Absolute Configurations of Antipodes of γ-Chloro-β-carboxy-β-hydroxybutyric Acid, Chlorocitramalic Acid

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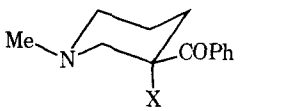
Abstract □ A new four-step synthesis of γ-chloro-β-carboxy-β-hydroxybutyric acid, chlorocitramalic acid, from β-carboxybut-2-enoic acid is described. The absolute configuration of the chlorocitramalic acid antipodes was established.

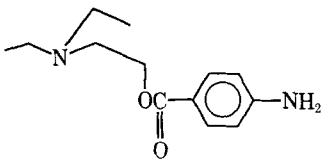
Keyphrases □ Chlorocitramalic acid—synthesis from itaconic acid □ Antipodes of γ-chloro-β-carboxy-β-hydroxybutyric acid, chlorocitramalic acid—absolute configurations □ NMR spectroscopy—structure □ IR spectrophotometry—structure

The molar potency ratio (8:1) of the local anesthetic, I*a*, relative to procaine (II) (1), the novel rotatory dispersion characteristics of I*b* (2), and the speculative nature of the interpretations of the rotatory dispersion curves of I*b*, although consistant with studies conducted to date (2–4), prompted an investigation of the absolute configuration of I. Wilcox *et al.* (5) claimed the synthesis and resolution of III and the conversion of (−)-III to asymmetrically tagged citric acid which, upon degradation by arsenite-treated rat liver homogenate, afforded α-ketoglutaric acid tagged in the γ-carboxyl group. This and later studies on the stereochemistry of intermediates and reactions of the tricarboxylic acid cycle, recently reviewed (6), permitted assignment of the R-configuration to the Wilcox citric acid precursor, which was to be used as the configurational reference in the projected investigation (Scheme I).



I*a*: X = *p*-H_2N-$C_6H_4CO_2$

I*b*: X = Cl, H, Me, MeO, HO, AcO, Et



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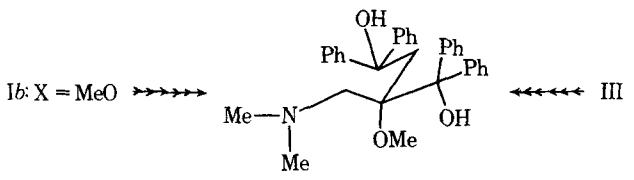
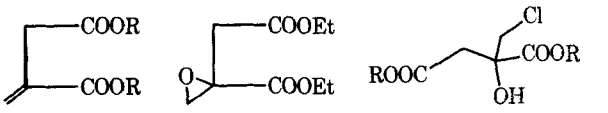


Scheme I

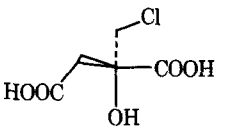
The *abbau* sequence, the subject of a later communication (7), failed. While that work was in progress, attempts to reproduce the synthesis and resolution cited by Wilcox *et al.* (5) also ended in failure (*cf.*, 8). The procedure afforded crude, viscous, acid products containing nitrogen in varying amounts (*cf.*, 8). Attempts to resolve these materials repeatedly led to optically inactive, viscous, halogen-free, acid products of indeterminate composition, containing nitrogen in varying amounts (*cf.*, 8). Because it was hoped that attempts to resolve III would prove more fruitful starting with a demonstrably pure sample of III, and because the Wilcox synthesis required the use of cyanide, powerful vesicants, and lachrymators (such as ethyl chloroacetate and γ-chloroacetoacetate) and afforded only crude acid from which it was not possible to isolate pure III, a new synthesis of III was developed. Again, pure crystalline III failed to resolve, as cited by Wilcox (5, *cf.*, 8), but the corresponding, optically active lactone (IV) was obtained. While these results do not contradict the main conclusion regarding the biochemical stereospecificity of the aconitase system or the ultimate deduction of the R-configuration for a Wilcox precursor of citric acid, there existed some doubt as to the structure of the precursor and, thus, as to the sign of rotation of R-III.



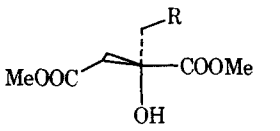
V: R = H
VI: R = Et

VII

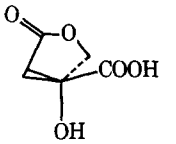
III: R = H
VIII: R = Et



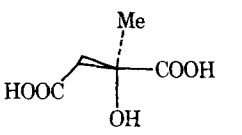
S-(+)-III



R-(+)-IX: R = OH
R-(+)-X: R = *p*-$CH_3C_6H_4SO_3$
S-(+)-XI: R = Cl
S-(+)-XII: R = I
XIII: R = SH
S-(+)-XIV: R = H



R-(+)-IV



S-(+)-XV

This paper deals with an unequivocal synthesis of III and a sequence of reactions which permitted assignment of absolute configuration to its antipodes.

After esterification of itaconic acid (V), the ester (VI) was converted to the epoxide (VII) which, as expected (9), afforded the β-hydroxy-γ-chloro ester (VIII) exclusively by treatment with dry HCl. Acid hydrolysis

gave III, a solid, in 30% overall yield. While attempts to resolve III failed using (−)-brucine, the lactone, (+)-IV, was obtained in optically pure form from the (−)-brucine salt after 11 crystallizations. Hydrolysis of (+)-IV and esterification of the dibasic acid *via* the silver salt yielded (+)-IX, which was converted to the γ-tosylate, (+)-X. After treatment with LiCl, (+)-X afforded (+)-XI and then (+)-III by hydrolysis in acid. Correlation of configuration of (+)-III with S-(+)-XV (8, 10) by catalytic reduction of III and XI or by Zn/H^+ reduction failed. The assignment of configuration was made by conversion of (+)-IV of 86 ± 4% optical purity to S-(+)-XV of about the same degree of optical purity (94 ± 6%). The tosylate, (+)-X, prepared from the partially resolved lactone, (+)-IV, was converted to the iodo analog, (+)-XII [*cf.*, (+)-IV → (+)-IX → (+)-X → (+)-XI], from which the γ-sulfhydryl compound (XIII) was obtained. Raney nickel desulfurization and hydrolysis afforded (+)-XIV and S-(+)-XV, respectively. Since the intermediates and products of this sequence were liquids that resisted all efforts to effect crystallization, X was subjected to the same sequence and afforded crystalline XV, identified by melting point and mixed melting point with an authentic commercial sample of XV. The NMR spectra of both samples of XV were identical to that of the oil assigned the structure (+)-XV.

Accordingly, R-III is the levorotatory antipode, $[\alpha]_D^{25}$ −7.5°. The results achieved by Wilcox *et al.* (5) clearly require that their resolved acid, $[\alpha]_D^{25}$ −44.9°, an oil, contain γ-halo functionality, the quantity and structure of which must remain enigmatic.

EXPERIMENTAL[1]

Melting points were measured in a Thomas-Hoover unimelt apparatus and are uncorrected. Refractive indexes were measured on a calibrated, thermostated, Zeiss-Abbe refractometer and are believed accurate to within ±2 parts per 10^4. Optical rotations were determined with a Perkin-Elmer model 141 photoelectric polarimeter, using a 1.000-dm. cell. IR spectra were determined with a Perkin-Elmer model 421 spectrophotometer, using sodium chloride plates or cells. Assignments of absorption bands, believed accurate to within ±5 $cm.^{-1}$, were made by analogy with reported values (11). Proton NMR spectra were determined on a Varian A-60A spectrometer. Assignments of absorption bands, believed accurate to within ±1 Hz., were made by analogy with reported values (12). When absorptions of exchangeable protons fell within areas of other bands of prime interest, samples were treated with D_2O. Tetramethylsilane was used as the internal reference. Drying, clarifying, and decolorizing of solutions in organic solvents were carried out simultaneously with dry Na_2SO_4 and activated charcoal[2], respectively. After suction filtration through diatomaceous earth[3] pads in sintered-glass funnels, all solvents were removed in a rotatory evaporator under reduced pressure. Oils and solids were further dried (0.1 mm. Hg, room temperature, 4–12 hr.) prior to spectral analysis. Petroleum ether refers to the fraction boiling from 30–60°.

Ethyl β-Carbethoxybut-2-enoate (VI)—Itaconic acid (V), 780 g., in 2 l. of absolute EtOH containing 1.5 g. of hydroquinone and 95 g. of concentrated H_2SO_4, was boiled (18 hr.), cooled (5°), and then alkalized to pH 6–7 with cold (5°) 20% Na_2CO_3. The EtOH was evaporated, and the residue was extracted with $HCCl_3$. The extract was dried, filtered, and fractionally distilled to give 734 g. (67%) of VI: b.p.$_{0.23}$ 58°; n_D^{20} 1.4384; IR (film) 1730 (ester C=O) and 1635 $cm.^{-1}$ (C=C); lit. (13) b.p.$_{20}$ 118°; n_D^{20} 1.4383.

(±)-Ethyl β,γ-Epoxy-β-carbethoxybutyrate (VII)—To 140 ml. of 90% H_2O_2 in 1 l. of cold (5°) H_2CCl_2 was added (90 min.) 1260 g. of $(F_3CCO)_2O$ with vigorous stirring. This solution was added with stirring (5 hr.) through a pressure-equalizing funnel to 734 g. of VI and 2260 g. of dry Na_2HPO_4 in 4 l. of boiling H_2CCl_2. After the exothermic reaction subsided, the mixture was boiled (1 hr.) and then cooled (10°) while adding enough water to dissolve the salts. The H_2CCl_2 layer was separated and combined with 1.5 l. of H_2CCl_2 used to extract the aqueous phase. The H_2CCl_2 solution was washed with saturated $NaHCO_3$, dried, filtered, and fractionally distilled to give 531 g. (66%) of VII: b.p.$_{0.15}$ 75–76°; n_D^{20} 1.4372; IR (film) 1735 (ester C=O), 925, and 900 $cm.^{-1}$ (C—O—C); NMR (CCl_4) δ 4.4–3.9 (q,q,2H,2H,OCH_2,OCH_2), 3.25–2.75 (m,2H,-H_2C—O—C), and 1.23 p.p.m. (t,6H,CCH_3,CCH_3).

Anal.—Calcd. for $C_9H_{14}O_5$: C, 53.46; H, 6.93; O, 39.61. Found: C, 53.7; H, 6.93; O, 39.42 (by difference).

(±)-Ethyl γ-Chloro-β-carbethoxy-β-hydroxybutyrate (VIII)—Dry HCl (70 g.) was bubbled into a solution of 312 g. of VII in 1.1 l. of dry, cold (5°) Et_2O. After 5 hr., the solvent and excess HCl were evaporated. The residue, dissolved in Et_2O, was washed with cold (5°) saturated $NaHCO_3$. The Et_2O phase was dried and filtered, and the oil was distilled to give 338 g. (92%) of VIII: b.p.$_{0.07}$ 81–82°; n_D^{20} 1.4510; f.p. 27°; IR (film) 3510 (OH) and 1740 $cm.^{-1}$ (ester C=O); NMR ($DCCl_3$) δ 4.6–3.9 (q,q,2H,2H,OCH_2,OCH_2), 3.73 (s,2H,$ClCH_2$), 2.88, 2.83 (s,s,H,H,CH_2COO), and 1.6–1.1 p.p.m. (t,t,3H,3H,CCH_3,CCH_3).

Anal.—Calcd. for $C_9H_{15}O_5Cl$: C, 45.43; H, 6.34. Found: C, 45.7; H, 6.52.

(±)-γ-Chloro-β-carboxy-β-hydroxybutyric Acid (III)—After 410 g. of VIII and 600 ml. of concentrated HCl were boiled (4 hr.) and cooled (room temperature, 12 hr.), the solvent and HCl were evaporated and the residue was dried and dissolved in $HCCl_3$ to give an oil which crystallized overnight. The solid was recrystallized from Et_2O and then from EtOAc. Residues obtained from evaporation of the mother liquors were recycled to give a combined yield of 226 g. (73%) of III: m.p. 153–154°; IR (Et_2O) 3500 (OH) and 1740 $cm.^{-1}$ (acid C=O), (mineral oil) 3400, 3300 (OH) and 1750, 1680 $cm.^{-1}$ (acid C=O); NMR (Me_2CO-d_6) δ 3.86 (s,2H,CH_2Cl), 2.97, and 2.90 p.p.m. (s,s,H,H,CH_2COO).

Anal.—Calcd. for $C_5H_7O_5Cl$: C, 32.9; H, 3.87. Found: C, 33.0; H, 3.88. Neutral equivalent, 92.

Attempted Resolution of III—To 146 g. of III in 150 ml. of H_2O was added 335 g. of (−)-brucine in 450 ml. of hot MeOH. To this was added 450 ml. of H_2O (20°). The solution was cooled (10°) rapidly and then refrigerated (4°, 12 hr.). The salt was harvested, washed with cold (4°) H_2O–MeOH (1:1), dried, and redissolved in 400 ml. of MeOH. To this was added 800 ml. of H_2O (20°). The solution was cooled (4°, 12 hr.). After four crystallizations, 47 g. of salt was obtained: $[\alpha]_D^{25}$ (dimethylformamide) −11.4 ± 0.3° (c 10.00); m.p. 112–120°. Ten grams of salt was dissolved in 50 ml. of 50% H_2SO_4, and the solution was extracted in a lighter-than-water continuous liquid–liquid extractor using Et_2O (2 days). The Et_2O phase was dried and evaporated to give a crude oil, 0.9 g., which was chromatographed on a silicic acid column eluting with Et_2O–petroleum ether (1:4 → 4:1). One fraction, on evaporation, afforded III: m.p. 153–154°; undepressed on admixture with an authentic sample; no detectable rotation ±0.002° (c 2.50, H_2O). Another fraction afforded an oil: $[\alpha]_D^{25}$ (H_2O) +8.6 ± 0.2° (c 1.30); IR 3400 (OH), 1780 (γ-lactone C=O), 1730 $cm.^{-1}$ (acid C=O); NMR (Me_2CO-d_6) δ 4.72, 4.56, 4.40, 4.24 (4 line *AB* pattern, CH_2O), 3.36, 3.06, 2.80, 2.51 p.p.m. (4 line *AB* pattern, CH_2COO); assigned the Structure IV.

Synthesis and Resolution of (±)-β-Hydroxy-β-carboxybutyrolactone (IV)—A slurry of 45.5 g. of III and 50 g. of $CaCO_3$ in 150 ml. of H_2O was stirred (24 hr.), concentrated (0.5 volume), mixed with 63 g. of $(COOH)_2 \cdot H_2O$ in 50 ml. of H_2O, cooled (5°), and filtered. The filtrate was evaporated to give, after drying, 21 g. (86%) of IV, an oil that resisted all attempts to effect crystallization. The spectra of a chromatographically pure sample were identical to those of IV obtained in the attempted resolution of III.

To 40 g. of IV in 100 ml. of H_2O was added 109 g. of (−)-brucine in 40 ml. of hot MeOH. After 24 hr. at room temperature, the salt was harvested, washed with cold (4°) MeOH–H_2O (4:1), and dried.

[1] Analyses were performed by Weiler and Strauss, Oxford, England.
[2] Norit.
[3] Celite.

After 10 recrystallizations, the salt (3 g.), $[\alpha]_D^{25}$ (H_2O) $-12.5 \pm 0.2°$ (c 1.00), was dissolved in H_2O and passed through an acid ion-exchange resin[4] column. Evaporating the effluent and drying the residue gave 0.57 g. of (+)-IV: m.p. 87–89°; $[\alpha]_D^{23}$ (H_2O) $+45.2 \pm 0.2°$ (c 3.1); lit. (13) m.p. 86–90°, $[\alpha]_D^{16}$ (H_2O) $-45.6°$ (c 2.43); the IR and NMR spectra were identical to those of IV obtained from III.

Anal.—Calcd. for $C_{28}H_{32}N_2O_9$: C, 62.21; H, 5.96; N, 5.18. Found: C, 61.9; H, 5.64; N, 5.00.

(±)- and R-(+)-Methyl γ-(*p*-Toluenesulfonyloxy)-β-carbomethoxy-β-hydroxybutyrate [X and R-(+)-X]—A slurry of 1 g. of IV and 1.5 g. of $CaCO_3$ in 10 ml. of H_2O was stirred and heated (70°, 24 hr.), concentrated to 5 ml., mixed with 10 ml. of MeOH, and cooled (4°). The solid was harvested, washed with cold (4°) MeOH–H_2O (1:1), and mixed with 10 ml. of H_2O and 0.75 g. of $(NH_4)_2CO_3$. After stirring (room temperature, 12 hr.), the $CaCO_3$ was filtered off, and the filtrate and H_2O washings were concentrated to 5 ml. This was treated with 2.5 g. of $AgNO_3$ in 3 ml. of H_2O. The precipitate was harvested, washed with cold (4°) H_2O, dried (50°) in the dark, suspended in 10 ml. of dry C_6H_6, and treated with 6 ml. of MeI. After stirring (room temperature, 12 hr.), the solid was filtered off and washed with C_6H_6. The filtrate and wash liquor were mixed with activated charcoal, filtered, and evaporated to give, after drying, 0.82 g. (62%) of an oil assigned as Structure IX, (±)-methyl β,γ-dihydroxy-β-carbomethoxybutyrate: IR (film) 3490 (OH) and 1740 cm.$^{-1}$ (ester C=O); NMR ($DCCl_3$) δ 3.86 (s,H, OCH), 3.80 (s,3H,$COOCH_3$), 3.68 (s,s,4H,OCH,$COOCH_3$), 2.82, and 2.78 p.p.m. (s,s,H,H,CH_2COO).

To 1.13 g. of IX in 2 ml. of $HCCl_3$ and 1 ml. of dry pyridine was added (30 min.) dropwise a solution of 1.15 g. of *p*-$CH_3C_6H_4SO_2Cl$ in 2 ml. of $HCCl_3$. The reaction was stirred (room temperature, 6 hr.), mixed with 10 ml. of *N* HCl, and extracted with two 10-ml. portions of $HCCl_3$. The extract was washed with H_2O, mixed with activated charcoal, dried, filtered, and evaporated. The residue was recrystallized three times from 1:1 EtOAc–petroleum ether to give 1.3 g. (68%) of X: m.p. 74–75.5°; IR ($HCCl_3$) 3510 (OH), 3030 (ArH), 1750 (ester C=O), and 1175 cm.$^{-1}$ ($ArSO_3$); NMR (CCl_4) δ 7.9–7.2 (m,4H,C_6H_4), 4.16 (s,2H,SO_3CH_2), 3.78 and 3.68 (s,s,3H, 3H,$COOCH_3$,$COOCH_3$), 2.81 and 2.75 (s,s,H,H,CH_2COO), and 2.46 p.p.m. (s,3H,CH_3Ar).

Anal.—Calcd. for $C_{14}H_{18}O_8S$: C, 48.54; H, 5.23. Found: C, 48.4; H, 5.29.

In like manner, 0.57 g. of optically pure R-(+)-IV afforded, after drying, 0.53 g. (72%) of R-(+)-IX, an oil: $[\alpha]_D^{23}$ (MeOH) $+5.1 \pm 0.2°$ (c 5.30), and then 0.55 g. (58%) of S-(+)-X: m.p. 70–72°. The IR and NMR spectra of these antipodes were identical to those of the respective racemates.

(±)- and (+)-Methyl γ-Chloro-β-carbomethoxy-β-hydroxybutyrate [XI and S-(+)-XI]—A solution of 0.5 g. of X and 0.5 g. of LiCl in 5 ml. of dry Me_2CO, after stirring (room temperature, 6 days), was evaporated and the residue was dissolved in 10 ml. of H_2O. This solution was extracted with two 10-ml. portions of Et_2O. The extract was dried, mixed with activated charcoal, filtered, and evaporated to give 195 mg. (65%) of XI from 1:1 EtOAc–petroleum ether: m.p. 43–45°; IR ($HCCl_3$) 3520 (OH) and 1748 cm.$^{-1}$ (ester C=O); NMR (CCl_4) δ 3.85 and 3.70 (s,s,3H,3H,$COOCH_3$, $COOCH_3$), 3.67 (S,2H,CH_2Cl), and 2.83 and 2.77 p.p.m. (s,s,H,H, CH_2COO), lit. (5) m.p. 44–45°, (8) 44°.

In like manner, 0.55 g. of R-(+)-X afforded an oil purified by chromatography on a silica gel column using Et_2O–petroleum ether (1:4) as the eluant. One fraction yielded, after evaporating the solvent and drying the residue, 0.26 g. (80%) of an oil, S-(+)-XI: $[\alpha]_D^{25}$ ($HCCl_3$) $+4.0 \pm 0.3°$; the spectra were identical to those of XI.

S-(+)-γ-Chloro-β-carboxy-β-hydroxybutyric Acid [S-(+)-III]—A solution of 0.18 g. of S-(+)-XI in 2 ml. of concentrated HCl was stirred (room temperature, 12 hr.—90°, 4 hr.) and evaporated to give a residue, which was dried and dissolved in 3 ml. of H_2O. The solution was mixed with $HCCl_3$. The residue obtained by evaporation of the aqueous solution was dissolved in EtOAc. This was mixed with activated charcoal, dried, filtered, chromatographed, and evaporated to give, after drying, 130 mg. (65%) of an oil, S-(+)-III: $[\alpha]_D^{25}$ (H_2O) $+7.5 \pm 0.5°$ (c 1.00); the spectra were identical to those of III prepared from XI and from VIII.

(±)- and S-(+)-β-Carboxy-β-hydroxybutyric Acid [XV and S-(+)-XV]—A solution of 1 g. of NaI and 0.5 g. of partially resolved tosylate, R-(+)-X {$[\alpha]_D^{25}$ ($HCCl_3$) $+11.6 \pm 0.5°$ (c 5.00), [86.5 ± 4% (+)-X], prepared from the lactone, R-(+)-IV, $[\alpha]_D^{23}$ (H_2O) $+33 \pm 1°$ (c 6.70), [86.5 ± 4% (+)-IV]} in 5 ml. of dry Me_2CO was stirred (room temperature, 24 hr.) and then evaporated. The residue was dissolved in 4 ml. of H_2O, and the solution was extracted with two 10-ml. portions of Et_2O. The extract was dried, mixed with activated charcoal, filtered, and evaporated to give, after drying, 0.434 g. (98%) of an oil assigned the structure S-(+)-XII, S-(+)-methyl γ-iodo-β-carbomethoxy-β-hydroxybutyrate: $[\alpha]_D^{25}$ ($HCCl_3$) $+5.1 \pm 0.3°$ (c 4.30); IR (film) 3500 (OH) and 1740 cm.$^{-1}$ (ester C=O); NMR (CCl_4) δ 3.86 and 3.72 (s,s,3H,3H,$COOCH_3$,$COOCH_3$), 3.45 (s,2H,ICH_2), and 2.90 and 2.86 p.p.m. (s,s,H,H,CH_2COO).

A cold (4°) solution of 0.5 g. of dried NaSH in 5 ml. of absolute EtOH was saturated with H_2S, warmed to room temperature, mixed with 0.43 g. of S-(+)-XII in 4 ml. of absolute EtOH, stirred (2 hr.), acidified with 0.01 *N* HCl, and evaporated to give a residue which was mixed with 5 ml. of H_2O. The mixture was extracted with two 10-ml. portions of Et_2O. The extract was dried, mixed with activated charcoal, filtered, and evaporated to give, after drying, 0.238 g. (82%) of a vile-smelling, halogen-free oil assigned the structure methyl γ-mercapto-β-carbomethoxy-β-hydroxybutyrate, XIII: IR ($HCCl_3$) 3508 (OH), 2590 (SH) and 1700 cm.$^{-1}$ (broad, ester C=O); NMR ($DCCl_3$) principal absorptions at δ 3.89, 3.81, 3.68 (s,s,s, SCH_2,$COOCH_3$,$COOCH_3$), 2.88 and 2.75 p.p.m. (s,s,CH_2COO).

Freshly activated Raney nickel (2 g.) was boiled in 10 ml. of Me_2CO (2 hr.). The Me_2CO was displaced by several washings and decantations with absolute EtOH. The slurry was mixed with 0.238 g. of XIII, boiled (15 min.), and filtered. The filtrate and wash liquor were evaporated to give an oil, which was dried and dissolved in Et_2O. This solution was treated with activated charcoal, filtered, and evaporated to give, after drying, 0.144 g. (57%) of a sulfur-free, dextrorotatory ($HCCl_3$) oil assigned the structure S-(+)-methyl-β-carbomethoxy-β-hydroxybutyrate, S-(+)-XIV: NMR ($DCCl_3$) δ 3.80, 3.70 (s,s,3H,3H,$COOCH_3$,$COOCH_3$), 2.89, 2.78 (s,s,H,H, CH_2COO), and 1.44 p.p.m. (s,3H,CH_3C).

A solution of 0.114 g. of S-(+)-XIV in 1.5 ml. of concentrated HCl was stirred (room temperature, 24 hr.), diluted with 1.5 ml. of H_2O, heated (90°, 4 hr.), cooled to room temperature, alkalized with saturated $NaHCO_3$, extracted with two 3-ml. portions of $HCCl_3$, acidified with 0.01 *N* HCl, and evaporated. The dried residue was mixed with 5 ml. of EtOAc. The mixture was treated with activated charcoal, filtered, and evaporated to give, after drying, 27 mg. (30%) of an oil assigned the structure S-(+)-β-carboxy-β-hydroxybutyric acid, S-(+)-XV: $[\alpha]_D^{23}$ (H_2O) $+20.9 \pm 3°$ (c 0.45); lit. (8, 14) m.p. 110°, $[\alpha]_D^{22}$ (H_2O) $+23.7°$ (c 5.0); NMR (Me_2CO-d_6) δ 2.85, 2.76 (s,s,H,H,CH_2COO), and 1.44 p.p.m. (s,3H,CH_3C).

The NMR spectrum of XV, m.p. 114–116° from EtOAc–petroleum ether, obtained in like manner from X, was identical to that of S-(+)-XV and to that of an authentic commercial sample of XV, m.p. 115–116°, mixture m.p. 115–116°, lit. (14) m.p. 116.4–117.2°. The NMR spectra of the respective racemic and optically active intermediates (XII–XIV) were also identical.

[4] Dowex W50-X4.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 19, 1970, from the *Laboratory of Medicinal Chemistry, Division of Chemistry, College of Pharmaceutical Sciences, 115 W. 68th St., New York, NY 10023*

Accepted for publication January 27, 1971.

This investigation was supported by Grant NB-03593 from the U. S. Public Health Service, Bethesda, Md.

Abstracted from the M.S. thesis of S. K. Suh.

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Inhibitory Effect of Cholesteryl Nitrate on Interaction of Nitrogen Dioxide with Cholesterol in Monomolecular Films

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Abstract □ Cholesterol monomolecular films exhibit a loss in surface pressure on exposure to nitrogen dioxide due to the formation and subsequent desorption of cholesteryl nitrate. The cholesterol–nitrogen dioxide interaction is inhibited by the prior addition of cholesteryl nitrate, and the degree of inhibition is a direct function of the mole fraction of the nitrate ester. Total inhibition occurs when this mole fraction is about 0.75. The differential equations developed to describe the sequence of events involved in this process were programmed for solution on an analog computer. This simulation indicated that at least six molecules of cholesteryl nitrate are required for each remaining cholesterol molecule to produce a total inhibition of the nitrogen dioxide–cholesterol reaction.

Keyphrases □ Cholesteryl nitrate, inhibitory effect—nitrogen dioxide–cholesterol interaction, monomolecular films □ Monomolecular films, nitrogen dioxide–cholesterol interaction—inhibitory effect of cholesteryl nitrate

Recently, it was reported that cholesterol monomolecular films, when exposed to approximately 175 p.p.m. of nitrogen dioxide for 60 min., exhibited a condensation effect (*i.e.*, a decrease in surface area at all surface pressures tested) that corresponded to a loss of about 75% of the cholesterol (1). Moreover, continued exposure did not result in any further loss of cholesterol (1). Other studies demonstrated that the formation and subsequent desorption of cholesteryl nitrate were responsible for this loss of cholesterol from the film and the concurrent condensation effect (2).

Similarly, Bergström and Wintersteiner (3) noted that the extent of autoxidation of cholesterol dispersed in an aqueous soap solution did not exceed 70%, even after extended exposure to air. These workers demonstrated that this limiting effect was caused by the build-up of the reaction products. Furthermore, their data indicated that this effect was not due to reversibility of the reaction but rather to a physical phenomenon related to the state of the system. The accumulation of the oxidation products at the surface of the soap–cholesterol micelles apparently served to protect the remainder of the cholesterol from oxidation (4).

It is also very likely that the reaction between cholesterol and nitrogen dioxide, which leads to the formation of cholesteryl nitrate, is irreversible. Under such conditions, then, the limiting effect observed after a loss of 75% of the cholesterol on exposure to nitrogen dioxide could be due to a physical interaction between the cholesteryl nitrate and the unreacted cholesterol.

This paper presents data which support this postulation and presents a reaction scheme that accounts for the experimental observations.

EXPERIMENTAL

Materials—Cholesterol[1] and cholesteryl nitrate[2] were used for the film studies. Their purity was verified by TLC using samples of 250 mcg. Single spots were observed in both cases. Solutions of these lipids were prepared in spectroscopic grade hexane. All other chemicals were of reagent grade. The water used for the subphase was first deionized and then distilled from an all-glass still just prior to use.

A mixture of 0.5% nitrogen dioxide[3] (99.5% pure) and 99.5% prepurified grade nitrogen[3] was used as the source of nitrogen dioxide.

Apparatus and Methods—The gas mixture was allowed to pass through a flowmeter at a rate of 100 ml./min. and directed into a short length of perforated Teflon tubing, which was fixed to the underside of a Lucite trough cover, as previously described (5). This served to maintain the desired gaseous atmosphere over the film.

The film balance used to study the surface pressure–surface area (π–A) characteristics of the film was described previously (5). Surface pressures were measured by the Wilhelmy plate method (6).

An EAI TR20 analog computer[4] was used to simulate possible models descriptive of the experimental results.

Solutions of the lipids in hexane were spread on a 0.065 M phosphate buffer at pH 7.0, and the gas was allowed to flow over the film for 60 min. At the end of this time, the gas flow was discontinued; any nitrogen dioxide remaining over the surface was removed by use of the exhaust fan in the hood in which the film-balance unit was set. Manual compression of the film was then initiated, and surface pressure readings were obtained at various film areas.

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