($CHCl_3$-EtOH, 6:1); UV (EtOH) λ_{max} 260 nm; NMR (Me_2SO-d_6) δ 2.26-2.34 (m, 1 H, 2'-H_A), 2.38-2.48 (m, 1 H, 2'-H_B), 3.59-3.64 (m, 4 H, 5'-H and CCH_2Cl), 4.02 (m, 1 H, 4'-H), 4.11 [m, 2 H, $N(NO)CH_2C$], 4.52-4.60 (m, 1 H, 3'-H), 5.00-5.18 (br s, 1 H, 5'-OH, D_2O exchangeable), 5.66 (d, 1 H, 5-H), 6.23 (dd, 1 H, 1'-H, J = 6.41 and 6.50 Hz), 7.91 (d, 1 H, 6-H), 9.12 [d, 1 H, 3'-NHCON(NO), D_2O exchangeable], 11.3 (s, 1 H, 3-NH, D_2O exchangeable). Anal. ($C_{12}H_{16}N_5ClO_6$) C, H, N.

3'-[3-(2-Chloroethyl)-3-nitrosoureido]-2',3'-dideoxy-5-fluorouridine (8). To a solution of 6 (1.20 g, 3.40 mmol) in 10 mL of 80% aqueous HOAc was added sodium nitrite (0.48 g, 7.00 mmol) slowly at 0 °C. The reaction mixture was stirred at the same temperature for 4 h and then treated with AG 50W-X8 (H^+) resin (3.2 g) to remove Na^+. The resin was separated by filtration, and the filtrate was evaporated in vacuo to give a glassy residue. Compound 8 was isolated and purified by silica gel column chromatography using $CHCl_3$-EtOH (3:1) as eluting solvent system. The fractions containing pure 8 were combined, concentrated, and redissolved in a minimum amount of EtOH. The solution was then added slowly to a stirred mixture of ether-hexane (1:2). The resulting precipitate was collected by filtration and dried at room temperature under vacuum, to afford 0.5 g (34%). Compound 8 started to effervesce around 94 °C and decomposed at 162 °C: R_f 4.9 ($CHCl_3$-EtOH, 3:1); UV (MeOH) λ_{max} 2.69 nm (ϵ 10 435); NMR (Me_2SO-d_6) δ 2.28-2.38 (m, 1 H, 2'-H_A), 2.41-2.48 (m, 1 H, 2'-H_B), 3.59-3.69 (m, 4 H, 5'-H and CCH_2Cl), 4.12 [t, 2 H, $N(NO)CH_2C$], 4.54-4.62 (m, 1 H, 3'-H), 4.14-4.17 (m, 1 H, 4'-H) 5.27 (br s, 1 H, 5'-OH, D_2O exchangeable), 6.22 (dd, 1 H, 1'-H, J = 4.70 and 4.92 Hz), 8.31 (d, 1 H, 6-H, J_{HF} = 7.23 Hz), 9.13 [d, 1 H, 3'-NHCON(NO), D_2O exchangeable), 11.8 (s, 1 H, 3'-NH, D_2O exchangeable). Anal. ($C_{12}H_{15}N_5ClFO_6$) C, H, N.

Assays for the Determination of Alkylation and Carbamoylating Activity. Alkylation activity was determined by the method of Wheeler et al.[4] and carbamoylation activity by the method of Brubaker et al.[13] Determinations of half-life of the compounds were performed as follows: a 100 μM stock solution of the nitrosourea in absolute ethanol was prepared and the concentration measured by UV spectroscopy. A 100-μL portion of the stock solution was pipetted into a 3-mL glass vial containing 1900 μL of phosphate-buffered saline (pH 7.4) maintained at 37 °C in a water bath. Immediately following addition of the nitrosourea the solution was vortexed for 5 s. Aliquots (15 μL) were removed at t = 0, 1, 2, 3, 4, 5, 8 and 24 h and analyzed by reversed-phase liquid chromatography.[15] The plot of log [nitrosourea] vs. time for each nitrosourea was linear, indicating pseudo-first-order kinetics in each case.

Biological Test Procedures. Mouse L1210 cells were maintained as suspension cultures in Fischer's medium supplemented with 10% horse serum at 37 °C in a humidified atmosphere of 5% CO_2-95% air. Under these conditions the generation time for L1210 cells is approximately 12 h. The test compound at the given concentration was added to L1210 cells (2×10^4 cells/mL), which were in their exponential phase of growth. The increase in cell number of the drug-free culture (control), as well as that of the cultures supplemented with the test compound, was determined after 24, 48, and 72 h of growth.

Transplantation of L1210 ascites cells was carried out by withdrawing peritoneal fluid from donor CDF_1 female mice bearing 7-day growths. The suspension was centrifuged for 2 min (1600g), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was determined with a Coulter particle counter, and the cell population was adjusted to 10^6 cells/mL. A 0.1-mL portion of the resulting cell suspension (containing approximately 10^5 cells) was injected intraperitoneally into each animal. The drug was administered by a single intraperitoneal injection, beginning 24 h after tumor implantation. The test compound was injected as a solution in isotonic saline. The drug was administered intraperitoneally in a volume of 0.25 mL. For any one experiment, animals were distributed into group of five or six mice of comparable weight and maintained throughout the course of the experiment of a suitable diet and water ad libitum. Controls given injections of comparable volume of vehicle (saline) were included in each experiment. Mice were weighed during the course of the experiments, and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity.

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Registry No. 3, 84472-86-6; 4, 85236-95-9; 5, 96699-72-8; 6, 96699-70-6; 7, 96699-73-9; 8, 96699-71-7.

Synthesis and Biological Evaluation of Cyclic Analogues of *l*-Carnitine as Potential Agents in the Treatment of Myocardial Ischemia[1]

Patrick M. Woster and Wallace J. Murray*

Department of Biomedicinal Chemistry, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 68105. *Received August 12, 1985*

A series of cyclic rigid analogues of *l*-carnitine has been synthesized and examined for activity as substrates for the carnitine-acylcarnitine translocase, the enzyme that mediates transport of fatty acids into the mitochondria. Synthetic steps to seven of these analogues are described in this paper. Bioassay of these compounds is conducted in a preparation of isolated heart mitochondria that have been previously loaded with [^{14}C]-*l*-carnitine. Efflux of radioactivity from the mitochondria is then monitored in the presence of the compound being evaluated in order to assess the amount of enzyme activity initiated. The palmityl ester of *l*-*N*,*N*-dimethyl-*trans*-2-carboxy-4-hydroxypyrrolidinium chloride elicited a 13.63 and 63.07% efflux of [^{14}C]-*l*-carnitine at concentrations of 3 and 50 mM, respectively. This represents the first instance in which a nonnaturally occurring analogue of *l*-carnitine has been shown to undergo transport via this mitochondrial translocase, suggesting the possibility that cyclic carnitine analogues may find utility as agents in the treatment of myocardial ischemia.

It is well established that carnitine acts as a carrier molecule in the transport of fatty acids into the mitochondria and that this process is mediated by a mitochondrial enzyme known at the carnitine/acylcarnitine translocase.[2,3] This enzyme, acting in conjunction with two identical carnitine acyltransferases (CAT_1 and CAT_2) on opposite sides of the inner mitochondrial membrane,

(1) Presented at the 189th National Meeting of the American Chemical Society, Miami Beach, FL, Apr 28-May 3, 1985; MEDI 29.

(2) Pande, S. V. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 883.
(3) Ramsay, R. R.; Tubbs, P. K. *FEBS Lett.* **1975**, *54*, 21.

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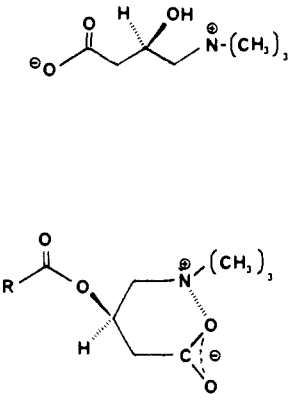
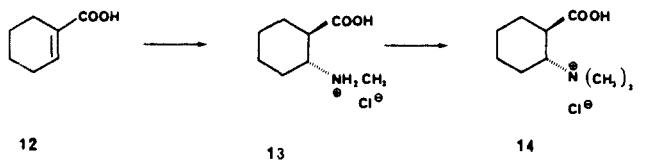


Figure 1. Preferred conformations of unesterified (−)-carnitine (top) and (−)-acylcarnitine (bottom).

Scheme I



12 13 14

exchanges extramitochondrial acylcarnitine for intramitochondrial carnitine with a 1:1 stoichiometry.[4] Exogenously administered carnitine has proven beneficial in the treatment of myocardial ischemia, since the buildup of acyl-CoA around the infarcted tissue, and the resulting metabolic imbalances that lead ultimately to an increase in the size of the infarct, are rapidly reversed.[5] In addition, CAT_1, associated with the carnitine/acylcarnitine translocase on the outer aspect of the inner mitochondrial membrane, is now known to be the major control point in fatty acid oxidation and ketone body production.[6,7] These facts taken together indicate that this enzyme complex is an excellent site for pharmacological manipulation of these crucial biochemical processes.

Although some of the structural features that a molecule must possess in order to undergo transport by the carnitine/acylcarnitine translocase are known,[8] the exact conformational and stereochemical requirements of the enzyme remain unexplored. EHT and CNDO/2 molecular orbital calculations conducted in our laboratory and supported by X-ray crystallography data[9] suggest that carnitine can assume two low-energy conformers. Unesterified carnitine prefers an extended, charge-separated conformation, while esterified carnitine assumes a folded "pseudo" six-membered ring conformer that brings the carboxylate anion and the onium head into close proximity (Figure 1). These observations led us to synthesize and evaluate a number of cyclic rigid analogues of carnitine in order to probe the spatial and stereochemical requirements of the translocase.

In light of the current interest in carnitine as a therapeutic agent in the treatment of myocardial ischemia,[10]

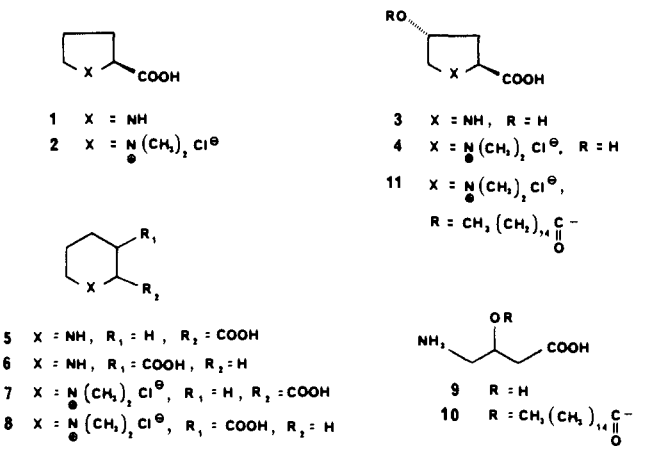


Figure 2.

such a series of compounds would represent a novel class of medicinal agents should they prove active. In addition, information about the structural requirements of the enzyme could lead to the rational design of additional series of compounds in this class.

Results

Chemistry. The structures for the carnitine analogues chosen for this study are outlined in Figure 2 and Scheme I. Compounds **2**, **4**, **7**, and **8** were prepared by a modification of the procedure of King.[11] Methylation of the corresponding *l* α-amino acid (or in the case of **8**, the racemic β-amino acid) was accomplished by treatment with methyl iodide and NaOH in methanol. The acylated analogues **10** and **11** were prepared by using a modification of the procedure described by Bremer.[12] The appropriate starting material (racemic 4-amino-3-hydroxybutyric acid (**9**) or compound **4**, respectively) was dissolved in trifluoroacetic acid and treated with palmitoyl chloride. Compound **12** was prepared in three steps from cyclohexanone, via formation and subsequent dehydration of cyclohexanone cyanohydrin and saponification of the resulting nitrile.[13] Michael addition of methylamine to **12** afforded the *trans*-2-(methylamino)-cyclohexanecarboxylic acid as the hydrochloride salt **13**, which upon treatment with methyl iodide and sodium bicarbonate in methanol was converted to the quaternary ammonium derivative **14** (Scheme I).

Biological Evaluation. The cyclic analogues of carnitine described in this report were evaluated by using the assay procedure developed by Pande.[14] The degree of affinity each compound has toward the carnitine/acylcarnitine translocase was determined by monitoring the efflux of [^{14}C]-*l*-carnitine from isolated rat heart mitochondria. The results of the evaluation of these analogues and their corresponding starting materials appear in Table I. Little or no transport was initiated in the presence of these compounds except in the case of **11**, which elicited a 13.63 and 63.07% efflux of radioactivity at concentrations of 3 and 50 mM, respectively. This is the first instance in which a synthetic analogue of carnitine has been shown to cause efflux of [^{14}C]-*l*-carnitine from isolated mitochondria via the carnitine/acylcarnitine translocase system.

Discussion. The important biochemical role played by the carnitine/acylcarnitine translocase system makes it desirable to determine the exact structural and stereochemical requirements of the enzyme. Currently it is

(4) For a recent review of carnitine-mediated transport of fatty acids into the mitochon ria, see: Bremer, J. *Physiol. Rev.* **1983**, *63*, 1420.
(5) Opie, L. H. *Am. Heart J.* **979**, *97*, 375.
(6) McGarry, J. D.; Foster, D. W. *Ann. Rev. Biochem.* **1980**, *49*, 395.
(7) Paulson, D. J.; Ward, K. M.; Shug, A. L. *FEBS Lett.* **1984**, *176*, 381.
(8) Pande, S. V.; Parvin, R. *J. Biol. Chem.* **1976**, *251*, 6683.
(9) Murray, W. J.; Reed, K. W.; Roche, E. B. *J. Theor. Biol.* **1980**, *82*, 559.
(10) Thomsen, J. H.; Shug, A. L.; Yap, V. U.; Pattel, A. K.; Karras, T. J.; DeFelice, S. L. *Am. J. Cardiol.* **1979**, *43*, 300.
(11) King, H. *J. Chem. Soc.* **1941**, 337.
(12) Bremer, J. *Biochem. Preps.* **1968**, *12*, 69.
(13) Moriconi, E. J.; Mazzocchi, P. H. *J. Org. Chem.* **1966**, *31*, 1372.
(14) Halperin, M. L.; Pande, S. V. *Methods Enzymol.* **1979**, *56*, 368.

Table I. Efflux of [^{14}C]-*l*-Carnitine from Loaded Mitochondria[a]

compd	final concn, mM	control DPM	exptl DPM	total DPM	percent trans
1	3.0	4867	5048	12246	2.45
			(6)	(342)	
2	3.0	5356	5574	12246	3.17
			(306)	(342)	
3	3.0	5065	5346	12246	3.91
			(253)	(342)	
4	3.0	4694	4933	12246	3.16
			(480)	(342)	
5	3.0	3982	4275	10870	4.25
			(160)	(206)	
6	3.0	3962	4130	10870	2.43
			(189)	(206)	
7	3.0	3991	3851	10870	0.00
			(241)	(206)	
8	3.0	4217	4027	10870	0.00
			(122)	(206)	
9	3.0	3936	4223	11957	3.58
			(101)	(439)	
10	3.0	3244	3467	12246	2.49
			(163)	(342)	
11	3.0	4892	5894	12246	13.63
			(284)	(342)	
11	50.0	3592	8868	11957	63.07
			(90)	(489)	
14	3.0	4321	4166	10870	0.00
			(274)	(206)	
carnitine	3.0	4867	11817	12246	94.19
			(289)	(342)	

[a] Each experimental data point represents the mean of three determinations. Values in parentheses are the standard errors of the mean. All DPM values are corrected for background, and counting efficiency is 95%.

known that a substrate must possess both a quaternary ammonium and carboxylate moeity in order to undergo transport and that (−) isomers are preferentially utilized.[8] To date, all of the compounds studied in this system have been free to rotate about carbon–carbon bonds, and hence no conclusions about the optimal orientation for interaction with the enzyme may be made. For this reason, cyclic rigid analogues of *l*-carnitine have been proposed in this laboratory to study the effect of spatial arrangement, conformation, and charge separation on the ability of a substrate to interact with the carnitine/acylcarnitine translocase. The results of this study indicate that cyclic analogues of carnitine are capable of stimulating efflux of [^{14}C]-*l*-carnitine from intact mitochondria by interacting with the carnitine/acylcarnitine translocase system.

Under normal conditions, carnitine appears in the rat heart predominantly as either free carnitine or its acetyl ester. However, a number of other acylcarnitines occur in heart tissue in considerably lower concentrations. It has been shown recently that acylcarnitines compete very effectively with carnitine at low concentrations for uptake by the mitochondria, resulting in a 2- to 4-fold increase in the K_m for carnitine influx.[15] Octanoyl (−)-carnitine completely inhibited carnitine influx into the mitchondria when both were present at a concentration of 2.0 mM. In view of these data, it is not surprising that compound **11**, an analogue of acylcarnitine, was the only compound that caused substantial efflux of [^{14}C]-*l*-carnitine from the mitochondria under the assay conditions described herein. There is not a sufficient amount of data to determine whether esterification of **4** leads to a conformational change resulting in a tight association of the charged groups, as has been shown in the case of carnitine itself.[9] However, this remains a possible explanation for the enhanced ability of acylcarnitines to be translocated into the mitochondria.

The data presented herein suggest that cyclic carnitine analogues may prove to be of value in enhancing transport of acyl-CoA into the mitochondria. Determination of the optimal orientation for a substrate of this enzyme will allow the design of agents that could be esterified to acyl-CoA in vivo and subsequently be transported into the mitochondria, thus reducing damage to the myocardium in an ischemic event. Synthesis and evaluation of additional cyclic analogues of carnitine are an ongoing concern in our laboratories.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Routine NMR and IR spectra were consistent with assigned structures and were obtained on a Varian EM 360 and a Perkin-Elmer 1420, respectively. ^{13}C NMR spectra were obtained on a Varian CFT-20 NMR spectrometer. All chemicals were reagent grade or better and were obtained from Aldrich Chemical Co. or Sigma Chemical Co. Ion-exchange resins were obtained from BioRad Laboratories, and [^{14}C]-*l*-carnitine was purchased from the Amersham Corp. Elemental analyses were performed by Micro Tech Inc., Skokie, IL.

***l*-*N*,*N*-Dimethyl-2-carboxypyrrolidinium Chloride (2).** A 5.0-g portion (0.043 mol) of *l*-proline and 5.17 g (0.129 mol) of NaOH were dissolved in 50 mL of previously dried methanol. To this solution was added 18.3 g (0.129 mol, 8.0 mL) of methyl iodide, and the reaction was allowed to reflux gently for 6 h. After this time, an additional 6.1 g (0.043 mol, 2.7 mL) of methyl iodide was added and the reflux was continued for an additional 6 h. The mixture was allowed to cool, the solvent was removed in vacuo, and the crude residue was dissolved in water and applied to a cation-exchange column (AG 50W-X8, 100–200 mesh, 50 × 1.5 cm). The column was washed with 500 mL of water and then eluted with 500 mL of 1.5 N aqueous HCl. The aqueous solution was evaporated under reduced pressure, and the product was freed from residual salt by taking it up in dry methanol several times. Recrystallization from methanol/ether then afforded pure **2** as a white solid: 6.8 g (88%); mp 234–235 °C dec (lit.[11] mp 235 °C dec).

***l*-*N*,*N*-Dimethyl-*trans*-2-carboxy-4-hydroxypyrrolidinium Chloride (4).** Compound **4** was prepared by the procedure described above in 93% yield; mp 225.5–226.5 °C (lit.[16] mp 227 °C).

(15) Idell-Wenger, J. A. *J. Biol. Chem.* **1981**, *256*, 5597.

(16) Kung, A.; Trier, G. *Z. Physiol. Chem.* **1913**, *85*, 209.

l-*N*,*N*-**Dimethyl-2-carboxypiperidinium Chloride** (7). Compound 7 was prepared by the procedure described above in 76% yield; mp 223.5 °C (lit.[17] mp 224–225 °C).

(±)-*N*,*N*-**Dimethyl-3-carboxypiperidinium Chloride** (8). Compound 8 was prepared by the procedure described above in 84% yield; mp 287–288 °C (lit.[17] mp 285–287 °C).

(±)-**3-(Palmitoyloxy)-4-aminobutyric Acid Hydrochloride** (10). A 1.0-g sample (0.0084 mol) of 3-hydroxy-4-aminobutyric acid was dissolved in 10 mL of trifluoroacetic acid and warmed with stirring to 60 °C on a water bath. Palmitoyl chloride (7.33 g, 0.0252 mol) was slowly added and the reaction allowed to stir for 6 h. A 2.0-mL portion of methanol was then added to quench the reaction, the mixture was cooled, and 100 mL of acetone was added. The mixture was refrigerated overnight to effect crystallization, and the product was collected by filtration and washed with hexane. The crude solid was purified on a cation-exchange column as previously described. Recrystallization from methanol/ether yielded 10 as a white solid: 2.66 g (80.4%); mp 178–180 °C. Anal. ($C_{20}H_{40}NO_4Cl$) C, H, N.

l-*N*,*N*-**Dimethyl-*trans*-2-carboxy-4-(palmitoyloxy)-pyrrolidinium Chloride** (11). Compound 11 was prepared by the procedure described for compound 10 and was isolated as a white solid in 94% yield: mp 107–108 °C. Anal. ($C_{23}H_{44}NO_4Cl$) C, H, N.

Cyclohexene-1-carboxylic Acid (12). Compound 12 was prepared by the method of Moriconi and Mazzocchi[13] in 68% overall yield; bp 91–93 °C (0.25 mm) (lit.[13] bp 97–99 °C (1.0 mm)).

trans-**2-(Methylamino)cyclohexanecarboxylic Acid Hydrochloride** (13). A 5.0-g sample (0.04 mol) of compound 12 and 30 mL (corresponding to 0.386 mol) of 40% aqueous methylamine were sealed in a steel bomb and heated at 140–160 °C for 48 h. The solvent was removed from the reaction mixture under reduced pressure, and the residue was dissolved in 50 mL of 1.0 N aqueous hydrochloric acid and extracted with three 50-mL portions of ether. The aqueous layer was then evaporated, and the residue was taken up in 20 mL of water and applied to a cation-exchange column as previously described. The column was eluted with 500 mL of 1.5 N aqueous HCl and the eluate evaporated to yield 13 as a viscous oil. Further purification was accomplished on a silica gel column (1.5 × 75 cm, eluted with chloroform/methanol/acetic acid (60:40:5). Repeated attempts at crystallization were unsuccessful; yield 3.21 g (51%). Anal. ($C_8H_{16}NO_2Cl$) C, H, N.

trans-**2-(Trimethylammonio)cyclohexanecarboxylic Acid Chloride** (14). A 3.0-g portion of compound 13 (0.0155 mol) and 5.21 g of sodium bicarbonate (0.062 mol) were dissolved in previously dried methanol, and to this mixture was added 6.6 g (0.0465 mol, 2.9 mL) of methyl iodide. The reaction mixture was allowed to reflux for 72 h, with an additional 2.2 g (0.0155 mol, 0.97 mL) of methyl iodide being added every 24 h. The solvent was removed in vacuo, and the residue was dissolved in water and applied to a cation-exchange column as previously described. The eluate was evaporated and the product was separated from the residual salt by taking it up several times in dry methanol. Purification on silica gel as previously described and recrystallization from methanol/ether gave pure 14 as colorless needles: 1.28 g (37.2%); mp 257–258.5 °C. Anal. ($C_{10}H_{20}NO_2Cl$) C, H, N.

Mitochondrial Assay. Hearts were excised from three of four male Sprague–Dawley rats that were allowed free access to food and water prior to sacrifice by cervical dislocation. The mitochondria were then isolated by differential centrifugation as described.[14] The resulting mitochondrial pellet was then resuspended in 200 μL of buffer (containing 200 mM D-mannitol and 50 mM Trizma HCl at pH 7.4, 1.5 μL of a 4 mg/mL solution of rotenone, 1 μL of a 10 mg/mL solution of oligomycin, and 0.5 μL of a 12 mg/mL solution of antimycin A), and the suspension was warmed on a water bath to 28 °C. A 50-μL portion of a 2.8 mg/mL solution of *l*-carnitine and 250 μL of a solution of [^{14}C]-*l*-carnitine (corresponding to 5 μCi) were added, and the suspension was swirled to mix for 3 min. The mitochondria were then washed as described and resuspended as evenly as possible in 250 μL of buffer per heart used. Control tubes were prepared by combining 125 μL of buffer and 100 μL of water. Experimental tubes contained 125 μL of buffer and 100 μL of either a 7.5 or 125 mM solution of the compound being evaluated. Efflux of radioactivity was initiated by adding 25 μL of the mitochondrial suspension to each tube, and the samples were gently stirred or shaken in ice for 1 min. A 10-μL portion of mersalyl solution (26 mM mersalyl, 200 mM D-mannitol, 50 mM Trizma base) was then added to quench the reaction. The tubes were spun in an Eppendorf microcentrifuge for 90 s, and 200 μL of the supernatant from each sample was added to 10 mL of Aquasol II for scintillation counting. Total radioactivity was determined by counting 25 μL of the mitochondrial suspension directly. All DPM values (except total radioactivity) were multiplied by 1.3, since only 200 of 260 μL were counted. A value for percent efflux was determined by applying the following formula:

$$\% \text{ efflux} = 100 \times \frac{\text{DPM(exptl tube)} - \text{DPM(control tube)}}{\text{DPM(total radioact sample)} - \text{DPM(control)}}$$

Scintillation counting efficiency was 95%.

Registry No. 1, 147-85-3; 2, 4136-37-2; 3, 51-35-4; 4, 101198-81-6; 5, 535-75-1; 6, 498-95-3; 7, 101312-27-0; (±)-8, 101198-32-7; (±)-9, 924-49-2; (±)-10, 101198-83-8; 11, 101198-84-9; 12, 636-82-8; 13, 101198-85-0; 14, 101198-86-1; palmitoyl chloride, 112-67-4; carnitine, 541-15-1; carnitine-acylcarnitine translocase, 56093-16-4.

(17) Yoshimura, K. *Z. Physiol. Chem.* **1912**, *78*, 156.

Allergenic α-Methylene-γ-butyrolactones. Study of the Capacity of β-Acetoxy- and β-Hydroxy-α-methylene-γ-butyrolactones To Induce Allergic Contact Dermatitis in Guinea Pigs

Pierre Barbier and Claude Benezra*

Laboratoire de Dermato-Chimie, Associé au CNRS (UA 31), Université Louis Pasteur, Clinique Dermatologique, CHU de Strasbourg, 67091 Strasbourg, France. *Received June 20, 1985*

(±)-Tulipalin B was prepared in six steps from phenyl sulfide and ethyl 2-bromopropionate. The sensitizing power in the skin of (±)-tulipalin A (1a) and B (1b) and of the β-acetoxy derivatives (1c) was studied. All are able to induce allergic contact dermatitis (ACD) and give cross-reactions. γ,γ-Disubstituted analogues (with a $-(CH_2)_5-$ chain in the γ-position) were synthesized and used to induce ACD in guinea pigs: they all were sensitizers and cross-reacted. However no cross-reaction was demonstrated between γ,γ-unsubstituted and γ,γ-substituted compounds showing a great specificity of ACD.

The α-methylene-γ-butyrolactone structural unit 1a characterizes a number of natural compounds[1] with a variety of biological properties including the induction of *allergic contact dermatitis* (ACD). Among these sub-

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