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INHIBITING STEROL BIOSYNTHESIS WITH RING C OXYGENATED CHOLESTEROL DERIVATIVES: A CHEMICAL SYNTHESIS APPROACH

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Abstract

Oxysterols, defined as sterols with an additional oxygen function, have emerged as compounds of significant interest due to their diverse and multifaceted biological properties. This review explores the various aspects of oxysterols, including their cytotoxicity, atherogenicity, carcinogenicity, mutagenicity, hypocholesterolemia, and effects on specific enzymes. These compounds are not only found widely distributed in nature but are also present in animal tissues and food items. Furthermore, oxysterols have been isolated from natural drugs employed in traditional medicine for cancer treatment. In this review, we delve into the biological implications and sources of oxysterols, shedding light on their potential impact on human health and offering insights into their relevance in both the natural world and therapeutic practices.

1. Introduction

In general, oxysterols are defined as sterols bearing second oxygen function, in addition to that at carbon-3, and having an iso-octyl or modified iso-octyl side chain. They have demonstrated a variety of biological properties, including cytotoxicity, atherogenicity, carcinogenicity, mutagenicity, hypocholesterolemia, and effects on specific enzymes [1-3]. They are found widely distributed in nature, have been found in animal tissues and food stuffs [1] and have been isolated from natural drugs used in folk medicine for the treatment of cancer [4-6].

Oxysterols derivatives of cholesterol and sterol intermediates in cholesterol biosynthesis have been found to be potent inhibitors of sterol biosynthesis in animal cell culture. The reported inhibition of cholesterol biosynthesis in mammalian cells by oxygenated derivatives of cholesterol and lanosterol has been shown in most cases to decrease cellular levels of HMG- -CoA reductase, a key regulatory enzyme in sterol biosynthesis [7-12]. These studies suggest a regulatory mechanism which, by analogy to steroid hormone receptors and bacterial induction-repression systems, requires a binding protein to recognize oxysterols and mediate subsequent cellular events.

Experimental result for the existence of a specific cytosolic receptor protein for oxysterols has been presented. These experimental results indicated a good correlation between the actions of certain oxysterols on HMG-CoA

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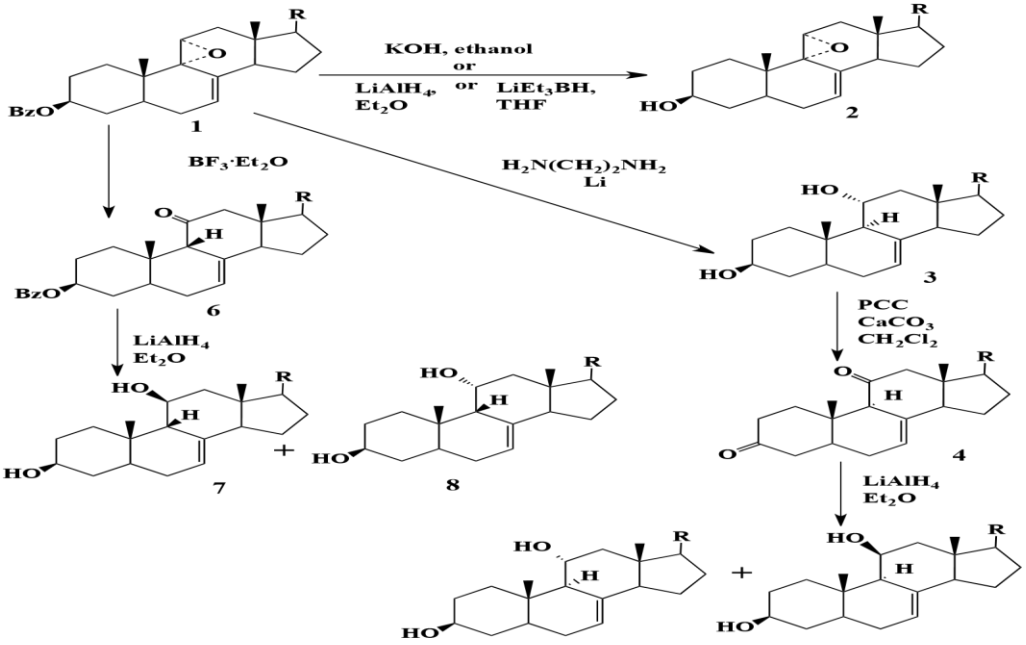
reductase in L cells and their affinity for an oxysterol binding protein [11,13,14]. This oxysterol model for the regulation of cholesterol biosynthesisproposes that oxygenated derivatives of cholesterol or lanosterol are produced in cells as signal molecules which feedback and regulate enzymes of the cholesterol biosynthetic pathway [15-18].

In the present study, we have continued our efforts to prepare new oxysterols and observe their activity as potential inhibitors of HMG-CoA reductase. Oxysterols in ring C have not been studied to the same degree as those found on the side chain and other regions of the steroid nucleus [11,18]. Since the C ring of steroids is associated biochemical pathways essential for mammalian growth and development it appeared to be worthwhile to further study to effects of additional examples of oxysterols of ring C and observe their inhibitory activity on HMG-CoA reductase.

2. Results and Discussion

The ring C oxysterols 5□,8□-cholest-7-ene-3□,11□-diol (**3**) and 3□-hydroxy-5□-cholest-9(11)-en-12-one (and their 3-keto derivatives) were previously known to be effective inhibitors of HMG-CoA reductase [11,19]. Lanosterol derivatives possessing a 9□-hydroxyl group were found to be less effective as inhibitors [19].

3□-Hydroxy-5□-cholest-8-en-11-one, while demonstrating poor reductase inhibition, was found to be an effective inhibitor of tumor cell growth and was shown to produce significant, but not sustained, hypocholesterolemic activity in laboratory animals [2,10,21]. These results encouraged us to prepare additional examples of these interesting compounds and observe their ability to inhibit reductase. Scheme 1 outlines our approach to this effort by using 3□-benzoyloxy-9□,11□-epoxy-5□cholest-7-ene (**l**) as a starting material. We have previously described the chemical synthesis of **1** by the selective epoxidation of 3□-benzoyloxy-5□-cholest-7,9(11)dienewith *m*-chloroperoxybenzoic acid (MCPBA) in ether [21]. Although the configuration of the epoxy group in **1** was assigned by analogy to other previously prepared □-epoxides in the steroid series, [22,23] it became necessary to demonstrate the correct assigned configuration by X-ray crystallographic studies. The results of these X-ray studies are presented elsewhere [24].



Scheme 1. Chemical Synthesis of Ring C Oxysterols from 3 5

3b-Benzoloxy-9a,11a-epoxy-5a-cholest-7-ene (1).

Many steroidal epoxides are susceptible to nucleophilic ring opening [25-30]. However, attempted ring opening of **1** by KOH, $LiAlH_4$, or $LiEt_3BH$ was unsuccessful and resulted in the 3□-hydroxy-epoxide (**2**). These phenomena might result from the restricted access of the nucleophiles to the □-face of the molecule at C-9 and C-11due to steric hinderance from the C-18 and 19 methyl groups. Previously, hindered steroidal epoxides have been successfully reduced by lithium in ethylenediamine and application of these methods to **1** resulted in the 3□,11□-diol (**3)** [31]. This steroid has been prepared previously by the selective hydroboration of 3□benzoyloxy-5□-cholest-7,9(11)-diene [19].

Selective oxidation of the homoallylic alcohol **3** with pyridinium chlorochromate (PCC) and calcium carbonate in methylene chloride gave the unsaturated diketone **4** [32]. Reduction of **4** with $LiAlH_4$ produced the epimeric diols

3 and **5**. Hydride attack from the less hindered □-face of **4** resulted in a higher yield of epimer **5**.

In an additional series of reactions, biologically unnatural steroids containing a *cis* B/C ring junction were prepared. The epoxide **1** was treated with $BF_3 \cdot Et_2O$ and rearranged to the C-9,□H 11-ketone **6**. Similar rearrangements of steroidal epoxides, resulting from a *cis*-1,2-hydride shift, have been observed previously [21,33,34]. Reduction of **6** with $LiAlH_4$ produced the epimeric C-9,□-H diols **7** and **8**. The altered configuration of the C ring projected the C-11 ketone towards the □-face of the steroid nucleus, resulting in hydride attack from the □-face, yielding the epimer **8** as the major product.

The epimeric diols **3** and **5** resulting from the hydride reduction of ketone **4**, were separated by column chromatography. The minor product **3** was identical to that derived from the lithium in ethylenediamine reduction of epoxide **l**. In the proton NMR spectrum, the □ protein on C-11 was split into a doublet of doublet of doublets (ddd) with J=5.3, 9.7 and 11.2 Hz, which were consistent with predicted values (Table 1). The melting point and spectral data were identical with **3** prepared from the selective hydroboration of 3□-benzoyloxy-5□-cholest-7,9(11)-diene [19]. The proton NMR of the major product **5** demonstrated coupling constants (ddd, J=3.0, 3.2 and

3.1Hz) for the □ proton on C-11which were consistent with calculated values.

Table 1: Calculated coupling constants (Hz) of diols 3, 5, 7 and 8[a]

Compound	J9,11	J11,12□	J11,12□
3 (9□H,11□H)	9.62	5.46	10.50
5 (9□H,11□H)	3.07	3.33	3.12
7 (9□H,11□H)	0.90	6.21	1.29
8 (9□H,11□H)	10.77	7.61	8.11

a) Molecular mechanics calculations using the program PCMODEL gave rise to coupling constants via a standard Karplus relation.

In a similar manner, the epimeric diols **7** and **8**, resulting from the hydride reduction of ketone **6**, were separated by column chromatography. The proton NMR of the major product **8** exhibited coupling constants (ddd, J=10.0, 6.7 and 8.0 Hz) for the □ proton on C-11which were in good agreement with calculated values (Table 1). In addition, the minor product of **7**, containing a proton on C-11, exhibited coupling constants (ddd, J=0.9, 6.2 and 1.2 Hz) which were also consistent with calculated values.

All four of the epimers **3, 5**, **7**,and **8** and the epoxide **2** were found to be active in reducing the level of HMG-CoA reductase activity in cultures of mouse L cells in (Table 2). Evaluation of the natural (*trans*-B/C-ring junction) epimers **3** and **5** indicated that the C-11, □-hydroxy epimer **3** possessed greater activity than its □-hydroxy counterpart. Evaluation of the unnatural (*cis*-B/C-ring junction) epimers**7** and **8** indicated that the C-11, □-hydroxy epimer **7** was more inhibitory than the □hydroxy epimer **8**. A similar trend in inhibitory activity has been observed in the hydroxyl epimers (both the natural and unnatural C/D ring junction) of C-15-hydroxy steroids [35,36].

The epoxide **2**, although exhibiting less inhibitory activity than the C-11epimer diols, was also shown to be a significant inhibitor of reductase. It is of interest to note that the degree of inhibition of epimers **3**, **5**, **7**, and **8** was somewhat similar (0.61-0.94 μM) when compared to the activities other oxysterols [3,10,11,12,18] the effects of the stereochemistry at C-9 *(cis/trans*-ring junction) was not a crucial factor in the activity of these oxysterols.

Table 2: Ring C oxysterol repression of HMG-CoA reductase activity in L cells

Compound	Concentrations (μ M) required for 50% inhibition
2	2.3
3	0.61
5	0.84
7	0.83
8	0.94

These results indicate that the ring C oxysterols examined in this study possessed inhibitory effects similar to those observed at other sites on the steroid nucleus [3,10,11,12,35,36]. Their significant inhibition of HMG-CoA reductase activity indicates the potential utility of this class of oxysterol in controlling normal and abnormal cellular replication.

3. Experimental

The synthesis of 3□-benzoyloxy-9□,11□-epoxy-5□-cholest-7-ene (**1**) has been described [21]. Procedures for recording melting points (MP) and of infrared (IR), ^{1}H NMR, and mass spectra (MS; electron impact, EI) together with details concerning column and thin layer chromatography (TLC) have been described [37,38].

Cell culture studies were conducted using mouse L cells (a subline of NCTC clone 929 mouse fibrobasts) and were grown in a serum-free medium. HMG-CoA reductase activity was determined in cell homogenates as previously described [39]. • The concentration of sterol, in the medium, which gave 50% repression of HMGCoA reductase after 5 h of incubation, was determined graphically from a plot of inhibitor activity (percentage of control value) versus at least 4 concentrations of sterol.

9□,11□-Epoxy-5□-cholest-7-en-3□-ol (2)

Epoxide **1** (4.0 g, 7.9 mmol) was dissolved in 650 ml of ethanol with gentle warming and a solution of 22 g of KOH in 80 ml of water was added. The resulting mixture was refluxed for 5 hrs, evaporated to 1/4 its initial volume under reduced pressure, and poured into 800 ml ice/water. The precipitate was collected and subjected to silica gel column chromatography using a gradient of ethyl acetate in chloroform as the eluting solvent. Compound **2** (2.4 g, 75.6%) was recrystallized from acetone-water.

MP 171.5-173.5°C; □□$_{max}$(KBr)/cm^{-1} 3357, 1653, 1470, 1046, and 885; □$_H$ (250 MHz; $CDCl_3$) 0.57 (3H, 18-H_3), 0.89 (3H, s, 18-H_3), 3.25 (lH, m, 11-H), 3.61(lH, m, 3H), 5.63 (lH, m, 7-H); m/z 400 (M^+, 100%), 385 (M^+-CH_3, 13%), 382 (M^+-H_2O 8%), 367 (M^+-CH_3-H_2O, 16%), 287 (M^+-side chain, 50%), 269 (M^+-H_2O-sidechain, 4%),

(Found: M^+, 400, 3554, $C_{27}H_{44}O_2$ requires M, 400, 3541).

Treatment of **1** with $LiAlH_4$ in ether or $LiEt_3BH$ in THF, using reaction conditions described previously, [33,34] produced **2** in 76 and 81% yields, respectively, with identical physical and spectral properties described herein.

9□-Cholest-7-ene-3□,11□-diol (3)

Epoxide **1** (5.0 mmol) was dissolved in 100 ml of ethylenediamine in a 1000 ml flask with gentle warming in a warm water bath (40ºC). Lithium metal (20 g) was added in small pieces. The flask was cooled if the temperature rose during the reaction. After approximately 30 min, methanol (100 ml) was slowly added to decompose the excess lithium. Water (200 ml) was then slowly added and the resulting product precipitate was collected, washed with water, dried in a vacuum desiccator, and subjected to silica gel column chromatography, using a gradient of ethyl ether in toluene as the eluting solvent, to yield 2.61g of 9□-Cholest-7-ene-3□,11□-diol (**3**, 65.4%).

MP 164-166ºC; $\square_{max}$(KBr)/cm^{-1} 3349, 1653, 1468, 1381and 968; $\square_H$ (250 MHz; $CDCl_3$) 0.55 (3H, s, 18-H_3), 0.86 (3H, s, 19-H_3), 3.60 (1H, m, 3□-H), 3.96 (1H, ddd, J=5.30, 9.65 and 11.18 Hz, 11□-H), 5.27 (lH, m, 7-H); m/z 402 (M^+, 2%), 387 (M^+CH_3, 6%), 384 (M^+-H_2O, 8%), 289 (M^+-side chain, 6%), 274 (M^+-CH_3-sidechain, 17%), 271 (M^+-H_2O-side chain, 100%), 253 (M^+-$2H_2O$-side chain, 9%), (Found: M^+, 402.3498, $C_{27}H_{46}O_2$ requires M, 402.3496).

Cholest-7-ene-3,11 dione (4)

9□-Cholest-7-ene-3□,11□-diol (**3**, 2.0 g, 5.0 mmol) was dissolved in 175 ml of methylene chloride and calcium carbonate (2.2 g) was added. The solution was stirred for 30 min at room temperature after 5.2 g of pyridinium chlorochromate (PCC) was added. Saturated NaCl solution 200 ml was added and the mixture was thoroughly extracted with ether. The extracts were filtered through anhydrous $MgSO_4$ and evaporated to dryness. The solid was then recrystallized from acetone-water to yield 1.54 g of diketone **4** (78% yield).

MP 158-160ºC; $\square_{max}$(KBr)/cm^{-1} 2942, 1712, 1681, 1670, 1382, and 1250; $\square_H$ (250 MHz; $CDCl_3$) 0.53 (3H, s, 18-H_3), 0.83 (3H, s, 19-H_3), 3.38 (lH, s, 9□-H), 5.36 (lH, m, 7-H); m/z 398(M^+, 100%), 383 (M^+-CH_3, 31%) 380 (M^+-H_2O, 20%), 365 (M^+CH_3-H_2O, 27%), 285 (M^+-side chain, 18%), (Found: M^+, 398.3181, $C_{27}H_{42}O_2$ requires M, 398.3185).

9□-Cholest-7-ene-3□,11□-diol (3) and 9□-Cholest-7-ene-3□,11□-diol (5)

Diketone **4** (2.5 g, 6.3 mmol) was dissolved in 300 ml of anhydrous ethyl ether. Lithium aluminum hydride (5.0 g) was slowly added and the mixture was stirred at room temperature for 8 hr. The solution was cooled to 0ºC and ice slowly added to decompose excess hydride. The mixture was poured into a saturated aqueous ammonium chloride solution and thoroughly extracted with ether. The extracts were evaporated to dryness at reduced pressure to yield 2.2 g of solid. TLC analysis (solvent: 40% ether-toluene) indicated two major components (R_f 0.29 and 0.16 respectively). The mixture was subjected to silica gel column chromatography using a gradient of ether in toluene as the eluting solvent. 9□-Cholest-7-ene-3□,11□-diol (**3**, R_f 0.16, 0.3 g, 12%). The physical and spectral properties were identical with **3** prepared from the reductive rearrangement of epoxide **l**. 9□-Cholest-7-ene-3□,11□diol (**5**, R_f 0.29, 1.2 g, 47%).

MP 160-163ºC; $\square_{max}$(KBr)/cm^{-1}3355, 1630, 1468, 1381 and 1034; $\square_H$(250 MHz; $CDCl_3$) 0.55 (3H, s, 18-H_3 0.86 (3H, s. 19-H_3), 3.60 (lH, m, 3□-H), 4.35 (lH, ddd, J=3.0, 3.2 and 3.1 Hz, 11□-H), 5.29 (lH, m, 7-H); m/z 402 (M^+, 5%), 387 (M^+CH_3, 8%), 384 (M^+-H_2O, 21%), 369 (M^+-CH_3-H_2O, 209%), 351 (M^+-CH_3-$2H_2O$, 8%), 289 (M^+-side chain, 14%), 274 (M^+-CH_3-side chain, 2%), 271 (M^+-H_2O-side chain, 100%), (Found: M^+, 402.3491, $C_{27}H_{46}O_2$ require M, 402.3496).

3□-Benzoyloxy-9□-Cholest-7-en-11-one (6)

3□-Benzoyloxy-9□,11□-epoxy-5-cholest-7-ene **1** (2.0g, 3.96 mmol) was dissolved in 10ml of THF and 200 ml of anhydrous ethyl ether. The solution was cooled to 0ºC and 10 ml of boron trifluoride etherate was slowly added with stirring. The mixture was kept at 0ºC for 30 minutes, then was poured into water and extracted with ether. The extracts of ether were evaporated to dryness at reduced pressure and recrystallized to yield 1.84 g of compound **6** (92% yield).

MP 155-156ºC; □$_{max}$ (KBr)/cm^{-1} 1716, 1653, 1277 and 131; □$_H$ (250 MHz; $CDCl_3$) 0.71 (3H, S, 18-H_3), 0.88 (3H, S, 19-H_3), 5.05 (lH M, 3□-H), 3.38 (lH, s, 9□H), 5.55 (lH, m, 7-H), 7.45 (3H, m, aromatic), 8.02 (2H, m, aromatic); m/z 504 (M^+, 12%), 489 (M^+-CH_3, 52%), 391 (M^+-side chain, 11%), 382 (M^+-B_zOH, 61%), 367 (M^+CH_3-B_zOH, 100%), 364 (M^+-H_2O-B_zOH, 12%), 352 (M^+-2CH_3-B_zOH, 21%), 349 (M^+-CH_3-H_2O-B_zOH, 32%), (Found: M^+, 504.3612, $C_{34}H_{48}O_3$ requires M, 504.3606).

9□-Cholest-7-ene-3□-diol (7) and **9□-Cholest-7-ene-3□,11□-diol (8)**

3□-Benzoyloxy-9□-cholest-7-ene-11-one (**6**) (5.0 g) was dissolved in 4 ml of anhydrous ethyl ether. Lithium aluminum hydride 5.0 g was slowly added and the mixture was stirred at room temperature for 8 h. The solution was cooled to 0ºC and ice was poured into a saturated aqueous ammonium chloride solution and thoroughly extracted with ether. The extracts were evaporated to dryness at reduced pressure to yield 3.7 g of solid. TLC analysis (solvent: 30% ether-toluene) indicated two major components (R_f 0.36 and 0.47, respectively). The mixture was subjected to silica gel column chromatography using a gradient of ether in toluene as the eluting solvent.

9□-Cholest-7-ene-3□,11□-diol (**8**, R_f 0.36, 3.3 g, 66%).

MP 191-193ºC; □$_{max}$(KBr)/cm^{-1} 3395, 1630, 1466, 1377 and 1047; □$_H$ (250 MHz; $CDCl_3$) 0.80 (3H, s, 18-H_3), 0.91 (3H, s, 19-H_3), 3.63 (lH, m, 3□-H), 4.25 (lH, ddd, J=l0.0, 6.7 and 8.0 Hz, 11□-H), 5.71 (lH, m, 7-H). M/e 402 (M^+, 41%), 387 (M^+CH_3, 4%), 384 (M^+-H_2O, 22%), 369 (M^+-CH_3-H_2O, 38%), 354 (M^+-2CH_3-H_2O), 4%, 351 (M^+-CH_3-2H_2O, 21%), 289 (M^+-side chain, 100%), 271 (M^+-H_2O-side chain, 29%), (Found: M^+, 402.3498, $C_{27}H_{48}O_2$ requires M, 402.3493).

9□-Cholest-7-ene-3□,11□-diol (**7**, R_f 0.46, 90.56 g, 12%).

MP 190-192ºC; □$_{max}$(KrBr)/cm^{-1} 3359, 1612, 1375, 1261 and 1047; □$_H$ (250 MHz; $CDCl_3$) 0.80 (3H, s, 18-H_3), 0.91 (3H, s, 19-H_3), 3.60 (lH, m, 3□-H), 4.03 (lH, ddd, J=0.9, 6.2 and 1.2 Hz, 11□-H), 5.60 (lH, m, 7-H). M/z 402 (M^+, 38%), 387 (M^+CH_3, 4%), (M^+-H_2O, 22%), 384 (H^+-H_2O, 22%), 369 (M^+-CH_3-H_2O, 37%), 354 (M^+2CH_3-H_2O, 3%), 351 (M^+-2H_2O-CH_3, 23%), 289 (M^+-side chain, 100%), 271 (M^+H_2O-side chain, 28%), (Found: M^+, 402.3497, $C_{27}H_{48}O_2$ requires M, 402.3493).

4. References

Smith, L. L. (1981). Cholesterol Autoxidation. Plenum Press, New York, (pp. 231256).

Parish, E. J., Nanduri, V. B. B., Kohl. H. H., &Taylor, F. R. (1986). Oxysterols: Chemical synthesis, biosynthesis and biological activities. Lipids, 21, 27-30.

Gibbons, G. F. (1983). The role of oxysterols in the regulation of cholesterol biosynthesis. Biochem. Soc. Trans., 11, 649-651.

Cheng, K. P., Nagano, N., Bang, L., &Ourisson, G. (1977). Chemistry and biochemistry of Chinese drugs. I. Sterol derivatives cytotoxic to hepatoma cells. J. Chem. Res., (S)217, (M)2521.

Nagano, J., Poyser, J. P., Cheng, K-P., Bang, L., Ourisson, G., & Beck, J. P. (1977) Chemistry and biochemistry of Chinese drugs. II. Cytotoxicity of hydroxysterols on tumor cells. Synthesis and biological activity. J. Chem. Res., (S)218, (M)2522.

Zander, M., Patrick, K., Bang, L., &Ourisson, G. (1977). Chemistry and biochemistry of Chinese drugs. III. Mechanism of action of hydroxylated sterols in cultured hepatoma cells. J. Chem. Res., (S)219, (M)2572.

Schroepfer, G. J. Jr. (1981). Sterol Biosynthesis. Annu. Rev. Biochem., 50, 585-611.

Schroepfer, G. J. Jr. (1982). Sterol Biosynthesis. Annu. Rev. Biochem., 51, 555-585.

Kandutsch, A. A., & Taylor, F. R. (1985). Lipoprotein and Cholesterol, Metabolism. Straus JF, Menon KMJ, editors. In: Steroidogenic Tissues, 194-219.

Gibbons, G. F. (1983). Molecular control of 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase: The role of oxygenated sterols. In J. R. Sabine (Ed.), 3-Hydroxy-3methylglutaryl Coenzyme A Reductase (pp. 153-232). West Palm Beach: CRC Press.

Taylor, F. R., Saucier, S. E., Shown, E. P., Parish, E. J., &Kandutsch, A. A. (1984). Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of hydroxymethylglutaryl coenzyme A reductase. J. Biol. Chem., 259, 12382-12387.

Panini, S. R., Sexton, R. C., Gupta, A. K., Parish, E. J, Chitrakorn, S., &Rudney, H. (1986). Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol biosynthesis by oxylanosterols. J. Lipid Res., 27, 11901204.

Kandutsch, A. A., Taylor, F. R., & Shown, E. P. (1984). Different forms of the oxysterol-binding protein. Binding kinetics and stability. J. Biol. Chem., 259, 12388-12397.

Morisaki, M., Sonoda Y., Makino, T., Ogihara, N., Ikekawa, N., &Sato, Y. (1986).

Inhibitory Effect of 15-Oxygenated Sterols on Cholesterol Synthesis from 24,25-Dihydrolanosterol. J. Biochem., 99, 597-600.

Kandutsch, A. A., Chin, H. W., &Heiniger, H. J. (1978). Biological activity of some oxygenated sterols. Science, 201, 498-501.

Spencer, T. A. (1994). The oxidation of crystalline cholesterol. Acc. Chem. Res., 27, 83-90.

Taylor, F. R. (1992). Oxysterol regulation of Cholesterol biosynthesis. In W. D. Nes, E. J. Parish, & M. Trzaskos (Eds.), Regulation of lsopentenoid Metabolism (pp. 81-93). ACS Symposium Series. American Chemical Society, Washington, D. C.

Parish. E. J, Parish, S. C., & Li, S. (1995). Side-chain oxysterol regulation of 3Hydroxy-3-methylglutaryl Coenzyme A reductase activity. Lipids., 30, 247251.

Schroepfer, G. J., Jr., Parish, E. J., &Kandutsch, A. A. (1988). Inhibitors of sterol biosynthesis. Synthesis and activities of ring C oxygenated sterols. Chem. Phys. Lipid, 46, 147-154.

Parish, E. J., Chitrakorn, S., Luu., B., Schmidt, G., &Ourisson, G. (1989). Studies of the oxysterol inhibition of tumor cell growth. Steroids, 53, 579-596.

Parish, E. J., Nanduri, V. B. B., Seikel, J. M., Kohl, H. H., &Nusbaum, K.E. (1986). Synthesis of 3□-hydroxy-5□-cholest-8-en-7-one and 3□-hydroxy-5□-cholest-8en-11-one: Evaluation as potential hypocholesterolemic agents. Steroids, 48, 407-418.

Parish, E. J., Spike, T. E., &Schroepfer, G. J. (1977). Sterol synthesis. Chemical synthesis of 3□-benzoyloxy-14□,15□-epoxy-5□-cholest-7-ene, a key intermediate in the synthesis of 15-oxygenated sterols. Chem. Phys. Lipids, 18, 233-239.

Conner, B. N., Parish, E. J., Schroepfer, G. J., Jr., &Quiocho, F. A. (1977). Synthesis and crystal structure of 3□-p-bromobenzoyloxy-14□,15□-epoxy-5□-cholest-7ene. Chem. Phys. Lipids, 18, 240-257.

Parish, E. J., Luo, C., Webb, T., &Gorden, J. D. (2007). Syntheses of ring C oxysterols: inhibitors of sterol biosynthesis. Lipids, 42, 35-40.

Henbest, H. B., & Wilson, R. A. L. (1956). Preparation of 1-oxygenated steroids. The reaction of cholest-1-en-3□-ol with thionyl chloride. J. Chem. Soc., 3289-3292.

Plattner, P. A., Fürst, A., Marti, L. &Schmid, H. (1949). Zur Kenntnis der Sesquiterpene und Azulene. 87. Mitteilung. Synthese des Guaj-azulens I. Helv. Chim. Acta, 32, 2137-2144.

Plattner, P. A., Heusser, H., &Kulkarni, A. B. (1948). Über Steroide und

Sexualhormone. 155. Mitteilung. Über 3□,5-Dioxy-koprostan und zwei epimere 3,4-Dioxy-cholestane. Helv. Chim. Acta, 31, 1822-1831.

Plattner, P. A., Heusser, H., &Kulkarni, A. B. (1948). Über Steroide und Sexualhormone. 156. Mitteilung. Über die reductive Aufspaltung von Steroidepoxyden mit Lithiumaluminiumhydrid, I. Synthese von 3□,5-Dioxykoprostan. Helv. Chim. Acta, 31, 1885-1890.

Plattner, P. A., Heusser, H., &Kulkarni, A. B. (1949). Über Steroide und

Sexualhormone. 158. Mitteilung. Über die reductive Aufspaltung von Steroidepoxyden mit Lithiumaluminiumhydrid III. Vereinfachte Synthesen von Derivaten des 5-Oxy-koprostans. Helv. Chim. Acta, 32, 265-269.

Plattner, P. A., Heusser, H., &Kulkarni, A. B. (1949). Über Steroide und Sexualhormone. 162. Mitteilung. Über die □-Oxyde des Allo-cholesterins und des Epi-allo-cholesterins. Helv. Chim. Acta, 32, 1070-1074.

Parish, E. J., &Schroepfer, G. J., Jr. (1981). Sterol synthesis. A simplified method for the synthesis of 32-oxygenated derivatives of 24,25-dihydrolanosterol. J. Lipid Res., 22, 859-868.

Parish, E. J., Luo, C., Parish, S., &Heidepriem, R. W. (1992). Selective Oxidation of Steroidal Homoallylic Alcohols Using Pyridinium Chlorochromate (PPC). Synth. Commun., 22, 2839-2847.

Parish, E. J., Newcomer, M. E., Gilliland, G. L., Quiocho, F. A., &Schroepfer, G. J., Jr. (1976). Synthesis and structure of 15-oxygenated 5□,14□-cholest-7-en-3□ol derivatives. Tetrahedron Lett., 17, 4401-4404.

Parish, E. J., &Schroepfer, G. J., Jr., (1977). Sterol synthesis. Synthesis of 15oxygenated 5□,14□-cholest-7-en-3□-ol derivatives. Chem. Phys. Lipids, 19, 107-113.

Schroepfer, G. J., Jr., Parish, E. J., Chen, H. W., &Kandutsch, A. A. (1977). Inhibition of sterol biosynthesis in L cells and mouse liver cells by 15-oxygenated sterols. J. Biol. Chem., 252, 8975-8980.

Schroepfer, G. J., Parish, E. J., &Kandutsch, A. A. (1979). Further studies on the inhibition of sterol biosynthesis in animal cells by 15-oxygenated sterols. Chem. Phys. Lipids, 25, 265-285.

Parish, E. J., Chitrakorn, S., Taylor, F. R., & Saucier, S. E. (1984). Chemical synthesis of 4,4'-dimethyl-7-oxygenated sterols. Inhibitors of 3-hydroxy-3methylglutaryl reductase. Chem. Phys. Lipids, 36, 179-188.

Parish, E. J., Wei, T.-Y., &Livant, P. (1987). A facile synthesis and carbon-13 nuclear magnetic resonance spectral properties of 7-ketocholesteryl benzoate. Lipids, 22, 760-763.

Saucier, S. E., Kandutsch, A. A., Phirwa, S., &Spenser, T. A. (1987). Accumulation of regulatory oxysterols, 32-oxolanosterol and 32-hydroxylanosterol in mevalonate-treated cell cultures. J. Biol. Chem., 262, 14056-14062.