port the results in detail elsewhere, a few general observations may be indicated here.

The efficiencies of the compounds vary among themselves as much as five-fold, and the toxicities even more widely. Of those active in moderate dosage, particular interest would seem to attach, as had been anticipated, to ethyl 1-methylbutylacetyl urea and the corresponding amide. These have respectively safety margins of 18 and 11, as compared with a range from 2 to 5 for known barbiturates, and 6 for the commercial analog tested allyl isopropylacetyl urea (Sedormid).

In the N-alkyl series it was observed that when the N-substituent group was methyl, prolonged mild sedation was secured. When it was larger, excitement rather than sedation resulted.

A very interesting point is the relatively great analgesic action shown by certain of the amides, this being produced without a correspondingly deep hypnotic effect as in the barbiturates. There are also definite variations among individual members of the series.

Two chemically related substances, diethyl malonic mono-allyl amide and the bis (diethylamide) of ethyl-1-methylbutylmalonic acid, showed no true hypnotic action; the second produced local anesthesia and on oral administration clonic convulsions.

Conclusions

An extended series of simple and substituted alkyl amides and ureas has been synthesized in connection with a study of their use as sedatives and hypnotics. Considered in connection with the N-aryl and N-alkyl barbiturates described in a companion paper the results indicate the wide value of secondary butyl, amyl and hexyl groups in conferring to such compounds valuable therapeutic properties.

NORTH CHICAGO, ILL. RECEIVED MAY 8, 1936

[CONTRIBUTION FROM THE ABBOTT LABORATORIES, NORTH CHICAGO, ILL.]

N-Alkyl and N-Aryl Substituted Barbituric Acids[1]

BY D. L. TABERN AND E. H. VOLWILER

Although Fischer and Dilthey described several simple N-substituted barbituric acids as long ago as 1904,[2] until recently little systematic study has been made of higher homologs. Dox and his group[3] reported upon certain intermediate members of the series, but did not prepare compounds containing certain secondary butyl, amyl and hexyl groups, which in the simple barbiturates[4] and thiobarbiturates[5] have been found frequently to confer valuable therapeutic properties. Such compounds are among the most effective yet produced and are characterized by a prompt and intense action of short duration.

Apparently the early N-alkyl and aryl barbiturates did not attract attention because preliminary studies failed to reveal anything of especial interest; they are in general but little more active than the analogs derived from urea itself, and in certain members tend to produce pre-anesthetic excitement, paraplegia and convulsions rather than hypnosis.

More recently, however, 5,5-ethylphenyl-N-methylbarbituric acid has been reported to possess a somewhat specific action in epilepsy without the production of pronounced hypnosis, and another, 5,5-methylcyclohexenyl-N-methylbarbituric acid, has been found to have an extremely short but intense period of hypnotic activity. These interesting facts pointed to the need for a more careful study of promising compounds containing one or more secondary alkyl groups in the 5-position with particular reference to rapidity of onset, duration of action, degree of sedation or hypnosis, route and rapidity of elimination, etc. It also seemed of interest to prepare certain members containing secondary and tertiary groups attached to the nitrogen in position "1," none of these ever having been described.

Chemical

The thirty-five barbiturates of this series were prepared by one or more of the following methods: (1) reaction of the dialkylmalonic esters with the appropriate substituted urea in the presence of sodium ethylate at 100–110°; (2) reaction of the dialkyl cyanoacetic esters with a substituted

(1) Presented at the Kansas City Meeting of the American Chemical Society, April 16, 1936.

(2) Fischer and Dilthey, *Ann.*, **335**, 334 (1904).

(3) (a) Dox and Hjort, *J. Pharmac.*, **31**, 455 (1927); (b) Hjort and Dox, *ibid.*, **35**, 155 (1929); (c) Dox and Jones, THIS JOURNAL, **51**, 316 (1929).

(4) Volwiler and Tabern, *ibid.*, **52**, 1676 (1930).

(5) Tabern and Volwiler, *ibid.*, **57**, 1961 (1935).

urea followed by hydrolysis with 15–20% sulfuric acid for ten hours; or (3) the action of allyl bromide on the sodium salt of the simple barbituric acid in water at 100°.[3e] The presence of copper sulfate as a catalyst seems greatly to increase the speed of the latter alkylation. The alkali soluble fraction in each case was extracted and distilled at 2 to 5 mm., passing over below 200°. In the case of reaction 3, there was always a small amount of unchanged dialkyl acid which boiled over 210° under the same conditions and could thus be separated with reasonable accuracy.

The intermediate primary alkyl ureas were best prepared by the action of the corresponding amines upon nitrourea in water or in alcohol at about 80°. After the evolution of nitrogen was at an end, the solvent was removed *in vacuo* and the urea dried and used directly or recrystallized from a suitable solvent, such as acetone or ethyl acetate. Tertiary butyl urea was synthesized by the action of tertiary butyl chloride on urea in the presence of lead carbonate.[6] It was found desirable to carry out the reaction with good stirring, adding the white lead slowly.

Many of the barbituric acids studied were very viscous liquids, insoluble in water but soluble in organic solvents, including petroleum ether; even after long standing, they could not be induced to crystallize and indeed prevented the crystallization of intentionally added portions of the corresponding dialkylbarbituric acids. They are soluble in alkalies, and solid alkali metal salts may be prepared by evaporation *in vacuo* of an absolute alcohol solution. Aqueous solutions of such salts are strongly hydrolyzed and have a *p*H of approximately 10 to 10.5.

Most of the malonic esters have been described previously. The purified barbiturates were analyzed for nitrogen and values obtained agreeing closely with the calculated.

Pharmacologic[7]

While the more promising of the N-substituted derivatives have been studied upon several species and on man, for sake of brevity and uniformity only the results of intravenous injections in rabbits will be indicated here. The figures given represent milligrams per kilogram body weight.

Barbituric acid derivatives			M. p., °C.	Minimum effective dose	Minimum lethal dose
Methyl	1-methylbutyl	N-methyl	105–108	15	90
Methyl	1-methylbutyl	N-ethyl	Oil	30	>100
Methyl	1-methylbutyl	N-allyl	Oil	30	>160
Ethyl	1-methylbutyl	N-methyl	Oil	15	30
Ethyl	1-methylbutyl	N-phenyl	Oil	200	...
Methyl	1-ethylpropyl	N-methyl	[e]	20	130
Ethyl	1-ethylpropyl	N-methyl	Oil	8	80
Methyl	Cyclohexenyl	N-methyl[a]	[e]	10	110
Methyl	*s*-butyl	N-methyl	81–83	20	200
Methyl	*s*-butyl	N-ethyl	[e]	40	>180
Methyl	*s*-butyl	N-2-methylallyl	130	30	...
Ethyl	*s*-butyl	N-methyl	Oil	20	85
Allyl	*s*-butyl	N-methyl	Oil	20	35
Methyl	Isopropyl	N-methyl	113–114	50	250–300
Methyl	Isopropyl	N-ethyl	106–107	60	100[b]
Ethyl	Isopropyl	N-methyl	124–125	30	135
Ethyl	Isopropyl	N-ethyl	[e]	30	> 80
Methyl	2-ethylbutyl	N-methyl	98–100	15	>120
Ethyl	2-ethylbutyl	N-methyl	63–65	10[b]	...
Methyl	1-methylamyl	N-methyl	Oil	20	70
Ethyl	Ethyl	N-*s*-butyl	83–85	>200	>200
Ethyl	Ethyl	N-*t*-butyl	98	>200	>200
s-Butyl		N-*n*-butyl	Oil	80	150[b]
Methyl	1-methylbutyl	N-*t*-butyl		Ineffective	
Methyl	1-methylbutyl	N-*n*-butyl	Oil	Ineffective	
Mono	1,3-dimethylbutyl		235	>165	...
Methyl	1,3-dimethylbutyl		205	45	170
Ethyl	1,3-dimethylbutyl		175	1–3[c]	7[b]
Methyl	1,3-dimethylbutyl	N-methyl[d]	Oil	12–16[b]	

[a] Evipal. [b] Convulsions. [c] Stimulation. [d] See Shonle, THIS JOURNAL, **58**, 585 (1936). [e] Partially crystallized material.

The hypnotic effect comes on very rapidly, with deep hypnotic doses the animal being asleep by the time of completion of the injection. Recovery is likewise rapid, the animal awakening in from fifteen minutes to two hours. Sufficient depth of hypnosis and analgesia may be secured to permit surgical procedures but in this respect the best members of the series seem in-

(6) Schneegans, *Arch. Pharm.*, **231**, 677 (1893).

(7) For the tests here described we are indebted to Mr. H. C. Spruth of the Pharmacologic Department of the Abbott Laboratories.

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).