The findings here reported explain the contradictory evidence presented by various workers from time to time regarding the influence of salts upon the activity of this enzyme and emphasize anew the importance of controlling the hydrogen-ion activities of the solutions in which the enzyme action occurs.

The influence of salts upon the activity of malt amylase appears to be specific.

These findings as a whole establish quite definitely a difference, in dependence of optimal activity upon neutral salts, between pancreatic and malt amylases, which are being studied in detail as representative and analogous enzymes of animal and plant origin, respectively.

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DIALKYL BARBITURIC ACIDS

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Nearly a hundred different 5,5-disubstituted barbituric acid derivatives have been prepared in the quarter of a century since Fischer and Dilthey[1] and Fischer and von Mering[2] found that certain of these derivatives could be used therapeutically as sedatives and hypnotics. These investigators were aware that certain of the compounds were more active as hypnotics than others and that the effect could be markedly changed by varying the chemical nature of the substituent radical.

Subsequent investigations[3] have shown that those barbituric acids in which the sum of the C atoms in the two substituent groups is 6, 7 or 8 are the most effective. When evaluated on rats, cats, rabbits or mice, by the intravenous or subcutaneous injection of their sodium salts, this group shows, in most instances, a wider margin of safety between the anesthetic or down dose and the toxic dose than does, for example, diethylbarbituric acid.

The investigation was started in 1927 for the purpose of studying the various isomeric amyl-ethyl and amyl-allyl derivatives of the barbituric acids because of the commercial availability of certain of the amyl alcohols, and to extend the study of the secondary alkyl-ethylbarbituric acid series.[4]

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

[2] Fischer and von Mering, *Therap. d. Gegenwart.*, **44**, 97 (1903); **45**, 145 (1904).

[3] (a) Carnot and Tiffeneau, *Compt. rend.*, **175**, 242 (1922); (b) Shonle and Moment, THIS JOURNAL, **45**, 243 (1923); (c) Volwiler, *ibid.*, **47**, 2236 (1925); (d) Nielson, Higgens and Spruth, *J. Pharmacol.*, **26**, 371 (1926); (e) Swanson and Page, *ibid.*, **31**, 1 (1927); (f) Dox and Hjort, *ibid.*, **31**, 455 (1927).

[4] (a) During the course of this investigation Dox and Jones, THIS JOURNAL, **50**, 2033 (1928), have reported on 5-*n*-amyl-5-ethylbarbituric acid. (b) Volwiler and Tabern described a number of amyl-ethyl and amyl-allyl derivatives at the Minneapolis Meet-

In view of Hsueh and Marvel's[5] statement relative to *sec.*-butylethylbarbituric acid we carefully repeated our work and found that this acid does not melt at 197°, as stated by them, but that after repeated crystallization *sec.*-butyl-ethylbarbituric acid melted at 164–165° (corr.). Our former figure[3b] of 158° (uncorr.) had been obtained with a limited amount of substance.[6]

In repeating this former work of ours we found that if we introduced the *sec.*-butyl group in ethylmalonic ester, we obtained a satisfactory yield of *sec.*-butylethylmalonic ester, which yielded *sec.*-butylethylbarbituric acid melting at 164°. However, if we reversed the procedure, we were able to introduce but an insignificant amount of the ethyl group into the *sec.*-butylmalonic ester, and could not separate any of the diethyl *sec.*-butylethylmalonate by fractional distillation. The barbituric acid prepared from the higher-boiling fraction of the ester was physiologically inert, melted at about 190° and proved on analysis to be *sec.*-butylbarbituric acid contaminated with a trace of *sec.*-butylethylbarbituric acid. It was possible to introduce the allyl group into this *sec.*-butylbarbituric acid, *sec.*-butylallylbarbituric acid having the correct melting point being obtained on recrystallization.

It was likewise found impossible to introduce the ethyl group into the *sec.*-amyl- or the cyclopentylmalonic esters or the allyl group into *sec.*-butylmalonic ester. To all outward appearance the reaction was normal, sodium bromide being deposited as usual. The reaction occurred, however, between the aliphatic bromide and the sodium ethylate instead of between the aliphatic bromide and the sodium *sec.*-butylmalonic ester, which indicates that the equilibrium was displaced toward the sodium ethylate since it reacted with the alkyl bromide. This may be interpreted as an instance of steric hindrance, or as an instance of the difference in the negativity of the secondary aliphatic substituted as compared to the primary aliphatic substituted malonic esters.

Experimental

Alcohols.—In view of the improbability of successfully purifying the commercial *sec.*-amyl alcohols by fractionation, the synthetic *sec.*-amyl alcohols prepared by the Grignard method at the Eastman Kodak Laboratories were used. Di-*n*-propylcarbinol and methylbutylcarbinol were likewise obtained from the same source. Cyclo-

ing of the American Chemical Society, September, 1929, THIS JOURNAL, **52**, 1676 (1930), including propylmethylcarbinyl- and *sec.*-butylcarbinyl-ethylbarbituric acids and the esters from which they were prepared.

[5] Hsueh and Marvel, *ibid.*, **50**, 855 (1928).

[6] Dr. Marvel was kind enough to send us a sample of his acid and the ester from which it was prepared. Investigation showed that the 197° melting acid was almost physiologically inert, and analyzed as *sec.*-butylbarbituric acid which melts at 197°. The ester had a boiling point and refractive index much closer to diethyl *sec.*-butylmalonate than to diethyl *sec.*-butylethylmalonate.

pentanol was prepared by reducing, in the presence of a platinum catalyst,[7] cyclopentanone obtained from adipic acid.[8] The other alcohols, obtained from various sources, presented no unusual aspects.

Alkyl Bromides.—The primary alkyl bromides were prepared according to the method of Kamm and Marvel,[9] while the secondary alkyl bromides were prepared by the Norris[10] method.

When the work was initiated, the authors had not considered that rapidly distilling a secondary alcohol with hydrobromic acid would produce other than the corresponding bromide in a degree of purity satisfactory for synthetic work. It was noted at that time, however, that the malonic esters and barbituric acids prepared from 2-bromo- and 3-bromopentane which had been made by the above method had similar physical characteristics. Dr. M. S. Kharasch, in discussing these reactions with one of us, pointed out that even rapidly distilling either of the 2 or 3-pentanols with hydrobromic acid should result in a rearrangement tending to give the same equilibrium mixture of 2 and 3-bromopentane, the exact proportions of the isomers depending on the conditions of the reaction.

We were later informed of the quantitative determination made by Dr. Sherrill and her co-workers,[11] now published, in which the values of the D line of 2-bromopentane and 3-bromopentane were shown to be at 20°, 1.4412 and 1.4443, respectively. Since Lucas, Simpson and Carter[12] have shown that there is a linear relation between the composition and refractive index, one can determine by calculation that the bromide obtained by Sherrill from diethylcarbinol and hydrobromic acid was a mixture of 71% 2-bromopentane and 29% 3-bromopentane, while that obtained from propylmethylcarbinol was a mixture of 81% 2-bromopentane and 19% 3-bromopentane.

It is evident that the preparation of *sec.*-amyl bromides by the usual methods results in mixtures and this circumstance doubtless obtains for the higher secondary bromides. In order to be sure then of the homogeneity of secondary bromides, one should use the method of Kharasch as described in detail by Sherrill, which gave as judged by the refractive index a 2-bromopentane slightly purer than that obtained by Lucas and Moyse.[13]

When the bromopentane obtained by rapidly distilling propylmethylcarbinol with 48% hydrobromic acid was washed and dried as directed by Sherrill, and fractionated,

[7] Adams, Voorhees and Shriner, "Organic Syntheses," John Wiley and Sons, Inc., New York, **1928**, Vol. VIII, p. 92.

[8] Thorpe and Kon, *ibid.*, **1925**, Vol. V, p. 37.

[9] Kamm and Marvel, *ibid.*, **1921**, Vol. I, p. 1.

[10] Norris, *Am. Chem. J.*, **38**, 626 (1907); THIS JOURNAL, **38**, 1075 (1916); McCullough and Cortese, *ibid.*, **51**, 225 (1929).

[11] (a) Sherrill, Otto and Pickett, *ibid.*, **51**, 3023 (1929); (b) Sherrill, Baldwin and Haas, *ibid.*, **51**, 3034 (1929).

[12] Lucas, Simpson and Carter, *ibid.*, **47**, 1462 (1925).

[13] Lucas and Moyse, *ibid.*, **47**, 1459 (1925).

two main cuts were obtained, one boiling at 117–118°, having a refractive index n_D^{20} 1.4416, the other boiling at 118–118.5°, with a refractive index n_D^{20} 1.4418, corresponding, respectively, to 87 and 81% of 2-bromopentane. In another trial, a 118–119° cut from technical propylmethylcarbinol was used. The two main cuts of the bromide boiled at 116.5–117°, with a refractive index n_D^{20} 1.4413, and 117–117.5°, with a refractive index n_D^{20} 1.4415, corresponding, respectively, to 97 and 90% of 2-bromopentane.

On distilling diethylcarbinol with 48% hydrobromic acid, a bromopentane is obtained which on fractionating gave a main fraction boiling at 117°, with a refractive index n_D^{20} 1.4433, corresponding to 68% of 3-bromopentane. However, when diethylcarbinol, obtained from technical alcohol, was used, the resulting bromides could be separated into two main fractions, one boiling at 115–116°, having a refractive index n_D^{20} 1.4419, and the other boiling at 116–117°, having a refractive index n_D^{20} 1.4425, corresponding, respectively, to 77 and 58% of 2-bromopentane.

This explains then why malonic esters prepared from these secondary alkyl bromides had a wider range of refractive indices and boiling points than did the malonic esters containing primary alkyl groups. Since the *sec.*-hexyl and -heptyl bromides used in this research were prepared in an analogous manner, similar rearrangements may have occurred.

When isopropylmethylcarbinol is distilled from 48% hydrobromic acid, the distillate is a mixture of amylene, *tert.*-amyl bromide and some 2-bromo-3-methylbutane.[14]

Mono-alkyl Malonic Esters.—The usual methods of preparing the mono-alkyl substituted malonic esters were employed,[15] no marked differences being observed in the yields from the primary and secondary aliphatic bromides. In actual practice it is unnecessary to use an amount of alcohol more than 10 times the weight of the sodium. The yields of the mono-substituted esters were generally greater than of the corresponding disubstituted esters and the reaction was completed in less time.

Dialkyl Malonic Esters.—The usual method of preparing the disubstituted malonic esters was employed when primary bromides were used. The use of iso-amyl chloride instead of iso-amyl bromide in the preparation of diethyl iso-amylethylmalonate did not lower the yield. When the *sec.*-amyl or -hexyl bromides were used the yields by the above methods were quite low, although the secondary group was introduced last. If, however, most of the alcohol was removed from the sodium salt of the mono-substituted ester before the bromide was added, the yields were bettered.

Diethyl hydroxyethylethylmalonate was obtained in a 27% yield when ethylene chlorohydrin was caused to react in the usual manner with sodium diethyl ethylmalonate. In contrast to diethyl hydroxyethylmalonate, it can be distilled under vacuum without decomposition. A better yield would undoubtedly be obtained if benzene were used in place of the alcohol.

Mono-substituted Barbituric Acids.—The mono-substituted barbituric acids were prepared by condensing the mono-substituted malonic esters with urea in absolute alcohol in the presence of sodium ethylate,[1] gently refluxing for two to three hours. Too prolonged refluxing causes a poor yield. They were isolated and purified in the usual manner.

Disubstituted Barbituric Acids.—Certain of the allyl-alkyl barbituric acids were prepared by the interaction of allyl bromide on the sodium salt of the desired mono-

[14] Wischnegradsky, *Ann.*, **190**, 328 (1878), obtained tertiary halides instead of secondary halides on treating isopropylmethylcarbinol with aqueous hydrochloric and hydriodic acids at 40°.

[15] Adams and Kamm, "Organic Syntheses," John Wiley and Sons, Inc., New York, **1924**, Vol. IV, p. 11.

substituted barbituric acid.[16] The most satisfactory yields were obtained when the barbituric acid was dissolved in a molecular quantity of 25 to 35% aqueous potassium hydroxide, to which was added somewhat in excess of a mole of allyl bromide and alcohol to about 10% of the total volume. The reaction was carried out in a shaking machine, from forty to fifty hours' time being required. Without the alcohol the reaction proceeded more slowly. The disubstituted acids were isolated and purified in the usual manner.

Most of the disubstituted barbituric acids were prepared by Fischer's method with the exception that a prolonged refluxing in a boiling water bath or on the sand-bath advantageously replaced the briefer period of heating in an autoclave. In some instances, after refluxing for one hour, most of the alcohol was removed under vacuum and the solid residue was heated in a boiling water-bath for four hours.

Technique.—In carrying out the fractional distillation under vacuum it was found that the refractive index was a better measure of purity than the pressure and boiling point. Consequently fractionation was repeated until a middle portion having a constant refractive index (or relatively constant in the instance of esters having a secondary group) was obtained.

The refractive indices were determined with an Abbé refractometer, with the temperature held constant to 0.2°. Duplicate observations taken on different days checked within one point in the fourth place.

The fractional distillation was carried out with the Fischer and Harries[17] apparatus which permits the fractionation to proceed while samples are being removed. Instead of the Claisen flask, a 200- or 500-cc. round-bottomed pyrex flask was used to which was attached a 30-cm. column.[18] The temperature was recorded by an Anschütz thermometer, the stem of which was entirely contained within the top of the fractioning column. During the course of the fractional distillation, even though the temperature was constant, repeated small cuts were made in order to obtain more data on the refractive index of the distillate.

No attempt was made to obtain the ultimate analysis of the esters since they were to be used as intermediates. The constancy of boiling point and refractive index served as the basis for making the cuts. Two or more preparations of each of the esters and barbituric acids have been made during the course of this investigation.

The mono- and disubstituted malonic esters prepared are given in Tables I and II. The yields are reported only for the ester having the described physical properties. In every instance refractionation of the lower cuts would have increased the yield.

[16] (a) Volwiler, THIS JOURNAL, **47**, 236 (1925); (b) Preiswerk, U. S. Patent 1,444,802 (1923); (c) Dox, U. S. Patent 1,615,870 (1927).

[17] Fischer and Harries, *Ber.*, **35**, 2158 (1902).

[18] This column had an internal diameter of 2 cm. and is indented as described in THIS JOURNAL, **39**, 2718 (1917).

TABLE I

MONO-SUBSTITUTED MALONIC ESTERS

Diethyl malonate derivatives	Yield, %	Boiling point °C.	Mm.	n_D^{20}
β-Hydroxyethyl[a]	8.3	136–137	15	1.4312[b]
Sec.-butyl[3c]	50.8	94–95	3	1.4248
n-Amyl[3]	44	121–123	6	1.4253
Iso-amyl[3c]	54.4	102	3	1.4255
Diethylcarbinyl	35.5	100–102	3	1.4275
Propylmethylcarbinyl[c]	50.5	103–104	4	1.4273
Cyclopentyl	56	113.8	4	1.4434

[a] Traube and Lehmann, *Ber.*, **32**, 720 (1899), first described this ester. Cretcher, Koch and Pittenger, THIS JOURNAL, **47**, 3083 (1925), were unable to distil hydroxyethylmalonic ester prepared from ethylene oxide and malonic ester without decomposition. We treated an absolute alcoholic solution of 1 mole of the sodium salt of malonic ester with 1.05 moles of ethylene chlorohydrin in the usual manner. The low yield obtained was due both the fact that most of the malonic ester had not entered into the reaction and that decomposition occurred during the fractionation. [b] Refractive index at 25°. [c] Boedecker, U. S. Patent 1,739,662, gives 122–125° at 13 mm.

TABLE II

DISUBSTITUTED MALONIC ESTERS

Diethyl malonate derivatives	Yield, %	Boiling point °C.	Mm.	n_D^{20}
n-Butyl-allyl	80	127–131	11–12	1.4387
Sec.-butyl-ethyl[3b]	39	100–103	3.5–4	1.4329
β-Hydroxyethyl-ethyl	27	128–131	6	1.4444
n-Amyl-ethyl[3]	39.7	136.5	10	1.4295[a]
Iso-amyl-ethyl[3b]	41	130.5	10	1.4295
Sec.-butylcarbinyl-ethyl[4b]	29.8	141–143	15	1.4316
Propylmethylcarbinyl-ethyl[4b]	27	111–112.5	4	1.4343
Diethylcarbinyl-ethyl	20	110–112	4	1.4329–1.4337[a]
Isopropylmethylcarbinyl-ethyl	10	110–116	7	1.4400[a]
Cyclopentyl-ethyl	48	115.4–116.2	4	1.4448
n-Butylmethylcarbinyl-ethyl	33–43	126–134	9	1.4331–1.4366[a]
Di-*n*-propylcarbinyl-ethyl	31	127–133	10	1.4302
Phenylethyl-ethyl	39	173–177	5	1.4829–1.4836[a]
n-Heptyl-ethyl	62	143.5	3.5	1.4343

[a] Refractive index at 25°.

Boiling Points.—The following boiling points were observed: for diethyl ethylmalonate, 92.2° at 10 mm., 106° at 19 mm., 115.5° at 30 mm., 121° at 40 mm.; for diethyl iso-amylethylmalonate, 121.5° at 6 mm., 130.5° at 10 mm., 143.5° at 20 mm., 154° at 30 mm.; for diethyl iso-amylmalonate, 104.4° at 4 mm., 119.5° at 8 mm., 125.5° at 10 mm., 137.5° at 19 mm. and 146.5° at 29 mm.

Tables III and IV describe the mono- and disubstituted barbituric acids prepared from the above described esters.

Isopropylmethylcarbinyl-ethylbarbituric acid was prepared in the usual manner from the corresponding malonic ester in an exceedingly low yield. It melts at 183–186° and is physiologically active. An amount of the barbituric acid sufficient for analysis and complete pharmacological testing was not prepared.

The di-*n*-propylcarbinyl-ethylbarbituric acid prepared gave a nitrogen value al-

TABLE III

MONO-SUBSTITUTED BARBITURIC ACIDS

Barbituric acid derivative	Yield, %	Melting point, °C. (corr.)	Nitrogen, % Calcd.	Found	
Propylmethylcarbinyl[a]	76	164–166	14.14	14.47	14.33
Diethylcarbinyl[b]	69	165–168	14.14	14.58	14.63
Cyclopentyl	51	221–223	14.29	13.97	13.95

[a] Boedecker, U. S. Patent 1,739,662 gives 162–163°, corr. [b] German Patent 293,163 describes a diethylcarbinyl barbituric acid having a melting point of 198°.

TABLE IV

DISUBSTITUTED BARBITURIC ACIDS

Barbituric acid derivative	Yield, %	Melting point, °C. (corr.)	Nitrogen, % Calcd.	Found	
Propylmethylcarbinyl-ethyl[a]	52	128.5–130	12.39	12.50	12.59
Diethylcarbinyl-ethyl[a]	36	127–129	12.39	12.00	11.88
Sec.-butylcarbinyl-ethyl[4b]	54.5	136–138	12.39	12.57	12.59
Cyclopentyl-ethyl	73	182–183	12.50	12.40	12.48
n-Butylmethylcarbinyl-ethyl	42	121–123	11.66	12.17	12.08
β-Hydroxyethyl-ethyl[b]	67	178	...	.....	
Propylmethylcarbinyl-allyl	50	86–88	11.76	12.01	12.04
Diethylcarbinyl-allyl	..	Wax-like	11.76	.....	
Cyclopentyl-allyl	23	161–163	11.86	11.63	11.58
n-Butyl-allyl[c]	58	125–125.5	...	.....	

[a] German Patent 293,163 describes both of these barbituric acids but gives a melting point of 162° for the diethylcarbinyl-ethylbarbituric acid. [b] It was found possible to prepare the β-hydroxyethyl-ethylbarbituric acid in the usual manner from diethyl hydroxyethylmalonate. Cretcher, Koch and Pittenger, THIS JOURNAL, **47**, 3083 (1925), using an indirect method, obtained a melting point of 176° (corr.) for this acid. [c] Prepared from diethyl *n*-butyl-allylmalonate instead of by the action of allyl bromide on potassium *n*-butylbarbiturate, as described by Volwiler.[3a]

most 1% above the theoretical and melted around 158°. An acid melting about 10° lower was also obtained; both were physiologically active.

n-Butylmethylcarbinyl-ethylbarbituric acid. When the crude acid was recrystallized from a benzene–gasoline solution and then from dilute alcohol, two fractions were obtained; the major fraction melted at 122.5°, and had 12.08 and 12.17% of nitrogen, while the minor fraction melted at 106° and had 11.70 and 11.95%. Both were equally effective as hypnotics. The higher-melting product is presumed to be the *n*-butylcarbinyl-ethylbarbituric acid isomer.

Discussion

The disubstituted barbituric acids described are readily soluble in hot benzene (in contrast to the slight solubility of the mono-substituted) and can be crystallized from benzene or dilute alcohol. The one exception was the diethyl-allylbarbituric acid which precipitated as an oil, hardening on standing. These barbituric acids are almost insoluble in water, but dissolve readily in dilute sodium hydroxide solutions.

That the aqueous solutions of the alkali metal salts of the disubstituted barbituric acids are not indefinitely stable does not seem to be generally

recognized outside of the chemical laboratory. Solutions of some of these salts show evidences of decomposition after a few days' standing. When a 5 or 10% solution of the sodium salt is boiled but for a short time, the ring is ruptured and ammonia is evolved. After a more prolonged boiling, a solid separates out as the solution cools. In the case of sodium iso-amyl-ethylbarbiturate, this solid was shown to be iso-amyl-ethylacetylurea. The amount of the precipitate which occurs after boiling an aqueous solution is not a measure of the full extent of the ring cleavage.

Initial decomposition can be detected by slowly adding a 1:10 solution of hydrochloric acid to the cold solution of the sodium barbiturate until the precipitation of the barbituric acid is complete, filtering off the precipitate, washing it with water and drying for a few hours at 70 and 80°. If there has been any decomposition the melting point will be lower than that of the pure barbituric acid initially used to prepare the salt. Since the difference may be only a few degrees, the melting points of the control and the test sample should be run at the same time.

It is essential in preparing solutions for biological study that any excess of alkali over one molar proportion should be avoided, that only a minimum amount of warming should be employed, and that only freshly prepared solutions be used.

Various salts of ammonia, mono-alkyl- and dialkylamines were prepared by dissolving the above-described barbituric acids in an excess of a solution of the volatile base and then evaporating the excess of the base and solvent until the solid salt was obtained. It was found that unless the salt was protected from exposure to air, the loss of the volatile base continued, leaving a mixture of the barbituric acid and the salt which was not water soluble.[19]

The addition of alcohol or glycerin to aqueous solutions of the various sodium barbiturates lessens the rate of decomposition. If, further, a weak organic acid is added to just short of the point of precipitation, the stability is further increased because of the lowering of the alkalinity. In this way solutions of the salts of more labile barbituric acids may be stabilized for some months. An absolute alcoholic solution of a sodium barbiturate does not decompose on boiling. The different barbituric acids exhibit varying rates of decomposition under the same conditions, those with a greater molecular weight usually being the more readily decomposed.

The alkali metal salts are best prepared by concentrating under vacuum non-aqueous solutions of equimolar proportions of the alkali metal hydrox-

[19] Kubli, U. S. Patent 1,316,047, has prepared various alkylamine salts of barbituric acids with the expectation of overcoming the decomposition caused by the strong alkaline solution of the sodium salts. We have observed that the ampouled solution of Somnifen, an alkylamine salt of allyl-isopropylbarbituric acid, has decomposed in standing, forming the usual alkali-insoluble precipitate.

ide and the barbituric acid until the salt is obtained in solid form. The alkali metal salts of the barbituric acids of higher molecular weight are more hygroscopic than those of a lower molecular weight.

The calcium and magnesium salts are less soluble than the sodium, potassium or lithium salts, and may be prepared by adding the calculated amount of a soluble salt of the desired alkaline earth metal to a solution of the sodium barbiturate, and concentrating until a precipitate of the alkaline earth barbiturate occurs.[20]

No attempt will be made to report extensive pharmacological data, nor will any theory be advanced as to the mode of action of the barbituric acids. However, the exceedingly prompt effect following intravenous injections of solutions of their sodium salts and the relatively delayed appearance of unchanged barbituric acid in the urine are presumptious of the functioning of the intact molecule.

The effective and fatal doses were obtained by injecting intraperitoneally into white rats freshly prepared 2% solutions of the sodium salts of the various barbituric acids. Animals of about 100 g. weight, which had been starved for twenty-four hours previous to the injection, were used. The effective dose reported (M. E. D.) is that amount which will abolish reflexes when the inner ear is touched with a thin wooden applicator. (This depth of anesthesia is not sufficient to permit indiscriminate operative procedures to be carried out.) The M. L. D. is that dose which caused the death of all or a majority of animals on that dose. Variations of as much as 75% in the amount of the identical lot of barbituric acid required to produce anesthesia and death have been noted when this lot was tested at varying periods throughout a year.[21] The percentage of the M. L. D. required to produce anesthesia at these various periods, however, was not materially different. The data reported in Table V were obtained under controlled conditions so that the doses may be compared with one another, taking into consideration that the usual biological variations obtain in these experiments in which the number of animals are employed is limited.

No sedative or hypnotic effect was noted when large doses of the monosubstituted barbituric acids were injected. The replacement of a hydrogen in the β-position in one ethyl group of diethylbarbituric acid by an hydroxyl group results in the loss of the hypnotic activity. The animals did not exhibit any toxic symptoms at the doses injected.

It will be noted that diethylbarbituric acid and phenylethylbarbituric acid give a similar value for M. L. D./M. E. D. and it is this fact that has

[20] Quade, U. S. Patent 1,461,831, prepared the calcium and magnesium diethylbarbiturate by digesting an aqueous solution of diethylbarbituric acid with calcium oxide or magnesium carbonate and concentrating the filtrate, a method quite satisfactory for the more soluble barbituric acids.

[21] These variations were probably due to differences in strains of rats, seasonal changes, etc.

TABLE V

PHARMACOLOGICAL DATA[a]

Barbituric acid derivative	No. of animals	M. E. D., mg. per kg.	M. L. D., mg. per kg.	% of M.L.D. required to abolish ear reflexes	M. L. D. / M. E. D.
Diethyl	32	300	400	75	1.33
Phenyl-ethyl	24	175	240	73	1.37
n-Amyl-ethyl	18	80	210	38	2.63
Iso-amyl-ethyl	18	72	180	40	2.50
Sec.-butylcarbinylethyl	18	80	210	38	2.63
Propylmethylcarbinylethyl	18	35	90	39	2.57
Diethylcarbinyl-ethyl	16	35	90	39	2.57
Iso-propylmethylcarbinyl-ethyl	11	80	180	44	2.25
Cyclopentyl-ethyl	18	80	180	44	2.25
n-Butyl-ethyl	24	100	200	50	2.00
Isobutyl-ethyl	24	110	260	42	2.36
Sec.-butyl-ethyl	16	50	140	36	2.80
Propylmethylcarbinyl-allyl[b]	24	35	90	39	2.57
Diethylcarbinyl-allyl	27	40	100	40	2.50
Cyclopentyl-allyl	23	50	130	38	2.60
n-Butylmethylcarbinyl-ethyl	20	60	140	43	2.33
β-Hydroxyethyl-ethyl	7	Absent	>2000		
Diethylcarbinyl	5	Absent	>4000		
Propylmethylcarbinyl	5	Absent	>5000		
Cyclopentyl	5	Absent	>1000		
Sec.-butyl	5	Absent	>1000		

[a] We are indebted to W. E. Fry for his careful and painstaking work in administering the solutions of the sodium salts and in observing the condition of the animals at regular intervals after the administration of each compound.

[b] Boedecker and Ludwig, *Arch. exp. path. Pharmakol.*, **139**, 361 (1929), mention this acid.

undoubtedly led to the many statements that the various barbituric acids are not essentially different in their action.

It is possible by varying the substituent groups to change or modify the pharmacological properties of the barbituric acids. Dimethyl and hydroxyethyl-ethylbarbituric acids are devoid of hypnotic activity, while di-iso-amylbarbituric acid causes only muscular incoördination. Propylmethylcarbinyl-ethylbarbituric acid is eight times as active as diethylbarbituric acid, and twice as active as iso-amyl-ethylbarbituric acid. *n*-Butylmethylcarbinyl-ethylbarbituric acid is three times as active as its isomer, di-*n*-butylbarbituric acid. *Sec.*-butyl-ethylbarbituric acid is twice as effective as *n*-butyl-ethylbarbituric acid. While three-fourths of the M. L. D. of diethyl and phenyl-ethylbarbituric acid is required to produce a state of anesthesia in rats, only two-fifths of the M. L. D. of the various amyl-ethylbarbituric acids are required. Death after the administration of a fatal dose of the amyl-ethylbarbituric acids occurs usually within the hour, while with diethylbarbituric acid it is delayed for ten to

twenty-four hours. On the other hand, animals recover from a sub-lethal dose of iso-amyl-ethylbarbituric acid in half the time required for recovery from diethylbarbituric acid, indicating that the body is able to destroy or eliminate the one more readily than the other. Eddy,[22] who administered various barbituric acids orally to cats, noted that they did not act in an identical manner. Loevenhart,[23] found that iso-amyl-ethylbarbituric acid was more than five times as effective as diethylbarbituric acid in protecting rabbits against death from procaine hydrochloride.

When considered from a molecular basis, the differences are even more significant; for example, a molecule of iso-amyl-ethylbarbituric acid is five times as effective as a molecule of diethylbarbituric acid, while a molecule of propylmethylcarbinyl-ethylbarbituric acid is ten times as effective in producing anesthesia in rats.

These changes, caused by varying the substituent groups, have been observed in mice, rats and dogs. It is quite probable that in man somewhat similar relationships hold qualitatively if not quantitatively. The selection of the most suitable member of that group having the highest therapeutic index, for medical use, must be dependent on additional pharmacological factors.

In considering the isomeric amyl-alkylbarbituric acids, slight variations only are noted in the therapeutic index. It is probable that a 10% variation in this ratio may occur due to biological variations beyond the control of the investigator. Consequently one could not ascribe the differences obtained in the therapeutic index to differences in the structure of the amyl groups. With the isomeric butyl-ethylbarbituric acids the differences in the therapeutic index seem too wide to be ascribed solely to biological variation and indicate the superiority of the *sec.*-butyl group in this series. In the butyl-alkyl series, Volwiler's[3c] data show that while the *sec.*-butyl-allylbarbituric acid was effective at a lower dose than was the iso- or *n*-butyl derivative, it had the lowest ratio for M. L. D./M. E. D. This is in contrast to the butyl-ethyl series in which the *sec.*-butylethylbarbituric acid gives the highest therapeutic ratio.

The most striking effect was the observation that the *sec.*-butylethyl and both the propylmethylcarbinyl and the diethylcarbinylethyl and allylbarbituric acids were effective in half the amount required for the primary isomers. In contrast to this, isopropylmethylcarbinyl and cyclopentylethylbarbituric acids were no more effective than the primary isomers, while the cyclopentyl-allyl-barbituric acid was almost as effective as the propyl-methylcarbinyl isomers. The pharmacological significance of this difference in the action of the secondary groups is being investigated.

The therapeutic indices of the *sec.*-butyl, *sec.*-amyl and *sec.*-hexyl deriva-

[22] Eddy, *J. Pharmacol.*, **33**, 43 (1928).

[23] Knoefel, Herrick and Loevenhart, *ibid.*, **33**, 265 (1928).

tives show a slight decrease as the molecular weight increases, although less of the *sec.*-amyl derivative is required to produce anesthesia than of the *sec.*-butyl or the *sec.*-hexyl derivatives.

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Summary

1. The following new esters of diethylmalonate have been prepared and described: diethylcarbinyl, cyclopentyl, *n*-butyl-allyl, β-hydroxyethyl-ethyl, diethyl-carbinyl-ethyl, isopropylmethylcarbinyl-ethyl, cyclopentyl-ethyl, *n*-butylmethylcarbinyl-ethyl, di-*n*-propylcarbinyl-ethyl, phenyl-ethyl-ethyl and *n*-heptyl-ethyl.

2. The following new barbituric acids have been prepared and described: cyclopentyl, cyclopentyl-ethyl, *n*-butyl-methylcarbinyl-ethyl, propylmethylcarbinyl-allyl, diethylcarbinyl-allyl, and cyclopentyl-allyl barbituric acids.

3. No marked difference was found in the ratio, M. L. D./M. E. D. of the various isomeric amyl-ethyl or allyl barbituric acids. The ratio obtained for the amyl group was, however, double that obtained for diethyl or phenyl-ethylbarbituric acid. The secondary isomers usually were effective at a much lower dose than were the primary.

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[CONTRIBUTION FROM THE LABORATORIUM FUER CHEMISCHE TECHNOLOGIE DER UNIVERSITAET WIEN, VIENNA, AUSTRIA, AND NORTHWESTERN UNIVERSITY MEDICAL SCHOOL, DEPARTMENT OF RESEARCH BACTERIOLOGY, CHICAGO, ILLINOIS]

TWO ISOMERIC QUINONEDITHIOGLYCOLIC ACIDS

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In the course of some work on the formation and properties of a variety of thioindigoid derivatives, the preparation of a 1,4-benzoquinone-2,5-dithioglycolic acid was attempted.

Usually the arylthioglycolic acids are prepared by alkaline condensation of the corresponding mercaptan with monochloro-acetic acid. As to the method of preparing the quinonedithioglycolic acids a general procedure was given by German Patent 175,070. According to this patent, *p*-quinones are found to react with substances represented by the general formula RSH (R representing an acid radical) as indicated by the equation