2. Studies made by the Thunberg method on the reducing power of such suspensions for methylene blue revealed differences in the speed of enzyme action which were dependent on the amount of deuterium oxide in the suspensions employed. As small a quantity of D_2O as 1:2000, added to water, produced a difference in the speed of decolorization of the dye, accelerating the reaction.

3. Similar differences in the speed of catalase activity of both muscle and *Lupinus albus* seed extracts were also noted.

4. The possible physiological importance of the phenomena was discussed.

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[FROM THE RESEARCH LABORATORY OF PARKE, DAVIS AND COMPANY]

Tertiary Alkylbarbituric Acids

BY A. W. DOX AND W. G. BYWATER

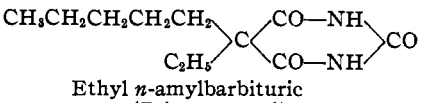
The hypnotic properties of 5,5-dialkylbarbituric acids have been the subject of extensive investigations for more than three decades. A goodly number of these products are now available for therapeutic use, and the number of unknown isomers and homologs with probable hypnotic properties is still greater. In general, those derivatives which have been found most satisfactory have contained one ethyl group and a second hydrocarbon radical with five or six carbon atoms. Within these limits are the well-known drugs Phenobarbital, Phanodorm, Amytal, Pentobarbital and Ortal.

Isomers may differ considerably in potency, duration of effect and therapeutic ratio. A notable instance is the great difference in potency between the isoamyl and the 1-methylbutyl derivatives, where the only variation in structure is a branching at the near or at the far end of the hydrocarbon chain. Further examination to include other isomeric amyl derivatives shows that the difference is a matter of primary *versus* secondary amyl groupings. Thus the primary amyls, *n*-amyl $EtCH_2CH_2CH_2$, isoamyl Me_2CH-CH_2CH_2, and 2-methylbutyl $EtCHMeCH_2$, have practically the same potency, whereas the secondary amyls, 1-methylbutyl $EtCH_2CHMe$ and 1-ethylpropyl Et_2CH, are about twice as effective. In lower homologs the difference is less striking, *e. g.*, propyl *vs.* isopropyl, and butyl or isobutyl *vs.* secondary butyl show less pronounced differences. In the hexyl series adequate data are not available. A comparison between the open-chain hexyls and the secondary hexyls of cyclic structure is hardly valid. With increasing number of carbon atoms the situation becomes increasingly complex. The three primary hexyls, *n*-hexyl, 2-ethylbutyl and 2-methylamyl have about the same potency, but the secondary hexyls are not yet available. Where two branchings occur in the chain, as in 1,2-dimethylpropyl and 1,3-dimethylbutyl, the situation is complicated by a convulsive action of the drug.

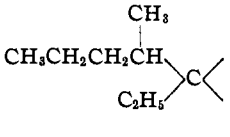
The wide difference in potency between the primary and secondary amyl derivatives suggested the possibility of a similar difference between the secondary and tertiary derivatives. No tertiary alkyl barbituric acids are described in the literature. It is hardly likely that no efforts have been made to prepare such derivatives. Probably the poor yields of the intermediate malonic esters have discouraged investigators from pursuing such attempts. Fischer and Dilthey were unable to obtain the diisopropylmalonic ester and attributed their failure to steric hindrance. The same reasoning would apply to esters of tertiary alkylmalonic acids. However, by recourse to other procedures not involving the use of malonic esters, diisopropyl- and diphenylbarbituric acids have been prepared. Their physiological properties were disappointing, aside from the difficulty of synthesis.

Our purpose was to obtain tertiary alkylethylbarbituric acids in quantities just sufficient for physiological testing, regardless of yields, in order to determine whether the increased potency of secondary over primary alkyls would be carried further into the tertiary series. Tertiary butyl- and tertiary amyl-ethylbarbituric acids were thus prepared in small yields and tested for hypnotic action. They differed in melting points from the isomeric primary and secondary butyl and amyl derivatives, all of which are known, hence a possible rearrangement into primary or secondary

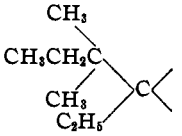
alkyls is precluded. The expectation that their physiological activity would be greater than that of the isomeric secondary derivatives was, however, not realized.



Ethyl *n*-amylbarbituric
(Primary amyl)



Ethyl 1-methylbutyl-
(Secondary amyl)



Ethyl 1,1-dimethylpropyl-
(Tertiary amyl)

Ethyl Tertiary Butylmalonate.—This ester has been prepared in small yield by Abderhalden and Rossner[1] but the physical constants have not been reported. By allowing molecular proportions of ethyl malonate, alcoholic sodium ethoxide and tertiary butyl bromide to react for twenty-four hours at 5°, then at room temperature for several days, and subjecting the reaction mixture to repeated fractionation, we obtained a 6.4% yield of the tertiary ester. It boiled at 205–210° (750 mm.), had $n^{20}D$ 1.4180 and d^{25}_{25} 1.0144. The smallness of the yield is due to a competing reaction whereby halogen acid is removed from the butyl bromide with formation of the unsaturated hydrocarbon.

Tertiary Butylbarbituric Acid.—The usual barbituric acid synthesis by condensing the malonic ester with urea in the presence of three equivalents of alcoholic sodium ethoxide for eight hours at 105° and precipitating the acid from its sodium salt yielded a white crystalline product with melting point 230°. After recrystallization from benzene it melted at 230–231°.

Anal. Calcd. for $C_8H_{12}N_2O_3$: N, 15.21. Found: N, 15.52. Like all known mono-alkylbarbituric acids it was physiologically inert.

Ethyl Tertiary Butylethylmalonate.—The condensation between ethyl ethylmalonate and tertiary butyl bromide with alcoholic sodium ethoxide was unsuccessful, no appreciable yield of a higher boiling ester being obtained. A small yield was obtained, however, by using sodium powder in dry toluene in place of alcoholic sodium ethoxide. Fractionation of the toluene solution then gave a 3.8% yield of an ester with boiling point 244–248°, $n^{20}D$ 1.4335 and d^{25}_{25} 0.9829.

Tertiary Butylethylbarbituric Acid.—The above ester was condensed with urea by the usual procedure and a barbituric acid with melting point 191.5–192.5° obtained. We did not succeed in purifying it to the theoretical nitrogen content. *Anal.* Calcd. for $C_{10}H_{16}N_2O_3$: N, 13.20. Found: N, 14.31, 14.34. The impurity which raised the nitrogen content was probably the mono-tertiary butylbarbituric acid, or diethylbarbituric acid, since ethyl ethylmalonate invariably contains small amounts of ethyl malonate and ethyl diethylmalonate. On this assumption the actual content of tertiary butylethylbarbituric acid in the preparation would be 46.5%.

(1) Abderhalden and Rossner, *Z. physiol. Chem.*, **163**, 177 (1926).

Ethyl Tertiary Amylethylmalonate.—The reaction was performed by allowing a solution containing ethyl ethylmalonate, sodium ethoxide and tertiary amyl bromide in molecular proportions to stand at room temperature for twenty-five days. The higher boiling product was then separated by fractionation. The yield was 4.6% of an oily liquid boiling at 93–96° at 1.5 mm., $n^{20}D$ 1.4435.

Tertiary Amylethylbarbituric Acid.—Condensation of the ester with urea by the usual procedure gave a white crystalline product with melting point 196.5–197.5°. Recrystallization from benzene–petroleum ether did not change the melting point. *Anal.* Calcd. for $C_{11}H_{18}N_2O_3$: N, 12.41. Found: N, 12.92.

Physiological Tests.—White mice were used for testing hypnotic action, the substance in each case being administered intraperitoneally as the sodium salt. In the table the isomeric primary and secondary alkylethylbarbituric acids are included for purposes of comparison. The results are expressed as minimum lethal dose (MLD), maximum tolerated dose (MTD) and minimum effective dose (MED) in mg. per g. body weight. The MED is taken as that dose which caused the animals to lie prostrate without attempting to rise.

TABLE I

Barbituric acid	MLD	MTD	MED
Ethyl *n*-butyl	0.4	0.3	0.15
Ethyl isobutyl	.4	.3	.125
Ethyl *s*-butyl	.2	.15	.05
Ethyl *t*-butyl (50%)	.5	.4	.2
t-Butyl	>1.2	inert	inert
Ethyl isoamyl	0.2	..	0.1
Ethyl 1-methylbutyl	.125	..	.05
Ethyl *t*-amyl	.15	0.1	.075

It appears that these tertiary alkylethylbarbituric acids are somewhat more effective than the isomeric primary derivatives, but not more so than the secondary isomers. The tertiary amyl had a much more prolonged effect than the secondary amyl. Aside from the very low yields obtainable by ordinary methods of synthesis, the tertiary alkyl derivatives seem to have no advantage over the secondary isomers and their commercial development is not recommended. The material available was insufficient for tests on larger animals.

Summary

Tertiary butyl- and tertiary amyl-ethylbarbituric acids were obtained in small yield through the corresponding malonic esters. The amyl derivative is believed to be reasonably pure, the butyl derivative about 50% pure. Tertiary butylbarbituric acid, like all known mono-alkylbarbituric acids, is physiologically inert. Contrary to expectation the tertiary alkylethyl derivatives are not more active physiologically than the secondary isomers.

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