Secondly, *N*-phenyl-*N'*-cyano-*S*-(triphenylstannyl)isothiourea (Compound 9) and its oxygen analog (1) both exhibited total inhibition of two of the 10 fungi tested. The former compound completely inhibited *Aspergillus niger* and *R. stolonifer* at a concentration of 100 μg/ml. The oxygen analog, on the other hand, was most active against *Myrothecium verrucaria* and *S. cerevisiae* at a similar concentration. The latter activity is noteworthy in that none of the 11 compounds reported in the present investigation completely inhibited *S. cerevisiae*.

The antibacterial activity of Compound 9 is shown in Table IV. This compound was remarkably active against the three Gram-positive species, displaying complete inhibition at a concentration of 1 μg/ml. In addition, partial inhibition of the Gram-negative organism, *Escherichia coli*, was evident. This latter activity contrasts with that of the oxygen analog, *N*-phenyl-*N'*-cyano-*O*-(triphenylstannyl)isourea, which was previously reported to be ineffective against *E. coli* (1). In addition, among the four Gram-positive species tested, total inhibition by the oxygen analog was accomplished only against *Micrococcus agilis* (1).

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 23, 1974, from the *Department of Chemistry and the ‡Department of Biology, St. John's University, Jamaica, NY 11439

Accepted for publication December 13, 1974.

The authors thank Professor Harold A. Horan for the IR spectral data.

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New Synthesis of *(RS)*-Carnitine Chloride

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Abstract □ A four-step synthesis of *(RS)*-carnitine chloride was developed using extremely mild reaction conditions and versatile intermediates. Crotyl chloride was converted to *tert*-butyl 3-butenoate using *tert*-butyl alcohol and triethylamine in ether. Treatment of *tert*-butyl 3-butenoate with *m*-chloroperbenzoic acid in chloroform afforded *tert*-butyl 3,4-epoxybutyrate. Reaction of this compound with trimethylamine hydrochloride in methanol, followed by mild acid hydrolysis of the *tert*-butyl ester, afforded *(RS)*-carnitine chloride.

Keyphrases □ *(RS)*-Carnitine chloride—four-step synthesis under mild reaction conditions from crotyl chloride □ Crotyl chloride—starting material in four-step synthesis of *(RS)*-carnitine chloride

The importance of *(R)*-(−)-carnitine chloride in the transport of fatty acids across membranes was demonstrated previously (1). Specifically, carnitine chloride catalyzes the oxidation of long chain fatty acids by participating in the enzyme-mediated transport of activated fatty acids from the cytoplasm to the β-oxidation sites within the mitochondria (2, 3). The objectives of this investigation were to develop a convenient and versatile synthetic route that could be readily adapted to the synthesis of not only carnitine chloride itself but also to carnitine analogs. The availability of carnitine analogs could provide valuable biochemical tools for investigating the structural requirements of carnitine chloride as a catalyst of fatty acid oxidation (4).

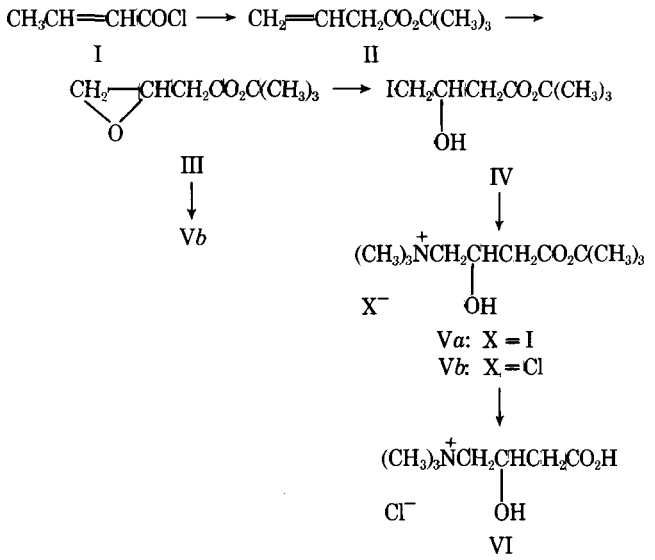
DISCUSSION

In 1966, Ozeki and Kusaka (5) described the synthesis of numerous 3-butenoate esters from crotyl chloride (I) and the appropriate alcohol in the presence of a variety of tertiary amines. It appeared that proper functionalization of the olefinic bond in one of the 3-butenoate esters would provide a convenient and relatively straightforward synthesis of *(RS)*-carnitine chloride (VI) and, thus, carnitine analogs. The *tert*-butyl ester was selected because of the extremely mild reaction conditions required for conversion to the free carboxylic acid (dilute hydrochloric acid at room temperature). Triethylamine was selected as the base because it provided the highest yield of the desired 3-butenoate ester, with only small amounts (3.1%) of the unwanted *cis*- and *trans*-2-butenoate esters (determined by GC).

Since the previous report (5) provided few experimental details and the reaction was not discussed from the standpoint of providing an extremely versatile synthetic intermediate, it was necessary to develop the appropriate reactions conditions to afford the desired *tert*-butyl 3-butenoate (II) in a yield that would be useful for completing the synthesis of VI. The small amount of the *cis*- and *trans*-2-butenoate esters produced during the preparation of II were of little concern because the following step in the synthetic sequence involved epoxidation of the olefinic bond. The mildest conditions possible were used that would eliminate the possibility of isomerization of the 3-butenoate ester to a mixture of the *cis*- and *trans*-2-butenoate esters during exposure to the *m*-chlorobenzoic acid.

It is also well known that α,β-unsaturated carbonyl compounds do not normally undergo epoxidation when subjected to the same reaction conditions as isolated olefinic bonds. The desired epoxy ester (III) was initially converted to the iodohydrin (IV) using the method of Cornforth (6). Compound IV was subsequently treated with trimethylamine in methanol to afford *tert*-butyl 4-dimethylamino-3-hydroxybutyrate methiodide (V*a*) which, when subjected to ion-exchange chromatography, afforded V*b*. The *tert*-butyl protecting group was then removed by the mild reaction conditions described to yield VI.

It was later found, after much experimental effort, that III could be directly converted into the ester V*b*, using trimethylamine hydrochloride in methanol, in a yield high enough to make the reaction synthetically feasible, thus eliminating one step in the synthetic sequence as well as the ion-exchange chromatography.



Scheme I

A four-step synthesis of VI is described (Scheme I). It uses extremely mild reaction conditions and provides versatile intermediates, which can readily be used for the preparation of a variety of carnitine analogs. No other published synthesis of VI (7) that the authors are aware of utilizes such mild reaction conditions and such versatile intermediates[1].

EXPERIMENTAL[2]

***tert*-Butyl 3-Butenoate (II)**—The procedure of Ozeki and Kusaka (5) was modified. To a solution of 28 ml (0.3 mole) of *tert*-butyl alcohol and 28 ml (0.2 mole) of triethylamine in 75 ml of ether was added dropwise with stirring, while maintaining the temperature at 25–30°, a solution of 18.8 ml (0.2 mole) of freshly distilled crotyl chloride in 10 ml of ether. After the addition was completed, the mixture was allowed to stir at 25° for 1 hr.

The mixture was then filtered, and the solid was washed thoroughly with ether. The filtrate was washed with a cold 5% sodium bicarbonate solution, water, a cold 5% hydrochloric acid solution, water, a cold 5% sodium bicarbonate solution, water, and then a saturated sodium chloride solution. The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed at atmospheric pressure with a minimal amount of heating.

The residue was distilled to give 16.9 g (60%) of a colorless liquid, bp 52–54° (20–24 mm); IR (liquid film): 1730 (ester C=O) and 1642 (C=C) cm^{-1}; NMR ($CDCl_3$): δ 4.9–6.2 (m, 3H, CH_2=CH—), 2.9–3.0 (m, 2H, —CH_2—), and 1.45 [s, 9H, —$C(CH_3)_3$] ppm.

Anal.—Calc. for $C_8H_{14}O_2$: C, 67.6; H, 9.9. Found: C, 67.8; H, 9.9.

***tert*-Butyl 3,4-Epoxybutyrate (III)**—To an ice-cold solution of 8.4 g (0.06 mole) of II in 40 ml of chloroform was added dropwise, with cooling and stirring, a solution of 18.2 g (0.09 mole) of 85% *m*-chloroperbenzoic acid in 250 ml of chloroform. The solution was allowed to stand at 4° for 4 days. The resulting precipitate was removed by filtration, and the filtrate was stirred with 300 ml of a freshly prepared aqueous 10% sodium sulfite solution for 30 min (negative starch-iodide test). The organic phase was washed with a 5% sodium bicarbonate solution, water, and a saturated sodium chloride solution and then dried over anhydrous sodium sulfate.

The solvent was removed under reduced pressure to afford a yellow liquid, which was distilled to give 6.3 g (66%) of III as a colorless liquid, bp 40–42° (0.3 mm); IR (liquid film): 1728 (ester C=O) cm^{-1}; NMR ($CDCl_3$): δ 2.38–3.38 (m, 5H, CH_2—$CHCH_2$—) and 1.47 [s, 9H, —$C(CH_3)_3$] ppm.

Anal.—Calc. for $C_8H_{14}O_3$: C, 60.8; H, 8.9. Found: C, 60.5; H, 8.8.

***tert*-Butyl 4-Iodo-3-hydroxybutyrate (IV)**—The procedure of Cornforth *et al.* (6) was used. A mixture of 0.53 g (3.5 mmoles) of sodium iodide, 0.24 g (2.5 mmoles) of sodium acetate, 0.45 ml of acetic acid, and 2.5 ml of propionic acid was cooled to −30°. A 0.375-g (2.5-mmole) sample of III was added to the cooled solution. The mixture was stirred at from −30 to −20° for 30 min and then at 0° for 30 min. The mixture was poured into ether and an ice-cold 5% sodium bicarbonate solution. The organic phase was washed with water, a 10% sodium thiosulfate solution, water, and a saturated sodium chloride solution and then dried over anhydrous sodium sulfate.

The solvent was removed under reduced pressure to afford 0.616 g (88%) of a pale-yellow liquid, which was used immediately without further purification due to its apparent instability; IR (liquid film): 3420 (OH) and 1725 (ester C=O) cm^{-1}; NMR ($CDCl_3$): δ 2.5–4.2 [m, 6H, —$CH_2CH(OH)CH_2$—] and 1.46 [s, 9H, —$C(CH_3)_3$] ppm.

***tert*-Butyl 4-Dimethylamino-3-hydroxybutyrate Methiodide (V*a*)**—To a solution of 0.616 g (2.2 mmoles) of IV in 10 ml of methanol was added 5 ml (16 mmoles) of a 25% solution of trimethylamine in methanol. The mixture was allowed to stand at 25° for 19 hr. The solvent was then removed under reduced pressure to afford a white solid. The solid was slurried with anhydrous ether, filtered, and then dried under high vacuum at 25° to yield 0.711 g (95%) of V*a*, mp 138–139°.

Recrystallization from acetone–ether afforded an analytical specimen as white prisms, mp 146–148°; IR (mineral oil): 1715 (ester C=O) cm^{-1}; NMR (D_2O): δ 4.30–4.80 (m, 1H, —CH—), 3.50 [d, 2H, $(CH_3)_3$—$^+$NCH_2—], 3.26 [s, 9H, $(CH_3)_3{}^+$N—], 2.60 [d, 2H, —$CH_2CO_2C(CH_3)_3$], and 1.47 [s, 9H, —$C(CH_3)_3$] ppm.

Anal.—Calc. for $C_{11}H_{24}INO_3$: C, 38.3; H, 7.0; N, 4.1. Found: C, 38.0; H, 6.8; N, 3.9.

***tert*-Butyl 4-Dimethylamino-3-hydroxybutyrate Methochloride (V*b*)**—A 0.500-g (1.45-mmole) sample of V*a*, dissolved in 5 ml of methanol, was passed through a column (2-cm diameter) containing 8.1 g (4.3 mEq/g, dry basis) of resin[3] (chloride form, 50–100 mesh), which had previously been washed with 100 ml of water and then with 100 ml of methanol. Elution with 50 ml of methanol afforded 0.350 g (95%) of V*b* as a white solid. All attempts to purify this material by recrystallization failed because the isolated crystalline form proved to be extremely hygroscopic. (See later experimental details for analytical and spectral data for this compound.)

Preparation of V*b* Directly from III—A mixture of 0.656 g (4.2 mmoles) of III, 0.320 g (3.30 mmoles) of trimethylamine hydrochloride, and 2.0 ml of anhydrous methanol was allowed to stand at 25° for 5 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 white solid was dried for 19 hr at 25° under high vacuum; the yield was 0.724 g (73%), mp 203–204°.

Recrystallization from methanol–acetone afforded an analytical specimen of V*b* as white prisms, mp 210–211°; IR (mineral oil): 1715 (ester C=O) cm^{-1}; NMR (D_2O): δ 4.30–4.60 (m, 1H, —CH—), 3.50 [d, 2H, $(CH_3)_3{}^+$NCH_2—], 3.26 [s, 9H, $(CH_3)_3{}^+$N—], 2.60 [d, 2H, —$CH_2CO_2C(CH_3)_3$], and 1.47 [s, 9H, —$C(CH_3)_3$] ppm.

Anal.—Calc. for $C_{11}H_{24}ClNO_3$: C, 52.1; H, 9.5; N, 5.5. Found: C, 52.1; H, 9.5; N, 5.5.

***(RS)*-Carnitine Chloride (VI)**—A solution of 0.114 g (0.45 mmole) of V*b*, 8 ml of water, and 0.4 ml of concentrated hydrochloric acid was stirred at 25° for 17 hr. The water was removed at 30–40° under high vacuum; then four 10-ml portions of *tert*-butyl alcohol were added, as already described, to remove the remaining water azeotropically. The resulting white solid was dried under high vacuum at 25° for 2 days; the yield was 0.0874 g (98%) of white prisms, mp 195–197° [lit. (8) mp 196°]. The IR (mineral oil)

[1] For a discussion of previous methods used for the synthesis of *(RS)*-carnitine chloride, see Ref. 7.

[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 either 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.

[3] Dowex 1-X2.

and NMR (D_2O) spectra were superimposable with an authentic specimen of *(RS)*-carnitine chloride[4].

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 8, 1974, from the *Department of Pharmaceutical Chemistry, School of Pharmacy, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, VA 23298*

Accepted for publication December 24, 1974.

Supported by General Research Support Grant FR-5697-02 from the General Research Support Branch, Division of Research Facilities and Resources, National Institutes of Health, and by a grant-in-aid from Eli Lilly and Co.

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COMMUNICATIONS

Bioavailability of Digoxin Tablets in Relation to Their Dissolution *In Vitro*

Keyphrases □ Digoxin tablets—dissolution data compared to bioavailability □ Bioavailability of digoxin tablets—relationship to dissolution

To the Editor:

The bioavailability of digoxin tablets has been claimed to be related to their dissolution *in vitro*. Many investigators have correlated the dissolution rate of digoxin tablets to concentration of digoxin in serum or plasma and to values for areas under the serum level–time curves of tablets (1–6). They have concluded that the dissolution rate *in vitro* is adequate for predicting the bioavailability of commercial digoxin tablets. It has been reported recently, however, that despite the different dissolution rates of two different brands of digoxin tablets, the total amount of digoxin absorbed from these tablets was quite similar to that from digoxin elixir USP (7). The present paper reports that the dissolution test according to the USP (8) is not always effective for evaluating the bioavailability of commercial digoxin tablets.

The experiment was carried out with eight healthy volunteer female subjects, ranging in age from 23 to 50 years and in weight from 57 to 68 kg. The subjects had no history of GI, liver, or kidney disease, and none admitted to taking any medication regularly. One digoxin tablet[1] (0.25 mg) or 10 ml of digoxin standard solution[2] (0.025 mg/ml) was given with 100 ml of water to each subject at 9 am for 10 days under laboratory supervision in a crossover manner. Tablets were determined to meet USP specifications as to purity, disintegration time, and content uniformity.

After taking the drug, the subjects were not allowed to eat for 2 hr and were asked to abstain from alcoholic beverages during the experiment. For measuring serum digoxin levels, venous blood samples were taken on the 8th, 9th, and 10th mornings just previous to the next dose of the drug. After the last dose, samples were drawn at intervals of 0.5, 1, 2, 4, 8, and 24 hr.

[1] Digoxin Novum, Star Ltd., Pinnink. 53, SF-33100 Tampere 10, Finland.

[2] Digoxin, 0.00025 g (International Chemical Reference Substance Digoxin, WHO Centre for Chemical Reference Substances, Apotekens Centrallaboratorium, Solna 3, Sweden); propylene glycol, 0.4 g; alcohol, 0.1 g; and sterile water, a sufficient quantity to make 10 ml.