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Mechanistic Studies of the Rearrangements of Steroidal 16,17-Ketols and Syntheses of 20→16-*cis*-γ-Carbolactones

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'The teacher who walks in the shadow of the temple, among his followers, gives not of his wisdom but rather of his faith and his lovingness.'
Dedicated with respect and admiration to the memory of Professor Sir Derek Barton.

Abstract—Utilization of 17-keto-androstanes as starting materials for the synthesis of α- or β-oriented steroidal 20→16-γ-carbolactones has been explored following two different strategies. A highly efficient, stereospecific protocol has been developed for the β-oriented *cis*-γ-lactone. A different approach, involving prior attachment of a 3-carbon side chain on C-17 of a 17-oxo-16β-acetoxy-androstane led to the epimeric, α-oriented lactone. The mechanism of the rearrangement of epimeric 16β- or 16α-hydroxy-17-keto-androstanes to 17β-hydroxy-16-keto-androstanes was studied by ^{13}C NMR spectroscopy. The former occurs through a 1,2-sigmatropic H-shift, while the latter is likely to take place by simple enolization–reprotonation.

Introduction

Our interest in steroidal *cis*-20→16-γ-carbolactones originated in the course of studies on the pathway by which tomato plants convert the steroidal alkaloid tomatine (**Ia**) or its aglycone tomatidine (**Ib**) into allopregnenolone (**II**, Scheme 1).[1] The isolation of 23ξ-hydroxy-tomatidine (**III**) suggested the possibility of C-23 being the site where initial enzymatic hydroxylation takes place, which would be followed by oxidative cleavage leading to putative intermediate lactone **IVa** (tigogeninlactone).[2] This might suffer further oxidation with loss of carbon dioxide to afford allopregnenolone **II**. In order to determine the fate of the atoms in the piperidine ring we required a specimen of tomatidine isotopically labeled within this ring. We felt that a lactone like **IVa** would be an appropriate synthetic precursor towards this goal, as addition of an organometallic reagent to the lactone ring, followed by functional group manipulations, might provide the desired labeled target.

Tigogeninlactone (**IVa**) had been used as a key intermediate in the preparation of a variety of sapogenins[3] and alkaloids.[4] Sir Derek Barton himself had reported a photochemical preparation of this type of compound.[5]

In addition, products containing the 20→16-*cis*-γ-lactone moiety (**IVb-c**) have more recently been isolated from different natural sources.[6]

Initial synthetic efforts

Our initial synthetic approach to this γ-lactone system is shown in Scheme 2. It involved the addition of a Reformatsky reagent (from ethyl 2-bromopropionate) on a 16β-acetoxy-17-keto-androstane (**V**). We obtained a mixture of four different β-hydroxy esters (Scheme 2). These were separated by HPLC, and characterized spectroscopically as compounds **VIa–b** and **VIIa–b**.[7]

We rationalized the formation of products alkylated on C-16 by calling for an isomerization of the 16β-hydroxy-17-keto androstane system to a 17β-hydroxy-16-keto, which would suffer addition of the Reformatsky reagent and yield esters **VIIa–b** after acetylation of secondary hydroxyl groups (Scheme 3). In support of this hypothesis, the transposition of carbonyl groups in steroids, from C-17 to C-16 as well as other positions such as C-3 to C-2, had been reported.[8]

From the synthetic standpoint, we solved this problem by switching to milder reagents. Lithium ester enolates at low temperatures afforded only products of addition on C-17.[7] However, we were curious as to the differences with earlier results reported in the literature. The same reaction carried out *on the 16α-epimer* (**VIII**) had previously been reported to afford a single product.[9]

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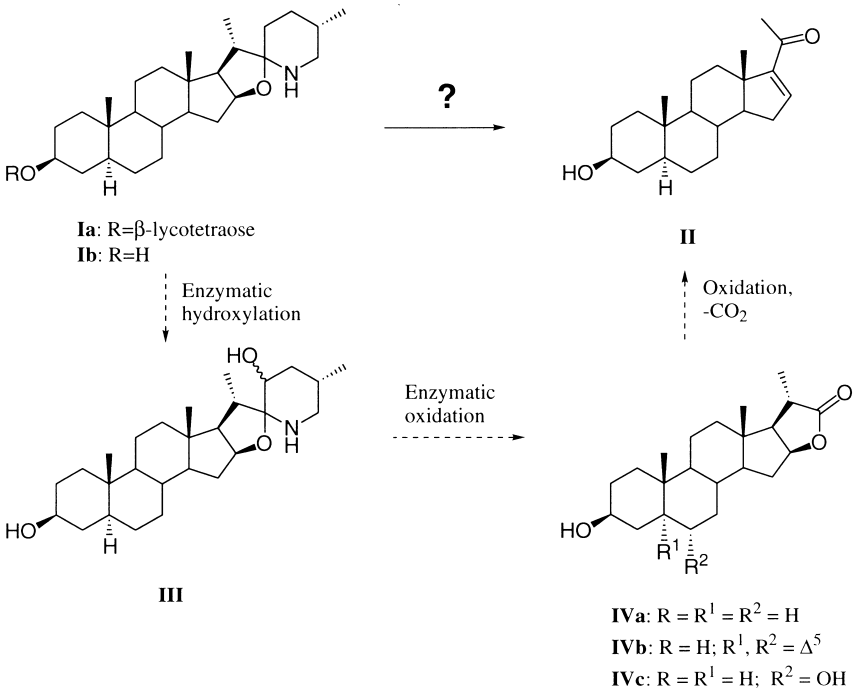
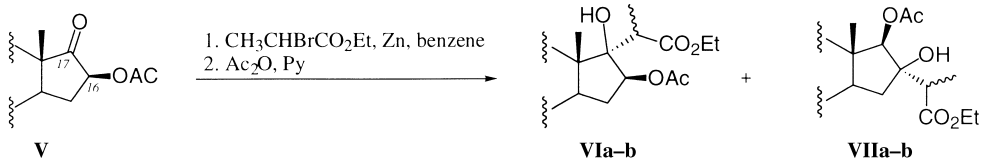
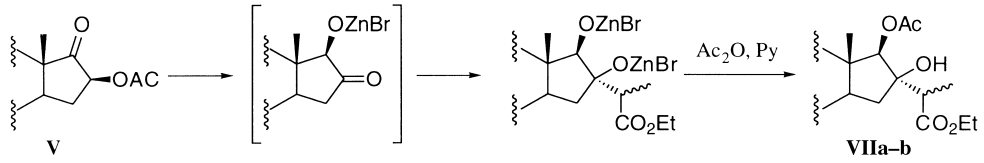


Scheme 1. Putative degradative pathway of tomatine.



Scheme 2. Reformatsky reaction on V.



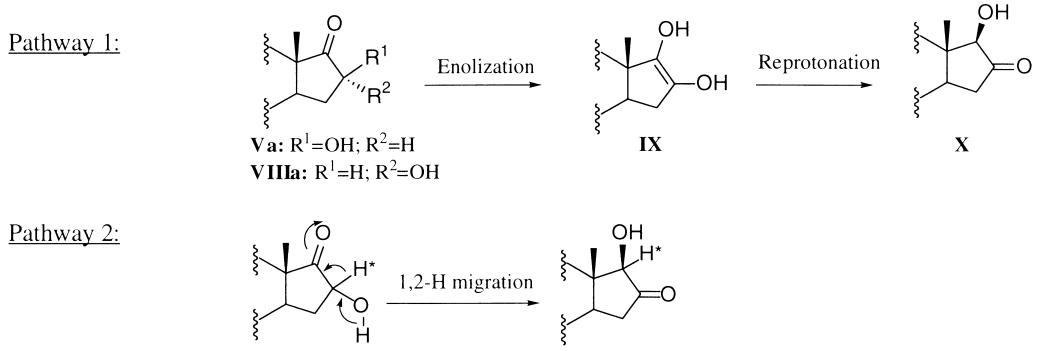
Scheme 3. Rationale for the formation of **VIIa–b**.

Indeed, we verified that under the same reaction conditions the 16α-epimer yields exclusively (HPLC analysis) a single product of addition on C-17.[10] Similarly, Reformatsky reactions of ethyl bromo*acetate* on both 16α- and 16β-acetoxy-17-keto-androstanes were reported to yield the expected β-hydroxy esters in good yields.[11]

Mechanistic studies on the interconversion of steroidal 16,17-ketols

We speculated that the different behavior of these C-16 epimers towards Reformatsky reaction conditions was due to differences in their rate of conversion to (the same) 17β-hydroxy-16-keto-androstane (**X**). In principle, we considered two different ionic pathways for these transformations. The first mechanism would involve enolization to an ene-diol (**IX**), followed by reprotonation on C-17 from the less-hindered α-face, to produce epimer **X**, which would presumably be the thermodynamically more-stable epimer. The second one would be a sigmatropic 1,2-intramolecular H-shift from C-16 to C-17 (Scheme 4).

^{13}C-NMR spectroscopy experiments were designed to explore these hypotheses.[12] First, we prepared a solution of compound **V** in CD_3OD/C_6D_6. Upon addition of KOD in D_2O, we verified the complete transformation to **X** after a few minutes. At no point in time were we able to detect the resonance for C-17. This fact was indicating the presence of a deuterium atom on C-17, and thus confirmed the possibility of using the presence



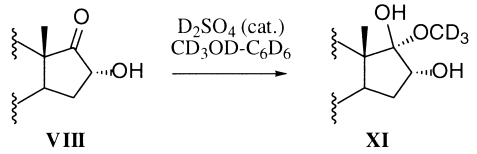
Scheme 4. Postulated pathways for ketols interconversion.

(or absence) of a resonance for C-17 as a diagnostic tool for the incorporation of a deuterium atom (rather than hydrogen) on a given carbon atom of the steroid molecule.[13]

More interesting results were obtained by treatment of the CD_3OD/C_6D_6 solution of **V** with catalytic amounts of D_2SO_4. The conversion of the starting material was much slower than under basic conditions, and indeed we detected the presence of rearranged product **X** *showing the C-17 resonance* for a few hours. As time went by, this resonance decreased in intensity and eventually disappeared. Since solvents and catalyst are fully deuterated there is no source of H atoms. Therefore, we interpret these results as showing that under acidic catalysis the conversion of **V** to **X** involves a 1,2-migration of $H16_\alpha$ to C-17. This is followed by a slower enolization–reprotonation (which introduces a deuterium on C-17 of **X**). Similar conclusions were reached by Numazawa et al. in experiments using deuterated substrates and mass spectrometry analysis.[14]

For the 16α-epimer **VIII**, experiments using stoichiometric amounts of KOD as a promoter were also too fast to allow for the detection of any intermediate, and only fully-deuterated reaction product **X** was seen. However, under acidic conditions (D_2SO_4) an interesting result was obtained, as no formation of rearranged product was seen and instead, a new product was formed. This was isolated by reverse-phase HPLC, and characterized as hemiketal **XI** (Scheme 5).[12b]

Thus, both 16-epimers show indeed a different behavior under acidic conditions (Scheme 6). In both cases reactivity is triggered by protonation of the oxygen on the C-17 carbonyl group, resulting in reaction intermediates which are diastereomeric at C-16. In the case of the 16α-hydroxy epimer (**A**), the intermediate's most favorable pathway is the formal addition of CD_3OD to the carbonyl group, resulting in the hemiketal being the only



Scheme 5. Fate of **VIII** under acidic conditions.

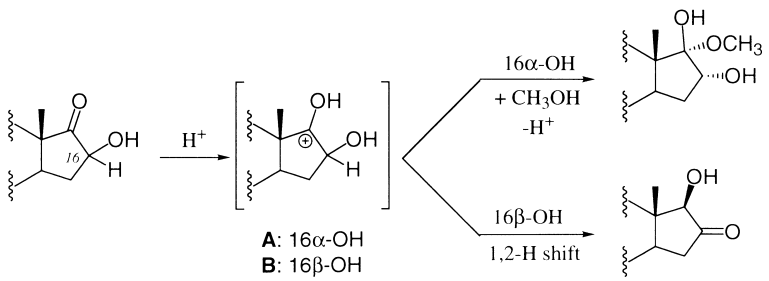
reaction product (stabilized by intramolecular hydrogen bonding). However, for the 16β-hydroxy epimer protonated intermediate (**B**), the parallel orbital alignment between the C16–$H16_\alpha$ σ-orbital and the empty C-17 *p*-orbital allows a fast 1,2-migration that leads to the 17β-hydroxy-16-keto-epimer. This migration is not preferred for the 16α-epimer, as the orbital alignment upon protonation of the carbonyl oxygen would not be favorable.

Molecular mechanics calculations on the epimeric starting materials and reaction intermediates **A** and **B** have provided support for this explanation.[15] As shown in Figure 1, the calculated dihedral angles formed by H-16β/C-16/C-17/O-17 for intermediate **A** is ca. 106°, while the calculated dihedral angles formed by H-16α/C-16/C-17/O-17 for intermediate **B** is ca. 93°. Thus, this difference could be significant, and intermediate **B** could provide substantially better orbital overlapping, which is the driving force for the intramolecular migration process.

Synthesis completion

We achieved addition specifically on C-17 of **V** by using the lithium enolate of *t*-butyl propionate at low temperatures (Scheme 7). After exchange of the acid-labile *t*-butyl group for a methyl group, methyl ester **XIV** was dehydrated by treatment with catalytic PTSA in toluene. This yielded a mixture of lactone **XV** and ester **XVI**, easily separable by flash chromatography. It is noteworthy that in forming lactone **XV**, inversion of stereochemistry at C-16 accompanies dehydration, as determined by ^1H NMR nOe experiments. Hydrogenation of lactone **XV** occurred smoothly, affording the α-oriented γ-lactone **XVII**, which was epimerized under basic conditions to the more stable lactone **XVIII**.[14] On the contrary, catalytic hydrogenation on conjugated ester **XVI** was extremely slow, and double bond saturation was observed only after hydrogenolysis of the 16β-acetoxy group. This presumably relieves steric hindrance around the tetra-substituted double bond, and saturated ester **XIX** could be obtained.

In order to obtain the desired β-oriented γ-lactone the synthetic strategy was modified as shown in Scheme 8.[16] Wittig reaction on 3β-(dimethyl-t-butylsilyloxy)-17-oxo-5α-androstane **XX** yielded stereo-specifically olefin **XXI**.



Scheme 6. Different fates for 16-epimeric ketols.

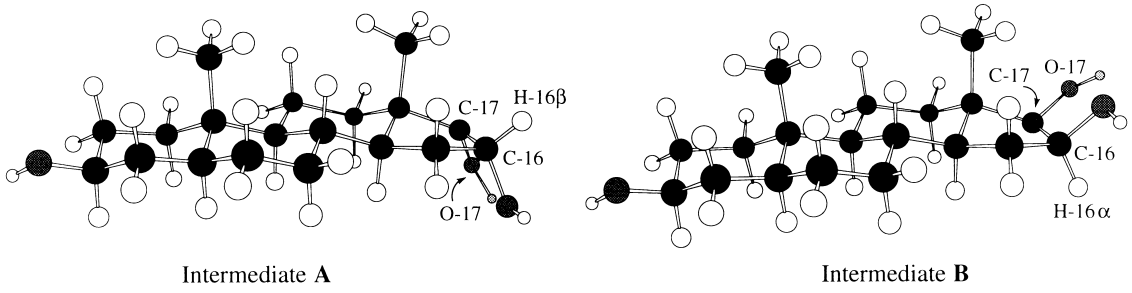
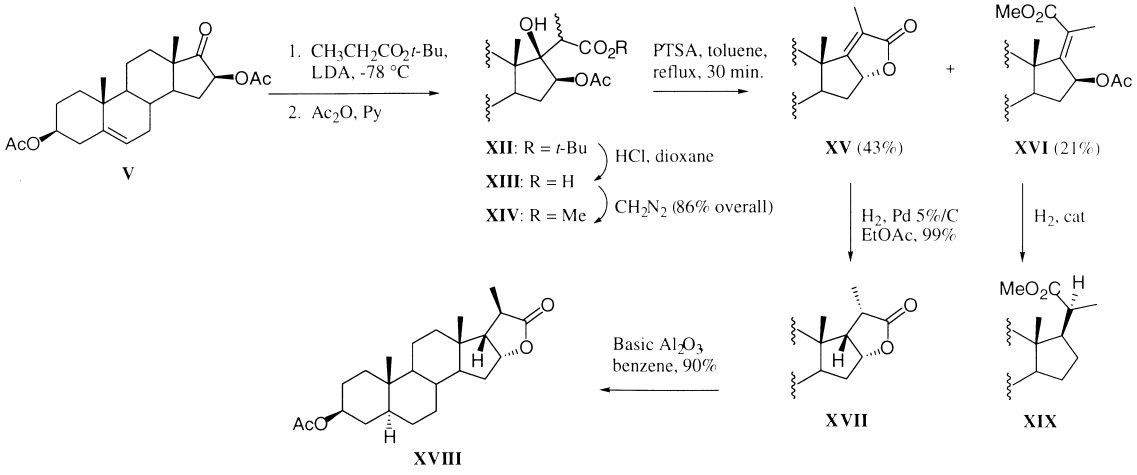
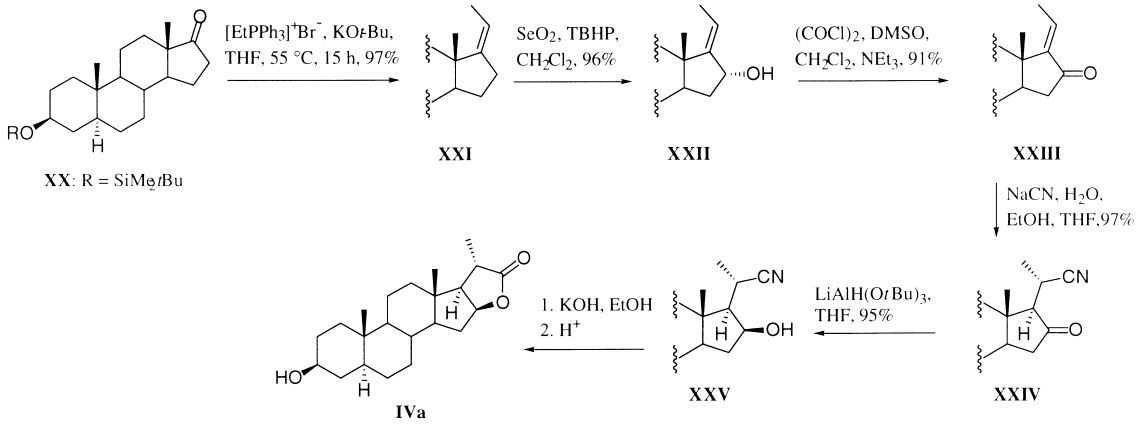


Figure 1. Calculated geometries for intermediates **A** and **B**.



Scheme 7. Synthesis of α-oriented 20→16-*cis*-γ-carbolactone.



Scheme 8. Synthesis of β-oriented 20→16-*cis*-γ-carbolactones.

Allylic hydroxylation with TBHP in the presence of catalytic amounts of SeO_2, followed by Swern oxidation produced in very good yield the conjugated ketone **XXIII**. Michael addition of NaCN in a THF–EtOH–H_2O mixture afforded nitrile **XXIV** as a single diastereoisomer. Stereospecific reduction of the ketone **XXIV** with lithium tri-*t*-butoxy aluminumhydride afforded the 16β-hydroxy-derivative **XXV**. As expected, the bulky hydride approaches the carbonyl group from the less-hindered α-face of the steroid nucleus, producing in excellent yield and selectivity the needed β-orientation for the hydroxy group on C-16. Alkaline hydrolysis of nitrile **XXV**, including an acidic work-up, produced lactone **IVa**, where lactonization as well as deprotection of the 3β-OTBDMS group took place during acidic work-up. This lactone resulted identical with authentic specimens obtained by degradation of tigogenin and tomatidine.

In summary, we developed a facile, high yielding protocol for the preparation of steroidal β-oriented 20→16-*cis*-γ-carbolactones. The different reactivity of epimeric 16-hydroxy (or acetoxy)-17-keto-androstanes has been explained based on the different pathways they have available to rearrange to the more-stable 17β-hydroxy-16-keto isomer. This type of ketol isomerizations have been reported previously, and different reaction mechanisms were postulated, including enolization and hydration–dehydration.[17] The generality of the synthetic protocol presented is being tested with vespertilin (**IVb**) and solanolide (**IVc**).

Acknowledgements

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