Preparation of Aromatase Inhibitors. Synthesis of 19,19-Difluoro-4-hydroxyandrost-4-ene-3,17-dione and Related Compounds

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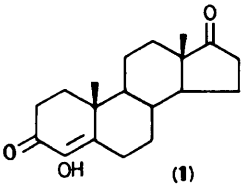
A new route to 19,19-difluoroandrost-4-ene-3,17-dione (**2**) has been devised, in which the key step is the reaction of (diethylamino)sulphur trifluoride (DAST) with 3,19-dioxoandrost-4-en-17β-yl benzoate (**5**). A novel rearrangement product (**7**) was also produced in this reaction. Compound (**2**) and its 4-hydroxy derivative (**3**; R = H) inhibited human placental aromatase *in vitro*, but were not as potent as 4-hydroxyandrost-4-ene-3,17-dione (**1**).

In a recent publication[1] we described novel routes to 4-hydroxyandrost-4-ene-3,17-dione (**1**) (HAD), and to various fluorosteroids. The rationale for this work was the known activity[2] of HAD as an irreversible inhibitor of the enzyme aromatase. This enzyme catalyses the final stages of estrogen biosynthesis and inhibitors may thus be of value in the treatment of estrogen-dependent breast cancer.[3] HAD is currently undergoing clinical evaluation, and initial results are encouraging.

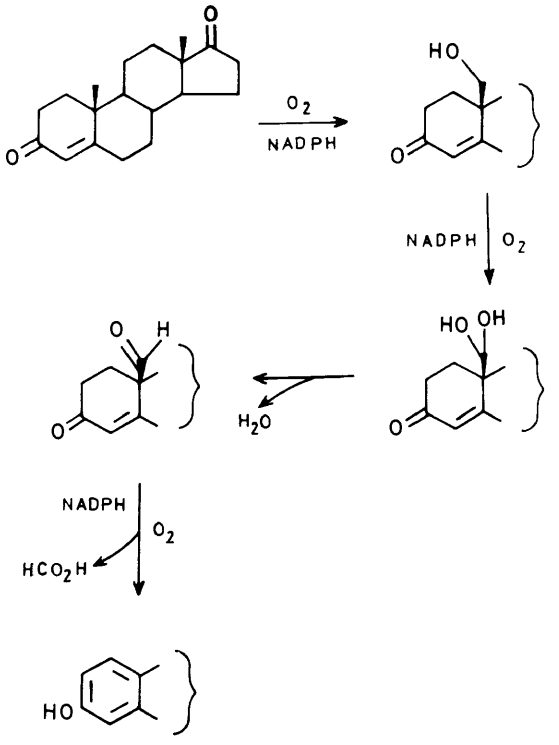


(**1**)

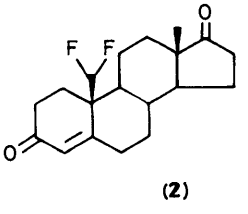
The biosynthetic pathway to estrogens is now fairly well understood,[4] and it has been established that the 19-methyl group of androst-4-ene-3,17-dione is removed *via* the oxidative sequence shown in the Scheme. One strategy for the inhibition would thus involve interference with these oxidative processes. To this end we sought to prepare 19,19-difluoroandrost-4-ene-3,17-dione (**2**)† and also its 4-hydroxy derivative (**3**; R = H).

The synthesis commenced with an oxidation of 3-oxoandrost-4-ene-17β,19-diol 17-benzoate (**4**)[6] (kindly supplied by Dr. J. Redpath of Organon Laboratories) to the corresponding aldehyde (**5**) using pyridinium chlorochromate (PCC). Reaction of this compound with DAST[7] in a mixture of dichloromethane and trichlorofluoromethane (1:1) at room temperature provided acceptable yields (*ca.* 60%) of 19,19-difluoro-3-oxoandrost-4-en-17β-yl benzoate (**6**). In addition, the rearrangement product (**7**) was also obtained, and we have already described[8] how the structure was elucidated (^{13}C n.m.r. and *X*-ray crystallography). To our knowledge this mode of steroid rearrangement is unique, though several related rearrangements are known.[9,10]

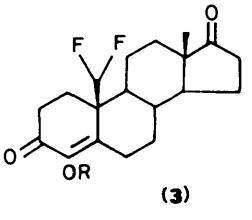
The synthesis of the dione (**2**) was completed by hydrolysis of the benzoate (**6**), then oxidation. The



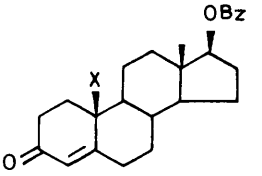
Scheme.



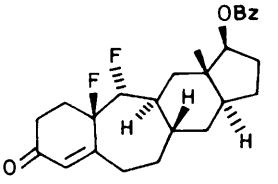
(**2**)



(**3**)



(**4**) X = CH_2OH
(**5**) X = CHO
(**6**) X = CHF_2



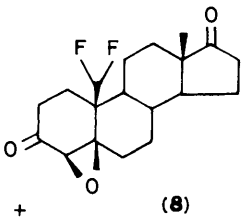
(**7**)

† During the course of our work, an alternative route to this compound was published,[5] but from the experimental details provided it would appear that our route is shorter and more efficient. A number of other 19-substituted steroids have also been described recently (B. W. Metcalf, C. L. Wright, J. P. Burkhart, and J. O. Johnston, *J. Am. Chem. Soc.*, 1981, **103**, 3221; J. A. Lovett, M. V. Darby, and R. E. Counsell, *J. Med. Chem.*, 1984, **27**, 734; P. J. Bednarski, D. J. Porubek, and S. D. Nelson, *ibid.*, 1985, **28**, 775; J. N. Wright, M. R. Calder, and M. Akhtar, *J. Chem. Soc., Chem. Commun.*, 1985, 1733), and several of these possessed activity as aromatase inhibitors.

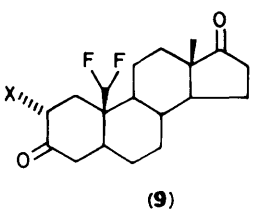
corresponding 4-hydroxy derivative (**3**; R = H) was prepared *via* epoxide (**8**). Epoxidation of enone (**2**) produced predominantly the β-epoxide (**8**), which upon treatment with methanolic sodium hydroxide formed 19,19-difluoro-4-methoxyandrost-4-ene-3,17-dione (**3**; R = Me). This ether was cleaved using BBr_3 in dichloromethane to yield the alcohol **3**; R = H).

Our other standard method for preparation of 4-hydroxyandrost-4-ene-3,17-dione derivatives,[1] namely reaction of the epoxide with acids, failed with epoxide (**8**), and either 19,19-difluoro-2α-hydroxyandrost-4-ene-3,17-dione (**9**; X = OH) (using H_2SO_4), or the corresponding formate (**9**; X = OCO·H) (using HCO_2H) were formed instead.

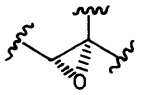
Compounds (**2**) and (**3**; R = H) were evaluated as aromatase inhibitors using human placental aromatase,[11] and they produced 50% inhibition of enzyme activity at concentrations of 1.3 μM and 3.3 μM respectively. The corresponding value for HAD (**1**) is 0.2 μM, and these new compounds are thus seen to be considerably less potent. Full details of this evaluation and others will be provided elsewhere.



(**8**)



(**9**)



Experimental

I.r. spectra were recorded with a Perkin-Elmer 157 double-beam grating spectrophotometer (solution spectra using chloroform as solvent); ^1H n.m.r. spectra were recorded with a Varian T-60 (60 MHz) or a Varian HA 100 (100 MHz) instrument (tetramethylsilane as internal standard); ^{13}C and ^{19}F n.m.r. spectra were recorded by Dr. Brian Wood at the City of London Polytechnic on a Jeol FX90Q (90 MHz) instrument; and mass spectra were recorded on an A.E.I. MS12 spectrometer. Kieselgel GF_{254} (Merck) was used for analytical t.l.c., and flash chromatography was performed with Merck silica gel (230—400 mesh). Organic solvents were distilled from calcium hydride when required anhydrous. Light petroleum refers to that fraction boiling in the range 40—60 °C.

3,19-*Dioxoandrost*-4-*en*-17β-*yl Benzoate* (**5**).—A solution of 3-oxoandrost-4-ene-17β,19-diol 17-benzoate (**4**) (6 g, 14.7 mmol) in dry dichloromethane (200 ml) was added to a stirred suspension of PCC (6.47 g, 30 mmol) in dry dichloromethane (50 ml at 0 °C). The reaction mixture was allowed to warm to ambient temperature and, after being stirred overnight, was diluted with diethyl ether (100 ml) and filtered. The filtrate was passed through a column (5 cm diameter) comprising silica gel (60—120 mesh) (25 cm) topped with 'Hyflo Super Cel "filter aid"' (12 cm), with diethyl ether (4 000 ml) as eluant. After removal of the solvent the crude product was purified by flash chromatography with diethyl ether–tetrachloromethane (2:1) as eluant. The pure *aldehyde* (**5**) was obtained as needles (5.73 g, 96%), m.p. 192—194 °C (decomp.); $[\alpha]_D^{20}$ +100° (*c* 7.05 in $CHCl_3$); $\nu_{max.}$ 3 300, 3 170 (olefin), 2 980, 2 950, 2 930, 2 883, 2 860, 1 717 (ester C=O), 1 675 (C-3 ketone), 1 455, 1 317, 1 270, and 1 170 cm^{-1} (both ester C–O); $\delta_H(CDCl_3)$ 0.95 (3 H, s, 18-H_3), 4.72—5.0 (1 H, m, 17-H), 6.0 (1 H, br s, 4-H), 7.29—7.69 (3 H, m, Ph ring), 7.97—8.17 (2 H, m, Ph ring), and 9.95 (1 H, s, 19-H); $\delta_C(CDC_3)$ 201.12 (C-19), 197.85 (C-3), 166.42 (benzoate carbonyl), and 160.95 (C-5) (Found: C, 76.9; H, 7.5. $C_{26}H_{30}O_4$ requires C, 76.8; H, 7.4%).

19,19-*Difluoro*-3-*oxoandrost*-4-*en*-17β-*yl Benzoate* (**6**) *and the Rearrangement Product* (**7**).—DAST (4.5 ml, *ca.* 36.9 mmol) was added at room temperature to a stirred solution of the aldehyde (**5**) (3.6 g, 8.87 mmol) in a mixture of dry dichloromethane (25 ml) and dry trichlorofluoromethane (25 ml). The solution was stirred for 44 h, diluted with dichloromethane (20 ml), poured onto crushed ice (*ca.* 100 g), and the organic layer was collected. The aqueous layer was extracted with dichloromethane (4 × 30 ml), and the combined organic phases were washed successively with water (2 × 30 ml), aqueous sodium hydrogen carbonate (5%; 30 ml), and water (30 ml). After the solution had been dried, and the solvent removed, the residue was repeatedly flash chromatographed with diethyl ether CCl_4 (2:1) as eluant. The *rearrangement product* (**7**) eluted from the column first and was obtained as needles (0.70 g, 24% based upon the quantity of aldehyde consumed), m.p. 160 °C; $[\alpha]_D^{20}$ +147° (*c* 6.49 in $CHCl_3$); $\nu_{max.}$ 2 980, 2 950, 2 880, 2 860, 1 715 (ester C=O), 1 693, 1 677 C-3 C=O), 1 453, 1 317, 1 280, and 1 125 cm^{-1}; $\delta_H(CDCl_3)$ 1.00 (3 H, s, 18-H_3), 4.36 (1 H, m, 17-H), 4.53—5.34 (1 H, m, J_{FH} 49.6 and 17, $J_{9,9a}$ 8 Hz, 9_a-H), and 6.04 (1 H, m, 4-H); $\delta_C(CDCl_3)$ 198.28 (d, J_{CF} 3.7 Hz, C-3), 166.37 (benzoyl C=O), 156.88 (dd, J_{CF} 14.5 and 7.2 Hz, C-5), 131.37 (d, J_{CF} 7 Hz, C-4), 97.35 (dd, J_{CF} 184.6 and 24.2, J_{FF} 16 Hz, C-9a), 93.77 (dd, J_{CF} 173.7 and 24.2, J_{FF} 16 Hz, C-10), and 82.62 (C-17); δ_F ($CDCl_3$, rel. to $CFCl_3$) −146.7 and −200.8 (2 d, J_{FF} 16 Hz) p.p.m. (Found: C, 72.5; H, 7.2. $C_{26}H_{30}F_2O_3$ requires C, 72.86; H, 7.06%).

Continued elution gave needles of the *difluoro compound* (**6**) [1.73 g, 60% based on the quantity of aldehyde (**5**) consumed in the reaction], and an analytical sample was obtained by recrystallization from CCl_4–light petroleum, m.p. 174.5—176 °C (decomp.); $[\alpha]_D^{20}$ +70° (*c* 7.16 in $CHCl_3$); $\nu_{max.}$ 3 330 and 3 170 (olefin), 2 990, 2 950, 2 937, 2 885, 2 860, 1 715 (ester C=O), 1 675 (C-3 ketone), 1 455, 1 317, and 1 270 and 1 117 cm^{-1} (both ester C–O); $\delta_H(CDCl_3)$ 0.98 (3 H, s, 18-H_3), 4.74—5.0 (1 H, m, 17-H), 6.0 (1 H, br s, 4-H), 6.08 (1 H, t, *J* 55 Hz, 19-H), 7.3—7.68 (3 H, m, Ph ring), and 7.95—8.2 (2 H, m, Ph ring); $\delta_C(CDCl_3)$ 198.43 (C-3), 166.42 (benzoate carbonyl), 160.75 (C-5), and 118.37 (t, J_{CF} 249.3 Hz, C-19); $\delta_F(CDCl_3$; relative to $CFCl_3$, ^1H-decoupled) −115.9 (q, J_{FF} 284.3, J_{FH} 55.5 Hz, 19-F) (Found: C, 73.0; H, 7.2%).

Finally, unchanged aldehyde (**5**) (0.86 g) was eluted from the column.

19,19-*Difluorotestosterone*.—A suspension of the difluorobenzoate (**6**) (7.0 g, 16.4 mmol) in methanolic potassium hydroxide (3%; 700 ml) was stirred at room temperature overnight. The solution was neutralized with acetic acid, the solvent was removed, and the residuum was suspended in water (100 ml). The mixture was extracted with dichloromethane (5 × 50 ml) and the extracts were washed successively with aqeuous sodium hydrogen carbonate (5%; 2 × 50 ml), water (2 × 50 ml), and brine (50 ml). After the extract had been dried and the solvent removed, the crude product was flash chromatographed with diethyl ether–dichloromethane (3:2) as eluant and was subsequently recrystallized from diethyl ether–light petroleum to afford the *title alcohol* as needles (4.3 g, 81%), m.p. 122—123 °C (decomp.); $[\alpha]_D^{20}$ +95° (*c* 5.33 in $CHCl_3$); $\nu_{max.}$ 3 620, 3 460 (br, OH), 3 010 (olefin), 2 950, 2 880, 2 860, and 1 650 cm^{-1} (C-3 ketone); $\delta_H(CDCl_3)$ 0.79 (3 H, s, 18-H_3), 1.88 (1 H, br s, OH), 3.45—3.85 (1 H, m, 17-H), 5.99 (1 H, br s, 4-H), and 6.08 (1 H, t, J_{HF} 56 Hz, 19-H); $\delta_C(CDCl_3)$ 198.66 (C-3), 161.23 (C-5), 128.04 (C-4), and 118.33 (t, J_{CF} 249.3 Hz, C-19; δ_F

($CDCl_3$; relative to $CFCl_3$, ^1H-decoupled) −115.7 (q, J_{FF} 284.4, J_{FH} 55.5 Hz, 19-F) (Found: C, 70.5; H, 8.2. $C_{19}H_{26}F_2O_2$ requires C, 70.33; H, 8.08%).

19,19-*Difluoroandrost*-4-*ene*-3,17-*dione* (**2**).—Jones' reagent (*ca.* 1 ml) was added dropwise to a stirred solution of 19,19-difluorotestosterone (1 g, 3.1 mmol) in AnalaR acetone (50 ml) at 0 °C. The solution was stirred at 0 °C for a further 15 min, poured into ice–water (*ca.* 100 g), and extracted with dichloromethane (5 × 20 ml). The combined extracts were washed with aqueous sodium hydrogen carbonate (5%; 25 ml). After removal of the solvent, the residuum was flash chromatographed with diethyl ether–dichloromethane (3:2) as eluant to afford the *dione* (**2**) as needles (0.97 g, 98%) from which an analytical sample was obtained by recrystallization from diethyl ether–light petroleum, m.p. 104.5—107 °C (decomp.) (lit.,[5] 109—110 °C); $[\alpha]_D^{20}$ +167° (*c* 6.78 in $CHCl_3$); $\nu_{max.}$ 3 030 and 3 010 (both olefin), 2 970, 2 950, 2 865, 1 737 (C-17 ketone), and 1 677 cm^{-1} (C-3 ketone); $\delta_H(CDCl_3)$ 0.92 (3 H, s, 18-H_3), 6.02 (1 H, br s, 4-H), and 6.09 (1 H, t, J_{HF} 56 Hz, 19-H); $\delta_C(CDCl_3)$ 219.72 (C-17), 198.25 (C-3), 160.27 (C-5), 128.42 (C-4), and 118.45 (t, J_{CF} 249.8 Hz, C-19); $\delta_F(CDCl_3$; relative to $CFCl_3$, H-decoupled)—115.6 (q, J_{FF} 284.7, J_{FH} 55.5 Hz, 19-F) p.p.m.

4,5-*Epoxy*-19,19-*difluoroandrostane*-3,17-*dione* (**8**).—Aqueous sodium hydroxide (4M; 8 ml) and hydrogen peroxide (30% 15 ml) were added dropwise to a stirred ice-cold solution of 19,19-difluoroandrost-4-ene-3,17-dione (**2**) (2.76 g, 8.57 mmol) in AnalaR methanol (300 ml). After 16 h at 4 °C, the mixture was poured onto crushed ice (150 g). The mixture was filtered, and the filtrate was extracted with dichloromethane (5 × 25 ml), acidified with hydrochloric acid (2M), and the acid washes extracted with dichloromethane (4 × 25 ml). The extracts were combined, dried, and the solvent was removed. Flash chromatography of the residues from the extracts and the filtration pad with diethyl ether–dichloromethane (1:1) as eluant afforded a *ca.* 4:1 mixture of 4β,5β- and 4α,5α-epoxides as needles (1.69 g, 58%); $\nu_{max.}$ 2 970, 2 957, 1 740 (C-17 ketone), 1 715 (C-3 ketone), 1 457, 1 410, 1 380, and 1 055 cm^{-1}; $\delta_H(CDCl_3)$ 0.9 (3 H, s, 18-H_3), 2.92 (1 H, s, 4α-H), 3.19 (1 H, s, 4β-H), 6.15 (1 H, t, J_{HF} 54 Hz, 19-H in 4α,5α-epoxide), and 6.22 (1 H, t, J_{HF} 56 Hz, 19-H in 4β,5β-epoxide) (Found: C, 67.3; H, 7.2. $C_{19}H_{24}F_2O_3$ requires C, 67.42; H, 7.15%).

19,19-*Difluoro*-2α-*hydroxyandrost*-4-*ene*-3,17-*dione* (**9**; X = OH).—Sulphuric acid (5M; 2.5 ml) was added to a solution of 4,5-epoxy-19,19-difluoroandrostane-3,17-dione (**8**) (a mixture of epimers; 1.2 g, 3.55 mmol) in AnalaR acetone (50 ml) and the reaction mixture was refluxed for 3 h. The bulk of the solvent was removed under reduced pressure, the residue was treated with water (100 ml), and the product was extracted into dichloromethane (4 × 30 ml). The combined extracts were washed in turn with water (30 ml), aqueous sodium hydrogen carbonate (5%; 2 × 30 ml), water (2 × 30 ml), and brine (30 ml). After the solution had been dried and the solvent removed, the residue was flash chromatographed with diethyl ether–tetrachloromethane (3:1) as eluant to yield the *title compound* as a froth (0.83 g, 69%) which could not be crystallized. Trituration of a solution of the product with light petroleum afforded a gel, and subsequent removal of the solvent under reduced pressure gave a solid, m.p. 97—98.5 °C (decomp.); $[\alpha]_D^{20}$ +154° (*c* 6.10 in $CHCl_3$); $\nu_{max.}$ 3 460 (br, OH), 3 070 (olefin), 2 955, 2 885, 2 650, 1 736 (C-17 ketone), and 1 717 cm^{-1} (C-3 ketone); $\delta_H(CDCl_3)$ 0.90 (3 H, s, 18-H_3), 3.45 (1 H, br m, OH), 4.45 (1 H, dd, *J* 3.25 and 6.75 Hz, 2-H), 6.05 (1 H, br s, 4-H), and 6.15 (1 H, t, J_{HF} 55 Hz, 19-H) [Found: M^+, 338.1694 (53.25%). $C_{19}H_{24}F_2O_3$ requires *M*, 338.1693].

19,19-*Difluoro*-3,17-*dioxoandrost*-4-*en*-2α-*yl Formate* [**9**; X = OC(O)H].—Formic acid (7 ml) was added to 4,5-epoxy-19,19-difluoroandrostane-3,17-dione (**8**) (a mixture of epimers; 0.3 g, 8.875 mmol) and the resulting solution was refluxed for 45 min, then poured into warm water (35 ml), and the mixture was allowed to cool to room temperature. The mixture was extracted with dichloromethane (5 × 15 ml) and the combined organic extracts were then washed successively with water (2 × 20 ml), aqueous sodium hydrogen carbonate (20 ml), water (2 × 20 ml), and brine (20 ml). After the solution had been dried and the solvent removed, the residue was purified by flash chromatography with diethyl ether–dichloromethane (1:1) as eluant to afford the *title formate* (0.18 g, 55%) as crystals, m.p. 157 °C (decomp.); $[\alpha]_D^{20}$ +113° (*c* 5.65 in $CHCl_3$); $\nu_{max.}$ 2 970, 2 950, 2 885, 2 863, 1 736 (ester carbonyl and C-17 ketone), and 1 175 cm^{-1} (C-3 ketone); $\delta_H(CDCl_3)$ 0.95 (3 H, s, 18-H_3), 5.65 (1 H, dd, *J* 3.0 and 7.0 Hz, 2-H), 6.05 (1 H, br s, 4-H), 6.17 (1 H, t, J_{HF} 55 Hz, 19-H), and 8.17 (1 H, s, formyl proton) [Found: M^+, 366.1644 (7.59%). $C_{20}H_{24}F_2O_4$ requires *M*, 366.1644].

19,19-*Difluoro*-4-*methoxyandrost*-4-*ene*-3,17-*dione* (**3**; R = Me).—Aqueous sodium hydroxide (4M; 5 ml) was added to a solution of 4,5-epoxy-19,19-difluoroandrostane-3,17-dione (**8**) (a mixture of epimers; 0.5 g, 2.22 mmol) in AnalaR methanol (17 ml) and the reaction mixture was then refluxed for 3 h. The bulk of the solvent was removed under reduced pressure, the residue was treated with water (15 ml), and the product was extracted into dichloromethane (4 × 15 ml). The combined extracts were washed in turn with water (2 × 15 ml) and brine (15 ml), and were then dried over sodium sulphate and purified by flash chromatography with diethyl ether–tetrachloromethane (8:1) as eluant. The title vinyl ether (0.25 g, 48%) was obtained as crystals which rapidly darkened in air, $\nu_{max.}$ 2 970, 2 940, 2 880, 2 860, 1 735 (C-17 ketone), 1 683 (C-3 ketone), and 1 055 cm^{-1}; $\delta_H(CDCl_3)$ 0.95 (3 H, s, 18-H_3), 3.65 (3 H, s, OMe), and 6.05 (1 H, t, J_{HF} 56 Hz, 19-H).

19,19-*Difluoro*-4-*hydroxyandrost*-4-*ene*-3,17-*dione* (**3**; R = H).—A solution of boron tribromide in dichloromethane (1M; 0.72 ml) was added to a stirred solution of 19,19-difluoro-4-methoxyandrost-4-ene-3,17-dione (**3**; R = Me) (0.25 g, 0.71 mmol) in dichloromethane (7 ml) held at −20 °C under an atmosphere of nitrogen. After 2 h at −20 °C the reaction mixture was poured into water (20 ml) and the organic phase was collected and washed in turn with water (2 × 10 ml), aqueous sodium hydrogen carbonate (5%; 10 ml), water (10 ml), and brine (10 ml). Flash chromatography with diethyl ether–tetrachloromethane (8:1) as eluant gave the title product (0.16 g, 67%) as a bright orange crystalline solid, which was purified by dissolution in diethyl ether and treatment with decolourizing charcoal; m.p. 185—187 °C; $[\alpha]_D^{20}$ +149° (*c* 5.12 in $CHCl_3$); $\nu_{max.}$ 3 450 (br, OH), 2 975, 2 940, 2 863, 1 737 (C-17 ketone), 1 677 (C-3 ketone), 1 647, 1 385, and 1 165 cm^{-1}; $\delta_H(CDCl_3)$ 0.9 (3 H, s, 18-H_3), 6.05 (1 H, t, J_{HF} 57 Hz, 19-H), and 6.4 (1 H, s, OH); $\delta_C(CDCl_3)$ 220.03 (C-17), 193.11 (C-3), 144.29 (C-5), 129.25 (C-4), 118.84 (t, J_{CF} 245.7 Hz, C-19), and 44.69 (t, J_{CF} 16.9 Hz, C-10); $\delta_F(CDCl_3$; rel. $CFCl_3$) −115.07 (dq, J_{FF} 284.4, J_{FH} 55.5 Hz) p.p.m. (Found: C, 67.4; H, 7.1. $C_{19}H_{24}F_2O_3$ requires C, 67.42; H, 7.15%).

Acknowledgements

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