



Steroid and sterol 7-hydroxylation: ancient pathways

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Abstract

B-ring hydroxylation is a major metabolic pathway for cholesterol and some steroids. In liver, 7 α -hydroxylation of cholesterol, mediated by CYP7A and CYP39A1, is the rate-limiting step of bile acid synthesis and metabolic elimination. In brain and other tissues, both sterols and some steroids including dehydroepiandrosterone (DHEA) are prominently 7 α -hydroxylated by CYP7B. The function of extra-hepatic steroid and sterol 7-hydroxylation is unknown. Nevertheless, 7-oxygenated cholesterol derivatives are potent regulators of cell proliferation and apoptosis; 7-oxygenated derivatives of DHEA, pregnenolone, and androstenediol can have major effects in the brain and in the immune system. The receptor targets involved remain obscure. It is argued that B-ring modification predated steroid evolution: non-enzymatic oxidation of membrane sterols primarily results in 7-oxygenation. Such molecules may have provided early growth and stress signals; a relic may be found in hydroxylation at the symmetrical 11-position of glucocorticoids. Early receptor targets probably included intracellular sterol sites, some modern steroids may continue to act at these targets. 7-Hydroxylation of DHEA may reflect conservation of an early signaling pathway. © 2002 Published by Elsevier Science Inc.

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1. 7-Oxygenated steroids and sterols

Steroid and sterol oxidoreduction governs biological activity and metabolic fate. Oxidative loss of the cholesterol side-chain generates steroids; oxidoreduction of the steroid nucleus (3-, 11- and 17-positions in particular) dictates activity and specificity. Recent work now points to an important role for B-ring (6- and 7-positions) modification.

7-Oxygenated steroids and sterols are widespread in mammals, birds, fish, and plants. Sterol processing in liver provides the best example of B-ring oxygenation. Hepatic 7 α -hydroxylation of cholesterol is the rate-limiting step for bile acid synthesis and elimination (Fig. 1). B-ring hydroxylation of sex steroids in liver may also represent metabolic elimination.

Nevertheless, prominent B-ring hydroxylation is also seen in diverse extra-hepatic tissues. This could argue against simple substrate inactivation. The major 3 β -hydroxysteroids including dehydroepiandrosterone (DHEA), pregnenolone, and androstane-3 β ,17 β -diol (A/enediol) are efficiently 7 α -hydroxylated in diverse tissues including brain [1–16]

Abbreviations: OH, hydroxy; OOH, hydroperoxy; A/enediol, androstane-3 β ,17 β -diol; A/enediol, androstene-3 β ,17 β -diol; DHEA, dehydroepiandrosterone; HSD, hydroxysteroid dehydrogenase; oxo, equivalent to keto

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(Figs. 2 and 3), with some 6 α and 7 β modification depending on the substrate conformation. Other steroids are modified: testosterone is 7 α -hydroxylated in testis [17,18] while 5 α -3 α steroids give rise to 6 α -hydroxy (OH) derivatives in prostate and lymphocytes [7,13,19].

With the exception of hepatic bile acid formation via 7 α -hydroxylation, almost nothing is known of the biological role of B-ring oxygenated sterols and steroids. One insight is provided by studies on the enzymes that catalyze their synthesis.

2. B-ring hydroxylated molecules: enzymes mediating their formation

2.1. Sterol 7-hydroxylation

At least three enzymes mediate sterol B-ring hydroxylation in liver. (1) CYP7A, whose expression is restricted to liver, hydroxylates cholesterol at the 7 α -position [20,21]; the enzyme has not been reported to metabolize steroids. (2) Studies on mice lacking CYP7A revealed an alternative pathway for bile acid synthesis via a related enzyme, CYP7B [22,23], expressed in liver and multiple other tissues (below). (3) A hepatic 7 α -hydroxylase specific for 24(S)-hydroxycholesterol (24(S)-OH-cholesterol), CYP39A1, has also been described [24]. In brain, but

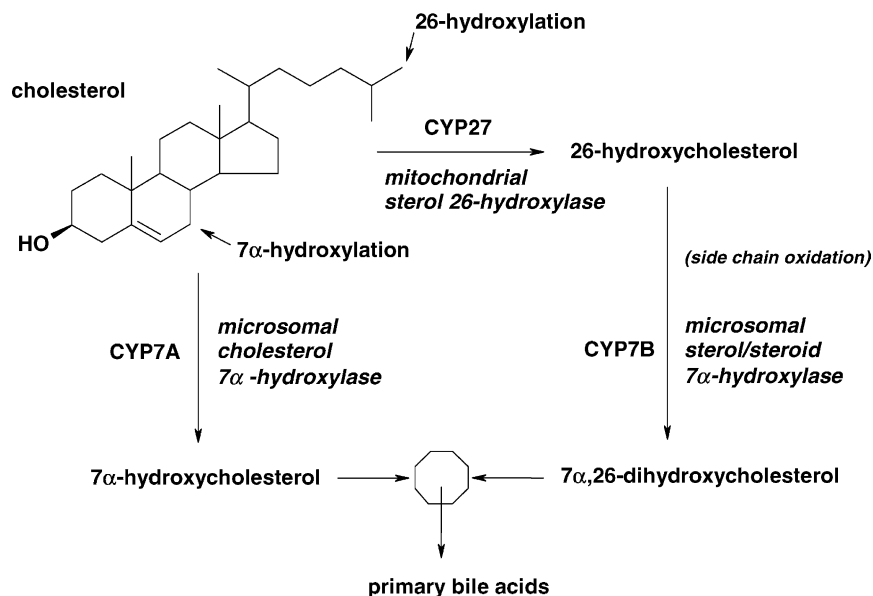


Fig. 1. Co-action of CYP7A and CYP7B on cholesterols to form bile acids. Figure redrawn from Schwarz et al. [30].

64 not in other tissues, 24(*S*)-hydroxylation of cholesterol
65 is a major export pathway [25–27]; brain-derived 24(*S*)-
66 OH-cholesterol is further metabolized in liver by CYP39A1.

67 2.2. Steroid 7-hydroxylation

68 In brain, several different B-ring hydroxylase enzymes
69 were suspected. Although DHEA is primarily 7α-hydro-
70 xylated, inhibitor studies pointed to a second enzyme with

activity at 7β [12,14–16]. A/enediol is principally 6α-
hydroxylated in brain and prostate [6,7,9,28], suggestive of
a further enzyme.

We reported molecular cloning of an enzyme from ro-
dent hippocampus, CYP7B, with sequence similarity to
CYP7A [29]. The enzyme differs from CYP7A in a number
of significant respects. First, in addition to catalyzing the
7α-hydroxylation of sterols (25- and 26-OH-cholesterols;
[30–32]), it is robustly active against steroids including

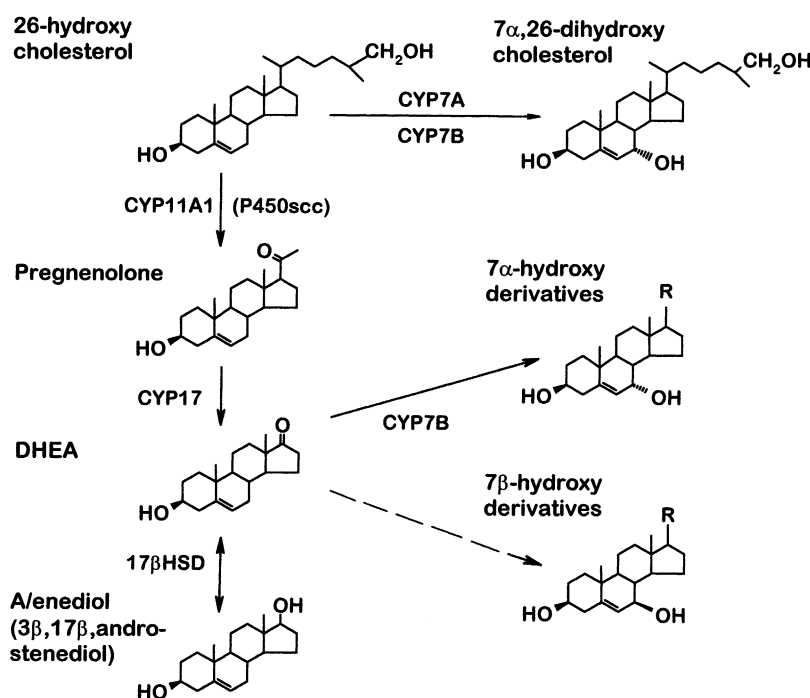


Fig. 2. 7-Hydroxylation of OH-cholesterol and 3β-hydroxysteroids.

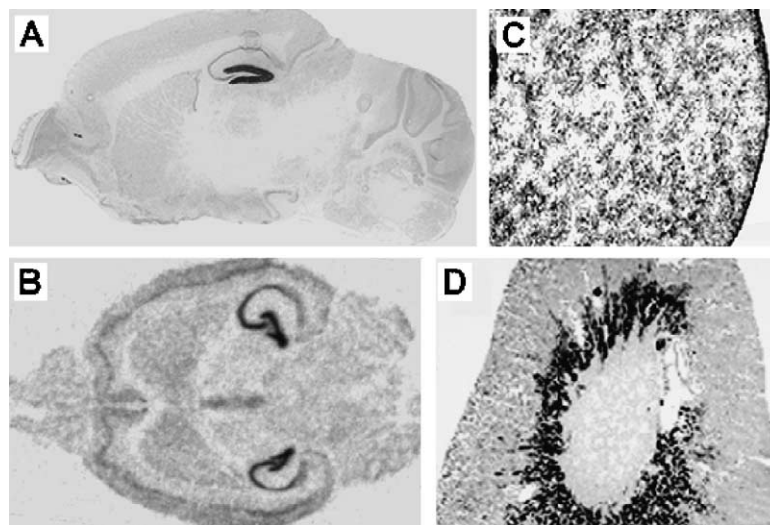


Fig. 3. CYP7B expression in brain and liver and kidney. Panels A, C, and D depict reporter gene expression (dark coloration), in transgenic mice, under control of CYP7B regulatory elements (A, brain, sagittal section; C, liver; D, kidney). Panel B shows in situ hybridization (dark/grey coloration) using a CYP7B probe (brain, horizontal section) [33]. Expression in neonates is very much more widespread and abundant [138].

80 DHEA, pregnenolone, and androstene-3 β ,17 β -diol (A/ene-
 81 diol); 17 β -estradiol was also modified at a lower rate [31].
 82 Second, CYP7B is not restricted to liver, and is expressed
 83 widely in the brain and other tissues [29,33], Fig. 3. In addition to
 84 affecting sterol 7 α -hydroxylation in liver [34], CYP7B gene
 85 disruption abolished steroid and sterol hydroxylation in di-
 86 verse tissues including brain, spleen, thymus, heart, lung
 87 (in male), prostate, uterus, and mammary gland [33]. Third,
 88 CYP7B may modify other positions including 6 α and 7 β :
 89 knockout mice fail to modify A/enediol (that is normally
 90 6 α -hydroxylated) while recombinant CYP7B enzyme ex-
 91 pressed in vaccinia or in yeast generates minor secondary
 92 metabolites including 7 β -hydroxysteroids ([33]; Vico et al.,
 93 Yeast, in press); 7 β metabolites are also abolished in CYP7B
 94 knockout mice [33] though onwards metabolism from 7 α
 95 was not excluded (see Lardy, this volume).

96 2.3. Other B-ring hydroxylases

97 Hepatic steroid metabolism includes hydroxylation at
 98 the 6 β -position by members of the CYP3A family [35,36].
 99 Outwith liver, CYP7B appears to be the primary steroid
 100 and sterol hydroxylase, though a distinct testosterone
 101 7 α -hydroxylase has been described in testis (CYP2A9/
 102 15 [17,18]) and in human (but not rodent) prostate,
 103 6 α -hydroxylation of 5 α -3 α steroids is performed by a
 104 non-P450 enzyme [7,13]. Other enzymes may exist.

105 3. Signaling by B-ring oxygenated sterols

106 Sterol hydroxylation at the 7 α -position is central to bile
 107 acid synthesis in liver; the role of steroid and sterol B-ring
 108 hydroxylation in other tissues is unknown. There is evidence

that they may play regulatory and/or signaling roles, ex- 109
 110 emplified (below) by effects on cholesterol regulation and
 111 apoptosis; other diverse effects of B-ring modified sterols
 112 have been noted (not reviewed).

113 3.1. Cholesterol homeostasis

114 Excess cholesterol represses its own synthesis and up-
 115 regulates hepatic CYP7A expression to promote elimina-
 116 tion. Conversely, bile acid excess can repress expression of
 117 CYP7A. Complex transcriptional regulation acts via a series
 118 of nuclear hormone receptors including LXR α , LXR β , FXR
 119 and LRH-1 [37–39] that mediate responses to oxygenated
 120 sterols and bile acids. B-ring modification does not appear
 121 to be essential for this regulation. While 6 α -OH-cholesterol
 122 may activate LXR [40] and 6 α -epoxycholesterols and
 123 7-oxocholesterol can inhibit [41], LXR is most efficiently
 124 activated by 22(R)- and 24(S)-OH-cholesterols [42,43].
 125 Though 6- or 7-hydroxylated sterols could contribute to this
 126 regulation, B-ring hydroxylated sterols may exert effects
 127 via other pathways.

128 3.2. Apoptosis control

129 One pathway links cholesterol supply to cell proliferation
 130 and/or programmed cell death. Oxysterols are inhibitors
 131 of cell activation and proliferation, and can induce cell
 132 death, particularly in lymphocytes [44–47]. 7 β -OH and
 133 7-oxocholesterols are neurotoxic [48]. The most potent
 134 apoptosis-inducing activity found in oxidized LDL was iden-
 135 tified as 7 β -hydroperoxycholesterol (7 β -OOH-cholesterol)
 136 [49,50]. Death in these models can be via classical apoptotic
 137 pathways [51,52].

138 Cell death may be a consequence of cholesterol biosyn-
139 thesis inhibition; however, the specific pathways by which
140 sterols can induce apoptosis remain to be elucidated.

141 4. Signaling by B-ring hydroxylated steroids

142 4.1. Brain function

143 The major metabolic route for DHEA in extra-hepatic
144 tissues is via 7α -hydroxylation [31–33]. The metabolism
145 of DHEA is of some interest. DHEA (and pregnenolone)
146 promote synaptic plasticity and memory function in ex-
147 perimental animals [53–59]. Further, blood DHEA levels
148 fall markedly with age in primates [60–65]. Cognitive de-
149 cline in old age could be causally linked to DHEA decline
150 [66–69]. However, oral DHEA replacement has not brought
151 the hoped-for improvements in cognitive function [66,70,71]
152 although beneficial effects are reported in adrenal dysfunc-
153 tion [72].

154 DHEA may require metabolism in target tissues. It is
155 of note that the DHEA metabolizing enzyme CYP7B is
156 particularly well-expressed in the hippocampus [29,33], a
157 brain region centrally involved in memory formation. Lardy
158 et al. [73] suggested that 7α -hydroxylation of DHEA is on
159 a metabolic pathway to more potent derivatives and recently
160 reported that 7-oxoDHEA (that may interconvert with 7-OH
161 derivatives) is more active in promoting brain function than
162 DHEA [74]. We have observed that 7α -OH-DHEA is more
163 active than DHEA in preventing hypoxic cell death of neu-
164 rones in vitro (Sundström, Martin, Lathe, Seckl, and Wulfert,
165 unpublished data). In the brain, therefore, 7-oxygenation seems to
166 be associated with activation of DHEA.

167 4.2. The immune system

168 DHEA and its metabolites promote the immune response
169 in experimental animals [75–84]; however, attempts to boost
170 immune-responsiveness in the elderly by DHEA replace-
171 ment have not been entirely promising [85].

172 As in brain, DHEA may require metabolism for bioactiv-
173 ity. CYP7B is expressed in thymus and in lymphocytes ([33];
174 our unpublished data). There is debate about the stereocon-
175 figuration of the active metabolite. 7α -OH-DHEA is a ma-
176 jor immunity-promoting derivative of DHEA [86,87] others
177 have argued that 7β -OH derivatives of A/enediol are most
178 effective ([78,82,88,89]; Loria, this volume).

179 4.3. Origins of 7β -hydroxylated molecules

180 Both 7α - and 7β -modified molecules have biological
181 activity, particularly in the immune system, but the origin
182 of 7β -OH molecules is enigmatic (see Lardy, this volume).
183 Several routes are possible. (1) Enzymatic hydroxylation:
184 trace 7β -modified molecules are seen in CYP7B reac-
185 tions [31]; allosteric modulation could favor 7β modifi-
186 cation [12]. (2) Epimerization: 7α -hydroperoxycholesterol

(7α -OOH-cholesterol) and 7α -OH-cholesterol may spon- 187
taneously epimerize to their 7β counterparts [88,89]; a 188
7-epimerase similar to the 3-epimerase enzyme [90] could 189
contribute. (3) Dehydrogenation and reduction: 11β -HSD 190
activity against 7α -OH-cholesterol [91] generates 7-oxo 191
molecules that could in turn generate 7β -OH derivatives. 192
All three are consistent with abolition of both 7α and 7β 193
derivatives by disruption of the CYP7B [33]. 194

5. Did signaling by B-ring hydroxylated molecules 195 predate conventional steroid signaling? 196

5.1. Dearth of conventional receptor targets 197

No dedicated conventional (nuclear) receptor has been 198
identified for 7-OH steroids. These could then act through 199
via gating (ligand inactivation) of typical nuclear receptors, 200
through the modulation of cell-surface ion channels (partic- 201
ularly in brain), or at atypical receptors. 202

A/anediol and DHEA are modest agonists of the es- 203
trogen and androgen receptors (ER and AR) [92,93]. 204
7-Oxygenation reduces activity of both molecules [1,94]. 205
Clearly hydroxylation can gate nuclear receptor access, but 206
the significance in vivo is unclear. 207

Hydroxylation of steroids (and possibly sterols) may mod- 208
ulate activity at cell-surface ion channels. Diverse channels 209
respond to steroids [95], but the GABA_A receptor has re- 210
ceived most attention. DHEA and related steroids are an- 211
tagonists of GABA_A, promoting neuronal activity (while 212
 3α - 5α steroids are agonists with potent anaesthetic proper- 213
ties). B-ring hydroxylation of DHEA and related steroids 214
could gate access to these receptors. 215

Gating of either sex steroid receptors or ion channels 216
such as GABA_A does not easily explain the apoptotic regu- 217
latory action and brain/immune system effects of these 218
molecules. For instance, GABA agonists can inhibit apopto- 219
sis, but steroids are orders of magnitude more effective than 220
the classic GABA agonist, muscimol [96]. This implies that 221
they are binding to other receptors. This could make sense if 222
these targets predated both the development of ion-channel 223
sensitivity to steroids and the radiation of the steroid hor- 224
mone receptor superfamily. 225

5.2. Late emergence of steroid signaling 226

Traditional wisdom depicts the evolution of intercellu- 227
lar steroid signaling from intracellular sterol signaling by 228
an evolutionary breakthrough—the oxidative removal of the 229
long hydrophobic side-chain of cholesterol via the action of 230
the P450_{scc} (side-chain cleavage; scc) enzyme, CYP11B. 231
This interpretation may be incomplete. 232

Steroid signaling proper emerged late in eukaryotic evo- 233
lution. The genome of the yeast, *Saccharomyces cerevisiae*, 234
contains no homolog to the vertebrate steroid hormone re- 235
ceptor family. Steroid signaling proper has been placed with 236

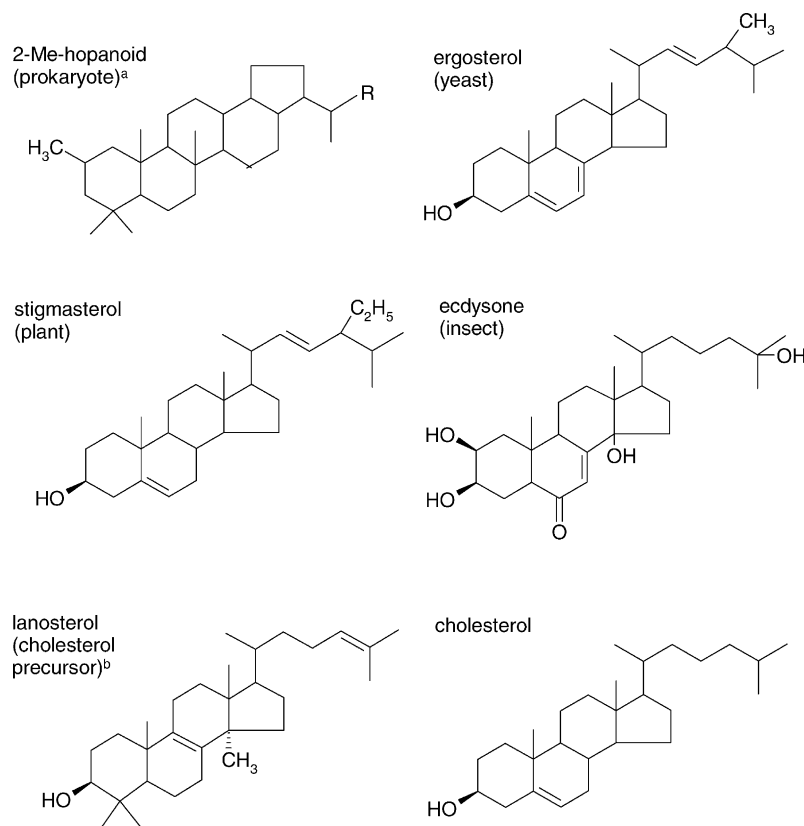


Fig. 4. Sterols/steroids from different organisms: (a) 2- (and 3-) methyl-bacteriohopanoids are commonly substituted (R in the figure) with a long side-chain (C8 outwith the hopanoid nucleus) bearing clustered OH groups (e.g. bacteriohopantetrol [103]); (b) cycloartenol rather than lanosterol is the sterol precursor in plants.

237 primitive fishes during the massive radiations taking place
 238 in the Cambrian period [97–100]. Ion-channel sensitivity
 239 to steroids only appears late in chordate evolution [101].
 240 Therefore, the full spectrum of growth, differentiation, and
 241 reproduction was achieved, in precursors to the vertebrate
 242 lineage, without conventional steroid signaling at either nu-
 243 clear or ion-channel receptors. These processes might have
 244 been subserved by sterols (rather than steroids) acting at
 245 atypical receptors.

246 5.3. Emergence of sterol-derived messengers: B-ring 247 modified derivatives can be generated non-enzymatically

248 Membrane sterols probably arose from terpenoids in-
 249 cluding the hopanoids of bacteria [102,103] with which
 250 they share axial and longitudinal dimensions required
 251 for membrane stabilization (but not the 3 β -OH group of
 252 steroids/sterols). Sterols of modern eukaryotes generally
 253 contain the 3 β -OH group, including ergosterol, and lanos-
 254 terol of fission and budding yeasts (*S. cerevisiae*, *Schizosac-*
 255 *charomyces pombe*), plant phytosterols such as stigmasterol,
 256 and the insect (and crab) hormone ecdysone (Fig. 4).

257 Signaling molecules can arise from abundant cell compo-
 258 nents. Membrane sterols are relatively insoluble; chemical
 259 oxidation of cholesterol primarily generates the more solu-

260 ble 7 α -OH, 7 β -OH and 7-oxo derivatives (Fig. 5). Lower
 261 amounts of 6 α -OH molecules, 5 α -6 α epoxides, and 7 α - and
 262 β -hydroperoxides are also produced, as are side-chain oxi-
 263 dized cholesterols. Oxygenation at the 5-6 unsaturated
 264 bond (perhaps facilitated by the 3 β -OH group) may pro-
 265 duce 5-6 epoxides that convert to 7-hydroperoxides,
 266 followed by thermal degradation to produce 7 α -OH, 7 β -OH,
 267 and 7-oxocholesterols [88,89,104–107]. Products of other
 268 membrane sterols may be similar [108]. B-ring oxidation is
 269 promoted by horseradish peroxidase, lipoxygenases, gamma
 270 irradiation, and metal ions (most particularly copper ion) and
 271 reduced in the presence of metal chelating agents (reviewed
 272 by Schroepfer [109]).

273 5.4. What do we know about the earliest sterol messengers?

274 Through increased solubility, and non-enzymatic produc-
 275 tion, 7-oxygenated sterols have considerable signaling po-
 276 tential. First, oxidized cholesterols are toxic, can bind to
 277 DNA and have mutagenic activity [110–112] possibly pro-
 278 viding an early driving force for inducible elimination (a
 279 relic of which may be found in the CYP7A export path-
 280 way). Second, they have the potential to signal both sterol
 281 abundance (growth) or sterol oxygenation (oxidative stress),
 282 suggestive of early growth and stress signals.

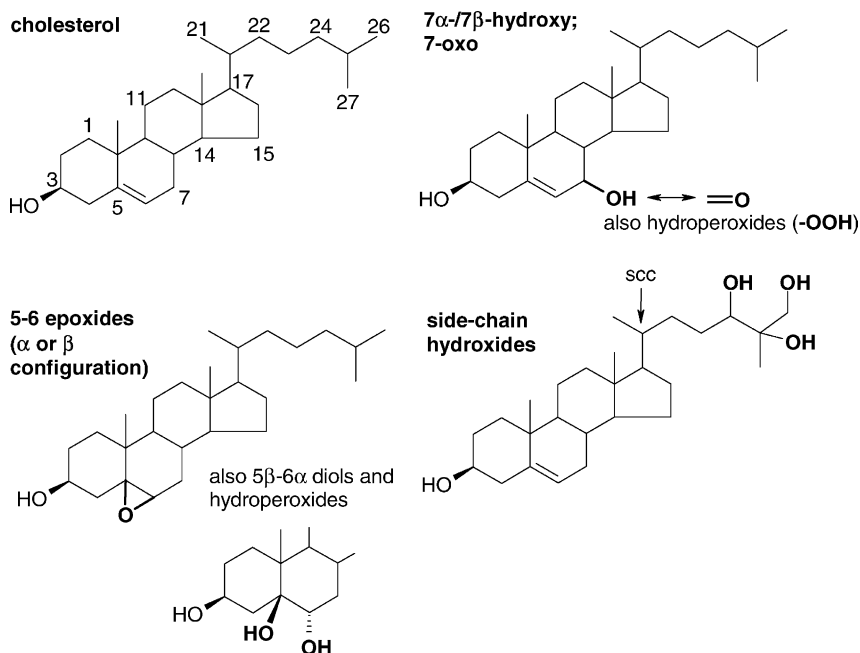


Fig. 5. Non-enzymatic oxidation products of cholesterol.

283 This latter idea finds some support in the structure of
 284 modern steroid receptors. The earliest steroid-type nu-
 285 clear receptor most resembled the present estrogen re-
 286 ceptor; this primordial receptor subsequently diverged to
 287 generate the estradiol/growth ($ER\alpha$, $ER\beta$, ERR) and glu-
 288 cocorticoid/stress families (GR , MR , PR , and later AR)
 289 [113–115].

290 Early ligands could have included 7-modified molecules.
 291 (1) Estrogen receptors: these respond to diverse 3β -hydroxy-
 292 lated steroids [116,117]; ligand binding to modern $ER\alpha$ is
 293 promoted by small 7α -substitutions that fit into an unoc-
 294 cupied cavity in the receptor [117]. (2) Glucocorticoid re-
 295 ceptors: these are activated by 11β -hydroxylated steroids.
 296 Crucially, the 11β - and 7α -positions are rotationally sym-
 297 metrical (Fig. 6): emphasized by an 11β -hydroxysteroid de-
 298 hydrogenase (HSD) with dual 11β - and 7α -dehydrogenase
 299 activity [91] and promotion of ligand binding to $ER\alpha$ by

11 β -substitutions [117]. Early 11β modifications may have
 exploited receptor targets binding 7α molecules. Thus, ex-
 isting 7-modified molecules (produced non-enzymatically)
 could have been early ligands for the joint precursor to ER
 and GR .

6. Early receptors may have included intracellular sterol sites

If signaling by sterols, possibly 7-modified sterols, pre-
 dated steroid signaling proper, what were the earliest targets
 for regulatory sterols? Molecular cloning experiments have
 begun to reveal a class of intracellular sterol-responsive tar-
 gets (see Moebius, this volume; reviewed in [118]). These
 include the emopamil binding protein (EBP), the sigma
 site, and the peripheral benzodiazepine receptor (PBR). (1)
 EBP encodes a sterol C8-C7 isomerase that catalyzes the
 penultimate step in the synthesis of cholesterol [119–123].
 (2) Sigma-1 shares significant homology with yeast $ERG2$
 (ergosterol synthesis; C8-C7 sterol isomerase) enzyme
 [122,124,125] but its catalytic activity has not yet been elu-
 cidated; related sigma-2 and -3 receptors have been discussed.
 (3) The PBR participates in translocating cholesterol from
 the outer to the inner mitochondrial membrane [126–128].
 These sites emerged early in evolution. Sigma finds a strict
 equivalent in the *S. cerevisiae* $ERG2$ gene product. PBR
 has only distant relatives in *S. cerevisiae* but a close coun-
 terpart in the fission yeast *S. pombe* ($SPBC725.10$); EBP
 has no obvious match in either yeast but the EBP -related
 protein $EBRP$ is highly homologous to a *S. cerevisiae* gene
 product, $YDL222C$, of unknown function.

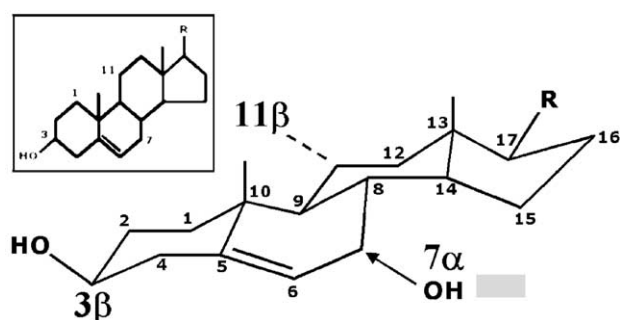


Fig. 6. Rotational symmetry between the 7α - and 11β -positions of the steroid nucleus. Some binding sites for 7α -modified molecules may accept 11β -modified equivalents.

329 These and other enzymes and transporters accompanied
330 the evolutionary switch to sterol-rich membranes, and are
331 contenders for the early regulatory targets for oxygenated
332 sterols. However, it is not known which, if any, were mod-
333 ulated by B-ring oxygenated molecules.

334 Some major drugs target intracellular sterol sites. Ligands
335 have marked effects on apoptosis and the immune system.
336 An anti-estrogen (tamoxifen) used in hormone-responsive
337 breast cancer may act via sterol sites; important brain-active
338 drugs, including anti-epileptics (diazepam), anti-ischemics
339 (emopamil), and neuroleptics (haloperidol) are ligands for
340 sterol sites. Sterols modulate the risk of Alzheimer's disease.
341 An understanding of these primitive pathways is vital.

342 6.1. Did the first steroids act at sterol sites?—the oxysterol 343 hypothesis

344 The first steroids, sterols lacking the hydrophobic
345 side-chain of cholesterol, may have targeted existing sterol
346 sites. In support, steroid action at oxysterol targets has been
347 demonstrated. Some sterol sites have significant affinity for
348 natural steroids including glucocorticoids, estrogens (and
349 anti-estrogens) and DHEA [129–137]. Different steroids
350 can have markedly different downstream effects at the same
351 sterol site. Functional overlap between sterols and steroids
352 is emphasized by present-day enzymes (CYP7B, 11 β -HSD)
353 that can modify both types of molecule.

354 Modern systemic steroids (including DHEA, estradiol,
355 and glucocorticoids and their metabolites) continue to target
356 sterol sites, acting in concert or in competition with endoge-
357 nous sterols. By this means steroids could, and can, con-
358 trol cell life and death at a systemic level. 7-Oxygenation
359 of 3 β -hydroxysteroids including DHEA may reflect conserva-
360 tion of early signaling pathways. In the search for targets
361 for B-ring modified steroids, intracellular sterol sites may
362 deserve some attention.

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