Reaction of Steroidal 17-Acetylenic Alcohols with Diethylamidosulfur Trifluoride and the Structural Assignments of 17-Fluorosteroids

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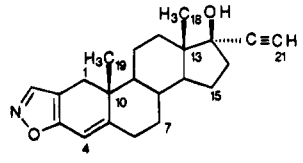
Reaction of diethylamidosulfur trifluoride (DAST) with C-17 tertiary alcohols of A-ring-fused steroids, such as Danazol (**1**), did not furnish the stereochemically coveted C-17β-fluoro derivatives. The C-17α-fluoro compounds were isolated as the predominant component of the reaction mixture along with the rearrangement and elimination products. It is postulated that the attack of fluoride ion to the intermediary carbonium ion occurs from the least hindered side of the steroid to provide only the α-fluoro derivatives. The formation of rearrangement and elimination products supports the proposed mechanism for the DAST reaction. Reaction products are definitively characterized by one- and two-dimensional (1D and 2D) NMR methods.

Introduction

Endometriosis is defined by the presence of ectopically located endometrial glands and stroma and is characterized by pain and infertility. Danazol (**1**), an orally active gonadotropin inhibitor, is an accepted treatment, but its use has been limited principally because of androgenic side effects.[1] From a pharmacokinetic perspective, attention was focused on chemical modification and variation of the heterocyclic ring of Danazol as a means of reducing the adverse biological activity of the drug.[1] The literature indicates that the C-17 substituent plays a significant role in the binding of the compounds to the steroid receptor site.[1,2] More recently, we have considered the influence of stereochemistry on drug activity and have focused our efforts toward an understanding of the effect of the substituent at the C-17 position.



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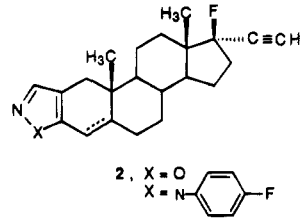

2, X = O
X = N–F

As a part of our investigations of the orally active gonadotropin inhibitors, we previously reported on the synthesis of compounds in which C-17 substitutions were substantially altered with bulky substituents such as aryls, heteroaryls, and terminally substituted acetylene derivatives.[3,4] The absence of any enhanced gonadotropin inhibitory activity in the previously reported series could be rationalized in terms of a stereochemically based intolerance on the part of the steroid receptor to the bulky substituents at the C-17 site of the drug substance. From both a biological and a chemical perspective, it is well-known that substitution of the hydroxyl group with fluorine does not alter the participation in the hydrogen bonding at the various receptor sites.[5,6] This paper describes the synthesis of a series of C-17 fluoro derivatives of the parent compound Danazol (**1**); fluorination was achieved using diethylamidosulfur trifluoride (DAST). Definitive characterization of the reaction products is also described.

Chemistry

A number of methodologies for the fluorination of organic compounds and for the replacement of the hydroxyl groups with fluorine have been developed; DAST or bis(dialkylamino)sulfur trifluorides are generally the reagents of choice.[6] Rozen and co-workers[7a] have reported on the reaction of various steroids with DAST, and the reaction has been reported to proceed through either an S_N2 mechanism or an elimination reaction involving a carbonium ion. Fluorination consequently proceeds either through retention of the stereochemistry or inversion, depending upon the structure of the steroids.[7b]

Danazol (**1**) was reacted with DAST at −60 to −70 °C in CH_2Cl_2, yielding two major products (Scheme 1) which

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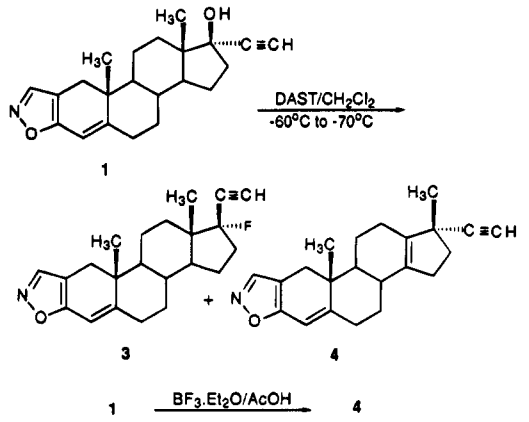
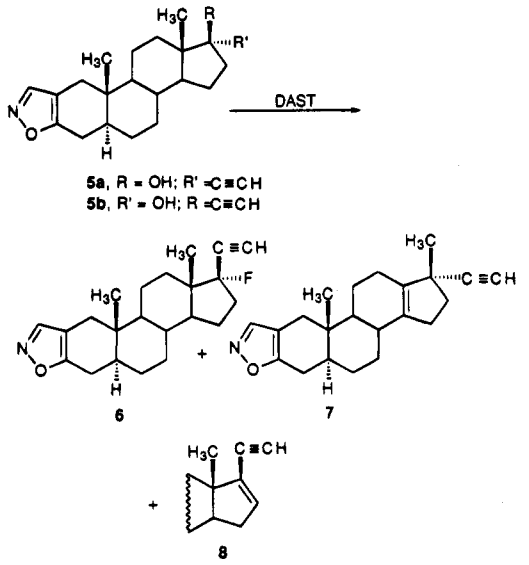
Scheme 1



Scheme 2

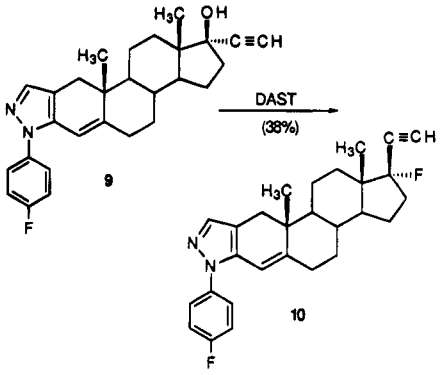


were separated by either fractional crystallization or HPLC to give **3** (37%) and **4** (58%). Analyses of the products indicate that fluorination of **1** occurred with inversion at the C-17 position to give **3**, while the olefin **4** was produced by a Wagner–Meerwein type rearrangement of the carbonium ion. The structural assignment of **3** is described below. An authentic sample of **4** was prepared from **1** using a procedure reported in the literature for C-17 acetylenic alcohols (Scheme 1).[8]

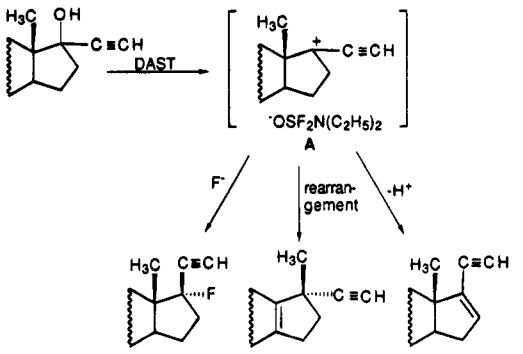
Similar reaction of DAST with a 4,5-dihydro derivative of Danazol, **5a**, gave the C-17α-fluoro **6** (60%) and the rearrangement product **7** (22%). The reaction is depicted in Scheme 2. A minor product (<5%) was also isolated from the reaction mixture and has been shown to be the elimination product, **8**. It was apparent that the reaction of DAST resulted in the inversion at C-17, and we postulate therefore that the acetylenic alcohols having the inverted center at C-17, such as **5b**, should provide the desired 17β-fluoro derivative when treated with DAST. However, reaction of isomer **5b** with DAST did not yield the expected 17β-fluoro compound; instead, the rearranged moiety, **7**, was isolated as the major product (70%), accompanied by the 17α-fluoro compound, **6** (15%), and only a trace amount of the 16,17-olefin, **8**, was observed in the crude reaction mixture.

Identical reaction of an A-ring-substituted pyrazolo steroid **9** (Scheme 3) with DAST resulted in only the

Scheme 3



Scheme 4



fluorinated species **10** in 38% yield. No rearrangement or elimination products were detected, indicating that the DAST reaction was highly dependent upon the structure of the steroids. Unreacted starting material was also isolated from the reaction mixture.

A proposed mechanism to account for the formation of the compounds described above is summarized in Scheme 4. The fluorination of the alcohols can be accounted for through formation of an ionic or ion-pair intermediate, similar to **A**.[5] The intermediate **A** which is formed from starting materials with either 17β-OH or 17α-OH (**5a** and **5b**) stereochemistry undergoes F^- attack from the least hindered side of the steroids to give the α-F-substituted compound. A carbocation rearrangement, or the loss of H^+, may account for the other two products depicted in Scheme 4. After rearrangement of the carbocation **A**, fluorination at the C-13 position of the steroid is possible, but this product was excluded on the basis of the NMR data. Again, the occurrence of the side products is governed by the structural type and stereochemical considerations, and no generalization can be assumed. But in cases where the potential carbocationic intermediate is stabilized in some fashion such as homoallylic carbocation, the retention of configuration in fluorination with DAST has been reported.[9]

Structure Elucidation: Protocol and Assignments

Plausible steroid structure assignments can be hypothesized using classical chemical shift arguments.[10] However, as the steroid literature indicates, such structural

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Table 1. Comparison of ^{1}H NMR and ^{13}C NMR Spectra of C-18 and C-19 Methyl Groups of the Various Steroids

	^{1}H NMR (δ)		^{13}C NMR (ppm)	
compound	C-18	C-19	C-18	C-19
1a	0.89	1.02	12.53	18.58
3 (α-F)	0.90	1.05	15.46	18.48
5a (β-OH)	0.78	0.90	12.54	11.37
5b (α-OH)	0.78	0.88	16.24	11.43
6 (α-F)	0.75	0.86	15.66	11.37
9 (β-OH)	0.91	1.03	12.47	18.19
10 (α-F)	0.90	1.02	15.60	18.19
3-oxo-5α-androstan-17β-ol	0.60	0.76	11.03	11.24
3-oxo-5α-androstan-17α-ol	0.50	0.75	16.95	11.21

assignments are often the subject of reinvestigation and reinterpretation.[11] As Table 1 illustrates, the predictability of the stereochemistry of a particular substituent at the C-17 position, based upon the proton chemical shifts of the C-18 and C-19 angular methyl groups, is at best tentative. The substitutions at C-17 in the Danazol derivatives reported in this paper, typified by **5a** (17β-OH) and **5b** (17α-OH) for example, clearly have very little interpretable effect on the proton chemical shifts of the C-18 and C-19 methyl groups. Several papers have appeared in the literature however which would appear to support the utility of carbon-13 chemical shifts in the determination of substituent stereochemistry in steroidal systems.[12-14] The carbon-13 chemical shift data shown in Table 1 for the reference compounds, 3-oxo-5α-androstan-17α-ol and 3-oxo-5α-androstan-17β-ol, would appear to confirm that a substantial methyl carbon shift to higher frequency may be indicative of an α-substituent at the adjacent C-17 position. Similar directional shifts for the C-18 methyl groups of the fluoro compounds, **3**, **6**, and **10**, suggest that the DAST reaction with C-17-substituted alcohols generates the undesired α-product. The generality that significant shifts of the C-18 methyl are evidence of α-substitution may however be compromised by substituent or solvent effects, and more unambiguous assignment methods are required.

In order to definitively assign the structure and the stereochemistry of the fluorinated analogs of Danazol, a series of one- and two-dimensional (1D and 2D) NMR experiments were implemented utilizing compound **3** as an example. The HMBC (^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence) experiment[15] shown in Figure 1 was used primarily to distinguish the methyl-18 and -19 groups; long range (two-bond and three-bond) coupling from the methyl protons to adjacent carbons allowed for assignment of carbons 12, 13, 14, and 17 (from methyl-18) and 1, 5, 9, and 10 (from methyl-19). Further carbon and proton assignments were revealed through careful analysis of the HMQC (^{1}H-detected heteronuclear multiple-quantum coherence)[16] experiment (shown in Figure 2), the COSY experiment,[17]

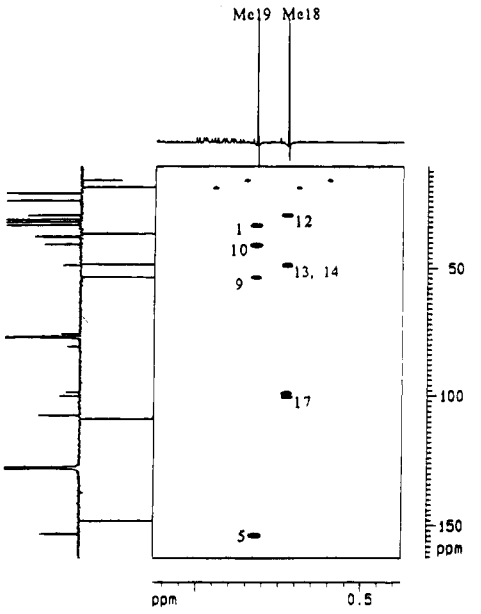

Figure 1. HMBC spectrum of compound **3** (500 MHz). Only the proton−methyl correlations are shown for the sake of clarity. The 1D proton and *J*-modulated 1D carbon spectra are included for reference purposes.

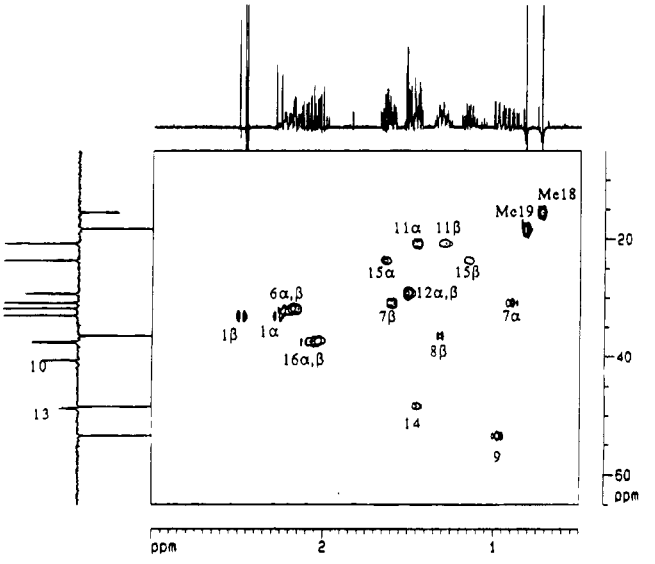

Figure 2. HMQC spectrum of compound **3** (500 MHz). The 1D proton and *J*-modulated 1D carbon spectra are included for reference purposes. All the relevant proton−carbon correlations are identified; the "spectroscopically silent" quaternary carbons (10 and 13) are also identified.

the TOCSY experiment,[18] the TOCSY−HMQC experiment,[19] and the carbon-detected *J*-modulation spin echo experiment.[20] Finally, proton spatial proximities and relative stereochemical displacements were probed with the NOE experiment.[21] The 1D NOE difference experiment was used, following the multiple irradiation method originally proposed by Saunders,[22] to definitively assign

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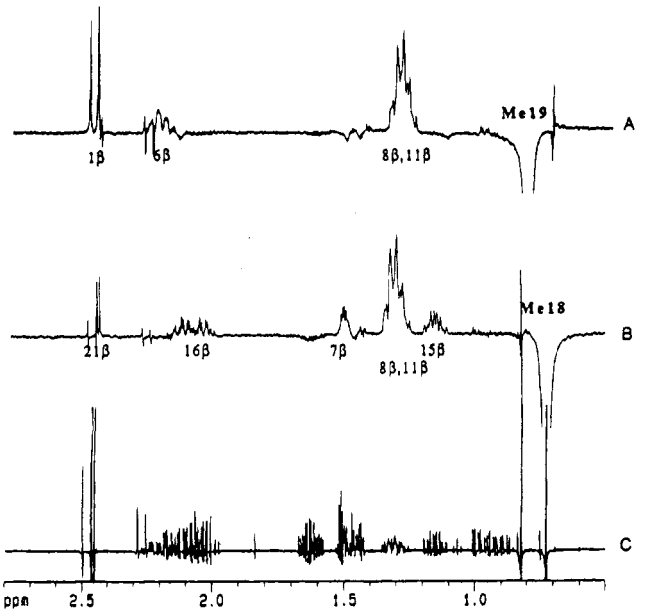

Figure 3. Homonuclear difference NOE spectra for compound **3** (500 MHz). Irradiation of methyl-19 (A), irradiation of methyl-18 (B), and a resolution-enhanced reference spectrum (C) are shown. Appropriate responses are identified.

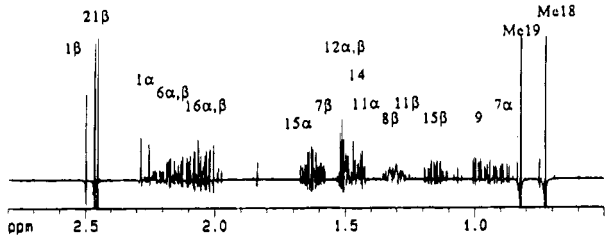

Figure 4. Resolution-enhanced 1D proton spectrum of compound **3** (500 MHz). Chemical shift assignments and stereochemical assignments are discussed in the text.

relative stereochemistry of the α,β-substitution for each steroidal ring proton; as Figure 3 shows, careful irradiation of the C-18 methyl group (0.73 ppm) clearly indicates a response from the adjacent ethyne proton (2.41 ppm), thereby indicating a β-orientation of this substituent at position 17. Several representative NOE difference spectra are shown in Figure 3. The complete proton assignments of compound **3** are presented schematically in Figure 4.

Conclusion

We have shown that reactions of A-ring-fused heterocyclic acetylenic 17α- and 17β-tertiary alcohols with the fluorinating agent, DAST, give rise primarily to α-fluoro products with inversion at the C-17 position when compared with the normal β-orientation of the hydroxyl group. Fluorination and the resulting side products were highly dependent upon the steroidal structure.

Experimental Section

NMR samples were dissolved in a 2:1 chloroform-d_1/benzene-d_6 solvent system. Standard data collection and data-process-ing methods were used throughout. Elemental analyses were performed by Galbraith Laboratories of Knoxville, TN, Instranal Laboratories of Rensselaer, NY, or QTI of Whitehouse, NJ. Analytical results are within ±0.4% of the theoretical values. Melting points are uncorrected.

General Procedure for DAST Reaction (example): (17β)-17-Fluoropregna-2,4-dien-20-yno[2,3-*d*]isoxazole (3) and (17α)-17-Methyl-18-norpregna-2,4,13-trien-20-yno-[2,3-*d*]isoxazole (4). To a cold solution of DAST (5.28 g, 0.032 mol) in CH_2Cl_2 (150 mL) at −60 to −70 °C under an argon atmosphere was added dropwise a solution of **1** (10.0 g, 0.03 mol) in CH_2Cl_2 (100 mL) over a period of 10 min. When addition was complete, the temperature of the reaction mixture was raised to −30 °C. The progress of the reaction was monitored by TLC, and after completion of the reaction (1−2 h), the reaction mixture was poured onto an ice-cold solution of 10% $NaHCO_3$. The organic layer was washed with water and a saturated salt solution and dried over anhydrous $MgSO_4$. Removal of the solvent gave the crude product which was either fractionally crystallized from ethyl acetate or separated by chromatography to give **3** (37%) as a pale yellow powder: mp 216−218 °C; MS *m/z* 339 (M^+), 324 ($M^+ - CH_3$); IR (KBr) 3295, 3060, 2220, 1635, 1610, 1472 cm^{-1}; ^1H NMR (300 MHz) δ 0.73 (s, 3H), 0.82 (s, 3H), 0.90−2.48 (m, 23H), 2.41 (d, $^4J_{H,F}$ = 5.4 Hz, 1H), 5.98 (s, 1H), 7.78 (s, 1H). Anal. Calcd for $C_{22}H_{26}FNO$: C, 77.84; H, 7.72; N, 4.13; F, 5.60. Found: C, 77.88; H, 7.70; N, 4.05; F, 5.52.

Compound **4** was isolated from the above reaction and recrystallized from EtOAc, 5.5 g (58%) as a white powder with mp 93−95 °C, and the spectral data were identical to those of the product isolated from the reaction below.

General Procedure for BF_3·Et_2O Reaction (example): (17α)-17-Methyl-18-norpregna-2,4,13-trien-20-yno[2,3-*d*]-isoxazole (4). To a suspension of **1** (20.0 g, 0.06 mol) in glacial acetic acid (100 mL) was slowly added freshly distilled BF_3·Et_2O (23.08 g, 0.16 mol) at ambient temperature. The brown-colored reaction mixture was stirred at ambient temperature for 5 h and poured onto a large volume of ice-cold water (1 L). The product was extracted with CH_2Cl_2 (3 × 300 mL). The organic layer was washed with water till neutral, dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure. The product was purified on a silica gel column (CH_2Cl_2:hexanes, 1:1) and recrystallized from ethyl acetate:hexanes (1:1) to give **4**, 9.0 g (47%): mp 94−96 °C; MS *m/z* 319 (M^+), 304 ($M^+ - CH_3$); IR (KBr) 3250, 3045, 2200, 1602, 1470 cm^{-1}; ^1H NMR (300 MHz) δ 1.01 (s, 3H), 1.29 (s, 3H), 0.80−2.95 (m, 19H), 2.15 (s, 1H), 6.22 (s, 1H), 8.02 (s, 1H). Anal. Calcd for $C_{22}H_{25}NO$: C, 82.72; H, 7.89; N, 4.38. Found: C, 82.66; H, 8.03; N, 4.40.

(5α,17β)-17-Fluoropregna-2-en-20-yno[2,3-*d*]isoxazole (6) was derived from **5a**: yield 60%; mp 184−186 °C; MS *m/z* 341 (M^+); IR (KBr) 3310, 3045, 2090, 1643, 1470 cm^{-1}; ^1H NMR (300 MHz) δ 0.50 (s, 3H), 0.70 (s, 3H), 0.63−2.41 (m, 26H), 2.35 (d, $^4J_{H,F}$ = 5.4 Hz, 1H), 7.77 (s, 1H). Anal. Calcd for $C_{22}H_{28}FNO$: C, 77.38; H, 8.26; N, 4.10; F, 5.56. Found: C, 77.41; H, 8.33; N, 4.16; F, 5.41.

(5α,17α)-17-Methyl-18-norpregna-2,13-dien-20-yno[2,3-*d*]isoxazole (7) was isolated from the DAST reaction of **5a**: yield 22%; mp 130−132 °C; MS *m/z* 321 (M^+), 306 ($M^+ - CH_3$); IR (KBr) 3255, 2840, 1642, 1445 cm^{-1}; ^1H NMR (300 MHz) δ 0.74 (s, 3H), 1.28 (s, 3H), 0.90−2.80 (m, 21H), 2.14 (s, 1H), 8.00 (s, 1H). Anal. Calcd for $C_{22}H_{27}NO$: C, 82.20; H, 8.47; N, 4.36. Found: C, 82.03; H, 8.53; N, 4.30.

(17β)-17-Fluoro-2′-(4-fluorophenyl)-2′H-pregna-2,4-dien-20-yno[3,2-*c*]pyrazole (10) was derived from **9**: yield 38%; mp 197−199 °C; IR (KBr) 3300, 3060, 2840, 1617, 1519 cm^{-1}; MS *m/z* 432 (M^+), 417 ($M^+ - CH_3$); ^1H NMR (300 MHz) δ 0.76 (s, 3H), 0.87 (s, 3H), 0.89−3.20 (m, 23H), 2.42 (d, $^4J_{H,F}$ = 5.44 Hz, 1H), 5.89 (s, 1H), 6.90 (m, 2H), 7.32 (m, 2H), 7.34 (s, 1H). Anal. Calcd for $C_{28}H_{30}F_2N_2$: C, 77.75; H, 6.99; N, 6.48; F, 8.78. Found: C, 78.04; H, 7.22; N, 6.38; F, 8.35.

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