Enantiomeric Steroids: Synthesis, Physical, and Biological Properties

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Received October 28, 2002

Contents

Jean-Francois Biellmann was born in 1934 in Strasbourg, France. During his final year in high school, he was drawn to chemistry under the influence of his teacher in Chemistry. He received his B.S. degree in Chemistry in 1957 from the University of Strasbourg and an Engineering degree from Ecole Nationale Superieure de Chimie de Strasbourg. His Ph.D. (1961) thesis under the supervision of Professor Guy Ourisson at the University of Strasbourg was on the stereochemical problems in steroids and triterpenes. From 1959 to 1960, he carried out research with Professor William S. Johnson at the University of Wisconsin. After a military service, he received postdoctoral training with Professor D. Arigoni at the ETH Zurich. He joined the Chemistry Department at the University of Louis Pasteur, Strasbourg, in 1963 as a Research fellow of the CNRS. J.-F.B. is interested in synthetic methodologies, enzyme inhibition, enzyme structure modification of enzymes, use of enzymes in organic synthesis, and chromophoric reagents. He has studied a number of enzymes and synthesized their inhibitors including xylose isomerase, horse liver alcohol dehydrogenase, cholesterol oxidase, pig lens, and human aldose reductases. Structures of the native enzyme, aldose reductases from pig and human, and inhibitor−enzyme complexes were determined not only by single-crystal X-ray analyses, but also by electrospray mass spectrometry in collaboration. These structures have provided organic and medicinal chemists a direction for designing new inhibitors which could prevent diabetic complications. Biellmann is one of the pioneers in the development of poly(ethylene glycol) (PEG) modified enzymes and their uses in organic synthesis. A method discovered in his laboratory for the synthesis of squalene was named Biellman's coupling. Later he became interested in nucleoside analogues for the inhibition of reverse transcriptase and of ribonucleotide reductase. Biellmann is directeur de recherche emerite and presently holds a position of communicating research fellow at the Chemistry Department Academia Sinica in Taiwan. Dr. Biellmann is also an artist and enjoys woodcut printing (by Prof. Duy Hua, Kansas State University, Manhatten, KS). (Photo by Gabriel Goerger.)

I. Introduction

It remains puzzling that the triterpenes that serve as precursors of steroids have been found only as one enantiomer in nature. In contrast, both enantiomers of the lower terpenes can be found in nature, sometimes even in the same organisms. As the steroids derive from triterpenes, so they too are found in nature as one enantiomer. Different cyclization mechanisms do not explain the different stereochemical outcome. For most of the monoterpenes and for the sesquiterpenes the cleavage of a carbon−oxygen bond of the precursor phosphate ester is the initial step of the cyclization. Instead, for most of the diterpenes and for the triterpenes the cyclization proceeds by protonation of a double bond or by opening of an epoxide. Despite their similar mechanisms, both enantiomers of diterpenes are present in nature while the triterpenes are present in one enantiomer. The structures of the cyclases will shed some light on this matter.

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10.1021/cr020071b CCC: $44.00
Published on Web 04/10/2003

The total synthesis of steroids has given hold of the unnatural enantiomers which have been submitted to the same activity tests as *nat*-steroids and have been found to be of lower if any activity than the natural steroids. However, it remains to be examined whether the unnatural enantiomers may have biological activities different from those of the natural steroids. Indeed, if one looks at the steroid as a skeleton bearing functional groups interacting with a receptor, there is no reason the unnatural enantiomer should not interact with some receptor.

The steroid skeleton has some symmetry which makes both enantiomers more related than one may think at first look. On one hand, the central part of the steroid has a symmetry center as shown in formula **1** ($R_1 = R_2 = H$), Figure 1.

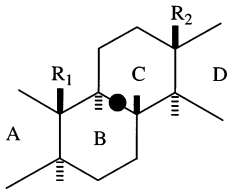

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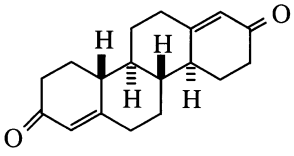
Figure 1.

This symmetry could be used in the total synthesis of steroids.[1−3] For instance, the tetracyclic skeleton **2**, Figure 2, and related compounds have received



2

Figure 2.

little attention in the total synthesis. A dissymmetrization in a later stage of the synthesis could be envisioned, and thus, the resolution would be avoided.

This partial symmetry of **1** ($R_1 = H$, $R_2 = Me$) has been utilized to prepare *ent*-19-nor-testosterone **30** from 19-nor-testosterone **29**.[4]

On the other hand, if one looks from C-3 of the steroid skeleton, one sees that rings A and C have a plane of symmetry. This is illustrated for testosterone **3** and *ent*-testosterone **4**. The *ent*-testosterone **4** may be drawn as **5**. There is a certain similarity of the *ent*-steroid **5** to the *nat*-steroid **3**, Figure 3.[5−7]

It remains puzzling that the following skeletons related to steroids **6** and **7**, Figure 4, appear to have attracted little attention with respect to their synthesis and their biological properties.

II. Nomenclature

The names given to the basic skeletons of steroids imply the stereochemistry at C-8, C-9, C-10, C-13, and C-14 but not at C-5. To describe their antipode, the prefix *ent*- is added for the enantiomer, *nat*- for the naturally occurring steroid, and *rac*- for the racemic steroid.[8−10]

The signs α/β indicate the relative stereochemistry. The α and β signs assume a relative sense: the substituent indicated by α (or β) is on the same side of the molecule as the α (or β) substituent in the basic

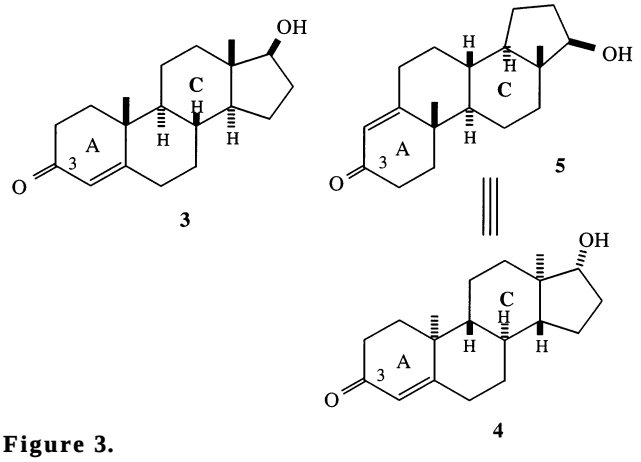
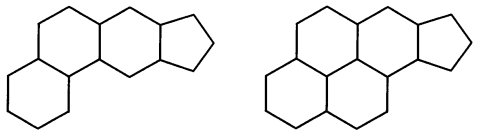


Figure 3.



6 **7**

Figure 4.

skeleton. The operation of naming an unnatural enantiomer amounts to naming the natural enantiomer and adding the prefix *ent*- to the name. This can be applied to the trivial names. The antipode of testosterone is *ent*-testosterone. In this review I will use this nomenclature and use the prefix *nat*- only in specific cases in order to avoid any confusion. The trivial name always refers to the *nat*-steroid. The prefix *rac*- may be useful to indicate a racemic steroid. Thus, testosterone **3** is 17β-hydroxyandrost-4-en-3-one and its enantiomer is *ent*-testosterone **4**, *ent*-17β-hydroxyandrost-4-en-3-one.

The (+) and (−) notation for the *nat*- and *ent*-steroids, respectively, has been used. The reason could be that the *nat*-steroids tend to be dextrarotatory. Indeed, a statistical survey of Table de Rotation shows that the *nat*-steroids tend to be dextrorotatory but the exceptions are numerous: *nat*-cholesterol is levorotatory. This proposal as well as the (D) and (L) notation should be avoided.

The synthesis of *ent*-steroids, their physical properties, and their biological effects will be treated in the following.

III. Synthesis

In this part I present the synthesis of *ent*-steroids from resolved precursor but not the synthesis of *rac*-steroids which were resolved. However, I will present the synthesis of *ent*-equilenin **8** by resolution because it is the first time that an *ent*-steroid has been prepared and its biological activity tested. The resolution of steroids by the action of microorganisms or enzymes will be discussed in section V, Biological Properties. Enantioselective synthesis of *nat*-steroids could be transferred to the preparation of *ent*-steroids. The *ent*-steroids will be presented in the order of increasing number of carbon atoms.

A. Synthesis of *ent*-Equilenin 8

Starting from 7-methoxy-1-keto-1,2,3,4-tetrahydrophenanthrene **9**, the diester **10** was built in the

following sequence: introduction of methyloxalo group converted to carbomethoxy group by pyrolysis in the presence of glass, methylation of the β-keto-ester enolate, Reformatsky reaction, dehydration, reduction, partial saponification, and Arndt−Eistert homologation. The Dieckmann cyclization of the diester **10** afforded the keto-ester **11**, which was converted to *rac*-equilenin **12** by acid treatment, Figure 5. The

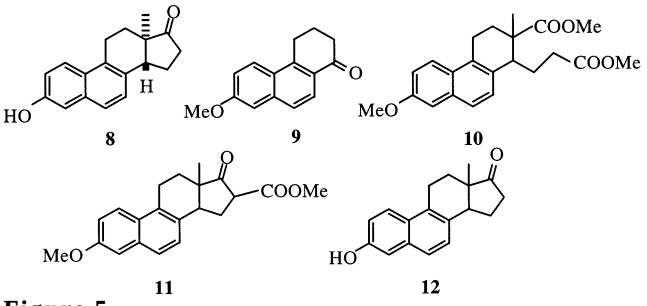


Figure 5.

use of L-menthoxyacetyl chloride for the resolution gave *nat*-equilenin on saponification, and the use of D-menthoxyacetyl chloride gave *ent*-equilenin **8**.[11]

B. Synthesis of *ent*-Estrone 23

*1. Preparation of ent-Estrone **23** and ent-Estrone Methyl Ether **24***

(+)-Camphor **13** was converted to (+)-9,10-dibromocamphor **14,** and ring fragmentation under basic conditions lead to the monocyclic acid **15** in a high yield, Figure 6. This acid **15** may be regarded as ring

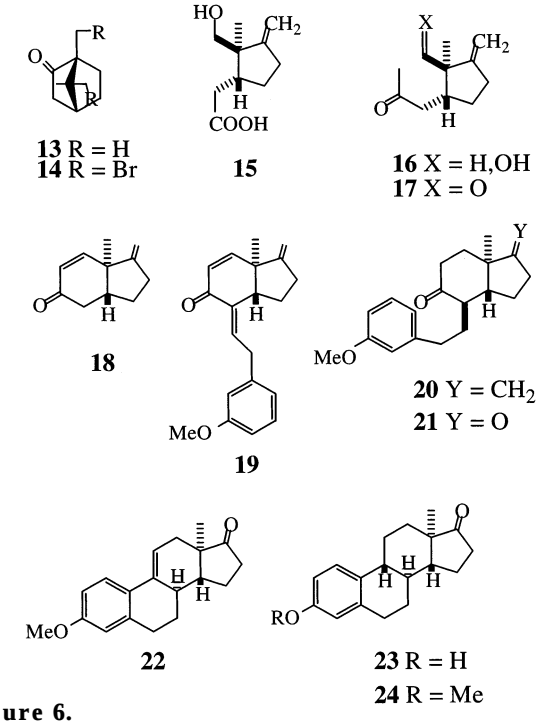


Figure 6.

D of *ent*-estrone **23**. Ring C is added by reaction sequence: action of methyllithium converts the acid **15** to the methyl ketone **16,** oxidation of the primary alcohol to the aldehyde **17**, and aldol condensation to transbicyclic dienone **18**. Reaction with Brederick reagent (t-BuOCH[NMe_2]$_2$), treatment with *m*-methoxybenzylmagnesium chloride, and acidification gave compound **19**. Reduction by lithium in ammonia gave ketone **20**, which was converted to the diketone **21** by ozonolysis. The diketone **21** was cyclized to *ent*-9,11- and *ent*-8,9-dehydroestrone methyl ether **22**. Catalytic hydrogenation gave the *ent*-estrone methyl ether **24**, which was demethylated by boron tribromide to *ent*-estrone **23**.[12,13]

Synthesis of *ent*-estrone **23**, *ent*-19-nor-androst-4-en-3,17-dione, and *ent*-19-nor-testosterone **30** makes use of cyclization of *o*-quinodimethane derivatives generated photochemically. Great care was taken to obtain the products in high ee.[14]

C. Synthesis of *ent*-3-Methoxy-16α-methylamino-estra-1,3,5(10)-trien-17β-ol 28

Reaction of bromhydrin **25** prepared from *ent*-3-methoxy-estra-1,3,5(10)-trien-17-one **24** (obtained by resolution[68]) with sodium azide in DMSO gave *ent*-3-methoxy-16α-azido-estra-1,3,5(10)-trien-17β-ol **26**, Figure 7. The reduction of the azido group with lith-

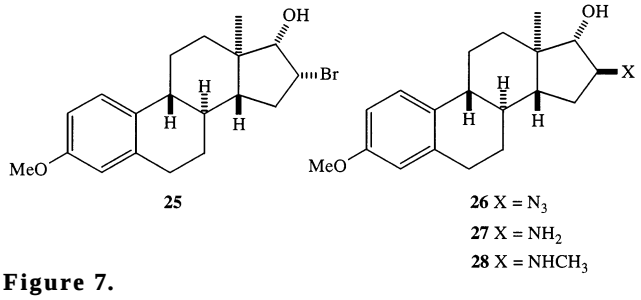


Figure 7.

ium aluminohydride gave the of *ent*-3-methoxy-16α-amino-estra-1,3,5(10)-trien-17β-ol **27**, and its formamide prepared by aminolysis of ethyl formate was reduced with lithium aluminohydride to the *ent*-3-methoxy-16α-methylamino-estra-1,3,5(10)-trien-17β-ol **28**.[15]

D. Synthesis of *ent*-19-Nor-testosterone 30

A 180° rotation of the molecule of 19-nor-testosterone **29** about the axis perpendicular to the skeleton shows that the rotated structure is enantiomeric to the original one except for rings A and D. The problem with preparing *ent*-19-nor-testosterone **30** from 19-nor-testosterone **29** is interchanging ring A and ring D, and this is not an easy task, Figure 8.

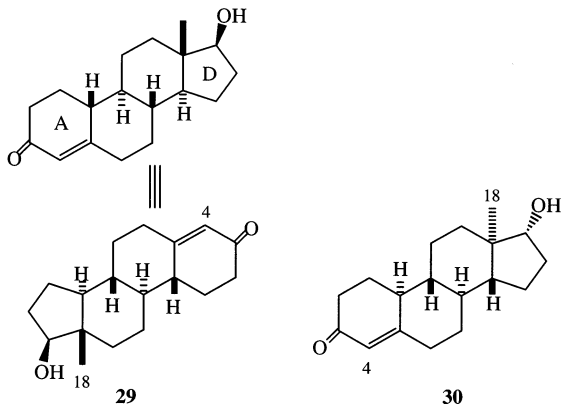


Figure 8.

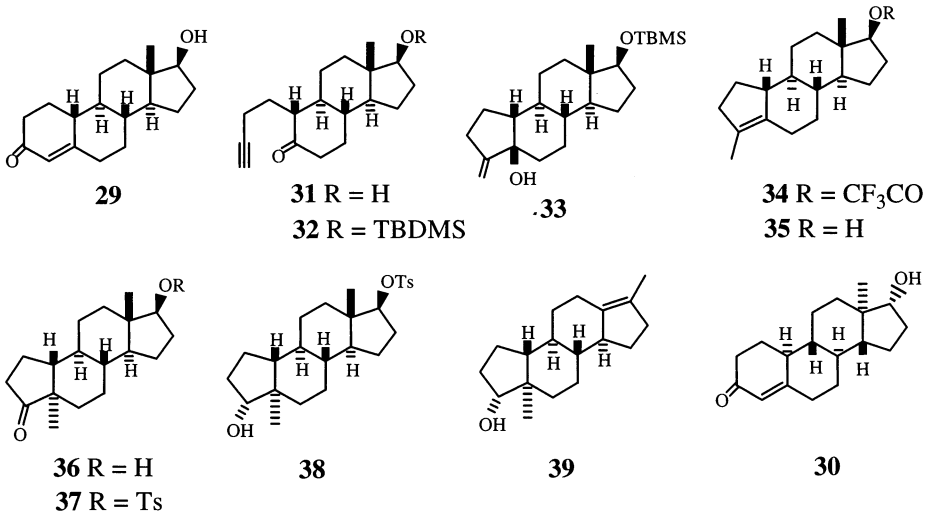


Figure 9.

Indeed, carbon C-4 has to be converted to the methyl group C-18 and the methyl group C-18 to carbon C-4.

19-Nor-testosterone **29** was converted to the epoxyketone by alcaline hydrogen peroxide. The corresponding tosylhydrazone fragmented into acetylenic ketone **31**, Figure 9. This acetylenic ketone **32** was cyclized to the allylic alcohol **33** by sodium naphthalenide. The allylic cation generated from alcohol in the presence of trifluoroacetic acid and of its anhydrid was reduced to olefin **34** by triethylsilyhydrid. The β-epoxide prepared from alcohol **35** with *tert*-butylperoxide in the presence of $Mo(CO)_6$ was converted to the ketone **36** by trifluoroborane. The C-17 hydroxyl group was converted to the tosylate **37,** and the keto group was reduced to the alcohol **38**. The reaction with ethylmagnesium bromide induced migration of the C-18 methyl group to C-17 and generated alkene **39**. Then alkene **39** was converted by cleavage and cyclization of the diketone to enone which is *ent*-19-nor-testosterone **30**.[4]

E. Synthesis of *ent*-Testosterone 4

To explore the structural dependence of the sensory process of steroids, *ent*-testosterone **4** and a number of *ent*-steroids were prepared. The *ent*-testosterone **4** was prepared in 15 steps following the total synthesis of testosterone. Great care was taken to obtain the products in high ee by recrystallization of two intermediates until constant melting point and rotation. By using the Hajos proceedure with (*R*)-proline,[16] diketone **40** was obtained and converted in seven steps to the tricyclic compound, which was converted to *ent*-testosterone **4** by described reactions. Enolate of diketone **40** was alkylated with 1,3-dichloro-2-buten, and hydrolysis of the chorovinylic group to the triketone **41** was done under acidic condition. This triketone **41** was converted to dienic-hemiketal **42** and the keto group reduced with lithium aluminohydride in order to obtain a high yield in the trans-hydrindan system. Catalytic hydrogenation gave hemiketal **43**. Acid hydrolysis of the hemiketal **43** was followed by cyclization of the diketone to the tricyclic compound **44** under basic condition. Alkylation with 1,3-dichloro-2-butene, acidic hydrolysis, and formation of ketal **45** was followed by methylation of the enolate, hydrogenation, hydrolysis of the ketal, and cyclization to *ent*-testosterone **4**, Figure 10.[17]

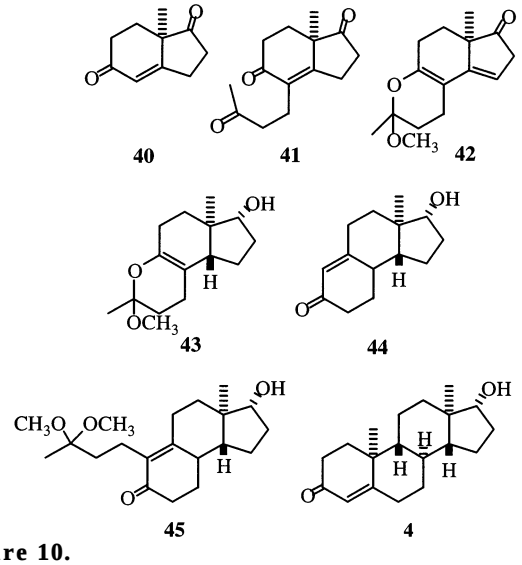


Figure 10.

This synthesis of *ent*-testosterone **4** was improved in order to prepare *ent*-cholesterol. The diketone **40** was reduced selectively to alcohol **46**, and the hydroxyl group was protected as *tert*-butyl ether **47**, Figure 11. Stiles' reagent gave product **48**. Catalytic reduction and reaction with formaldehyde afforded the *trans*-methylenketone **49**. A Robinson annulation with keto-ester **50** gave the tricyclicenone **51**. Reduction of the conjugated double bond by lithium in ammonia was followed by the alkylation of the resulting enolate by methyl iodide to give ketal-diketone **52**. At this stage recrystallization to constant rotation was performed. Reaction in acidic condition yielded the *ent*-testosterone **4**, which was used to prepare *ent*-cholesterol.[18,19]

F. Synthesis of *ent*-Androsta-4,9(11)-dien-3β,-17β-diol 53 and *ent*-Androsta-5,9(11)-dien-3β,-17β-diol 54

ent-Androsta-4,9(11)-dien-3β,17β-diol **53** and *ent*-androsta-5,9(11)-dien-3β,17β-diol **54** were prepared

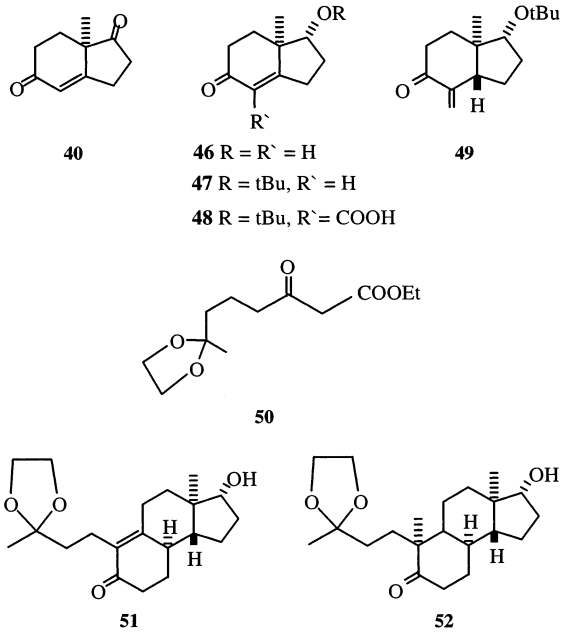


Figure 11.

from chiral dione **55** by described reactions whose yields were improved, Figure 12.[7]

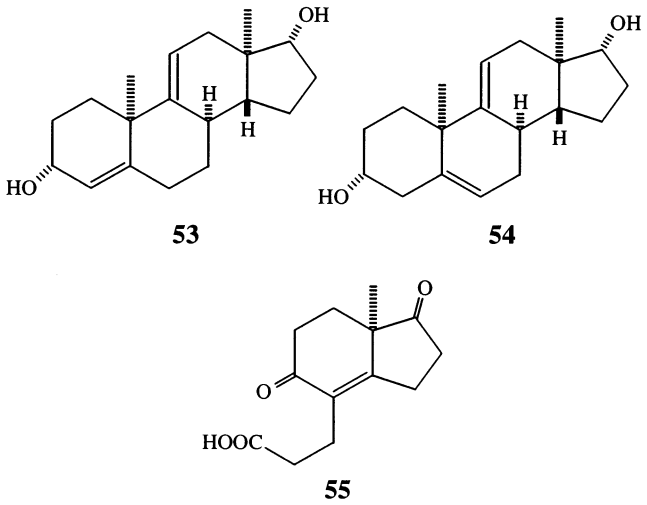


Figure 12.

G. Synthesis of D-Homo-18-nor-androsta-4,13-(17a)-dien-3,17-dione (or *ent*-13α-Methyl-D-homogon-4,13(17a)-dien-3,17-dione) 56

D-Homo-18-nor-androsta-4,13(17a)-dien-3,17-dione **56** is interesting with respect to the symmetry discussed in the Introduction. It can be regarded as an *ent*-steroid as shown in formula **56b**, Figure 13. Then this compound could be named as *ent*-13α-methyl-D-homo-gon-4,13(17a)-dien-3,17-dione. Its synthesis will be reported here.

Beckmann rearrangement of the oxime of dehydroepiandrosterone (3β-hydroxy-androsta-5-ene-17β-ol) **57** gave lactam **58**, Figure 14. This lactam **58** was saponified to the amino acid, which was permethylated with methyl iodide. Hofmann elimination gave the acid **59**. After classical modifications in rings A and B it was found that the acid chloride produced with oxallyl chloride had undergone, in the presence

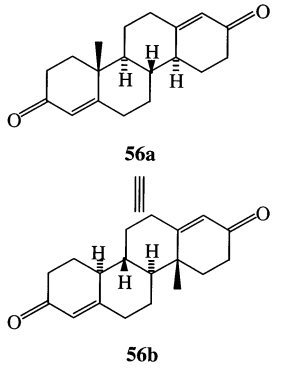


Figure 13.

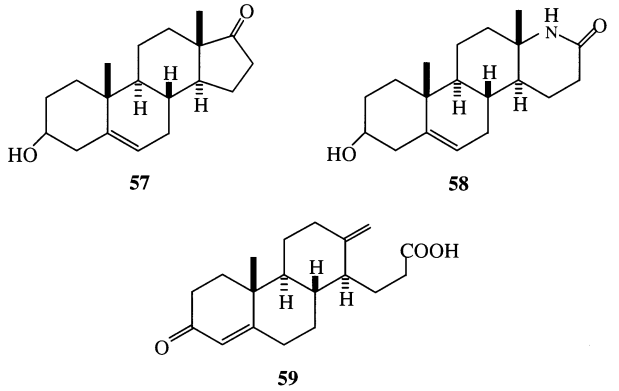


Figure 14.

of methylmagnesium−cadmium chloride, an intramolecular Friedel−Crafts reaction to give the tetracyclic compound **56**.[20−22]

H. Synthesis of *ent*-Progesterone 62

ent-Progesterone **62** was prepared frome *ent*-testosterone **4**, which was transformed to ketal **60**, Figure 15. After saponification of the acetate at C-17,

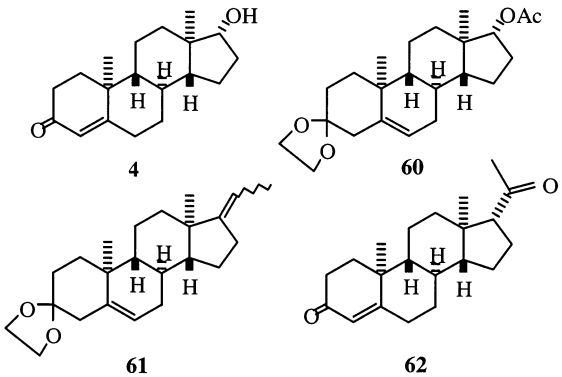


Figure 15.

the hydroxyl group was oxidized and a Wittig reaction of the ylid derived from ethyltriphenylphophonium iodide performed. Hydroboration of olefin **61** followed by hydrogen peroxide treatment gave alcohol at C-21. Oxidation and removal of the protecting group gave *ent*-progesterone **62** in 21% yield from *ent*-testosterone **4**.[23]

I. Synthesis of *ent*-Steroidal Bisammonium Salt 65

To explore the different factors involved in the neuromuscular blocking activity, the authors pre-

pared the enantiomers of steroidal diammonium salt starting from the *ent*-estrone methyl ether **24**. Beckmann rearrangement of the corresponding oxime yielded the lactam, which was subsequently reduced by lithium aluminum hydride to the amine. This amine was methylated by formaldehyde−sodium cyanoborohydride to the methylamine **63**, Figure 16.

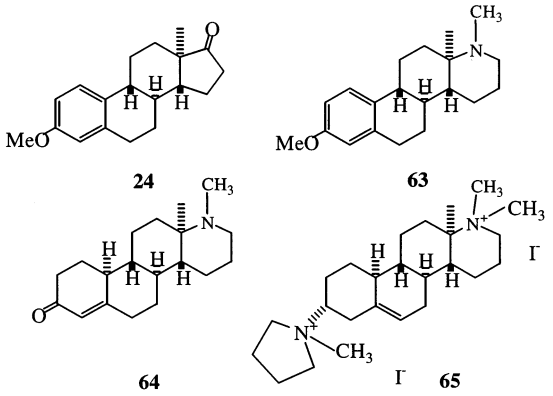


Figure 16.

The reduction by lithium in liquid ammonia afforded the enone **64**, and the dienamine prepared with pyrrolidine was reduced by sodium borohydride to diamine. This diamine *ent*-3β-(*N*-pyrrolidinyl)-D-homo-17a-methyl-17a-aza-estra-5-ene was converted to the diammonium salt **65** by treatment with methyl iodide. The ee was estimated to be 98−100% by Pirkle's method on the diamine.[24]

J. Synthesis of *ent*-Desmosterol 76

ent-Androst-4-en-3,17-dione **66**[25] was deconjugated, the ketone at C-3 selectively reduced to *ent*-3β-hydroxy-androst-5-en-17-one **67**, and the product purified as acetate **68**, Figure 17. Addition of two

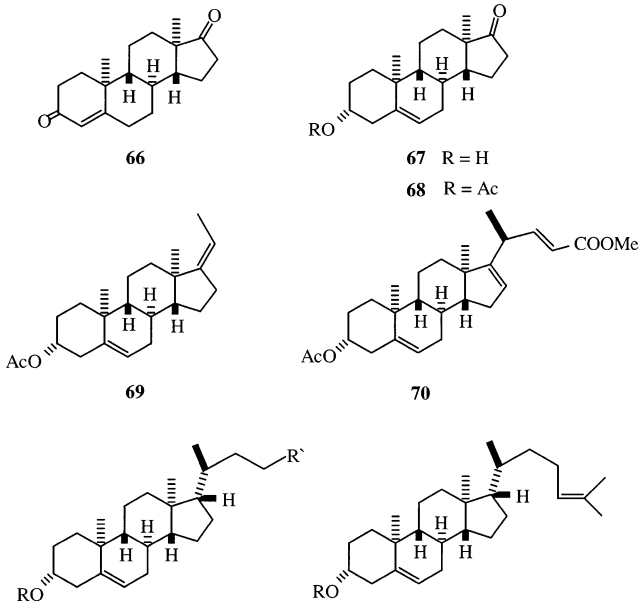


Figure 17.

carbon atoms was done with the ylid derived from ethyl triphenylphosphonium bromide, giving olefin **69** (*Z*/*E* ratio 9/1). After reacetylation of the hydroxyl group at C-3, an ene reaction with methyl propiolate was performed in the presence of diethylaluminum chloride. At this stage the minor isomer resulting from *E* olefin was easily separated from major product **70**. Reduction by hydrogen in the presence of platinum on charcoal gave the saturated ester **71**. The acetate group at C-3 of was replaced by the *tert*-butyldimethylsilyl group compatible with the next steps. Ester **72** was transformed to aldehyde **74** by reduction to alcohol **73** by lithium aluminohydride and oxidation by pyridinium chlorochromate. Wittig reaction with the ylid derived from isopropyltriphenylphosphonium iodide occurred in the highest yield of product with a phase-transfer catalyst. Removal of the silyl ether **75** gave *ent*-desmosterol **76**.[26]

K. Synthesis of *ent*-1α,25-Dihydroxyvitamin D_3 81

Ring A of *ent*-1α,25-dihydroxyvitamin D_3 **81** was prepared from vitamin D_2 **79** and rings C and D from a bicyclic system, Figure 18. The preparation of ring A from natural vitamin D_2 **79** involved a Mitsunobo reaction for inversion of the configuration of the hydroxyl group at C-3, the introduction of the hydroxyl group at C-1 by selenium dioxide, and selective cleavage to aldehyde **80**. The building of rings C and D started from the optically active thioester **77** prepared by Sharpless asymmetric dihydroxylation. A diastereoselective Mukaiyama−Michael reaction of this thioester with methyl-2-pentenone and further reactions gave the hydrindane sulfone **78**, which was coupled according to Julia's method with aldehyde **80** to *ent*-1α,25-dihydroxyvitamin D_3 **81**.[27]

L. Synthesis of *ent*-Cholesterol 86

The enone of *ent*-testosterone **4** (see above), whose ee was shown to be higher than 97% by Mosher's ester, was converted to the β,γ-unsaturated ketone by kinetic protonation of the dienenolate. Reduction of the ketone gave the equatorial alcohol **82**, which was protected as *tert*-butyldimethylsilyl ether **83**, Figure 19. The 17-hydroxyl group was oxidized to ketone, which reacted with the ylide derived from triphenylethylphosphonium iodide to give the *Z* olefin **84**. Hydroboration with 9-BBN (9-borabicyclo[3.3.1]nonane) gave the borane with the proper stereochemistry. Its reaction with chloroacetonitrile gave the nitrile **85** as a single isomer. This nitrile **85** was alkylated with 1-bromo-3-methylbutane. The cyano group was removed by reduction and the silyl group hydrolyzed as usual to give the desired *ent*-cholesterol **86**.[19]

This synthesis was improved, in part because the hydroboration of olefin **84** with 9-BBN could not be reproduced in another laboratory. A new method for the introduction of the side chain was devised. Ene reaction on (*Z*)-olefin **84** with 4-methyl-1-pentanal with total control of the stereochemistry at C-20 gave alcohol **87**, Figure 20. Hydrogenation of the 16−17 double bond to compound **88** occurred with control

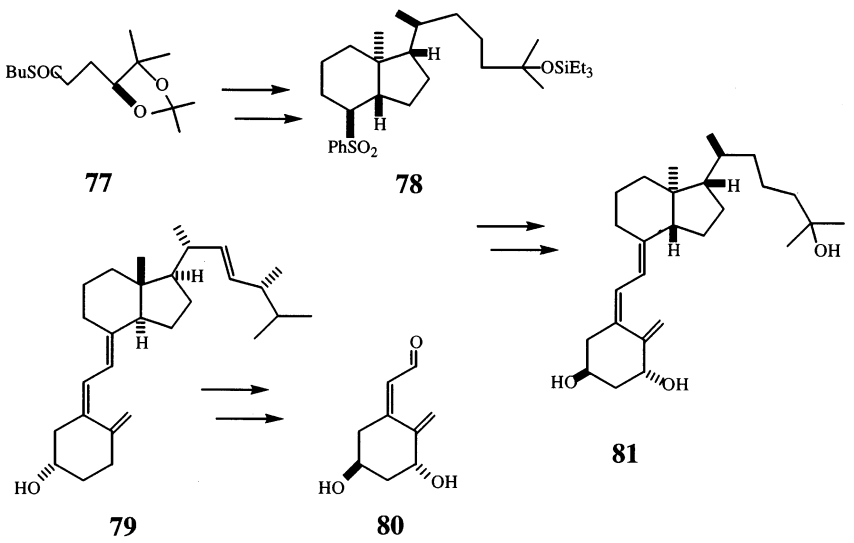


Figure 18.

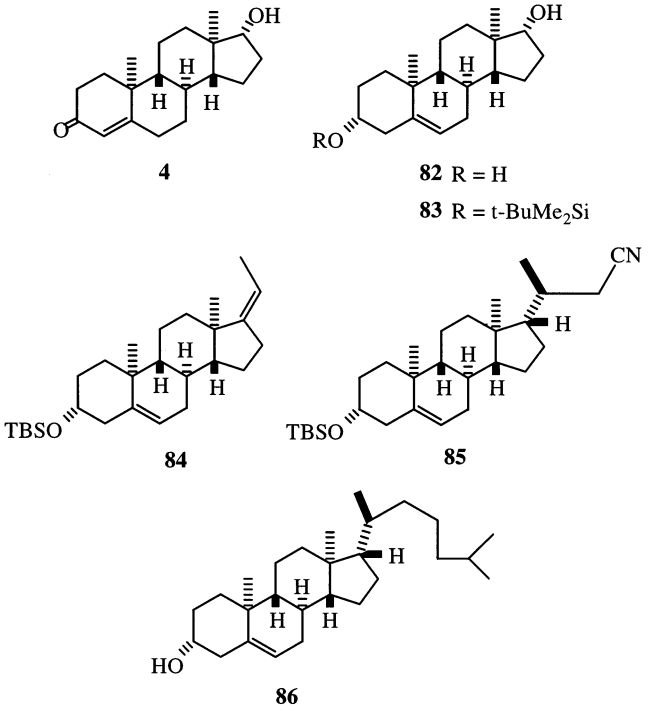


Figure 19.

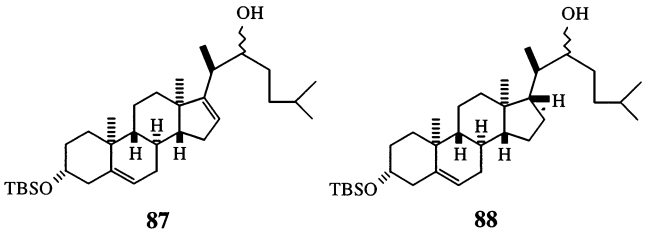


Figure 20.

of sterochemistry by the C-18 methyl group. The hydroxyl group as a mesylate was reductively removed with lithium triethylborohydride. The conversion of *ent*-testosterone **4** to *ent*-cholesterol **86** was done in a 9.7% overall yield.[28]

A synthesis where the side chain was introduced in an early step has recently been described.[29] Using the scheme explored,[30] the side chain was built on the racemic bicyclo[3.1.0]hexane keto-ester **89** with the proper relative stereochemistry, Figure 21. The

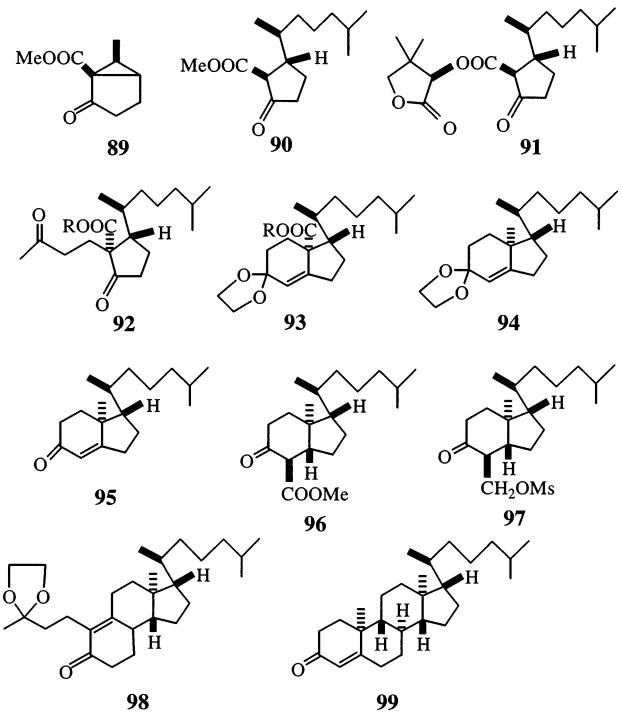


Figure 21.

racemic methyl ester **90** was converted to the diastereomeric esters with (*R*)-pentolactone, which were separated by chromatography. Keto-ester **91** yielded on reaction with methyl vinyl ketone compound **92** in 59% yield. After acid cyclization to indenone, ketal **93** was prepared and the ester was reduced by LAH to the hydroxymethyl group, which was converted to a methyl group of **94** in the following sequence: preparation of the mesylate, conversion of the mesylate to iodide, and reduction of the iodide by superhydride. Ketal **94** was hydrolyzed to enone **95**. After carbonation and esterification, the double bond was reduced to give the *trans*-indanone **96** and the carbomethoxy group was converted by LAH to a hydroxymethyl group, the keto group being protected as ketal. The correponding mesylate **97** was reacted

with the enolate of β-keto-ester **50**,[19] and cyclization and decarboxylation produced the tricyclic compound **98**. Methyl group was indroduced by reduction with lithium−ammonia and reaction with methyl iodide. Hydrolysis of the ketal group and cyclization gave *ent*-cholestenone **99**, converted to the *ent*-cholesterol **86** in a well-known reaction scheme. The enantiomeric purity was determined by rotation.[29]

ent-Deuteriocholesterol was prepared by catalytic deuteration of silyl ether of *ent*-desmosterol **76**. Vinylic and allylic isotopic exchanges resulted in a large isotopic distribution.[26]

M. Preparation of *ent*-Steroid Sulfates

The preparation of *ent*-steroid sulfates has been described. *ent*-Dehydroepiandrosterone, *ent*-pregnenolone, and *ent*-3α-hydroxy-5β-pregnan-20-one were prepared by known routes, and their sulfates were obtained by treatment with sulfur trioxide−trimethylamine complex in pyridine. The corresponding ammonium salts were obtained from methylene chloride solution of their sulfate esters with a stream of anhydrous ammonia.[25]

N. Synthesis of *ent*-RU 486 101

It was of interest to prepare the *ent*-RU 486 **101**, the enantionmer of RU 486 **100** (RU 38486, Mifepristerone, 11β-[4-(*N*,*N*-dimethylamino)phenyl]-17α-(prop-1-ynyl)-17β-hydroxy-estra-4,9-dien-3-one), and to verify that only the natural enantiomer **100** was active, Figure 22. The synthesis of *ent*-RU 486 **101**

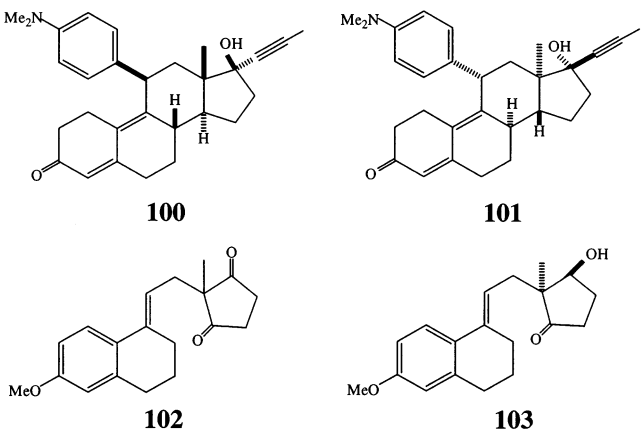


Figure 22.

made use of enantio- and stereoselective microbial reduction of diketone **102** by *Bacillus thuringiensis* to hydroxyketone **103**. Acid-catalyzed cyclization and catalytic reduction produced compound **104**, Figure 23. Birch reduction and acid hydrolysis yielded the enone **105**. This enone **105** was oxidized to dienone **106** by bromination−dehydrobromination. The ketalization occurred with isomerization to dieneketal **107**. Selective epoxidation gave the 5,10-epoxides, which were separated. The α-epoxide (as *ent*-steroid) **108** underwent an anti S_N2' opening with 4-(*N*,*N*-dimethylamino)phenylmagnesium bromide to give compound **109**. Oxidation of the hydroxyl group at C-17 and addition of propynyllithium gave the adduct

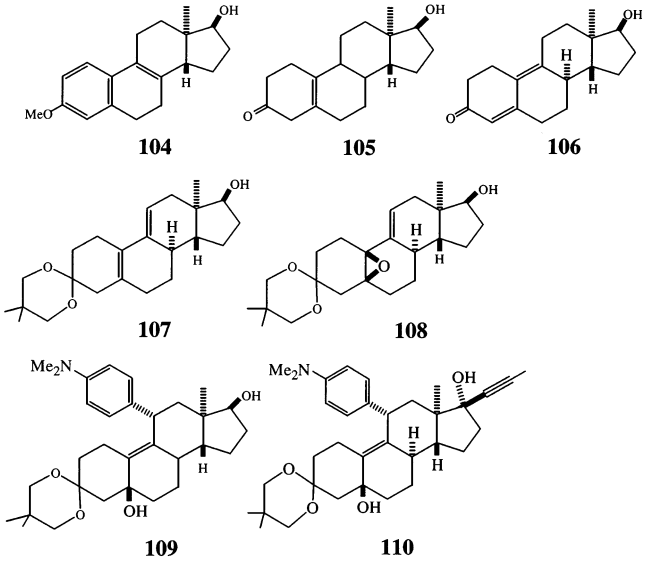


Figure 23.

110. Cleavage of the ketal by acid and dehydration gave *ent*-RU-486 **101**.[31]

O. New Entry to *ent*-Steroids

An interesting way to prepare *nat*- and *ent*-steroids from the same chiral precursor has been described. Diels−Alder reaction of the ketal of enantiopure 3-bromo-*cis*-1,2-dihydrocatechol obtained by microbial hydroxylation with benzoquinone gave compound **111**, Figure 24. Proper modifications of the ene−

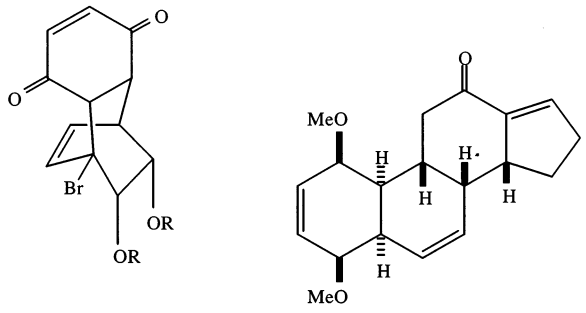


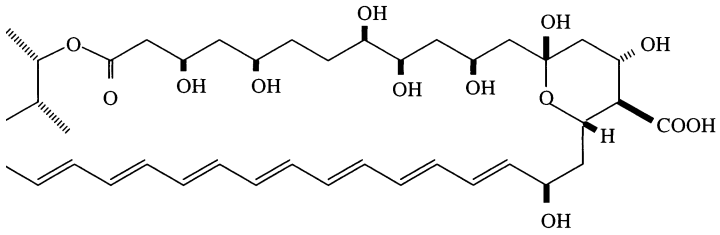
Figure 24.

dione and of the diol and reductive removal of the bromine and an anionic oxy-Cope rearrangement gave compound **112** or its enantiomer.[32]

IV. Physical Effects

I will review the physical studies on the effects of the *ent*-steroids compared to those of *nat*-steroids. These studies are restricted to *ent*-cholesterol **86**. To the best of my knowledge, no study of the behavior of 20-isocholesterol in membranes has been reported.

The earliest study on the physical properties was done on the interaction of cholesterol and *ent*-cholesterol with Amphotericin B **113**. The antifungal activity of Amphotericin B **113** is attributed to the formation of complexes with cholesterol or ergosterol **114** present in cell eukaryotic membranes, Figure 25. These complexes assemble and form in the membrane channels, causing loss of small molecules and



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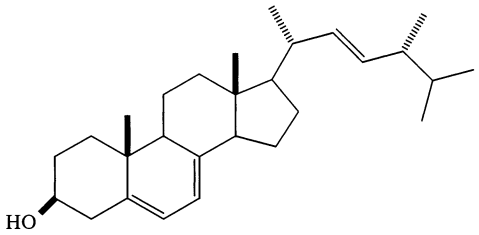


114

Figure 25.

ions from the eukaryotic cells but not from prokaryotic cells, where these steroids are not present. The formation of such complexes involves interactions between Amphotericin B **113** and cholesterol or ergosterol.[33] The interaction of Amphotericin B **113** with the *ent*-cholesterol **86** will be different and could be reflected in different properties. Indeed, cholesterol forms a conducting channel at low Amphotericin B **113** concentration in membranes containing cholesterol. In contrast, *ent*-cholesterol **86** does not form conducting channels at these concentrations. However, at 10 times higher concentration of Amphotericin B **113**, channels of higher conductance were detected. It was concluded that it is the interaction of cholesterol and Amphotericin B **113** that leads to the formation of the channels.[18,34]

The interaction of steroids with proteins should distinguish the enantiomers. Surprisingly, however, the monoclonal antibody raised against cholesterol monohydrate interacted strongly not only with cholesterol but also with *ent*-cholesterol **86** inserted in monolayers and weakly with epicholesterol (3α-hydroxyl group). Cholesterol was found to be oriented in compressed monolayers with an angle 75° to the interface. Therefore, the cholesterol part exposed to water is ring A and the chirality of the sterol is not expressed at this level. This is not the case with epicholesterol, where the hydroxyl group is axial while in cholesterol it is equatorial.[35] It has been shown that *ent*-cholesterol **86** replaces cholesterol in the P-glycoprotein, a membrane protein.[36]

Earlier investigations on the enantioselectve interactions of lipid/lipid and sterol/ lipid did not detect any enantioselectivity. The assumption that the chirality of cholesterol would not affect the membrane properties where *nat*- or *ent*-cholesterol was incorporated may not be true. Indeed, it has been shown that the monolayers spingomyelin (−)-*ent*-cholesterol **86** were more condensed than those with *nat*-cholesterol. In the monolayers with dipalmitoylphosphophatidylcholine, (−)-*nat*-cholesterol, or (−)-*ent*-cholesterol **86** the interactions were not enantioselective. This is the first evidence that the interaction of lipids with steroids may be enantioselective.[37]

The first evidence that the interactions of *N*-palmitoyl-D-erythro-sphingomyelin with cholesterol and *ent*-cholesterol **86** were enantiospecific at 20−30% sterol monolayer concentration has appeared recently. An additional phase was detected by Brewster microscopy during the compression of *ent*-cholesterol containing monolayers. This phase was not detected with *nat*-cholesterol. The nature of this phase remains to be investigated.[38]

Chirality of cholesterol is reflected in some physical properties. I will now discuss the biological effects where the enantioselectivity is supposed to be the rule.

V. Biological Properties

The fact that the biological activity is in general restricted to one enantiomer is well documented and is also the rule for the steroids.[39−41] However, the symmetry of the steroidal skeleton gives rise to the possibility that the *ent*-steroid may have some activities of the *nat*-steroid. Furthermore, *ent*-steroids could show some activities not found for the *nat*-steroids.

For the steroids, the total synthesis gave access to the *rac*-steroids, some of which may have been submitted to physiological testing. In general, the results were that the *rac*-steroid had 50% of the activity of the *nat*-steroid. It was concluded that the *ent*-steroids had either no activity or a much lower activity, so that under the experimental conditions their eventual activity would have gone undetected. If the *ent*-steroid is available, the question of its enantiomeric excess should be addressed. Even if the compounds results from a total synthesis where the resolution has been carried out in an early stage of the synthesis, the ee of the final compound may not be 100%, even if the purification of the intermediates were performed by crystallization. Rarely the biological activity of *ent*-steroid was checked for the presence of the active *nat*-enantiomer, like extent of the activity versus ee. Certainly the high activity of the *nat*-steroid, the sensivity of the test, and the difficulty to determine the ee in the range 99−100% make such experiments difficult. There are now chromatographic methods available to determine ee's in this range. The conditions for the separation of steroid enantiomers by liquid reversed-phase chromatography on Chiralpak AD have been published. For instance, this method allows the determination of *ent*-estradiol in drugs in the range 0.05−4.8%.[42]

While studying the enantiospecificity of enzymes, the same problem is found. When, for instance, the *ent*-steroid is a substrate, a quantitative transformation may be carried out or the reaction may be followed for longer reaction times. If the *nat*-steroid is present in trace amounts and is the only enantiomer transformed by the enzyme, the yield of the reaction product will not be quantitative or the reaction comes to an end before expected.

However, *ent*-steroids may have other activities than those shown by the *nat*-steroids, and this needs

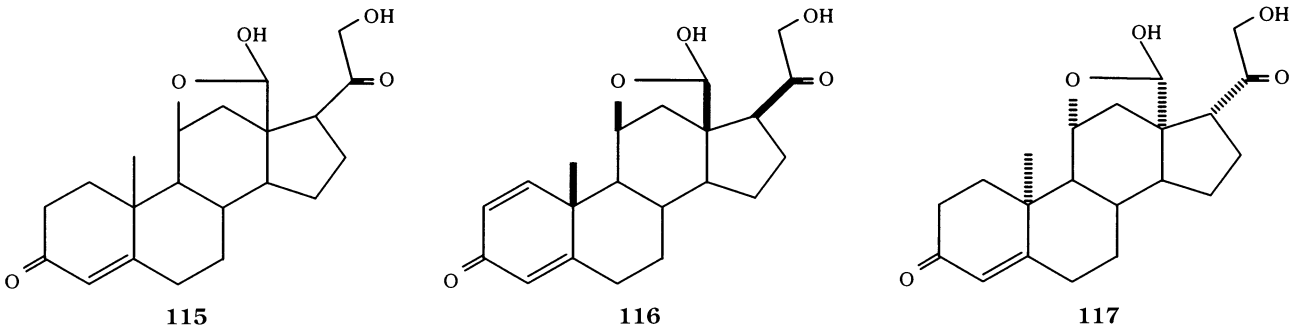


Figure 26.

to be explored further. In particular, if the hormonal activity is restricted to the *nat*-steroid, the active *ent*-steroids should not have hormonal activity as secondary activity.

The present section will be devoted to the transformations by living systems and by enzymes and to the physiological effects of *ent*-steroids.

A. Transformations by Living Organisms

The transformations of the steroids by living organisms have raised the interest of chemists since early times. Furthermore, the use of living systems for the resolution of the *rac*-steroids obtained by total synthesis was reported with the claim to be the first resolution by living organisms that described the isolation of both enantiomers: one untouched and other transformed.[43] In previous resolutions with living organisms, the transformed enantiomer was totally degraded and it was the untransformed enantiomer which was isolated. I will describe these transformations in the following order: transformation of the carbon−carbon bond, reduction of carbonyl compounds, and hydroxylations.

In this founding paper is described the resolution of the *rac*-aldosterone **115** by *Didymella lycopersici*, which introduces a double bond at C-1 in the natural enantiomer, to produce *nat*-1-dehydro-aldosterone **116** and *ent*-aldosterone **117**, Figure 26. When the racemic cortisone was given to the same organism, the *ent*-cortisone was left unchanged and *nat*-1-dehydro-cortisone was isolated. These transformations occur in high yield ('nahezu theoretische Ausbeute') and in high enantiomeric excess and may constitute a step in the synthesis.[43]

Microbiological dehydrogenation by *Corynebacterium simplex* of ring A of *rac*-13β-ethylgon-4-en-3-one and *rac*-13β-propylgon-4-en-3-one gave the *nat*-corresponding phenols in a yield of 15−20%. These phenols were taken to the 4-en-3-one, whose rotatory dispersion spectra were superimposable with the one of 19-nor-testosterone. The residual product was the partially resolved starting material. Other products and transformation by other strains are also reported. Clearly the system is enantioselective, but some total yields of extracted products are low.[44] Definite conclusion about the enantioselectivity of the enzymes involved awaits further work.

Transformation of 19-nor-testosterone **29** and *ent*-19-nor-testosterone **30** by *Mycobacterium smegmatis* has been reported, Figure 27. This system (resting cells) reduces the 4−5 double bond of 4-en-3-one. 19-

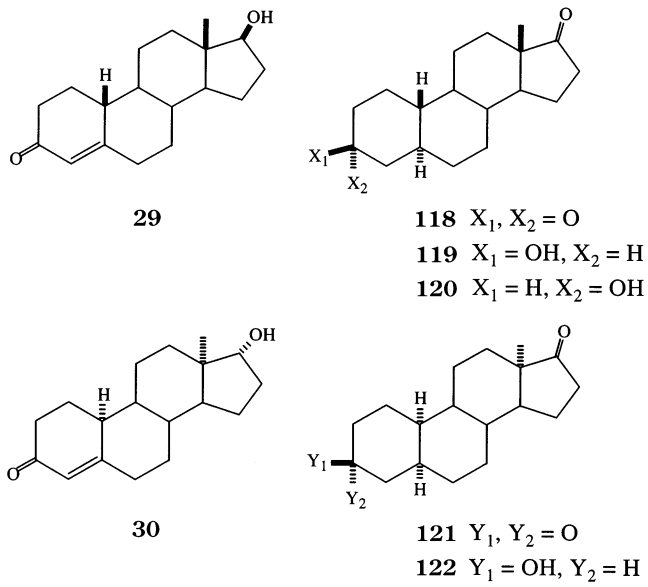
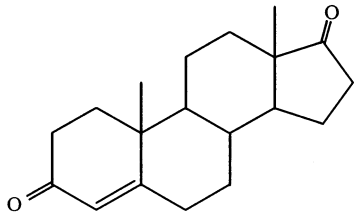


Figure 27.

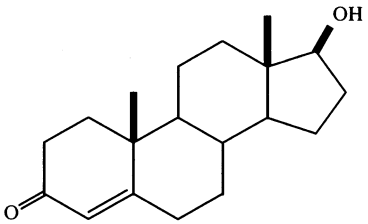
Nor-testosterone **29** was transformed into 19-nor-androstan-3,17-dione **118** and to both epimers, 3α-hydroxy- and 3β-hydroxy-19-nor-androstan-17-one **119** and **120**, while *ent*-19-nor-testesterone **30** was reduced to *ent*-19-nor-5β-androstane-3,17-dione **121** and *ent*-19-nor-5β-androstane-3α-ol-17-dione **122**. This suggests that reduction of the enone occurred from the same face and was not dependent on the chirality of the other centers, and the reduction of the carbonyl group at C-3 in the trans position gave both epimers, while for *ent*-steroid A/B cis to the reduction was stereoselective.[45] Again, this should now be checked with purified enzymes. *rac*-Estrone was enantiospecifically reduced by yeast at C-17 giving estradiol and *ent*-estrone **23**.[43]

Total synthesis of *rac*-testosterone was completed by the resolution of rac-androst-4-en-3,17-dione **123** by fermenting yeast, giving *nat*-testosterone **3** identical to the natural hormone and *ent*-androst-4-en-3,-17-dione **124**, Figure 28. The latter, **124**, was converted by lithium aluminohydride reduction and selective oxidation of the hydroxyl group at C-3 to *ent*-testosterone **4**. The *rac*-13α-androst-4-en-3,17-dione (13-isoandrosten-dione) was not reduced by the fermenting yeast.[46]

Resolution of *rac*-estra-4,9-dien-3,17-dione **125a** (and of *rac*-13β-ethyl-gona-4,9-dien-3,17-dione **125b** with the same results) by fermentation with *Hansenunla capsulata* (IFO 984) and *Saccharomyces chev-*



rac- 123



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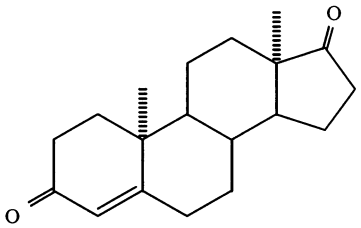

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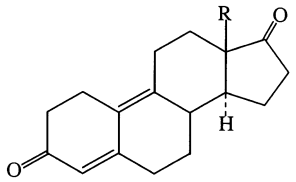
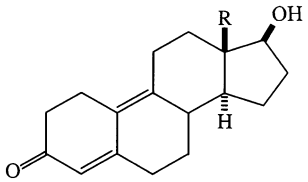
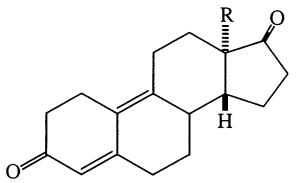
Figure 28.



125 a) rac R = Me
b) rac R = Et



126 a) R = Me
b) R = Rt

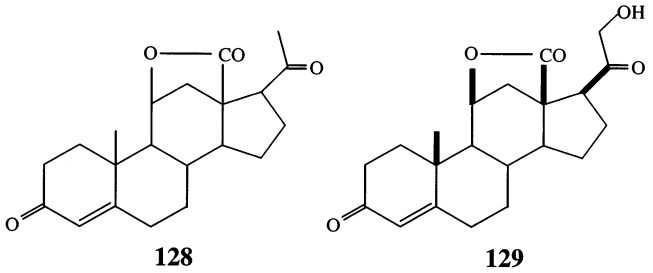


127 a) R = Me
b) R = Et

Figure 29.

alieri (NCYC 91) has been reported, Figure 29. The *nat*-steroid was reduced to the 17β-alcohol **126a** by both yeasts in high yield, and the *ent*-steroid **127** was not or very slowly reduced to the epimer at C-17. Most of the other microorganisms reduced both ketones to the racemic products.[47] Are some of the enzymes involved in the reduction not enantiospecific or are there different enzymes with opposite enantioselectivity? These questions do not seem to have been addressed.

Vischer, Schmidlin, and Wettstein described the resolution by *Ophiobolus herpotrichus* (fr) Sacc. of an intermediate **128** of the total synthesis of aldosterone, Figure 30. This organism hydroxylated at position

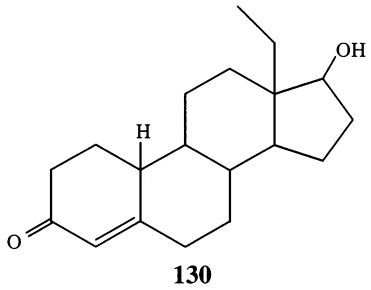


128 **129**

Figure 30.

C-21 the *nat*-enantiomer and *ent*- remained untouched. This *nat*-enantiomer **129** was converted to aldosterone, and this constitutes the total synthesis of aldosterone.[43]

Hydroxylation by *Aspergillus ochraceus* was carried out on the following *rac*-steroids: 19-nor-testosterone, 13β-ethyl (and 13β-propyl)-17β-hydroxy-gon-4-en-3-one **130**. Reaction occurred at different positions with different enantioselectivity. *rac*-19-Nor-testosterone gave the *nat*-11α-hydroxy derivative along with minor transformation products: *ent*-10β-hydroxy and *ent*-1β-hydroxy compounds. *rac*-13β-Ethyl (and propyl)-17β-hydroxy-gon-4-en-3-one **130** gave mainly the *ent*-1β-hydroxy product **131** besides *nat*-10β- and *nat*-6β-hydroxylated products, Figure 31. The purification



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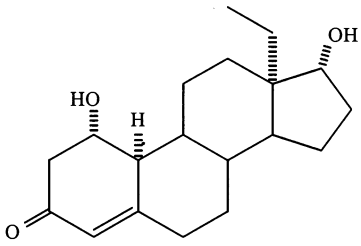

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Figure 31.

was performed by recrystallization, and the question of the ee of the hydroxylation reaction was not raised.[48]

In humans it was shown that the *nat*-aldosterone was converted to glucoronides with partial conversion

to tetrahydroaldosterone, while *ent*-aldosterone **117** was neither converted to glucoronide nor reduced.[49]

B. Enzymes

Only the oxido-reductases seemed to have been studied on the steroids and *ent*-steroids. In general, oxido-reduction and hydroxylation seem to be the only reactions reported with purified enzymes or with living organisms for the resolution of steroids. This limited range of reactions should be extended in the future.

The earliest report on the enzyme activity on the *rac*-steroids is that in 1960 by Johnson and coworkers. They mention the action of 3α- and 3*β*-hydroxysteroid dehydrogenase from *Comamonas testosteroni (Pseudomonas Testosteroni*) on two racemic substrates obtained by total synthesis: *rac*-18-nor-5*β*-androstan-3α,17*β*-diol **132** and *rac*-18-nor-5*β*,13α-androstane-3α,17α-diol **133**, Figure 32. They found

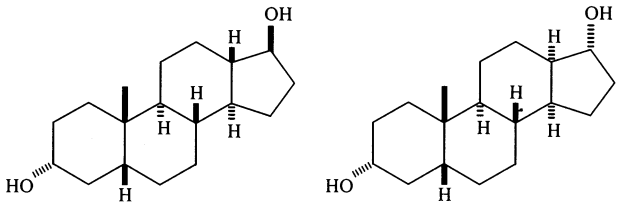


Figure 32.

that the α-dehydrogenase oxidizes the C-3 hydroxyl group of both racemates, so the α-dehydrogenase is not enantioselective, and *β*-dehydrogenase oxidizes only one enantiomer at C-17, the *nat*-steroid as interfered from the enzyme specificity.[50] The kinetics parameters remain to be determined.

The dehydrogenases from the same source have been studied extensively since, and their structure has been established. The α-hydrogenase is likely identical with the enzyme 3α-hydroxysteroid dehydrogenase, which belongs to the short-chain dehydrogenase-reductase superfamily. The substrate specificity of the 3α-dehydrogenase has been studied. It oxidizes the 3α-hydroxyl group in steroids with ring A/B cis and trans, such as androsterone and cholic acid. It has been shown to be active on fusidic acid and nonsteroidal substances. Therefore, the fact that the 3α-dehydrogenase could not be enantioselective is not surprising. However, this awaits further study with the purified enzyme. This enzyme may be involved in the metabolism of xenobiotics.[51−53] The *β*-hydrogenase may be 3*β*,17*β*-hydroxy steroid dehydrogenase belonging also to the short-chain dehydrogenase-reductase superfamily.[54]

By using homogenate of human placenta containing the 17*β*-hydroxysteroid and NAD^+, *rac*-estradiol **134** was resolved into *ent*-estradiol **135** and *nat*-estrone **136**, Figure 33. *ent*-Estradiol **135** is not a substrate of 17*β*-hydroxysteroid dehydrogenase. This enzyme has been used to resolve estradiol.[55]

By using the partially purified Δ^4-3-keto-steroid reductase from rat liver in the presence of glucose-6-phosphate and the glucose-6-phosphate dehydrogenase and $NAPD^+$, *rac*-aldosterone-21-acetate was

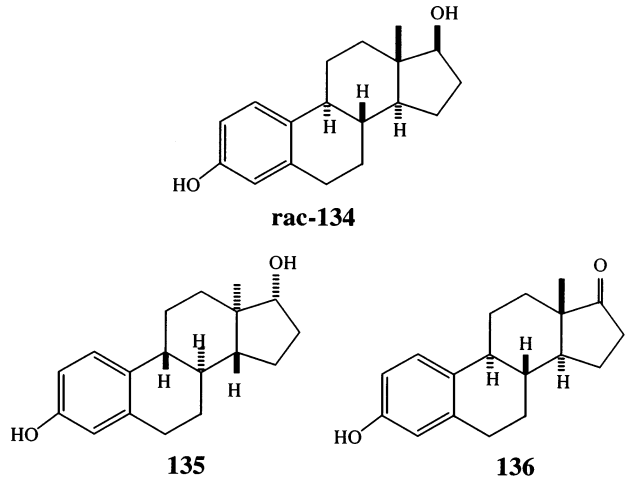


Figure 33.

resolved. The *ent*-aldosterone **102** in a yield close to 50% was recovered and submitted again to reduction. Only 2% was reduced. The reduction was highly enantioselective in favor of the *nat*-enantiomer, and the hydrolysis of the acetate at C-21 was not.[56]

Cholesterol oxidase, a flavine-dependent enzyme, oxidizes the hydroxyl group of cholesterol and of related steroids **137** and isomerizes the homoallylic double bond to the conjugated 4-en-3-one **138**, Figure 34. The reduced coenzyme is regenerated by oxygen,

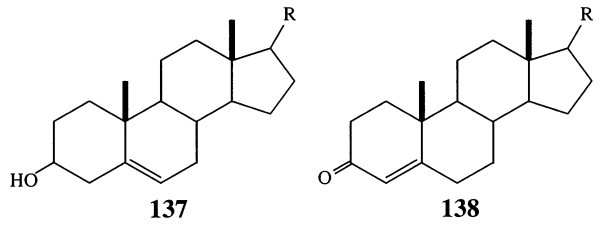


Figure 34.

which is converted to hydrogen peroxide. This enzyme is often induced by cholesterol and seems to be involved in the biodegradation of cholesterol. *ent*-Androsta-4,9(11)-dien-3*β*,17*β*-diol **53** and *ent*-androsta-5,9(11)-dien-3*β*,17*β*-diol **54** were oxidized by cholesterol oxidase from *Rhodococcus erythorpolis* with rate constants close to those of the corresponding *nat*-enantiomers (see Table 1). Not only was the oxidation

Table 1. Kinetic Parameters of the *nat*- and *ent*-Androstenediols for Cholesterol Oxidase from *Rhodococcus erythropolis* (in 0.1 M Phosphate Buffer pH 7.4 at 30 °C) (Taken from Ref 7)

	K_m (mM)	k_{cat} (μmol min^{-1} mg^{-1})
androst-4-ene-3*β*-17*β*-diol	66	0.37
androsta-4,9(11)-dien-3*β*,17*β*-diol	383	0.32
ent-androsta-4,9(11)-dien-3*β*,17*β*-diol **53**	525	0.20
androst-5-en-3*β*,17*β*-diol	6.5	0.05
androsta-5,9(11)-dien-3*β*,17*β*-diol	36	0.04
ent-androsta-5,9(11)-dien-3*β*,17*β*-diol **54**	52	0.03

of the hydroxyl group catalyzed, but so was the isomerization of the double bond. On the preparative transformation oxidation of the hydroxyl group at C-17 of the *ent*-steroid was observed, whereas oxidation at C-17 does not occur for the *nat*-steroids. This

oxidation at C-17 does not occur with all the cholesterol oxidases: it was observed also with the enzyme from *Pseudomonas fluorescens* but not with the enzymes from *Brevibacterium* Sp. and *Streptomyces* Sp.[7] The isomerization of the double bond is catalyzed by the Glu-361 in the enzyme from *Brevibacterium sterolicum*, and this residue has been found to be quite mobile so that the C-4β position of both enantiomers can be attained by this residue.[57,58] It has been mentioned that *ent*-cholesterol was oxidized by an unspecified cholesterol oxidase at a lower rate than that of cholesterol.[36]

The 5-en-3-ketosteroid isomerase from bacterial origin has been extensively studied.[59] Structures of complexes of this enzyme with substrate analogues have been established, so that I speculate on the action of this enzyme on *ent*-steroids. With equilenin two binding mode are observed. In the first, carboxylate of Asp-38 is close to C-4 and C-6 of equilenin. This carboxylate is the base implicated in the isomerization. The second mode in part stabilized by interaction with another enzyme molecule corresponds to an inverted steroid turned by 180° about the C-3−O bond of equilenin.[60] Then the carboxylate of Asp-38 is close to C-1 and C-10. This binding could be similar to the one of *ent*-steroid bound to this enzyme.

Interestingly, this enzyme from *Comamonas testosteroni* had been inactivated by 3β-oxirane. The reaction occurred with the carboxylate of Asp-38 involved in the proton transfer.[61] This residue should not be reacting with the epoxide when the steroid is bound in the expected manner. If bound in the second binding mode detected with equilenin, the carboxylate should be in position to react with the epoxide. Hence, the second binding mode as for equilenin could also occur in solution. Thus, isomerization of *ent*-steroid remains to be attempted with this enzyme in solution. For certain, the fate of the proton in the isomerization of *ent*-steroid should then to be examined.

Human cytochrome P450c17 (17α-hydroxylase,17,-20-lyase) and cytochrome P450c21 (21-hydroxylase) have been challenged with *ent*-progesterone **62**. Neither enzyme acted on this substrate, but *ent*-progesterone **62** proved to be a potent competitive inhibitor of P450c17 (K_i 0,2 μM) and a weaker inhibitor for P250c21 9 (K_i in the range of 1 μM).[23]

It is surprising that enzyme inhibition by an *ent*-steroid apparently has only been reported in one case.

C. Physiological Effects

It is to be expected that the *ent*-steroids are devoid of the activities of *nat*-steroids or that their activity is much lower. With some exceptions this proved to be the case, that the *ent*-steroids had definitely higher and more selective activity than the *nat*-steroids. These are directions to be explored in the future, especially for the steroids in the nervous system. The *ent*-steroids offer the advantage that they and their metabolites should not present any hormonal activities.

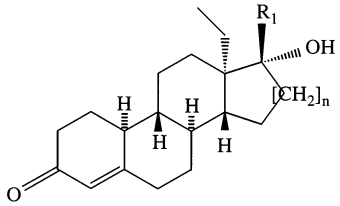
The results are presented in the order of increasing number of carbon atoms.

Likely in the first assay on *ent*-steroid activity, *ent*-equilenin **8** has been shown to produce estrus in rats at doses 13 times higher than *nat*-equilenin. The sample was obtained by resolution of the *rac*-equilenin prepared by total synthesis.[11]

ent-Estradiol **135** showed definitely lower activity than estradiol. On reproductive tissues in rodents, *ent*-estradiol compared to estradiol had only very weak estrogenic effects.[62,63] In a modifed Allen−Doisy test, *ent*-estradiol **135** showed estrogenic effects on reproductive tissues in rodents at 10 times higher dose than estradiol.[64] *ent*-Estradiol **135** antagonized uterotrophic effects of estradiol.[65,66] *ent*-Estradiol **135** interacts weakly with uterine-derived estrogen receptors.[67,68] It does not stimulate uterine growth or vaginal opening in rats but shows an activity close to that of estradiol in the neuroprotection. Both enantiomers are potent at the same level on attenuating oxidative stress-induced death in HT-22 cells and hydrogen peroxide toxicity in human SK-N-SH neuroblastoma cells at a 10 nM concentration. In addition, both enantiomers reduce to the same extent the lesion volume in the middle cerebral artery occlusion.[69] This absence of enantioselectivity should be further investigated. *ent*-Estradiol **135** showed activity comparable to that of estradiol in antilipemic tests in rats without feminizing activity.[70]

ent-Estradiol methyl ether is more selective in the inhibition of rat liver micorsomal P-450 than estradiol methyl ether.[71] Clearly, these are areas for further research on *ent*-steroids.

ent-Steroids **139** (R_1 = H, Et, ethynyl; n = 1, 2) showed no activity in an anti-estrogenic test and Hershberger or Clauberg tests, Figure 35. In the



139

Figure 35.

same test the response of the corresponding *rac*-compounds were not significantly different from those of the natural enantiomer at one-half dose.[72] In related studies it was shown that 17β-hydroxy-13β-ethyl-gon-4-en-3-one, 17β-hydroxy-13β-ethyl-17α-ethynyl-gon-4-en-3-one, and 17β-hydroxy-13β,17α-diethyl-gon-4-en-3-one had an anabolic effect and the unnatural enantiomers had none.[73,74]

nat- and *ent*-3-methoxy-16α-methylamino-estra-1,3,5(10)-trien-17β-ol **28** showed equal potency as anti-arrythmic agents. The *nat*-enantiomer has plasma cholesterol lowering activity, whereas the *ent*-enantiomer **28** did not show any effect. The idea that the *ent*-steroids devoid of hormonal activities could present more selective nonhormonal activity than the *nat*-steroids has been put forward for the first time in this paper.[15]

3β-(*N*-Pyrrolidinyl)-D-homo-17a-methyl-17a-aza-estra-5-ene dismethiodide and the enantiomer **65** have the same activity as a neuromuscular blocker. This

shows that the structural effects are not a major factor in determining the activity, but the 1.0 nm distance of the two cationic centers and a sufficiently high lipophilicity play a definite role for this activity.[24]

Steroid sulfates are negative modulators on $GABA_A$ receptors present in cultured rat hippocampal neurons. The enantioselectivity was detected with dehydroepiandrosterone ammonium sulfate and its enantiomer. However, no enantioselectivity with pregnenolone ammonium sulfate and 3α-hydroxy-5β-pregnan-20-one ammonium sulfate was observed. The authors suggest that their function may not be on the receptor itself but indirectly through membrane steroid-induced perturbation.[75−77] Later work showed that the enantioselectivity of pregnanolone-derived anesthetic steroids depends on the test and on the steroid structure and varies from none to 10 in favor of the *nat*-steroid.[78−80]

Pregnenolone sulfate is active on the retention performance in rats and mice. *ent*-Pregnenolone sulfate is active at 10-fold lower concentration, and its action is not antagonized by *rac*-2-amino-5-phosphonovaleric acid. Different mechanisms are proposed by the authors. Clearly, further work is needed for a better understanding. The *ent*-steroids may have an interest for memory enhancement.[81] The same *ent*-pregnenolone sulfate is active also in reversing scopolamine-induced amnesia at 10-fold higher dose than *nat*-pregnenolone sulfate.[82]

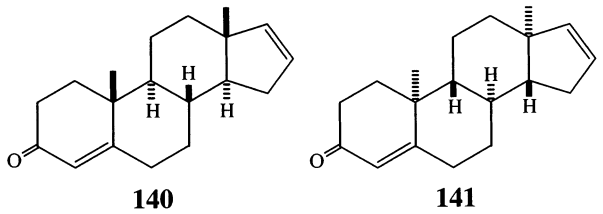
rac-Aldosterone was resolved by an enzyme active only for the natural form. It was found that the mineralocorticosteroid activity of *ent*-aldosterone was reduced by further treatment with the enzyme. Any activity of *ent*-aldosterone **117** appears to be due to contamination with the *nat*-form. Thus, the mineralocorticosteroid activity of *ent*-aldosterone **117** is quite low.[83]

ent-RU 486 101 (*ent*-RU 38486) had no abortive activity and did not antagonize the induction of the tyrosine aminotransferase by dexamethasone. To conclude, *ent*-RU 486 **101** did not show any RU 486 **100** activity but should undergo further testing to explore any other activity.[31]

ent-1α,25-Dihydroxyvitamin D_3 **81** showed no significant affinity to the vitamin D receptor and is inactive in tests for differentiation of promyelocytic leukemia HL-60 cells.[27]

Sterols are required for viability of nematode *Caenorhabditis elegans*. To test this question in vivo, *ent*-cholesterol was substituted for cholesterol. Sufficient cholesterol was carried over to the first generation on *ent*-cholesterol with mild behavioral defects. However, the second generation on *ent*-cholesterol died at an early stage of developpement. This shows that cholesterol cannot be replaced by *ent*-cholesterol **86**. Feeding a mixture of cholesterol and *ent*-cholesterol revealed the antagonism of *ent*-cholesterol **86** to cholesterol. The authors showed that labeled *ent*-cholesterol was incorporated and replaced cholesterol. Several explanations were proposed for the effects of *ent*-cholesterol. Differences in the metabolism of *nat*- and *ent*-cholesterol to a metabolite of specific and critical function by an enantiospecific processes may be one.[84,85] Also, one may add the enantiospecificity of the receptor. The enantiospecific interaction of cholesterol within the membranes is not to be rejected. Yet unknown steroids seem to play an important role in the development of the nematodes. This point is open to further investigation.

Even human senses can differentiate the steroid enantiomers. Androsta-4,16-dien-3-one **140** has a strong urine odor at 1 ppb, and the enantiomer **141** has no detectable odor, Figure 36.[17]



140 **141**

Figure 36.

To my surprise, I could not find any publication mentioning the results of tests of *ent*-testosterone. The advantage of the high input screening is that *rac*-steroids will be tested, and so unexpected activities for *ent*- as well for *nat*-steroids may be found.

VI. Conclusion

What are the challenges in steroids and *ent*-steroids research? In part, due to the molecular biology, the biochemistry of steroids is going to be better understood. In addition, on one hand, the enzymes involved in the metabolism will be better known and inhibitors can then be designed. On the other hand, the steroid receptors are going to be better explored. Analogues will replace the *nat*-steroids with the aim of more selective action. Since *ent*-steroids seem to be devoid of hormonal activity, the field of the *ent*-steroids is open for any other activities. See the *ent*-steroids in the future.

VII. References

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CR020071B