ferior to the secondary substituted thiobarbiturates previously reported by us.[5] Given orally they are relatively ineffective in animals and in man.

In general, most satisfactory compounds are secured where one group attached to the 5-carbon is secondary, the other is methyl, and the N-substituent also methyl. The attachment of large primary or of secondary and tertiary groups on the nitrogen leads to compounds of low hypnotic power. There is some indication that the N-alkyl acts additively in the production of the convulsions characteristic of barbiturates containing certain 6- and 7-carbon atom groups (benzyl, etc.). For instance, methyl-(1,3-dimethylbutyl)-barbituric acid could be given in a dosage of 170 mg./kg. without producing convulsions while both its N-methyl derivative and ethyl (1,3-dimethylbutyl)-barbituric acid produced convulsions in the range of 7 to 15 mg.

Conclusion

A series of N-alkyl and N-aryl barbiturates containing secondary and tertiary groups has been prepared. Pharmacologically, certain members offer some promise as short acting intravenous hypnotics and anesthetics.

NORTH CHICAGO, ILL. RECEIVED MAY 8, 1936

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

The Effect of the Composition of the Medium upon the Growth of Yeast in the Presence of Bios Preparations. I. The Effect of Magnesium Salts[1]

BY ELLIS I. FULMER, L. A. UNDERKOFLER AND JAMES B. LESH

Schopmeyer and Fulmer[2] found that the growth of certain molds on synthetic media, with glycerol or sucrose as substrates, formed materials which accelerated the growth of yeast. Recently, in the study of the growth accelerants produced by *Aspergillus niger* upon sucrose media, attempts were made to separate the active substances into the Bios I and Bios II of Miller and co-workers[3-5] who identified Bios I as *i*-inositol.

The properties of the fractions obtained were determined by their effect upon the growth of yeast in Medium C developed by Fulmer, Nelson and Sherwood.[6] When the activity of the Bios II fraction in Medium C was compared with its activity in Clark's medium, which was used by Miller, it was found that the same amount of stimulant gave a much larger growth in the latter medium. Medium C contains, per 100 cc.: 0.188 g. ammonium chloride, 0.100 g. dipotassium phosphate, and 10 g. of sucrose. Clark's medium contains, per 100 cc.: 0.834 g. of ammonium nitrate, 0.417 g. of potassium dihydrogen phosphate, 0.071 g. of calcium chloride, 0.208 g. of magnesium sulfate and 10 g. of sucrose.

Preliminary results showed that the addition of magnesium sulfate to Medium C had a marked effect upon the growth of yeast in the presence of the bios preparation. The addition of calcium chloride, or the replacement of ammonium chloride by ammonium nitrate had no influence upon the bios activity. The effect of magnesium salts was then further investigated.

The yeast employed was a strain of *Saccharomyces cerevisiae* isolated several years ago from a cake of Fleischmann yeast, and deposited with the American Type Culture Collection as No. 4226. The numbers of cells were determined by means of the Thoma-Zeiss counting chamber. The initial inoculation was made to a count of one (250,000 cells per cubic centimeter) from an actively growing culture. The final counts were made after twenty-four hour incubation at 30°. The Bios II added was equivalent to 2.0 cc. of the original extract per 100 cc. of medium. Inositol, where added, was used in concentration of 3.2 mg. per 100 cc. of medium.

In Table I are given data showing the effect of magnesium sulfate upon the growth of the yeast in several media. The inositol was Eastman's ash-free *i*-inositol. The Bios II preparations were made according to the procedure given by Lucas.[3]

(1) This research was supported in part from a grant received from the Rockefeller Fluid Research Funds administered by the Iowa State College.

(2) H. Schopmeyer and E. I. Fulmer, *J. Bact.*, **22**, 23 (1931).

(3) G. H. W. Lucas, *J. Phys. Chem.*, **28**, 1180 (1924).

(4) Edna V. Eastcott, *ibid.*, **32**, 1094 (1928).

(5) W. L. Miller, Edna V. Eastcott and J. E. Maconachie, THIS JOURNAL, **55**, 1502 (1933).

(6) E. I. Fulmer, V. E. Nelson and F. F. Sherwood, *ibid.*, **43**, 191 (1921).

Series A involved Bios II prepared from malt sprouts and in series B the Bios II was prepared from a synthetic medium which had supported the growth of *Aspergillus niger* on sucrose as substrate. It is evident that the addition of magnesium sulfate to Medium C, in the same concentration as that in Clark's medium, very markedly increases the growth of the yeast in the presence of Bios II. The addition of the salt to Medium C, without the bios, does not increase the growth, a result in harmony with previous reports from this Laboratory.[6,7]

TABLE I

The effect of Bios II and of inositol upon the growth of yeast in several media. Series A involves Bios II from malt sprouts; in series B the Bios II was prepared from a medium which supported the growth of *Aspergillus niger*. The control contained neither Bios II nor inositol.

	Count					
	Medium C		0.20%	Medium C + $MgSO_4 \cdot 7H_2O$	Clark's medium	
	A	B	A	B	A	B
Control	9	7	9	5	1	6
Control + inositol	8	..	9	...	1	...
Control + Bios II	52	71	191	194	248	255
Control + Bios II + inositol	54	74	284	215	256	311

The data of Table II show that the salt is effective in very low concentrations. The addition of inositol alone does not increase the growth of the yeast, but the growth is increased by the addition of inositol to the medium containing Bios II. This result agrees with the findings of Miller and co-workers.[3-5]

TABLE II

The effect of varying concentrations of magnesium sulfate upon the growth of yeast in Medium C in the presence of Bios II from malt sprouts.

Molarity of $MgSO_4 \cdot 7H_2O$	Count	
	Inositol absent	Inositol present
0	29	34
8×10^{-6}	44	53
8×10^{-5}	150	157
8×10^{-4}	142	147
8×10^{-3}	172	215
8×10^{-2}	192	313

The question at once arises as to whether the above phenomenon is common to all magnesium salts or whether the sulfate radical is involved. Data in Table III show that magnesium chloride and magnesium nitrate are practically without effect, while potassium sulfate gives some increase in growth. The combination of the potassium sulfate with magnesium sulfate is no more effective than is the latter salt alone. In adding the potassium sulfate the concentration of potassium is likewise increased. When ammonium sulfate is substituted for the ammonium chloride, the former salt gives about the same increase in growth as was evidenced by the potassium sulfate. Addition of magnesium chloride or of magnesium nitrate to the medium containing ammonium sulfate gives the same increase in activity of the bios as that shown by magnesium sulfate alone. The above data show that both magnesium and sulfate are involved in the phenomenon.

TABLE III

The effect of several salts upon the growth of yeast in Medium C in the presence of Bios II from malt sprouts.

Salt added (0.017 molar)	Count			
	Medium C		Medium C	NH_4Cl replaced by $(NH_4)_2SO_4$
	Inositol absent	Inositol present	Inositol absent	Inositol present
Control	28	34	47	89
$MgSO_4$	161	257	163	228
$MgCl_2$	30	34	146	204
$Mg(NO_3)_2$	27	31	155	210
K_2SO_4	68	80		
$MgSO_4 + K_2SO_4$	153	228		

Miller and co-workers state that neither Bios I (inositol) nor Bios II produce much increase in the growth of their yeast when used alone, but that the combination gives greatly enhanced growth. It is evident that Bios II alone increases the growth of the yeast employed by us although the effect is further enhanced by the addition of *i*-inositol (Bios I). Lucas,[3] Williams, Warner and Roehm,[8] Stantial,[9] Williams and Saunders[10] and Farrell[11] have called attention to great differences in the response of various strains of yeast toward bios preparations. Preliminary experiments in our laboratory with various strains of yeasts, show that with some yeasts the addition of magnesium sulfate does not increase the activity of the Bios II but actually decreases the growth. However, if inositol be added in the presence of the Bios II a marked increase in growth is evident with all the strains tested. Full details will be published in a later communication.

Summary

For the strain of yeast employed, the presence of magnesium sulfate markedly increases the growth of the yeast in the presence of a bios preparation

(7) F. F. Sherwood and E. I. Fulmer, *J. Phys. Chem.*, **30**, 738 (1926).

(8) R. J. Williams, M. E. Warner and R. R. Roehm, THIS JOURNAL, **51**, 2764 (1929).

(9) Helen Stantial, *Trans. Roy. Soc. Can.*, **28**, Sec. III, 163 (1932).

(10) R. J. Williams and D. H. Saunders, *Biochem. J.*, **28**, 1887 (1934).

(11) Leone N. Farrell, *Trans. Roy. Soc. Can.*, **29**, Sec. III, 167 (1935).

(Bios II). Magnesium chloride or nitrate does not show the above phenomenon while potassium or ammonium sulfate gives some increase in activity. Combinations of magnesium chloride or nitrate with potassium or ammonium sulfate give about the same increase in growth in the presence of the bios preparation as does magnesium sulfate.

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

Di- and Trialkyl Barbituric Acids

BY H. A. SHONLE AND WILBUR J. DORAN

During the past several years, a number of new dialkyl substituted barbituric acids have been prepared in this Laboratory for the purpose of studying the relationship of the pharmacological action to the chemical structure.[1] Since the intermediate malonic esters were available, it seemed advantageous to extend this study to include certain trialkyl substituted barbituric acids. During the course of the preparation of the trialkyl barbituric acids, several undescribed dialkyl barbituric acids were prepared.

A considerable number of 1-alkyl-5,5-dialkyl barbituric acids have been described since Fischer and Dilthey[2] prepared N-methyldiethylbarbituric acid.[3]

The various malonic esters were made in the usual manner by adding the alkyl halide, usually the bromide, to an absolute alcoholic solution of sodiomalonic ester or sodioalkylmalonic ester, refluxing until the reaction was completed and purifying the malonic ester by fractional distillation *in vacuo*. Table I summarizes some of the physical properties of the malonic esters.

Most of the barbituric acids were prepared by condensing the di-substituted malonic ester with urea, methyl urea, or ethyl urea, in the presence of an alcoholic solution of sodium ethoxide, after which they were precipitated and purified, usually by recrystallization from dilute alcohol. In some instances, however, the barbituric acid was an oil which would not readily crystallize, so that its purification had to be effected by fractional distillation *in vacuo*. Table II summarizes the properties of the various barbituric acids prepared.

TABLE I

	Ethyl malonate	B. p., °C.	Mm.	$n^{25}D$
1	3-Methylbutylmethyl[a]	103–104	3	1.4248
2	*n*-Hexylmethyl	125	3.5	1.4280
3	1-Methylpentylmethyl	126	6	1.4323
4	1-Methylpentylallyl	139	5	1.4442
5	2-Ethylhexylmethyl	126	1.5	1.4353
6	*n*-Pentylmethyl	99	8	1.4254
7	1-Methylbutylmethyl	124	10	1.4288
8	*n*-Propyl-2-methylbutyl	100	1	1.4319

[a] Sommaire [*Bull. soc. chim.*, **33**, 189–95 (1923)] describes this ester as boiling at 242–247°.

The di- and trialkyl barbituric acids were converted into their sodium salts by the addition of a 50% solution of sodium hydroxide to an alcoholic solution of the barbituric acid, followed by the removal of the alcohol by vacuum distillation. Solutions of the sodium salts of these barbituric acids were studied pharmacologically on several varieties of laboratory animals. The results obtained by the intraperitoneal injection into white rats are summarized in Table II, wherein the minimum anesthetic dose (M. A. D.) and the minimum lethal dose (M. L. D.) are reported. The detailed pharmacological study will be reported elsewhere.[4] From the pharmacological data, it appears, in general, that the introduction of a third alkyl group lessens the duration of the action. In some instances, alkylating the nitrogen group made the barbituric acids less effective.

We wish to thank Mr. E. E. Swanson and Mr. W. E. Fry for the pharmacological assays, and Mr. John H. Waldo, Miss Anna K. Keltch and Dr. E. C. Kleiderer for assistance in the preparation of several of these barbituric acids.

(1) Swanson, *Proc. Soc. Expil. Biol. Med.*, **31**, 961 (1934); U. S. Patent, 1,996,627; Shonle, Waldo, Keltch and Coles, THIS JOURNAL, **58**, 585 (1936).

(2) Fischer and Dilthey, *Ann.*, **335**, 334 (1904); U. S. Patent, 782,742.

(3) Among the various investigators who have reported in this field are: Dox and Hjort, *J. Pharmacol.*, **31**, 455 (1927); Hjort and Dox, *ibid.*, **35**, 155 (1929); Dox and Jones, THIS JOURNAL, **51**, 316 (1929); Kleiderer and Shonle, *ibid.*, **56**, 1772 (1934); Tabern and Volwiler, Kansas City Meeting, American Chemical Society, April 16, 1936.

(4) Swanson, in press.