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The Synthesis and Reactivity of β,γ-Acetylenic and Allenic Sulfoxides and Sulfones of Dehydroepiandrosterone and their Reactions with 1-Butanethiol and Glucose-6-Phosphate Dehydrogenase

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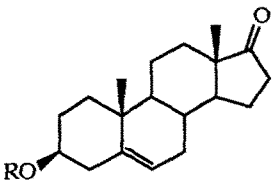
Abstract: The synthesis of the β,γ-acetylenic sulfoxide **2a** and sulfone **2b** of 3β-hydroxy-androst-5-en-17-one (dehydroepiandrosterone, DHEA) **1a** are described. Isomerization of the β,γ-acetylenes **2a,b** to their respective allenes **3a,b** and their reactions with the nucleophile 1-butane-thiol, and glucose-6-phosphate dehydrogenase were studied.

3β-Hydroxyandrost-5-en-17-one (Dehydroepiandrosterone DHEA), **1a** is a potent uncompetitive inhibitor of mammalian glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) thereby decreasing the formation of extramitochondrial NADPH and ribose phosphate.[1] The reduced production of these essential compounds may account for the anti-obesity and anti-carcinogenic effects of DHEA.[2] As part of a program of study of the allosteric inhibitors of G6PDH related to **1a,** we required reactive inhibitors with the potential to bind covalently to the G6PDH allosteric site. Since the sulfatide of DHEA, **1b**, has been shown to be the most potent natural inhibitor of G6PDH,[1] we chose to synthesize the β,γ-acetylenic sulfoxides **3b** and the β,γ-acetylenic sulfone **3c**, as potential masked reactive inhibitors. The design of the β,γ-acetylenic sulfoxides **3b** and sulfone **3c**, is based on the success of β,γ-acetylenic thiol esters[3] and β,γ-acetylenic ketones[4] as mechanism based enzyme inhibitors.



1a R = H

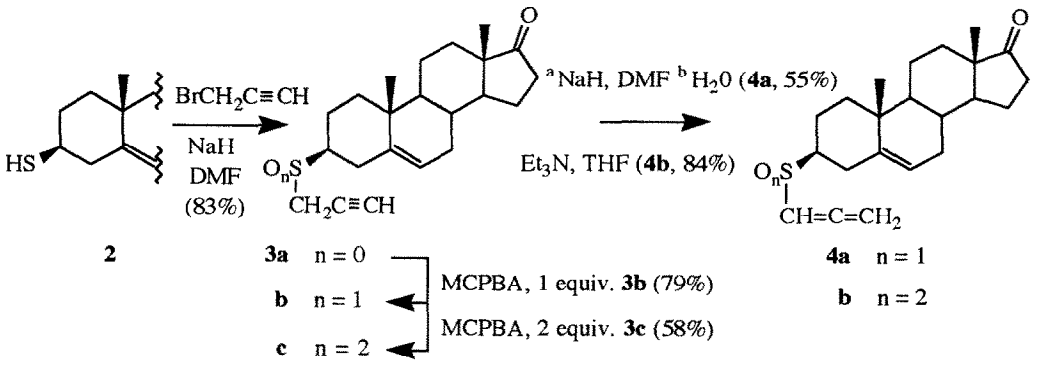
b R = SO_3CH_2–$CHOCOC_{15}H_{31}$–$CH_2OCOC_{15}H_{31}$

It was planned that the β,γ-acetylenic sulfone **3c** would bind strongly to the G6PDH regulatory site. Since the tautomerization of β,γ-acetylenic sulfones to the corresponding allenes with Et_3N has been reported,[5] it

was conceivable that a basic amino acid residue in the binding site would tautomerize the the propargylic moiety of **3c** to the electrophilic allenic sulfone **4b**, which would be rapidly trapped by a nucleophilic amino acid present in the steroid binding site of G6PDH, thereby effecting a selective labeling of the enzyme.

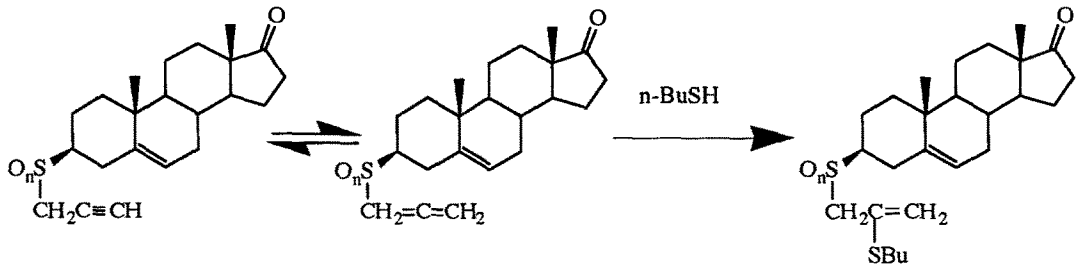
The steroidal allenic sulfoxide **3b** and steroidal allenic sulfone **3c** were synthesised from 3β-thioandrost-5-en-17-one **2** [6] as described in Scheme I. The C-3 thiol **2** was converted to the thiolate with sodium hydride and reacted with propargyl bromide to form the acetylenic sulfide **3a** in 83% yield. Treatment of **3a** with one or two equivalents of m-chloroperoxybenzoic acid (MCPBA) selectively oxidized the sulfur to either the diastereomeric acetylenic sulfoxides **3b** (79%) or the acetylenic sulfone **3c** (58%). There was no detectable formation of epoxides or ring D lactones. The above transformations were most easily confirmed by ^{13}C NMR spectra. For example the steroidal C-3 carbon in the sulfide **3a** appears at 44.1 ppm, in the sulfoxide **3b**, at 57.7 ppm and in the sulfone **3c**, at 60.0 ppm. Also the presence of the acetylene is readily apparent from the acetylenic carbon absorptions which are well separated from the other carbon resonances at 67-89 ppm.

Scheme 1. Synthesis of 3β-Propargylic and 3β-Allenic Sulfoxides and Sulfones of Dehydroepiandrosterone **3** and **4**



For covalent binding of the ligand to the substrate, the alkynyl sulfoxide **3b** and sulfone **3c** need to be tautomerized by base to the electrophilic allenic sulfoxide **4a** and sulfone **4b** respectively. The α–hydrogens of a sulfone are more acidic than those of a sulfoxide,[5] and this difference was exhibited in the conversion of **3b** and **3c** to their respective allenes. The alkynyl sulfone **3c** readily isomerized to the allenic sulfone **4b** using triethylamine in THF at room temperature. The isomerization resulted in an equilibrium between the propargyl sulfone **3c** and the allenic sulfone **4b** in the ratio of 13:87. In contrast the diastereomeric alkynyl sulfoxides **4a** were inert to the above conditions. However, treatment of **3b** with one equivalent of sodium hydride followed by quenching with water, effected the isomerization to the allenic sulfoxide **4a** in 55% yield.

As a test of the feasibility of relying on the spontaneous tautomerization of the propargyl sulfoxide **3b** and sulfone **3c**, to the electrophilic allenyl sulfoxide **4a** and sulfone **4b** respectively, and to test the electrophilicity of the allenes **4a,b**, each of the potential inhibitors, **3a,b,4a** and **b**, were reacted with an excess of the nucleophile n-butanethiol[7] in the absence of base. The results are summarized in Table 1.



3c n = 2 **4a** n = 1 **b** n = 2 **5a** n = 1 **b** n = 2

Table 1. Relative Reactivity of the Alkynyl- and Allenic Sulfoxides **3b,4a** and Sulfones **3c,4b** with 1-Butanethiol

Sulfoxides and Sulfones	n	Reaction Time	Michael Adduct	Yield
Alkyne **3b**	**1**	no reaction	--	--
Alkyne **3c**	**2**	30 h	**5b**	57 %
Allene **4a**	**1**	5 days	**5a**	46 %
Allene **4b**	**2**	5 min.	**5b**	98 %

The propargyl sulfoxide **3b** failed to react with 1-butanethiol whereas the electrophilic allenic sulfoxide **4a** reacted very slowly to yield the Michael adduct **5a**. The allenic sulfone **4b** proved to be highly reactive and afforded the Michael adduct **5b.** Of relevance towards the proposed mechanism for masked affinity labeling was the finding that the propargylic sulfone **3c**, in the absence of added base, gave the same product **5b** as the allenic sulfone **4b**.

The compounds **3b,c,4a,b** and the sulfide **3a**, were tested as inhibitors of mammalian G6PDH[9] and the results are found in Table 2. The inhibitory properties of these molecules correlate approximately with their lipophilicity, with the sulfide **3a**, being the best inhibitor, and the sulfones are better inhibitors than sulfoxides. Our lead structure for a masked affinity label, **3c**, proved to have relatively modest inhibitory activity.

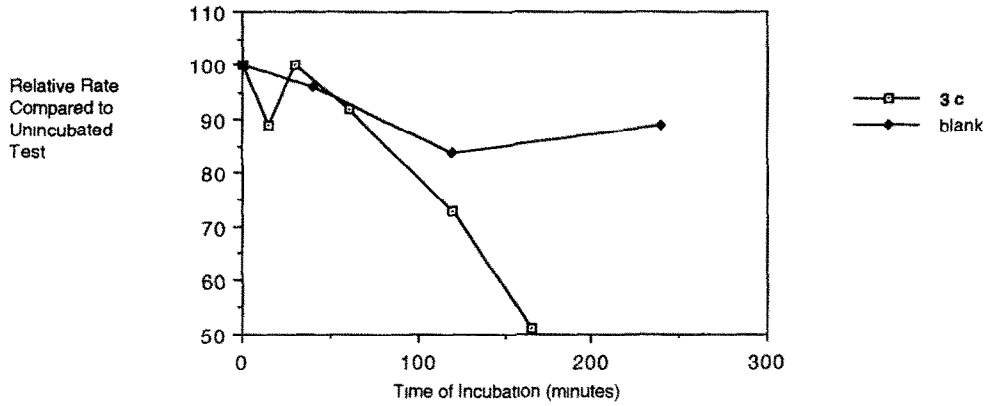
Table 2. Inhibition of Bovine Adrenal G6PDH by C-3 Substituted Androst-5-en-17-ones

C-3 Substituted Androst-5-en-17-one		Percent Inhibition at 10^{-5}M
Sulfide	**3a**	47
Sulfoxide	**3b**	< 5
Sulfone	**3c**	20
Sulfoxide	**4a**	< 5
Sulfone	**4b**	15

However, the observed percent inhibition of the enzyme with **3c** was shown to increase with the time of

incubation of this compound with G6PDH, NADP and the testing medium prior to initiating the enzymatic reaction by the addition of G6P. This time dependency of inhibition supports the proposal that **3c** is acting as a masked affinity label of G6PDH. These results are shown in Figure 1.

Figure 1. Comparison of the Relative Rate of Formation of NADPH with Time of Incubation



Based on the above results, we conclude that alkynyl sulfones may be isomerized to allenyl sulfones under mildly basic conditions, possibly equilibrating slowly at physiological pH. Since the allenic sulfone **4b** has been shown to be a potent electrophile, the slow formation of this molecule near the binding site could lead to a selective labeling of the steroidal binding site of G6PDH. This hypothesis was supported by the time dependency of inhibition of the enzyme, by **3c**. We have shown that allenic sulfoxides and sulfones, both react as Michael acceptors with nucleophilic mercaptans.

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References and Notes

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