A total of 150 mg (7.1%) of the hydrochloride was isolated. Recrystallization from isopropanol gave an analytical sample (Table II); TLC (free base) (5% methanol–benzene): R_f 0.44; IR (KBr): ν_{max} 3300 (OH), 2930 and 2850 (CH_2), 2750 (R_2NH^+), and 1165 and 1120 (CF_3) cm^{-1}. On standing, the melting point of the salt dropped to 120–130°, although TLC showed a single spot with the same R_f. TLC in two additional systems also showed a single component. The decrease in melting point may have been due to crystalline polymorphism.

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Synthesis and Biological Evaluation of Potential Hypoglycemic Agents I: Carnitine Analogs

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Abstract □ A series of 4-dialkylamino-3-hydroxybutyric acid hydrochlorides and methochlorides, analogs of carnitine, was synthesized by treatment of *tert*-butyl 3,4-epoxybutyrate with the appropriate amine or amine hydrochloride in methanol followed by mild acid hydrolysis. The compounds had no effect on blood glucose or serum fatty acid levels in rats.

Keyphrases □ Carnitine analogs—potential hypoglycemic agents, synthesis, biological evaluation, IR and NMR spectra □ Hypoglycemic agents, potential—carnitine analogs, synthesis, biological evaluation, IR and NMR spectra

For years, diabetes mellitus has been described as a disorder of carbohydrate metabolism and the changes observed in lipid metabolism were thought to be secondary. Recently, however, Randle (1) suggested that the primary event in the development of diabetes mellitus might be an abnormality of glyceride metabolism.

Randle suggested a possible mechanism involving an increased release of free fatty acids. This release, in turn, produces a resistance to the hypoglycemic action of insulin, thereby causing a rise in fasting glucose levels and eventual exhaustion of the pancreatic β-cells. As a result of the increased serum free fatty acid levels, a corresponding increase in the rate of fatty acid oxidation is noted. This increase causes the tissue concentration of acetyl coenzyme A to rise, which results in a disruption of carbohydrate metabolism (2) through inhibition of several enzymes (pyruvate dehydrogenase, phosphofructokinase, and hexokinase).

If this hypothesis is correct, a possible approach to the treatment of diabetes mellitus would involve the design of agents that would decrease the high rate of fatty acid oxidation and increase glucose oxidation. Evidence in support of this approach is provided by the hypoglycemic activity of α-bromopalmitic acid (3), a known inhibitor of fatty acid oxidation (4).

Using the Randle hypothesis as a basis, Stewart

Table I—Properties of Compounds III*a*–III*d* and IV*a*–IV*d*

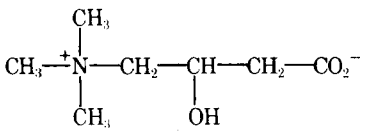
Compound	R_1	R_2	Yield, %	Physical Description (Melting Point when Crystalline)	IR Data, cm^{-1}	NMR Data, ppm	Formula	Analysis, % Calc.	Found
III*a*	CH_3CH_2	CH_3CH_2	41	Pale-yellow liquid[a]	Described under *Experimental*[b]		—	—	
III*b*	$(CH_2)_4$		81	Pale-yellow liquid[a]	3420 (OH) 1730 (C=O)	3.80–4.30 (m, 1H, —CH<)	—	—	
III*c*	$(CH_2)_5$		69	Pale-yellow liquid[a]	3420 (OH) 1730 (C=O)	3.80–4.15 (m, 1H, —CH<)	—	—	
III*d*	O<(CH$_2$)$_2$ / (CH$_2$)$_2$		49	Pale-yellow liquid[a]	3420 (OH) 1730 (C=O)	3.80–4.30 (m, 1H, —CH<)	—	—	
IV*a*	CH_3CH_2	CH_3CH_2	87	109–111°[c]	Described under *Experimental*[d]		$C_8H_{18}ClNO_3$	C 45.4 H 8.5 N 6.6	45.5 8.4 6.5
IV*b*	$(CH_2)_4$		92	83–88°[c]	1718 (C=O)	—	$C_8H_{16}ClNO_3$	C 45.8 H 7.6 N 6.7	45.8 7.6 6.5
IV*c*	$(CH_2)_5$		79	118–122°[c]	1718 (C=O)	—	$C_9H_{18}ClNO_3$	C 48.3 H 8.0 N 6.3	48.1 8.1 6.1
IV*d*	O<(CH$_2$)$_2$ / (CH$_2$)$_2$		85	168–170°[c]	1718 (C=O)	—	$C_8H_{16}ClNO_4$	C 42.6 H 7.1 N 6.2	42.8 7.1 6.1

[a] Attempted distillation of III*a*–III*d* led to extensive decomposition; therefore, no boiling-point or analytical data are reported. The spectral data are reported because this method was used for identification of these products. [b] IR spectra for III*a*–III*d* were run as liquid films. NMR spectra for III*a*–III*d* were run in deuterochloroform. [c] IV*a*–IV*d* were recrystallized from absolute ethanol–acetone to yield white prisms. [d] IR spectra for IV*a*–IV*d* were run as mineral oil mulls. NMR spectra for IV*a*–IV*d* were run in deuterium oxide.

and Hanley (5) suggested several sites where control of fatty acid metabolism is feasible. One possible point of pharmacological control would involve the inhibition of entry of long chain (>10 carbons) fatty acid coenzyme A derivatives into the mitochondria where oxidation occurs. Since long chain fatty acid coenzyme A derivatives cannot diffuse through the mitochondrial membrane, a transport system is required if they are to reach the enzymes involved in β-oxidation. This mechanism is called carnitine transport, and it is dependent on the presence of (−)-carnitine (I) and carnitine acyl transferases to transport long chain fatty acids into the mitochondria. The activity of this system has been shown to increase in livers of diabetic rats (6) and appears to be rate limiting for fatty acid oxidation.

Thus, the goals of this study were the design and synthesis of analogs of carnitine that would act as inhibitors of this transport system. Relatively few compounds have been studied as inhibitors of carnitine acyl transferases (7). Norcarnitine acts as a substrate in the absence of carnitine and as a competitive inhibitor in the presence of carnitine. Other related compounds, (*RS*)-β-hydroxy-γ-aminobutyric acid, *tert*-butylacetic acid, (*RS*)-β-hydroxy-γ-dimethylaminobutyramide, and choline, failed to act as inhibitors or substrates in this system.

A weak competitive inhibitor (3-deoxycarnitine) resulted when the β-hydroxyl group of carnitine is removed (7). The observed reduction of the affinity of



I

3-deoxycarnitine for the enzyme may result from a loss of the hydroxyl group as a binding site. Alternatively, the loss of the hydroxyl group may produce a change in the conformation of the inhibitor molecule, which alters its ability to bind to the enzyme.

These preliminary data indicate that the distance between the carboxyl group and the cationic function may be important. Therefore, initial synthetic efforts were directed toward compounds having the same chain length found in carnitine.

Thus, a series of dialkylaminohydroxybutyric acid hydrochlorides (IV*a*–IV*d*) and methochlorides (VI*a*–VI*c*), analogs of carnitine, was synthesized and evaluated with regard to their effects on blood glucose and serum free fatty acid levels.

DISCUSSION

tert-Butyl 3,4-epoxybutyrate (II) (8) was used as the starting material for the synthesis of the carnitine analogs IV*a*–IV*d* and VI*a*–VI*c* (Scheme I). The epoxy ester II readily reacted with the four secondary amines shown in Table I in methanol to provide the desired dialkylaminohydroxy esters (III*a*–III*d*). The yields were variable, and the major side reaction that occurred will be discussed in a future publication. The NMR spectral data afforded evidence that nucleophilic attack by the amine occurred at the desired 4-position and not at the 3-position. Hydrolysis of III*a*–III*d* with a dilute hydrochloric acid solution yielded the desired dialkylaminohydroxybutyric acid hydrochlorides (IV*a*–IV*d*) (Table I).

The synthesis of VI*a*–VI*c* was patterned after the synthesis of (*RS*)-carnitine chloride (8). It was found that II could be converted directly into the *tert*-butyl dialkylamino-3-hydroxybutyrate methochlorides (V*a*–V*c*) by treatment with the appropriate tertiary amine hydrochloride in methanol (Table II).

Hydrolysis of the esters V*a*–V*c* to the desired free acids VI*a*–VI*c* was again carried out by treatment with dilute hydrochloric acid. Although VI*a*–VI*c* (Table II) were all extremely hygroscopic (as were V*a*–V*c*), satisfactory NMR spectral data were obtained for all compounds. Since VI*c* could not be obtained in crystalline form, analytical data were obtained for the tetraphenylboronate salt (9).

Table II—Properties of Compounds Va–Vc and VIa–VIc

Compound	R_1	R_2	R_3	Yield, %	Physical Description (Melting Point when Crystalline)	NMR Data, ppm	Formula	Analysis, % Calc.	Found
Va	CH_3	CH_3	CH_3CH_2	90	Pale-cream semisolid[a,b]	Described under *Experimental*[c]	—	—	—
Vb	CH_3	CH_3CH_2	CH_3CH_2	67	Pale-yellow viscous liquid[a,b]	2.57 (d, J = 6 Hz, 2H, $-CH_2CO_2-$)	—	—	—
Vc	CH_3	CH_3	$CH_3CH_2CH_2CH_2$	81	Pale-yellow viscous liquid[a,b]	2.57 (d, J = 6 Hz, 2H, $-CH_2CO_2-$)	—	—	—
VIa	CH_3	CH_3	CH_3CH_2	44	White prisms[d]	Described under *Experimental*[c]	$C_8H_{18}ClNO_3$	C 45.4 H 8.5 N 6.6	45.2 8.4 6.4
VIb	CH_3	CH_3CH_2	CH_3CH_2	57	White prisms, 133–137°[e]	2.70 (d, J = 6 Hz, 2H, $-CH_2CO_2-$)	$C_9H_{20}ClNO_3$	C 47.9 H 8.9 N 6.2	48.1 8.8 6.3
VIc	CH_3	CH_3	$CH_3CH_2CH_2CH_2$	62	Thick viscous oil, 133–134°[f]	2.65 (d, J = 6 Hz, 2H, $-CH_2CO_2-$)	$C_{34}H_{42}BNO_3$	C 78.0 H 8.0 N 2.7	78.0 8.1 2.6

[a] Attempted purification of Va–Vc by crystallization was unsuccessful; therefore, no analytical data are reported. [b] IR spectra for Va–Vc and VIa–VIc could not be run because these compounds were extremely hygroscopic. [c] NMR spectra for Va–Vc and VIa–VIc were run in deuterium oxide. [d] Although satisfactory analytical data were obtained for this compound, it was too hygroscopic to obtain a melting point. [e] VIb was recrystallized from absolute ethanol–acetone. [f] VIc was analyzed as the tetraphenylboronate salt (Ref. 9) because the chloride could not be obtained in crystalline form.

RESULTS

Hypoglycemic Activity in Rats—Three control and three treated male rats, 140–160 g, from the local Harlan colony were fasted for 18 hr. All rats were given 100 mg of glucose in water subcutaneously, followed immediately by a single oral administration of the test compound in 5% aqueous acacia. Control rats received an equal volume of the vehicle. Blood samples were taken from the tail vein of all animals at 2 and 5 hr after administration of the test compound and were assayed for glucose with an automatic analyzer[1] using a modification of the method of Hoffman (10). Results for blood glucose are reported as the percent difference of the mean value for the treated animals from the mean value for the control animals.

No significant differences in the blood glucose levels relative to controls were observed for IVa–IVd and VIa–VIc when administered orally at a dose of 100 mg/kg.

Serum Free Fatty Acids—Three control and three treated male rats, 140–160 g, from the local Harlan colony were fasted for 18 hr. A single oral administration of the test compound in 5% aqueous acacia was given to the treated rats. Control rats received an equal volume of the vehicle. Blood samples were taken from the orbital sinus of all animals 1 hr after administration of the test compound for determination of serum free fatty acids. Serum free fatty acids were extracted using the method of Dole and Meinertz (11) and were assayed with an automatic analyzer[1] using the method of Baird *et al.* (12). Results for serum free fatty acids are reported as the percent difference of the mean value for the treated animals from the mean value for the control animals.

No significant differences in the serum free fatty acid levels relative to control levels were observed after IVa–IVd and VIa–VIc were administered orally at a dose of 100 mg/kg.

EXPERIMENTAL[2]

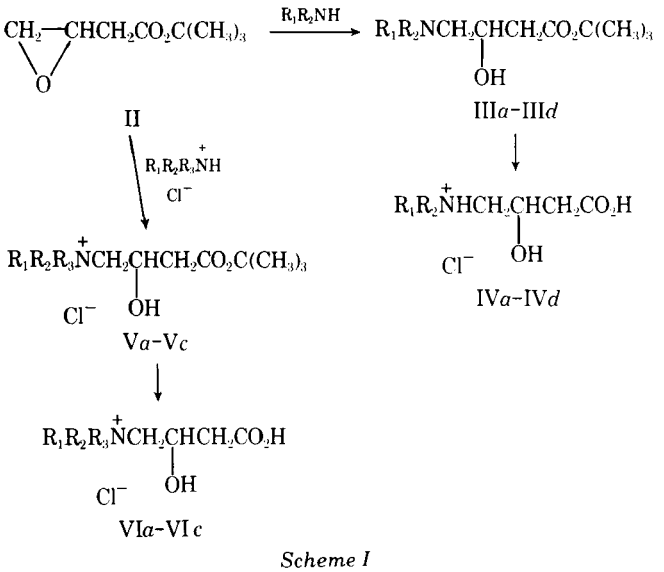
***tert*-Butyl 4-Diethylamino-3-hydroxybutyrate (IIIa)**—This example typifies the method used to prepare IIIa–IIId (Table I). To a solution of 1.58 g (0.010 mole) of *tert*-butyl 3,4-epoxybutyrate (II) (8) in 6 ml of methanol was added 1.3 ml (0.013 mole) of distilled diethylamine. The pale-yellow solution was stirred at 26° for 21 hr under a dry nitrogen atmosphere. The solvent was removed under reduced pressure to afford 1.91 g of a yellow liquid. This liquid was added to ether and an ice-cold 5% hydrochloric acid solution.

The acidic, aqueous extract was adjusted to pH 12 with 5% sodium hydroxide and then was extracted with ethyl acetate. The organic phase was washed with water and then a saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to afford 0.943 g (41%) of IIIa as a pale-yellow liquid.

Attempted distillation of IIIa, as well as IIIb–IIId, resulted in extensive decomposition, so these products were identified by spectral methods only; IR (liquid film): 3420 (OH) and 1730 (ester C=O) cm^{-1}; NMR (deuterochloroform): δ 1.04 (t, J = 7 Hz, 6H, CH_3 in ethyl groups), 1.48 [s, 9H, $-C(CH_3)_3$], 2.32–2.83 [m, 8H, $(-CH_2)_2NCH_2-$, $-CH_2CO_2-$], 3.82 (s, 1H, —OH), and 3.75–4.73 (m, 1H, >CH—) ppm.

4-Diethylamino-3-hydroxybutyric Acid Hydrochloride (IVa)—This example typifies the method used to prepare IVa–IVd (Table I). A solution of 0.943 g (0.004 mole) of IIIa in 20 ml of 7% hydrochloric acid was stirred at 25° for 20 hr under a nitrogen atmosphere. The water was removed under high vacuum at 50° to afford 0.863 g of a pale-yellow oil, which crystallized upon trituration with acetone followed by storage at 5° for 3 days.

The acetone was removed from the resulting white crystalline solid and the solid was dried, affording 0.723 g (83%) of IVa, mp



Scheme I

[1] Technicon Autoanalyzer.

[2] Melting points were determined with a Thomas-Hoover capillary melting-point apparatus and are uncorrected. Elemental analyses were obtained from Galbraith Laboratories, Knoxville, Tenn. IR spectra were determined using a Perkin-Elmer model 237 spectrophotometer. NMR spectra were determined on a Perkin-Elmer model R-24 spectrometer in deuterochloroform, using tetramethylsilane as the internal reference, or in deuterium oxide, using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal reference.

104–106°. An analytical specimen of IV*a*, mp 109–111°, was prepared by recrystallization from absolute ethanol–acetone; IR (mineral oil): 1718 (CO_2H) cm^{-1}; NMR (deuterium oxide): δ 1.28 (t, J = 7 Hz, 6H, CH_3 in ethyl groups), 2.65 (d, J = 6 Hz, 2H, $—CH_2CO_2—$), 3.05–3.50 [m, 6H, $(—CH_2)_2NCH_2—$], and 4.20–4.80 (m, 1H, >CH—) ppm.

***tert*-Butyl 4-Ethylmethylamino-3-hydroxybutyrate Methochloride (V*a*)**—This example typifies the method used to prepare V*a*–V*c* (Table II). A mixture of 0.656 g (4.2 mmoles) of *tert*-butyl 3,4-epoxybutyrate (II) (8), 0.361 g (3.30 mmoles) of dimethylethylamine hydrochloride, and 2.0 ml of anhydrous methanol was allowed to stand at 25° for 6 days in a glass-stoppered flask. The methanol was removed under reduced pressure, and the residue was added to ether and water. The organic phase was washed an additional time with water, and the aqueous extracts were combined. The water was removed under high vacuum at approximately 30–40°, and then the traces of water remaining were removed by azeotropic distillation with four 10-ml portions of *tert*-butyl alcohol under reduced pressure.

The resulting oil was dried for 19 hr at 25° under high vacuum (a pale-cream semisolid resulted after drying). All attempts at further purification by recrystallization failed, since all compounds listed in Table II were extremely hygroscopic; NMR (deuterium oxide): δ 4.30–4.60 (m, >CH—, no integration on this proton could be obtained because it was located underneath the HOD peak), 3.32–3.75 (m, 4H, $—CH_2NCH_2—$), 3.12 [s, 6H, $(CH_3)_2N—$], 2.57 (d, J = 6 Hz, 2H, $—CH_2CO_2—$), 1.45 [s, 9H, $—C(CH_3)_3$], and 1.35 (t, J = 6 Hz, 3H, CH_3— in ethyl group) ppm.

4-Ethylmethylamino-3-hydroxybutyric Acid Methochloride (VI*a*)—This example typifies the method used to prepare VI*a*–VI*c* (Table II). A solution of 0.760 g (2.86 mmoles) of V*a*, 50 ml of water, and 2.4 ml of concentrated hydrochloric acid was stirred at 25° for 17 hr. The water was removed at 30–40° under high vacuum, and then four 30-ml portions of *tert*-butyl alcohol were added as described for V*a* to remove azeotropically the remaining water. The resulting pale-yellow viscous oil (0.550 g, 91%) was triturated with acetone and a few drops of absolute ethanol. The white gummy oil crystallized slowly after standing at 5° for 5 days.

The resulting white prisms were dried under high vacuum at 25° for 1 day. The yield was 0.266 g (44%) of VI*a*, which was too hygroscopic to obtain melting-point or IR data; NMR (deuterium oxide): δ 4.55–4.82 (m, >CH—, no integration on this proton could be obtained because it was located underneath the HOD peak), 3.30–3.70 (m, 4H, $—CH_2NCH_2—$), 3.12 [s, 6H, $(CH_3)_2N—$], 2.67 (d, J = 6 Hz, 2H, $—CH_2CO_2—$), and 1.35 (t, J = 6 Hz, 3H, CH_3— in ethyl group) ppm.

Anal.—Calc. for $C_8H_{18}ClNO_3$: C, 45.4; H, 8.5; N, 6.6. Found: C, 45.2; H, 8.4; N, 6.4.

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