the green solid. In the Liebermann reaction a dark green color is imparted to the sulfuric acid.

Analyses. Calc. for $C_{18}H_{16}Se$: C, 69.45; H, 5.14. Found: C, 69.40; H, 5.39.

Acetophenone Dimethyl Acetal, $C_6H_5.C(OCH_3)_2.CH_3$.—In the course of the investigation, we had occasion also to prepare this compound.

Methyl orthoformate (22 g.), acetophenone (16.2 g.), anhydrous methyl alcohol (18.9 g.) and conc. hydrochloric acid (5 drops) were mixed in a flask equipped with a calcium chloride guard tube, and heated carefully for several hours at 40°, with constant shaking. After the mixture had stood for 16 hours, a few drops of alcoholic sodium methylate were added carefully to alkaline reaction, and the methyl alcohol distilled. On fractionating the residue at 20mm. pressure, the acetal passed over at 90°, as a colorless liquid of agreeable floral odor; yield, 20 g., or 89.3%.

This acetal is mentioned by Claisen[16] as formed from acetophenone and the hydrochloride of formiminomethyl ether in methyl alcohol solution, but he gives no details concerning the method of preparation or the properties of his product.

Summary

1. 2,4-Diaryl thiophenes and diaryl selenophenes may be produced in fair yields from ketone anils, of acetophenone anil type, by fusion with sulfur or selenium.

2. The properties of these thiophenes and selenophenes are recorded.

3. The following new compounds are described: acetophenone *o*-tolil, *p*-methyl-acetophenone anil, chloromercuri (HgCl) derivatives of 2,4-diphenylthiophene and of the corresponding selenophene, 2,4-diphenyl-selenophene, and 2,4-di-*p*-tolyl-selenophene.

4. The following compounds, already mentioned in the literature, have been prepared by new methods: 2,4-diphenyl-thiophene, acetophenone dimethyl acetal.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF ELI LILLY AND COMPANY]

SOME NEW HYPNOTICS OF THE BARBITURIC ACID SERIES

BY H. A. SHONLE AND A. MOMENT

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We know that the introduction of homologous groups in an identical way and position into the same or similar compounds tends to produce compounds having a similar therapeutic action, and that the hypnotic activity of the aliphatic hydrocarbons increases with increasing molecular weight until a chain of 6 or 8 carbon atoms is reached, after which the action decreases. Though the toxicity of isomeric alcohols decreases in the following order—primary, secondary and tertiary, the hypnotic activity increases.

Certain chemical substances which have specific affinity for particular groups of cells may be made to carry other groups therapeutically active

[16] Claisen, *Ber.*, 31, 1012 (1898).

toward these cells. It is possible in a number of instances to measure the relative activities of such added groups. In this investigation of the hypnotic activity of the alkyl hydrocarbons, the barbituric acid radical served as the carrier and the intensity of the hypnosis thereby produced was measured.

The relationship between physiological activity and chemical constitution has not been satisfactorily explained by any one theory, and it is probable that it will be found to be based on a number of overlapping considerations. With our present knowledge, the physiological activity of a series of homologous chemical compounds can be only approximately predicted. Quite often some minor change in structure produces an effect out of proportion to that expected.

As the data available relative to the activity of the 5,5-di-substituted barbituric acids do not cover the entire series and affords but rough comparisons, the investigators believed that in this series combinations might exist which would afford a better therapeutic action than do some of the products now in use. Consequently, various di-alkyl and alkyl-aryl barbituric acids were prepared and tested for hypnotic action, with results as follows.

The mono-alkyl and -aryl derivatives were found to be inactive in doses of 0.5 to 1 g. per kilogram of body weight when injected subcutaneously in rabbits.

Dimethyl-barbituric acid was without any apparent action on rabbits in a dose of 0.75 g. per kilogram of body weight. Then as the molecular weight increased the activity increased until a maximum was reached, after which it declined until the hypnotic activity is lost and the animal shows only muscular incoördination or no effect at all, as was the case with dibenzyl-barbituric acid which, in 0.5 g. doses, had no apparent pharmacological effect upon rabbits. However, the length and type of the carbon atom chains of the entering groups play an important part in modifying the activity. Branched chains are more active and less toxic than are the straight chains.

Of the aliphatic compounds investigated, *iso*-amyl-ethyl-barbituric acid was found to be the most active, as well as to possess the lowest toxicity; 0.03 g. per kg. of body weight was sufficient to put a rabbit to sleep in most cases, and when 0.04 g. was given the animal could not be aroused, while 0.15 g. per kg. of body weight caused death in 50% of the animals. Diethyl-barbituric acid (Barbital) in 0.15 g. dose per kg. of body weight was required to put rabbits to sleep, while 0.25 g. produced death. In dogs, *iso*-amyl-ethyl-barbituric acid in doses of 0.017 to 0.025 g. per kg. of body weight produced a deeper sleep than did 0.035 to 0.074 g. of barbital, while 0.025 g. of Luminal produced no apparent hypnotic action.

Fischer and Mering[1] state that dipropyl-barbituric acid is the most

[1] Fischer and Mering, *Med. Klinik*, 1, 1327 (1904–5).

active compound of the series which they investigated, having an activity twice that of barbital. In man they found that a dose of 0.15 to 0.5 g caused sleep within 15 to 40 minutes. In our investigations we found that *iso*-amyl-ethyl-barbituric acid is active in 0.10 g. doses, producing sleep in about 30 minutes (with no post-hypnotic effects whatever). Our tests with rabbits show that dipropyl-barbituric acid is somewhat less active than *iso*-amyl-ethyl-barbituric acid, 0.06 g. of the former being required to make the animal unconscious. Thus it will be seen that *iso*-amyl-ethyl-barbituric acid is a compound in which high activity is associated with low toxicity.

In view of the fact that Macht had found that benzyl alcohol possesses an antispasmodic or relaxant action, it was hoped that the introduction of this group into barbituric acid would accentuate the sedative or hypnotic action. However, benzyl-ethyl-barbituric acid, while a more active hypnotic than barbital, was found to cause tetanic convulsions when administered to dogs. As the molecular weight of the benzyl-alkyl derivatives increases, the hypnotic activity decreases. Tetanic convulsions were found by Dox and Yoder[2] to be a characteristic of the benzyl-alkyl-barbituric acids.

The mechanism of the action of the barbituric acid series is not known. Only part is decomposed in the body, as 90% of Barbital injected subcutaneously in dogs has been recovered in the urine, and 62% when given by mouth.[3] Compounds possessing alkyl groups which pass through the body unchanged seem to have little or no hypnotic activity.

Since we know that diethyl- and dipropyl-malonamide and the ureide of diethyl-malonic acid do not have hypnotic activity, it is evident that the barbituric acid is not hydrolyzed to either of these before it reaches the brain cell. The conversion of the ureide of diethyl-malonic acid to diethyl-acetyl-urea produces a compound which is active and very toxic and thus unlike the diethyl-barbituric acid. When diethyl-acetyl-urea is converted into diethyl-hydantoin we have a compound which is but slightly active as a hypnotic and which does not have the toxic action.

Presumably, the heterocyclic ring is decomposed in such a manner that the alkyl groups are free to function in the brain cells.

In the case of the benzyl-alkyl derivatives an additional convulsive action occurs. Since aromatic hydrocarbons have more effect upon the motor nerves than upon the sensory nerves, the benzyl group must function in some manner as an aromatic hydrocarbon. Inasmuch as convulsive action has not been noted when phenylethyl-barbituric acid was given, the phenyl group must function in a different manner.

The following table gives the relative activities of some of the barbi-

[2] Dox and Yoder, THIS JOURNAL, **44**, 1141 (1922).

[3] Fischer and Mering, *Therap. Gegenw.*, **6**, 145 (1904).

turic acid derivatives tested. The acids were dissolved in a molecular amount of dilute alkali, so that the solution contained 10% by weight of the acid. Subcutaneous doses were administered to rabbits. The dose is in grams per kilogram of body weight.

TABLE I

RELATIVE ACTIVITIES OF DERIVATIVES OF BARBITURIC ACID

Derivative of barbituric acid	Mol. wt.	Inability to rise when shaken Dose G.	Toxic Dose G.	Ratio	Remarks
Mono-ethyl	156	..	..	....	0.5 g. slight symptoms
Monobenzyl	218	..	..	....	0.5 g. ineffective
Monobutyl	184	..	..	....	1.0 g. ineffective
Dimethyl	156	..	..	....	0.75 g. ineffective
Diethyl	184	0.15	0.25	1 to 1.7	
Dipropyl	212	0.06	0.22	1 to 3.7	
Propyl-*iso*propyl	212	0.05	0.15	1 to 3	
Di-*n*-butyl	240	0.35	0.50	1 to 1.4	Animal dizzy and drunk next day
Dibenzyl	308	..	..	....	0.5 g. ineffective
*iso*Propyl-ethyl	198	0.10	0.20	1 to 2	
*iso*Butyl-ethyl	212	0.05	0.12	1 to 2.4	
n-Butyl-ethyl	212	0.04	0.10	1 to 2.5	
sec.-Butyl-ethyl	212	0.05+	0.20−	....	
iso-Amyl-ethyl	226	0.04	0.17	1 to 4.2	
iso-Amyl-propyl	240	0.08	0.22	1 to 2.8	
Benzyl-ethyl	246	0.04	0.06	1 to 1.5	Convulsions
Benzyl-*iso*propyl	260	0.20	0.35	1 to 1.8	
Benzyl-propyl	260	0.20	0.45	1 to 2.3	Convulsions
Phenyl-ethyl	232	0.08	0.15	1 to 1.9	

Experimental Part

The barbituric acids reported were prepared by the method of Fischer and Dilthey.[4] The alkyl and alkyl-aryl derivatives of malonic ester, prepared in the usual manner, were condensed with urea in an autoclave in the presence of sodium ethylate. The barbituric acids secured were purified by recrystallization from water, xylene or alcohol.

The alkyl bromides were prepared from the various alcohols either according to the method of Adams, Kamm and Marvel,[5] from sulfuric acid, sodium bromide, water and alcohol, or according to that of Weston[6] from sulfuric acid, sodium bromide and alcohol. The two methods gave similar yields, but the latter permitted a larger run to be made when the capacity of the still was a limiting factor.

The malonic ester was made according to the method of Noyes[7] with slight modifications.

The various alkyl derivatives of malonic ester were prepared by adding 1.25 to 1.5 moles of the alkyl bromide to 1 mole of the sodium salt of malonic ester or alkyl malonic

[4] Fischer and Dilthey, *Ann.*, **335**, 334 (1904).

[5] Adams, Kamm and Marvel, *Univ. of Ill. Bull.*, **18**, No. 6, p. 16 (1920).

[6] Weston, *J. Chem. Soc.*, 107, 1489 (1915).

[7] Noyes, THIS JOURNAL, **18**, 1105 (1896).

TABLE II

THE RELATIVE ACTIVITIES OF SOME OF THE BARBITURIC ACIDS, GIVEN BY MOUTH TO DOGS

Derivative of barbituric acid	Dog 1[a] G.	Dog 2 G.	Dog 3 G.	Dog 4 G.	Dog 5 G.
Diethyl.....	0.074 ++++	0.047 ++	0.035 ++	0.047 +[g]	0.034 0
*iso*Propyl-ethyl.....	0.025 ++	0.025 ++	0.035 ++	0.017 0	0.026 ++
*iso*Butyl-ethyl.....	0.025 +++	0.025 +++	0.029 ++++	0.017 ++	0.026 0
iso-Amyl-ethyl.....	0.025 ++++	0.025 ++++	0.029 ++++	0.017 ++	0.026 +++
n-Butyl-ethyl.....	... ..	0.044 ++++[c]	0.029 ++[f]	... ..	... ..
Benzyl-ethyl.....	0.049 +++[b]	0.047 ++++	0.029 ++	... ..	... ..
Phenyl-ethyl.....	0.025 0	0.025 0[d]	... ..	... ..	... ..
Di-*n*-butyl-ethyl.....	... ..	0.048 +++[e]	... ..	... ..	... ..

Dose: g. per kg. of body weight
O: no action
+: drowsy—light sleep
++: sleep, 1–2 hours
+++: sleep 3 hours
++++: sleep 4–5 hours to most of day

Weight of Dogs
Dog 1— 7.8 kg.
Dog 2—11.5 kg.
Dog 3—11.0 kg.
Dog 4— 5.6 kg.
Dog 5— 7.6 kg.

[a] Dog of nervous disposition. [b] Slight convulsions. [c] Trembles somewhat. [d] Unsteady for short time after administration. [e] Muscular incoördination marked during the following day. [f] A dose of 0.045 g. caused muscular incoördination during the following day. [g] A dose of 0.035 g. caused no drowsiness.

ester in absolute ethyl alcohol in the usual manner. The yields decreased somewhat with the increasing weight of the alkyl group. It was possible to secure 90–94% yields of ethyl-malonic ester, but only 80% yields of *iso*-amyl-ethyl-malonic ester. The use of absolute methyl, *iso*propyl and *n*-butyl alcohols instead of ethyl alcohol resulted in a lowered yield, the cause of which was not determined. Contrary to expectations, most of the substituted malonic esters, when perfectly dry, distilled at atmospheric pressure with little or no decomposition.

The esters were purified by fractional distillation in a vacuum and collected over a 5° to 10° range or less, as indicated below. Table III describes the new esters and gives additional data on several previously described.

TABLE III

NEW ESTERS

Compound malonic ester	Boiling point °C.	Mm.	d_{25}^{25}	n^{25}
sec.-Butyl-ethyl	155–160	60	0.9858	1.4264
n-Butyl-ethyl[8]	125–130	12	0.9756	1.4222
iso-Butyl-ethyl	119–120	8	0.9682	1.4228
iso-Amyl-ethyl	150	20	0.9540	1.4255
Propyl-*iso*propyl	143	42	0.9803	1.4239
n-Butyl-*iso*propyl	136	14	0.9742	1.4291
iso-Amyl-*iso*propyl[9]	140	25	0.9575	1.4273

The disubstituted malonic esters were condensed with urea in the presence of excess sodium ethylate according to the procedure of Fischer and Dilthey.

Sodium methylate in absolute methyl alcohol as recommended by Rising and Stieglitz[10] for phenyl-ethyl-barbituric acid was tried with *iso*-amyl-ethyl-malonic ester. The result was a sirupy product from which it was impossible to isolate any barbituric acid. Table IV gives the melting points of compounds prepared in the laboratory.

TABLE IV

MELTING POINTS

Compound derivative of barbituric acid	°C.	Compound derivative of barbituric acid	°C.
Mono-*n*-butyl[11]	210–215	*n*-Propyl-*iso*propyl	161–162
iso-Butyl-ethyl	174–176	*iso*-Amyl-*iso*propyl	173–175
iso-Amyl-ethyl	154–156	*iso*-Amyl-propyl	129–132
n-Butyl-ethyl[11]	126–128	*sec.*-Butyl-ethyl	155–157
n-Butyl-*iso*propyl	209–210		

Summary

1. A number of derivatives of barbituric acid have been synthesized and described.

2. Of the di-alkyl derivatives, *iso*-amyl-ethyl-barbituric acid was found to be the most active in its hypnotic properties.

3. The ratio of the toxic dose to the unconscious dose increases with the increasing molecular weight and then decreases. This ratio is affected

[8] Made by Raper, *J. Chem. Soc.*, **91**, 1837 (1907).

[9] Made by Nef, *Ann.*, **318**, 159 (1901).

[10] Rising and Stieglitz, THIS JOURNAL, **40**, 723 (1918).

[11] Dox and Yoder, *ibid.*, **44**, 1578 (1922).

by the length and type of the carbon chains of the entering groups where the molecular weight is constant.

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM THE RESEARCH LABORATORY IN ORGANOTHERAPEUTICS OF ARMOUR AND COMPANY]

A COMPARISON BETWEEN THE CHEMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF PEPSIN AND RENNIN

BY FREDERIC FENGER

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This investigation was carried out for the purpose of obtaining more specific knowledge regarding pepsin and rennin. In dealing with these two enzymes, whose physiological characteristics have been known and utilized for many years, subjects must unavoidably be touched which have been general knowledge for a long time. Such findings should merely be considered as confirmative evidence.

The glandular layer of the fundus portion of hog stomachs forms the basic material for all manufactured pepsin, while rennin is obtained from the mucus lining of the fourth stomach of young calves. These raw materials were examined first. The mucus linings, whether from hogs or calves, were dissected out and carefully freed from fat and muscular tissue, finely minced and desiccated in a vacuum at 35° to 40° to constant weight. Analyses of the desiccated substances for the material soluble in petroleum ether, moisture, ash, total phosphoric acid and total nitrogen were made; and the proteolytic, milk-curdling and hemostatic activities were also determined. The total chlorine was determined on the fresh minced linings and the inorganic chlorine on the ash. The difference between the two represents the amount of free hydrochloric acid and organic chlorine present in the normal fresh linings. The proteolytic activity was determined on all samples according to the assay method of the U. S. Pharmacopeia and the hemostatic properties estimated on oxalated beef plasma. The milk-curdling tests were carried out on fresh certified milk, on pasteurized milk and on milk acidified with lactic acid to correspond to an acidity of 0.2% of lactic acid with phenolphthalein as indicator.

The calf mucosa is quite tender, which may account for the considerable number of stomach ulcers encountered. In some cases the ulceration is superficial, although distinctly outlined; in others the muscular coat of the stomach is quite corroded.

Both hog and calf stomach linings possess considerable hemostatic properties. The importance of the ability to check capillary oozing