The rectal temperature fell rapidly during the first two hours, in one animal as much as 4.2° without the production of shivering or rigor.

All animals treated were fully recovered in 24 hours and showed no harmful after-effects.

In comparison with monocarbethoxy-ethyl-iso-urea the dicarbethoxy derivative shows more rapid and intense central depressant action and greater effect on body temperature. There is, however, a complete disappearance of muscular hypertonus with this compound.

Studies in oxygen consumption on animals treated with the iso-urea derivatives indicate that the fall of temperature must be due mainly to increased heat loss. This effect is similar to the antipyresis induced by acetanilide and allied compounds of the aromatic series.

Summary

A study of the methods of preparation of mono- and dicarbethoxy-guanidines, and of dicarbethoxy-ethyl-iso-urea, has been made and some modifications of existing methods have been introduced.

Preliminary observations on the pharmacological properties of these compounds are recorded.

SASKATOON, CANADA

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

PIPERIDINE DERIVATIVES. II. 1-ALKYL-3-CARBETHOXY-4-PIPERIDYL BENZOATES

BY S. M. MCELVAIN

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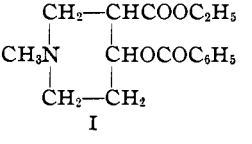
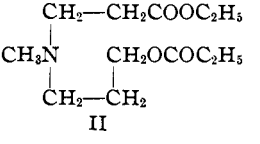
In an earlier paper[1] the preparation of a piperidine derivative (I) with essentially the same structure as the piperidine portion of the cocaine molecule was described. It was compared as to physiological action with the compound (II) which has the same characteristic groups but an open-chain structure.



I



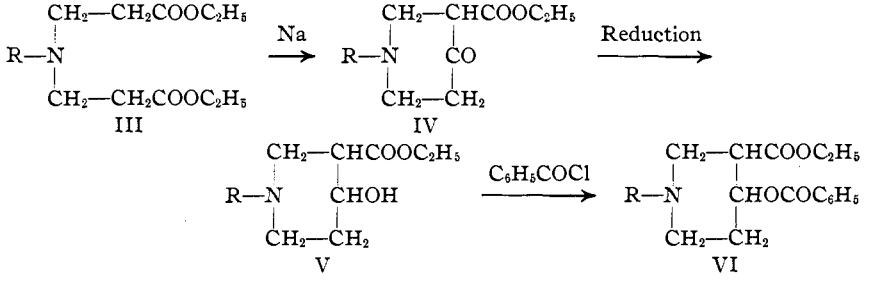
II

Inasmuch as the piperidine derivative possessed considerable physiological activity and the open-chain compound showed practically none, it appeared that a more detailed study of substances of Type I might bring to light some relationship between chemical constitution and physiological action.

[1] McElvain, THIS JOURNAL, **46**, 1721 (1924).

In the procaine series it is well known that the physiological action of the anesthetic is dependent to a considerable extent upon the alkyl groups which are attached to the nitrogen atom. Therefore it seemed that the first point of study of compounds of Type I should be in connection with the various alkyl groups which might be attached to the nitrogen atom.

This paper is concerned with the preparation and brief pharmacological report of a series of these 1-alkyl-3-carbethoxy-4-piperidyl benzoates. The preparation of these compounds is analogous to that of I. The β,β'-dicarbethoxy-diethyl alkyl amines (III) were condensed by sodium to the 1-alkyl-3-carbethoxy-4-piperidones (IV). These piperidones on catalytic reduction yielded the corresponding 4-hydroxy-piperidines (V), which on benzoylation gave the benzoates (VI). These reactions are represented as follows.



The compounds in which R is ethyl, *n*-propyl, *iso*propyl, *n*-butyl, *iso*-butyl, *sec.*-butyl, *n*-amyl and *iso*-amyl have been synthesized. The internal condensations of the diesters took place very smoothly, giving in most cases 50–60% yields of the piperidones. It was noticed that there was a tendency for the piperidone hydrochlorides to remain in a non-crystalline state as the size of the R group was increased. In the case of the *n*-butyl, *iso*butyl and *n*-amyl compounds this tendency lowered somewhat the yields of crystalline material. In the case of the *sec.*-butyl piperidone the hydrochloride could not be caused to crystallize.

None of the hydrochlorides of the 4-hydroxy-piperidines was crystalline. When these substances were purified they were isolated as the free bases by distillation. The butyl and amyl derivatives could not be purified satisfactorily because they decomposed to a very considerable extent on distillation at 1 mm. pressure.

With the benzoates also the tendency to remain in a non-crystalline state was quite marked. Salts of the *iso*propyl, butyl and amyl derivatives crystallized with difficulty and there was always considerable non-crystallizable material in the mother liquors. This difficulty in crystallization was probably due to stereoisomerism, the effect of which was more pronounced as the size of the groups in the 1 position was increased.

Experimental Part

β,β′-Dicarbethoxy-diethyl Alkyl Amines.—A solution of 0.5 mole of the primary alkyl amine in 400 cc. of 95% alcohol was treated with 90 g. (0.5 mole) of ethyl β-bromopropionate. The resulting solution became warm and was vigorously stirred until it cooled. While the stirring was continued, 80 g. of silver oxide was added, followed by 45 g. (0.25 mole) of ethyl β-bromopropionate. The mixture again warmed to some extent and after it had cooled, 40 g. of silver oxide was added. Finally, another 45 g. of ethyl β-bromopropionate and 40 g. of silver oxide were added and the mixture was stirred for two hours. The silver halide was filtered off and washed with 100 cc. of alcohol. The alcohol was removed from the filtrate by distillation and the remaining oil dissolved in 500 cc. of ether. The ether solution was washed twice with a dilute solution of potassium cyanide, after which the ether was removed and the remaining oil distilled under diminished pressure. The tertiary amine fraction was collected over a 10–15° range and was used as such in further syntheses. The yields were from 65 to 75%.

For analyses and physical constants the primary and secondary amines were removed from the tertiary amine by nitrous acid. This treatment gave a constant-boiling product.

TABLE I

β,β′-DICARBETHOXY-DIETHYL ALKYL AMINES

Alkyl group	Formula	B. p., °C. (2 mm.)	d_{20}^{20}	n_D^{20}	Analyses, N, % Calcd.	Found (Kjeldahl)
Ethyl	$C_{12}H_{23}O_4N$	126–128	1.0058	1.4385	5.71	5.87
n-Propyl	$C_{13}H_{25}O_4N$	132–134	0.9951	1.4393	5.40	5.60
*iso*Propyl	$C_{13}H_{25}O_4N$	128–130	.9960	1.4388	5.40	5.52
n-Butyl	$C_{14}H_{27}O_4N$	154–156	.9804	1.4400	5.12	5.21
*iso*Butyl	$C_{14}H_{27}O_4N$	153–156	.9766	1.4384	5.12	5.18
sec.-Butyl	$C_{14}H_{27}O_4N$	145–148	.9861	1.4415	5.12	5.27
n-Amyl	$C_{15}H_{29}O_4N$	164–168	.9669	1.4375	4.88	5.05
iso-Amyl	$C_{15}H_{29}O_4N$	160–164	.9689	1.4370	4.88	4.95

1-Alkyl-3-carbethoxy-4-piperidone Hydrochlorides.—A solution of 0.15 mole of the β,β′-dicarbethoxy-diethyl alkyl amine in 40 cc. of xylene was added to 3.5 g. of powdered sodium and the mixtureg ently warmed in an oil-bath until the reaction started. The condensation generated sufficient heat to cause the xylene to reflux. After this initial reaction subsided, the mixture was refluxed gently for 20 minutes until all of the sodium had disappeared. The reaction mixture was cooled and treated with 100 cc. of ice water and the resulting suspension extracted with 100 cc. of ether. The aqueous layer was separated, cooled by the addition of ice, and acidified to congo red with hydrochloric acid. Potassium carbonate was then added in excess and the free piperidone base extracted from the aqueous suspension by three 100cc. portions of ether. The

ether solution was then treated with dry hydrogen chloride in order to precipitate the hydrochloride. This salt was recrystallized from an alcohol-ether mixture. The yields were 50–60%.

TABLE II

1-ALKYL-3-CARBETHOXY 4-PIPERIDONE HYDROCHLORIDES

Alkyl group	Formula	M. p., °C.	Analyses, Cl, % Calcd.	Found
Ethyl	$C_{10}H_{18}O_3NCl$	143–145	15.10	15.21
n-Propyl	$C_{11}H_{20}O_3NCl$	138–140	14.23	14.37
*iso*Propyl	$C_{11}H_{20}O_3NCl$	144–146	14.23	14.36
n-Butyl	$C_{12}H_{22}O_3NCl$	127–129	13.48	13.78
*iso*Butyl	$C_{12}H_{22}O_3NCl$	124–126	13.48	13.37
sec.-Butyl	$C_{12}H_{22}O_3NCl$	Noncrystalline	...	...
n-Amyl	$C_{13}H_{24}O_3NCl$	141–143	12.80	12.73
iso-Amyl	$C_{13}H_{24}O_3NCl$	153–155	12.80	13.00

1-Alkyl-3-carbethoxy-4-hydroxy-piperidines.—A solution of 10 g. of the 1-alkyl-3-carbethoxy-4-piperidone hydrochloride in 100 cc. of alcohol was reduced catalytically in the manner which has been described[2] for 1-methyl-3-carbethoxy-4-hydroxy-piperidine. The free base was isolated because the hydrochlorides could not be caused to crystallize. Only the ethyl and two propyl derivatives were isolated, on account of the fact that the higher alkyl derivatives decomposed on distillation. The yields were about 70%.

TABLE III

1-ALKYL-3-CARBETHOXY-4-HYDROXY-PIPERIDINES

Alkyl group	Formula	B. p., °C. (2 mm.)	d_{20}^{20}	n_D^{20}	Analyses, N, % Calcd.	Found
Ethyl	$C_{10}H_{19}O_3N$	130–132	1.0754	1.4712	6.96	7.10
n-Propyl	$C_{11}H_{21}O_3N$	132–133	1.0502	1.4680	6.51	6.58
*iso*Propyl	$C_{11}H_{21}O_3N$	128–130	1.0522	1.4695	6.51	6.63

1-Alkyl-3-carbethoxy-4-piperidyl Benzoate Salts.—The semi-solid amorphous residue obtained after the removal of the solvent from the catalytic reduction of 10 g. of the 1-alkyl-3-carbethoxy-4-piperidone hydrochloride was treated with 20 cc. of benzoyl chloride. This mixture was heated in an oil-bath at 160–170° for 15 minutes. The benzoylation took place rapidly with the evolution of hydrogen chloride. The resulting solution was cooled and diluted with 300 cc. of ether, whereupon an oily precipitate of the hydrochloride of the benzoate formed. This ether suspension was left in an ice box for several hours for crystallization. The ether was then decanted and the hydrochloride recrystallized from an alcohol-ether mixture. The yields of recrystallized material were 40–50% of those theoretically possible from the piperidones.

The *iso*propyl and *sec.*-butyl derivatives could not be crystallized as the hydrochlorides. The free bases were liberated into ether and re-

[2] Ref. 1, p. 1725.

precipitated as the hydrobromides. These salts finally crystallized after several days of standing in an ice box.

TABLE IV

1-ALKYL-3-CARBETHOXY-4-PIPERIDYL BENZOATE SALTS

Alkyl group	Salt	Formula	M. p., °C.	Analyses, halogen, % Calcd.	Found
Ethyl	Hydrochloride	$C_{17}H_{24}O_4NCl$	214–216	10.39	10.37
n-Propyl	Hydrochloride	$C_{18}H_{26}O_4NCl$	208–210	10.00	10.15
*iso*Propyl	Hydrobromide	$C_{18}H_{26}O_4NBr$	161–162	20.00	20.05
n-Butyl	Hydrobromide	$C_{19}H_{28}O_4NBr$	194–196	19.32	19.40
n-Butyl	Hydrochloride	$C_{19}H_{28}O_4NCl$	175–177	9.60	9.64
*iso*Butyl	Hydrochloride	$C_{19}H_{28}O_4NCl$	197–199	9.60	9.80
sec.-Butyl	Hydrobromide	$C_{19}H_{28}O_4NBr$	162–164	19.32	19.48
n-Amyl	Hydrochloride	$C_{20}H_{30}O_4NCl$	164–166	9.26	9.24
iso-Amyl	Hydrochloride	$C_{20}H_{30}O_4NCl$	179–181	9.26	9.17

Pharmacological Studies of the 1-Alkyl-3-carbethoxy-4-piperidyl Benzoates[3]

The anesthetic efficiencies of these compounds were determined by applying a 2% solution of the drug to the rabbit's cornea for about one minute and testing the winking reflex every minute to determine the duration of anesthesia. The technique is described in detail by Schmitz and Loevenhart.[4]

Since only small amounts of these drugs were available, their toxicities were determined by subcutaneous injections near the root of the tail in white mice. The minimum lethal dose in Table V is the amount in milligrams per kilogram of body weight which kills at least 50% of the mice injected. The maximum tolerated dose is the largest amount which did not kill more than one out of five mice. The majority of the mice either died or recovered from all symptoms within a few hours and were observed for only 24 hours. Deaths occurring after 24 hours were not attributed to the drugs. The toxic symptoms caused by the drugs were similar to those caused by cocaine. They usually begin in five to ten minutes, the first symptom noted being marked depression. Following this the respiration becomes very rapid and the hind legs become paralyzed. Then the mouse becomes very excited and soon clonic convulsions develop, the mouse throwing itself about the cage or lying on its back or side exhibiting running movements. The convulsions last from ten minutes to two hours and are followed by depression and dyspnea. When the mouse recovered, the respiration gradually became faster and deeper, but in cases terminating in death, the respiration became slower finally ending in irregular gasps. Exophthalmos was observed in the majority of the cases.

[3] This section is contributed by Ralph E. Jones of the Department of Pharmacology of the University of Wisconsin. The author wishes to express his thanks and to acknowledge his indebtedness to Mr. Jones for this study.

[4] Schmitz and Loevenhart, *J. Pharm. Exptl. Therap.*, **24**, 159 (1924).

TABLE V

PHARMACOLOGICAL RESULTS

Alkyl group	Salt	Duration of anesthesia 2% solution applied to rabbit's cornea for 1 min. Expts.				Av. in min.	No. of mice used	Toxicity for white mice Maximum tolerated Mg. per kg.	Minimum lethal Mg. per kg.
Cocaine	HCl	36	31	28	24				
		30	33	29	24				
		32	29			29	18	100	150
Procaine	HCl	No complete anesthesia				..	15	800	900
Methyl	HCl	8	7	10	6				
			9	11	6	8	12	50	100
Ethyl	HCl	No complete anesthesia				..	15	100	150
n-Propyl	HCl	12	12	16					
		22	18	14		16	13	200	250
*iso*Propyl	HBr	13	15	15					
		14	16	18		15	15	120	150
n-Butyl	HCl	36	29	23					
		28	28			29	23	1500	1600
n-Butyl	HBr	27	28	35					
		32	44	35		33	14	1100	1200
*iso*Butyl	HCl	14	13	11					
		15	14	10		13	15	2400	2500
sec.-Butyl	HBr	10	16	17					
		10	17	15		14	13	450	500
n-Amyl	HCl	43	54	47					
		41	43			46	6	3000	4000
iso-Amyl	HCl	44	41	37					
		40	40			40	6	4000	4500

Discussion of Pharmacological Data

It will be noted from these pharmacological studies of Mr. Jones that the toxicity of the *iso*propyl derivative is considerably higher than that of the *n*-propyl derivative and that the *secondary* butyl derivative is likewise much higher in toxicity than the other butyl derivatives. This deviation is explained in part by the fact that the hydrobromide salts were used. As is indicated in the case of the *n*-butyl derivative, these salts show a higher toxicity than the corresponding hydrochlorides.

The striking feature of the series is the marked decrease in toxicity of these compounds as the size of the alkyl group attached to the nitrogen is increased. The increase in anesthetic action with the size of the alkyl group would be predicted, but the toxicity behavior deviates very widely from previous observations on the relationship of structure to physiological action. It is interesting to note that the amyl derivatives show about one-fifth of the toxicity of procaine or one-thirtieth of that of cocaine. At the same time their anesthetic power is considerably greater than that of cocaine.

The solutions of the salts were acid to litmus, and attempts to neutralize

them by the addition of sodium hydroxide resulted in precipitation of the free base. This acidity was responsible for the hyperemia and marked irritation which were noted with all of the compounds, and possibly tended to shorten the duration of the anesthesia because of the excessive lacrimation. It is hoped that further modification of the structure will eliminate this objectionable feature.

Summary

Thes yntheses of several 1,3,4-trisubstituted piperidines have been described and some of their properties noted.

The 1-alkyl-3-carbethoxy-4-piperidyl benzoates have very marked local anesthetic action. They show an increase in anesthetic action and a decrease in toxicity as the size of the alkyl radical attached to the nitrogen increases. The amyl derivatives possess a greater anesthetic power than cocaine and show only about one-thirtieth of its toxicity.

As pointed out, the salts of these compounds are acid in aqueous solution and cause irritation and hyperemia.

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[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMICAL RESEARCH, OKLAHOMA AGRICULTURAL AND MECHANICAL COLLEGE]

A MICRO-COLORIMETRIC METHOD FOR THE ESTIMATION OF PHOSPHOLIPINS IN SEEDS[1]

BY N. B. GUERRANT

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Introduction

The attention of the biological chemist has been attracted for a number of years to the presence of those phosphorus-containing lipoid substances found in both plant and animal tissues. The universal distribution of these substances seems to indicate that they play an important part in the metabolic processes of the living cell. There is little doubt that they serve as a source of the phosphorus required for building up the complex nucleoproteins of cell nuclei. Indeed it might be said that these substances play an important role in the making of the essential substratum of living matter.

This important class of substances has been designated by Leathes[2] as phospholipins. The exact chemical structure of none of the phospholipins has been definitely established, and the constituents of only a few of them have been approximated.[3] However, a considerable amount of

[1] Published with the permission of the Director of the Oklahoma Agricultural Experiment Station.

[2] Leathes, "The Fats," Longmans, Green and Co., London, 1910.

[3] Maclean, "Lecithin and Allied Substances," Monograph of Biochemistry, Longmans, Green and Co., 1918.