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Transformation of androstenedione into 17α-hydroxy-16β-methylpregn-4-ene-3,20-dione

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An efficient synthesis of 17α-hydroxy-16β-methylpregn-4-ene-3,20-dione from androstenedione has been studied. Structure of the product and its intermediates has been examined by spectral methods such as IR, MS, 1D and 2D NMR.

Keywords: Androstenedione, 17α-hydroxy-16β-methylpregn-4-ene-3,20-dione

Bioconversion of phytosterols mixture is a low-cost method for the industrial production of 17-ketosteroids such as androst-4-ene-3,17-dione (androstenedione, AD), androsta-1,4-diene-3,17-dione (ADD) and 9α-hydroxyandrost-4-ene-3,17-dione (9α-OH AD)[1-5], which are currently considered as the most attractive intermediates for synthesis of commercial corticoids[6-9], such as hydrocortisone, prednisolone, betamethasone, dexamethasone and triamcinolone, *etc*.

Phytosterols can be isolated from several inexpensive sources, mostly from tall-oil in paper industry[10] or filter cake in sugar cane industry[11]. Soybean is another very important source of phytosterols and constitutes a highly remarkable food additive in Asia and all over the world. Soybean oil has lately gained good acceptance in cuisine everywhere[12]. Paper and sugar cane as well as soybean industries are developing in Vietnam currently. In fact, soybean is one of the most important agricultural products. According to recent reports, hundreds of thousands (~million in the near future) tons of soybean oil are produced annually. On the other hand, this country has to import all steroidal drugs. Therefore it would be very profitable to use waste, a by-product of these industries, as raw materials for development of technology required for the synthesis of the steroid drugs. Soybean phytosterols can be extracted from by-product of soybean oil production in Vietnam, and purified by using a very efficient procedure with really good yield[13,14].

Vietnamese soy phytosterols can be bio-converted to AD, ADD[15], and 9α-OH AD with very good yields[16-18]. According to report of Andryushina *et al.*[14], the AD and 9α-OH AD technologies are very efficient and highly practical.

17α-Hydroxy-16β-methylpregna-4,9(11)-diene-3,20-dione is an important intermediate for the synthesis of betamethasone[16], and method for synthesis of this compound from androsta-4,9(11)-diene-3,17-dione is known[17].

We present here an improved method for transforming AD (**1**) into 17α-hydroxy-16β-methylpregn-4-ene-3,20-dione (**9**), which is the prospective alternative intermediate for produce of betamethasone and beclomethasone. To our best knowledge, no report on the synthesis of 17α-hydroxy-16β-methylpregn-4-ene-3,20-dione from androstenedione has been described so far. Therefore, in this study, a new route for the synthesis of 17α-hydroxy-16β-methyl-3,20-diketal derivative (**8**) from 16α,17α-epoxy-3,20-diketal (**7**) was performed using methyl magnesium bromide. The reaction of (**7**) with other methylating agents such as MeLi, MeMgCl will be studied and published in the near future.

As a result, the reaction of 16α,17α-epoxy-3,20-diketal (**7**) with MeMgBr gave 17α-hydroxy-16β-

methyl-3,20-diketal (**8**). Then removing the ketal protecting groups together with the regeneration of keto- functions in the 3- and 20-positions gave rise to 17α-hydroxy-16β-methyl-pregnan (**9**) in good yield by using known method[17].

Structure of the 17α-hydroxy-16β-methyl-pregnane (**9**) had been studied by IR, ^{1}H NMR, ^{13}C- NMR, and DEPT data.

The stereochemistry of substituents at carbon atoms C^{16} and C^{17} was determined by interpreting the results of the experiment using the NOESY method for the first time (Figure 1). In particular, Me-22 (attached to C-16) had cross peaks with Me-21 and Me-18, this indicated that they were β-oriented. The methods employed in this transformation are summarized in Scheme I.

Experimental Section

All reagents, solvents, adsorbents, *etc*. were purchased from Aldrich Chemical Co. (USA) and Merck Clevenot Co. (France). Solvents for column chromatography were purified and distilled prior to use. Melting points were determined on Boetius apparatus and are uncorrected. IR spectra were recorded on IMPACT-410 FT-IR with KBr pellets. ^{1}H and ^{13}C NMR, and NOESY spectra were recorded on Bruker AM 500 NMR spectrometer in $CDCl_3$ with TMS as internal reference. MS spectra were recorded on Agilent 6310 Ion Trap in $CHCl_3$. Analytical thin-layer chromatography was performed using Merck 60 GF_{254} plates.

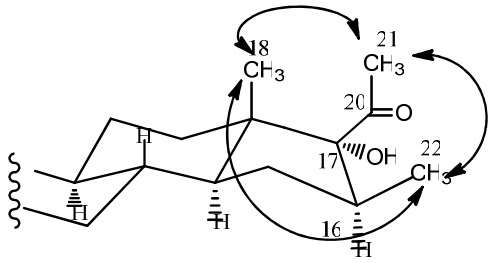
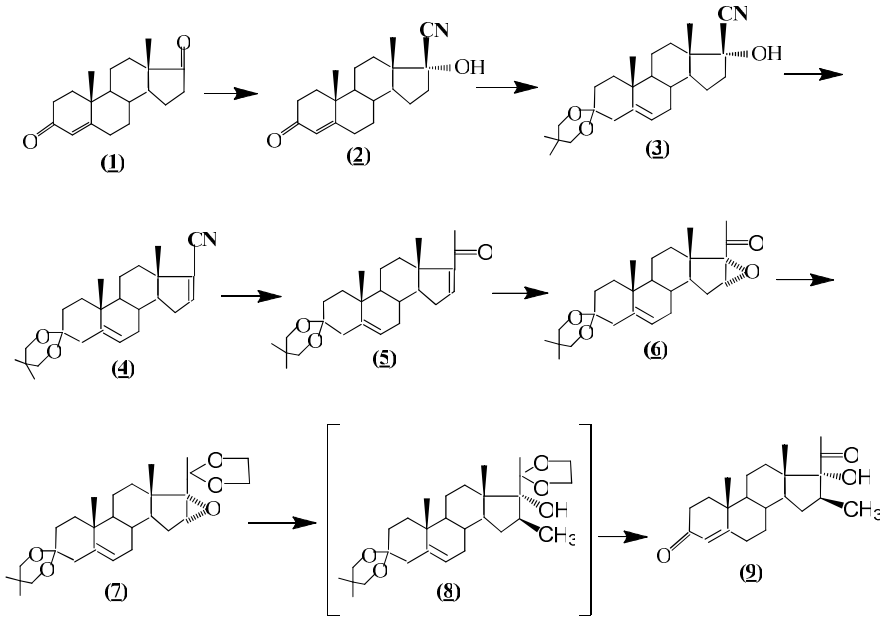


Figure 1 — Major NOESY correlations for compound **9**

17α-Hydroxy-17β-cyano-androst-4-en-3-one, 2

To a suspension of AD (**1**) (20.02 g, 0.07 mol) in methanol (56 mL) was added acetone cyanohydrin (12.75 mL, 0.14 mol) followed by NaOH (2.2 mL, 3.3M aqueous solution) and H_2O (12 mL). After being stirred at 36-40°C for 4 h, the reaction mixture was allowed to stand still overnight. Next, H_2O (48 mL) was added and the mixture was stirred at RT for 2 h. The resulting precipitate was filtered and washed with a methanol:water (1:2) mixture and then with water. Compound **2** was obtained by recrystallization from ethyl acetate (18.41g, 84%). m.p.210-13°C. IR (KBr): 3243 (O-H), 2233 (C≡N) and 1645 cm^{-1} (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.75 (s, 1H, H-4), 2.85 (s, 1H, O-H), 1.21 (s, 3H, CH_3-19) and 0.99 (s, 3H, CH_3-18).



Scheme I — Transformation of androstenedione into 17α-hydroxy-16β-methylpregn-4-ene-3,20-dione

17α-Hydroxy-3,3-(2,2-dimethylpropylene-dioxy)-17β-cyano-androst-5-ene, 3

To a suspension of **2** (23.50 g, 0.075 mol) in dichlomethane (92 mL) was added neopentyl glycol (35.40 g, 0.34 mol) and triethyl orthoformate (24 mL). The reaction mixture was stirred under nitrogen atmosphere at 0-5°C and then *p*-toluenesulfonic acid (2.33 g) was introduced. The reaction mixture was stirred for 8 h and triethylamine (2.33 mL) and H_2O (190 mL) were then added. The resulting precipitate was filtered and washed with water. The filtered liquid was extracted with $CHCl_3$. The organic phase was separated and the solvent evaporated under reduced pressure. The moist residue was triturated with a mixture of hexane (6.7 mL) and heptane (6.7 mL), filtered and washed with the above mixture twice, followed by water. After being dried, 25.16 g (84%) of the total product was obtained by recrystallization from acetone. m.p.225-28°C. IR (KBr): 3406 (O-H), 2254 (C≡N),; 1664 (C=C), 1102 cm^{-1} (C-O ketals); ^1H NMR (500 MHz, $CDCl_3$): δ 5.34 (t, 1H, H-6, *J* = 2.5 and 2.5 Hz), 3.59 (d, 1H, *J* = 11.0 Hz), 3.49 (d, 1H, *J* = 11.0 Hz), 3.48 (d, 1H, *J* = 11.5 Hz) and 3.44 (d, 1H, *J* = 11.5 Hz) (2 × CH_2-O-ketals), 1.04 (s, 3H, CH_3-19), 1.02 (s, 3H, CH_3-18), 0.94 (s, 3H, CH_3-ketals), 0.90 (s, 3H, CH_3-ketals).

3,3-(2,2-Dimethylpropylene-dioxy)-17-cyano-androst-5,16(17)-diene, 4

A mixture of **3** (24.74 g, 0.062 mol) and phosphorus oxychloride (18 mL, 0.19 mol) in anhydrous pyridine (177 mL) was stirred under nitrogen atmosphere at 40°C for 2 h and then at 45°C for ~8 h. The reaction mixture was poured into dilute hydrochloric acid solution (17.7 mL of concentrated HCl in 1416 mL of water) followed by continuous stirring at RT for 30 min. The solid product was filtered, washed with water and dried. Compound **4** was obtained by recrystallization from acetone (21.03g, 89%). m.p.188-90°C. IR (KBr): 2227 (C≡N), 1673 (C=C), 1104 cm^{-1} (C-O ketals); ^1H NMR (500 MHz, $CDCl_3$): δ 6.63 (dd, 1H, H-16, *J* = 2.0 and 3.0 Hz), 5.35 (d, 1H, H-6, *J* = 5.0 Hz), 3.59 (d, 1H, *J* = 11.5 Hz), 3.49 (d, 1H, *J* = 11.5 Hz), 3.46 (d, 1H, *J* = 12.0 Hz) and 3.43 (d, 1H, *J* = 12.0 Hz) (2 × CH_2-O-ketals), 1.06 (s, 3H, CH_3-19), 1.01 (s, 3H, CH_3-18), 0.95 (s, 3H, CH_3-ketals), 0.91 (s, 3H, CH_3-ketals).

3,3-(2,2-Dimethylpropylene-dioxy)-pregna-5,16(17)-diene-20-one, 5

To a suspension of **4** (19.05 g, 0.05 mol) in toluene (50.8 mL) was dropped a 3M solution of methylmagnesium bromide (50.8 ml, 0.15 mol) in diethyl ether while stirring under nitrogen. The mixture was then cooled to between −15 and −20°C. After that tetrahydrofuran (67 mL) and a mixture of ice (156 g), water (52 mL) and acetic acid 260 mL) were added. The solvent was evaporated under reduced pressure and the residue was poured into water (500 mL). The resulting precipitate was filtered, washed with water and dried. Compound **5** was obtained by recrystallization from ethyl acetate (15.72 g, 79%). m.p.203-207°C. IR (KBr): 1658 (C=O), 1103 cm^{-1} (C-O ketals); ^1H NMR (500 MHz, $CDCl_3$): δ 6.70 (dd, 1H, H-16, *J* = 1.5 and 3.0 Hz), 5.36 (d, 1H, H-6, *J* = 5.0 Hz), 3.59 (d, 1H, *J* = 11.5 Hz), 3.49 (d, 1H, *J* = 11.5 Hz), 3.46 (d, 1H, *J* = 12.0 Hz) and 3.43 (d, 1H, *J* = 12.0 Hz) (2 × CH_2-O-ketals), 2.26 (s, 3H, CH_3CO), 1.01 (s, 3H, CH_3-19), 1.01 (s, 3H, CH_3-18), 0.92 (s, 3H, CH_3-ketals), 0.89 (s, 3H, CH_3-ketals).

3,3-(2,2-Dimethylpropylene-dioxy)-16α,17α-epoxy-pregn-5-en-20-one, 6

A mixture of **5** (13.93 g, 0.035 mol), tetrahydrofuran (120 mL), methanol (60 mL), concentrated sodium hydroxide solution in MeOH (12 mL) and hydrogen peroxide H_2O_2 60% (12 mL, 0.32 mol) were stirred under the N_2 gas at 40-45°C for 20 h. The solvent was evaporated under reduced pressure, then a mixture of water and ice were added. The precipitated product was filtered *in vacuo*, washed with water, dried and recrystallized from ethyl acetate to afford compound **6** (13.16 g, 91%). m.p.211-15°C. IR (KBr): 1697 (C=O), 1664 (C=C), 1099 cm^{-1} (C-O ketals); ^1H NMR (500 MHz, $CDCl_3$): δ 5.34 (m, 1H, H-6), 3.67 (s, 1H, H-16), 3.58 (d, 1H, *J* = 11.5 Hz), 3.48 (d, 1H, *J* = 11.5 Hz), 3.46 (d, 1H, *J* = 11.0 Hz) and 3.43 (d, 1H, *J* = 11.0 Hz) (2 × CH_2-O ketals), 2.04 (s, CH_3CO), 1.05 (s, 3H, CH_3-19), 1.07 (s, 3H, CH_3-ketals), 1.03 (s, 3H, CH_3-18), 0.89 (s, 3H, CH_3-ketals).

3,3-(2,2-Dimethylpropylene-dioxy)-20-(1,2-ethylene-dioxy)-16α,17α-epoxy-pregn-5-ene, 7

To a solvent mixture of dichloromethane (112 mL) and ethylene glycol (109 mL, 2.1 mol) was added **6** (12.42 g, 0.03 mol) and the mixture was stirred

under nitrogen. Triethyl orthoformate (56 mL) and *p*-toluenesulfonic acid (0.84 g) were added. The mixture was stirred at RT for 6 h, and triethylamine (0.84 mL) was dropped. The solvent was evaporated under reduced pressure, then a mixture of water and ice were added to the residue. The resulting crystals were filtered *in vacuo* and washed with water, and recrystallised from ethyl acetate to obtain **7** (10.11 g, 81%). m.p.162-64°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.34 (t, 1H, H-6, J = 3.0 and 3.0 Hz), 3.88-3.99 (m, 8H, CH_2-O ketals), 3.37 (s, 1H, H-16), 1.44 (s, 3H, CH_3-21), 1.04 (s, 3H, CH_3-19), 0.99 (s, 3H, CH_3-18).

3,3-(2,2-Dimethylpropylene-dioxy)-20-(1,2-ethylene-dioxy)-17α-hydroxy-16β-methyl-pregn-5-en, 8

A 3M solution of methyl magnesium bromide (56 mL, 0.17 mol) in diethyl ether was cooled with ice under nitrogen atmosphere and then solvent was evaporated under reduced pressure at 60-65°C. Tetrahydrofuran (49 mL) and **7** (8.32 g, 0.02 mol) were introduced and the mixture was stirred for 16 h at 70-75°C. The above organo-magnesium (10 mL) and tetrahydrofuran (25 mL) were added and the reaction mixture was stirred for 7 h. After that, the mixture was cooled to 40°C and poured into saturated ammonium chloride solution (2100 mL) maintained at 0°C. The crude product (**8**) was filtered, washed with water, dried and was used for the next step without purification.

17α-Hydroxy-16β-methylpregn-4-ene-3,20-dione, 9

A suspension of **8** (6.05 g) in a mixture of acetone (45 mL), water (15 mL) and 70% H_2SO_4 (0.5 mL) was stirred at 60°C for 2 h. Solvent was then removed *in vacuo*, and ice water (60 mL) was added. The resulting precipitate was filtered, washed with water and dried. **9** was obtained by recrystallization from acetone (4.58 g, 67% from **7**). m.p. 199-201°C. IR (KBr): 3519 (O-H), 1710 and 1673 (C=O), 1618 cm^{-1} (C=C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.73 (br s, 1H, H-4), 2.69 (s, 1H, O-H), 2.26 (s, 3H, CH_3-21), 1.19 (s, 3H, CH_3-19), 1.18 (d, 3H, J = 7.5Hz, CH_3-22), 0.95 (s, 3H, CH_3-18); DEPT spectrum: 22 of peaks with 4 groups of CH_3, 7 groups of CH_2, 5 groups of CH and 6 quaternary C atoms; ^{13}C NMR (500 MHz, $CDCl_3$): δ 210.9 (s, C-20), 199.6 (s, C-3), 169.9 (s, C-5), 123.8 (d, C-4), 90.3 (s, C-17), 53.3 (d), 49.1 (d), 48.9 (s), 46.7 (d), 38.6 (s), 35.7 (t), 35.1 (t), 35.0 (d), 33.9 (t), 32.8 (t), 32.1 (t), 31.3 (t), 30.1 (q), 20.3 (t), 20.1 (q), 17.4 (q), 15.6 (q).

Conclusions

(i) 17α-Hydroxy-16β-methylpregn-4-ene-3,20-dione has been synthesized in high yield from androst-4-ene-3,17-dione. The structure of products has been examined by physical methods: IR, MS, 1- and 2D NMR.

(ii) The stereochemistry of substituents at carbon atoms C^{16} and C^{17} has been determined by interpreting the results of the experiment using the NOESY method for the first time.

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