ethyl-3-methyl-5-morpholinomethyl-4-keto-4,5,6,7-tetrahydrothionaphthene—synthesis □ GLC—analysis □ IR spectrophotometry—structure □ NMR spectroscopy—structure

Previously reported work (1) indicated that the Mannich bases of 4-keto-4,5,6,7-tetrahydrothionaphthene possessed some degree of biological activity. The marked activity (2) of molindone (I) led to the synthesis of the closely related bicyclic thiophene (II).



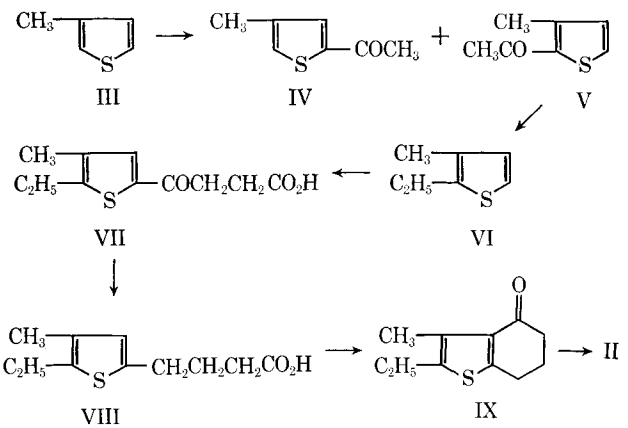
I



II

The preparation of IX was carried out according to procedures previously described in the literature (3). The separation of IV and V was accomplished on a 91.44-cm. (36-in.) stainless steel spinning band column and the purity of the fractions determined *via* GLC. Yields of IV–IX were comparable to those described in the literature.

Compound II was screened for CNS, cardiovascular, autonomic, endocrine, anti-inflammatory, antiallergic, and metabolic activities, however, no significant activity was noted.



III IV V VII VI VIII IX II

EXPERIMENTAL[1]

2-Ethyl-3-methyl-5-morpholinomethyl-4-keto-4,5,6,7-tetrahydrothionaphthene Hydrochloride (II)—A mixture of 3.0 g. (0.0155 mole) of 2-ethyl-3-methyl-4-keto-4,5,6,7-tetrahydrothionaphthene (4), 0.47 g. (0.0155 mole) of paraformaldehyde, 1.92 g. (0.0155 mole) of morpholine hydrochloride, 1 drop of concentrated hydrochloric acid, and 20 ml. of ethanol was refluxed for 4 hr. A solid precipitated upon cooling and was removed by filtration. After recrystallizing three times from ethanol, 3.1 g. (61%) of product, m.p. 223–223.5° (dec.) remained; $\lambda_{max.}$ (KBr) 2,450 and 1, 660 cm.$^{-1}$; NMR (D_2O), 1.01–1.40 (3H, t), 2.29–2.42 (3H, s), 2.55–2.98 (2H, m), 3.05–3.38 (4H, m), 3.40–3.65 (6H, m), 4.00–4.30 (5H, m).

Anal.—Calcd. for $C_{16}H_{24}ClNO_2S$: C, 58.25; H, 7.33; Cl, 10.78; N, 4.25; S, 9.72. Found: C, 57.96; H, 7.18; Cl, 11.05; N, 4.40; S, 9.78.

[1] All melting points were taken on a Thomas-Hoover melting point apparatus and are corrected. IR spectra were taken on a Perkin-Elmer 137B IR spectrophotometer using potassium bromide pellets. The NMR spectra were determined on a Varian A-60A spectrophotometer using tetramethylsilane as an internal standard. Chemical shifts are recorded as δ values (s = singlet, m = unresolvable multiplet, t = triplet).

REFERENCES

(1) J. Sam and G. G. Advani, *J. Pharm. Sci.*, **54,** 753(1965).

(2) A. A. Suguman and J. Herrmann, *Clin. Pharmacol. Therap.*, **8,** 261(1967).

(3) J. Lamy, D. Lavit, and Ng. Ph. Bui-Hoi, *J. Chem. Soc.*, **1958,** 4202.

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Quantitative Determination of Deoxyribonucleic Acid (DNA) in Normal and Abnormal Human Liver

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Abstract □ Normal and abnormal human livers from autopsy and biopsy specimens were analyzed for DNA content. It was found that the DNA content of young normal livers as well as cirrhotic livers was elevated above that obtained for normal adult livers.

Keyphrases □ Deoxyribonucleic acid (DNA) determination—normal, abnormal human livers □ Age, cirrhosis—liver DNA contents

The normal value of DNA content of human liver as well as animal liver is reported to be relatively constant (1). However, Leslie (2) has shown an elevation in DNA content in young livers of rats and other animals. Therefore, it was of interest to perform the quantitative determination of DNA from normal and abnormal human livers.

The determination of DNA can be based on the analysis of one of the three components; namely, the phosphoric acid, the purine or pyrimidine bases, or the sugar. The reactivity of the functional carbonyl group of the pentose has been utilized as the method of choice (3). Moreover, in cytochemistry the carbonyl group of 2-deoxyribose of DNA has been utilized in the feulgen staining reaction which is widely used in conjunction with microphotometric instrumentation to estimate the amount of DNA in nuclei (4).

EXPERIMENTAL

Human liver specimens were obtained from autopsy as well as biopsy from the State University of Iowa General Hospital and Veterans Administration Hospital of Iowa City, Iowa.

Liver specimens were stored in the freezer as soon as they were removed from the cadavers and from the patients, and the specimens preserved in cold showed reproducible results. In general procedure, DNA of the whole tissue is extracted with trichloroacetic acid (3) but in this study to isolate mitochondria, liver tissue was first homogenized in 0.88 *M* sucrose solution with a Teflon tissue homogenizer (5). The nuclei of the liver cells were separated by centrifuging the liver homogenate at 600 × *g* for 20 min. at 4°. The nuclear pellet was hydrolyzed in 20 ml. 5% trichloroacetic acid on a boiling-water bath for 30 min. using a sealed ampul bulb as a condensor. The hydrolyzed nucleic acid solution was cooled and filtered into a 25-ml. volumetric flask. It was diluted to volume with 5% trichloroacetic acid. A 2-ml. aliquot of this solution containing about 150 mcg. DNA was assayed by the method of Webb and Levy (3), which is a colorimetric procedure based on the reaction of 2-deoxypentose moiety of DNA with *p*-nitrophenylhydrazine. The concentration of DNA was determined by using as a reference standard calf thymus DNA (Mann Research Co.). The results of the analysis are shown in Table I and Table II. Statistical evaluation was by the Student *t* test.

RESULTS AND DISCUSSION

The data for 47 liver specimens are shown in Tables I and II. Table I shows the data from the analysis of young normal livers and adult normal livers. The data from the cirrhotic abnormal livers and noncirrhotic abnormal livers, regardless of age factor are shown in Table II. In reporting DNA content neither the sex (male or female) nor liver specimen (autopsy or biopsy) has been taken into consideration. Since any normal liver specimens between 20–30 years of age were not available, it was decided to use this period of age as a dividing line between young and adult normal human liver. It is very difficult to state that a given specimen of biological material is normal. However, by common agreement, the histological appearance of biological material is used to establish normal and abnormal categories. In this study, the patients were divided into these two major groups on the basis of the histological appearance of the liver specimens. Those cases in which liver cells were either