9(10→19)Abeo Steroids. Total Synthesis of *abeo*-Estradiol, *abeo*-Estradiol 3-Methyl Ether, and 17α-Ethynyl *abeo*-Estradiol 3-Methyl Ether

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9(10→19)Abeo steroids combine structural features of both normal and 19-nor steroids. Starting with the known seco steroid (1) the total synthesis of the title compounds is described. Stereochemical assignments in 9(10→19)*abeo*-estradiol (13) and its 3-methyl ether (9) are made by x-ray analysis of the 17-ketone (11).

One of the outstanding classes of oral contraceptives are the 19-nortestosterone derivatives. These are used in combination with estrogens and the formulated drugs suffer from side effects.[1] Steroidal estrogens are known to have postcoital antifertility effect, which could not be separated from the estrogenic activity in primates.[2] The 9(10→19)abeo steroids[3] represent a hybrid between normal and 19-nor steroids combining structural features of both types. It is hoped that abeo estrogens would retain the antifertility properties acting as modified estrogens with reduced hormonal effects. To that end the preparation of abeo analogues of estradiol was undertaken and the results are reported here.

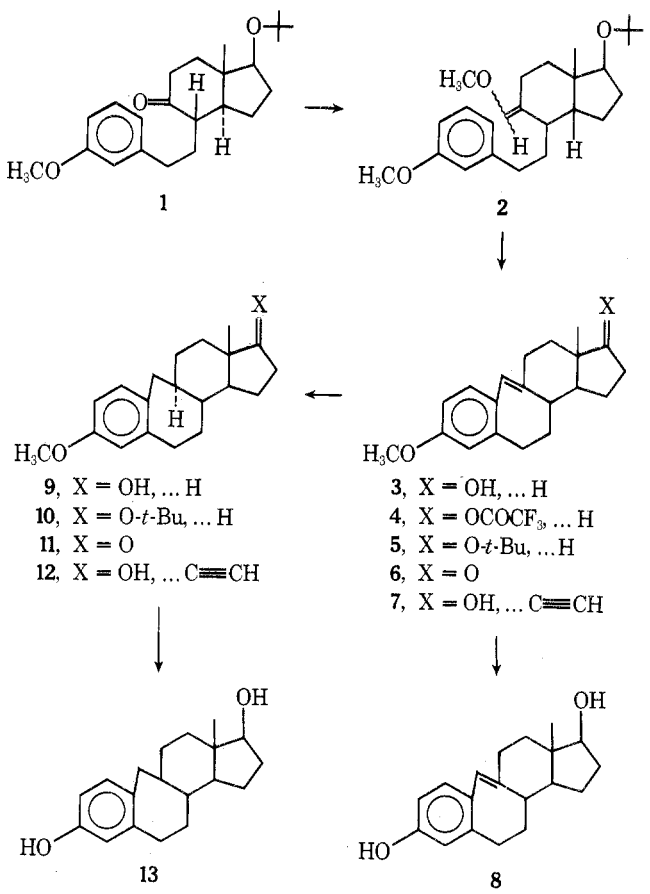
The two synthetic approaches reported[4-7] for the abeo-steroid nucleus are not general and do not lend themselves to preparing various types of abeo estrogens for biological evaluation. Both methods start with naturally occurring steroids where the 19-methyl group is functionalized and is subsequently incorporated into ring B by a rearrangement. Our total synthetic approach started with the known optically active seco steroid (1)[7,8] (Scheme I). Condensation of this ketone with the ylide derived from methoxymethylenetriphenylphosphonium chloride resulted in the formation of the enol ether (2) which consisted of a mixture of two geometric isomers as evidenced by the methoxy group signals in the ^{1}H NMR spectrum. Cyclization of 2 to the abeo-steroid nucleus was effected either by trifluoroacetic acid or *p*-toluenesulfonic acid. While the former furnished a mixture of the 17β-alcohol (3) and its trifluoroacetic ester (4), the latter furnished the uncleaved 17-*tert*-butyl ether (5).

Evidence for the cyclized structure rested upon spectral data as well as elemental analysis. The ultraviolet spectrum had a maximum similar to that reported for substituted styrenes.[9] In addition, the aromatic pattern in the ^{1}H NMR spectrum was similar to related steroid structures.[10] These data rule out cyclization at the position ortho to the methoxy group.

Oxidation of the alcohol (3) with chromic acid and 3,5-dimethylpyrazole[11] furnished the corresponding 17-ketone (6). This upon treatment with lithium acetylide-ethylenediamine complex[12] in dioxane gave the corresponding 17α-ethynyl derivative (7). Boron tribromide treatment of 5 resulted in the estratetraene (8).

Catalytic hydrogenation of 3 over palladium on charcoal resulted in the uptake of 1 mol of hydrogen and furnished a mixture of two isomeric compounds at C-9; the major isomer was suspected to be the 9α isomer (9) by analogy with results obtained on the steroid nucleus.[7] However, when 3 was reduced with sodium in liquid ammonia[13] a single product was isolated which was identical with the major isomer obtained earlier. Similarly, reduction of 5 furnished the corresponding dihydro compound (10) as the only product in good yield.

Scheme I



Confirmation of the stereochemistry at C-9 came from x-ray analysis of the 17-keto derivative (11) (vide infra). Treatment of 11 with lithium acetylide–ethylenediamine complex gave 17α-ethynyl 9(10→19)*abeo*-estradiol methyl ether (12). 9(10→19)*abeo*-Estradiol was obtained by simultaneous cleavage with boron tribromide of the 3-methyl and 17-*tert*-butyl ethers in compound 10.

X-Ray Analysis of Compound 11. Single crystals were grown by evaporation of a petroleum ether–ethanol solution. The crystal data follow: space group $P2_1$, a = 13.424 Å, b = 8.847 Å, c = 7.172 Å, β = 91.26°, V = 851.5 Å^3. The intensities of 2619 diffraction spectra were measured, of

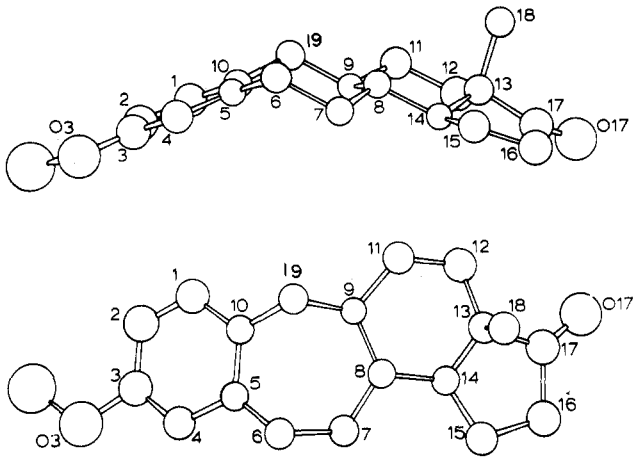


Figure 1.

which 1036 had intensity greater than twice the background. The crystal structure was solved by direct methods.[14,15] The structure was refined by full-matrix least-squares techniques and all hydrogen atoms were located in Fourier difference syntheses. The final reliability index (R) was 5.7%. The α configuration of the hydrogen substituent at C-9 was unambigously defined as illustrated in Figure 1. A complete structure report will be published elsewhere.

Experimental Section

Melting points (uncorrected) were determined with a Thomas-Hoover capillary apparatus. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter. ^{1}H NMR spectra were determined on a Varian A-60 or on a JEOLCO C-60-HL spectrometer using $CDCl_3$ and Me_4Si. Mass spectral fragmentations were obtained from either a Perkin-Elmer RMV-6E or CEC 24-104 mass spectrometer. Microanalyses were performed by Microanalysis, Inc., Marshallton, Del. Compounds **2–13** had acceptable carbon and hydrogen analyses. All evaporations were carried out in vacuo using a water aspirator and solutions were dried over anhydrous magnesium sulfate. Column chromatography utilized Brinkmann silica gel 60 (70–230 mesh ASTM).

(1*S*,3a*S*,4*S*,7a*S*)-1-*tert*-Butoxy-5-methoxymethylene-4-[2-(3-methoxyphenyl)ethyl]-7a-methylhexahydroindan (2). Methoxymethylenetriphenylphosphonium chloride (2.5 g, 7.3 mmol) was suspended in anhydrous ether (100 ml) under a stream of nitrogen. The suspension was cooled to −35 °C and phenyllithium (1.7 M, 4.23 ml) was introduced. A red color was formed instantly. After stirring at that temperature for 0.5 h, an ether solution (15 ml) of the ketone **1** (1.12 g, 3.0 mmol) was introduced and the reaction mixture was allowed to stir at −35 °C for 0.5 h, after which it was allowed to warm up to room temperature. Water (50 ml) was then added and the organic layer was separated and dried. The residue obtained after evaporation was placed on a dry silica gel column. Hexane elution furnished the product as an oil (0.98 g, 80%): NMR δ 0.98 (3 H, s), 3.2–3.5 (1 H, m), 3.53–3.56 (3 H, singlets), 3.78 (3 H, s), 5.83 (1 H, m), 6.66–7.42 (4 H, m).

(+)-17β-Hydroxy-3-methoxy-9(10→19)*abeo*-estra-1,3,5(10),9(19)-tetraene (3) and 3-Methoxy-17β-trifluoroacetoxy-9(10→19)*abeo*-estra-1,3,5(10),9(19)-tetraene (4). The enol ether (2, 110 mg, 0.28 mmol) was dissolved in trifluoracetic acid (1.0 ml) and the resulting dark solution was allowed to stand at room temperature for 0.5 h. Water (10 ml) was added and the solution was neutralized with aqueous sodium hydroxide. Ether extraction gave a gum (98 mg) whose thin layer chromatographic analysis (ether–hexane, 4:6) showed two spots, the upper minor compound (R_f 0.9) and a lower major spot (R_f 0.3). The pure compounds were obtained by column chromatography eluting with a 1:1 mixture of ether–hexane and collecting 10-ml fractions. Fraction 2 contained compound **4** (20 mg, 17%): mp 168–170 °C; NMR δ 1.0 (3 H, s), 4.5–5.0 (1 H, m), 3.78 (3 H, s), 6.3 (1 H, m), 6.5–7.1 (3 H, m).

Fractions 3–7 contained the parent alcohol **3** (80 mg, 95%): mp 90–92 °C; $[\alpha]^{25}D$ + 3.85° (*c* 0.26, $CHCl_3$); NMR δ 0.83 (3 H, s), 3.33–3.75 (1 H, m), 3.7 (3 H, s), 6.18 (1 H, m), 6.52–7.1 (3 H, m); uv (EtOH) λ_{max} 270 nm (log ϵ 4.29).

In later experiments the reaction mixture was treated with 10% aqueous methanolic sodium hydroxide with subsequent isolation of the alcohol **3** only.

(+)-17β-*tert*-Butoxy-3-methoxy-9(10→19)*abeo*-estra-1,3,5(10),9(19)-tetraene (5). To a solution of **2** (5.07 g, 13 mmol) in benzene (100 ml) was added a catalytic amount of *p*-toluenesulfonic acid (100 mg) and the solution was refluxed for 0.5 h. The reaction mixture was evaporated to dryness and the residue was dissolved in ether (300 ml). The ether solution was washed once with water, dried, and evaporated to dryness to give a yellowish gum (4.2 g, 90%). The analytical sample was obtained from methanol: mp 66–69 °C; $[\alpha]^{25}D$ +45.63° (*c* 1.03, $CHCl_3$); NMR δ 0.87 (3 H, s), 1.11 (9 H, s), 4.5–5.0 (1 H, m), 3.7 (3 H, s), 6.18 (1 H, m), 6.52–7.1 (3 H, m).

(+)-3-Methoxy-9(10→19)*abeo*-estra-1,3,5(10),9(19)-tetraen-17-one (6). 3,5-Dimethylpyrazole (580 mg) was added to a suspension of chromic acid (600 mg) in methylene chloride (40 ml), and the mixture was stirred at room temperature, under nitrogen, for 15 min. To this solution was added the alcohol **3** (617 mg, 2 mmol) dissolved in methylene chloride (10 ml) and the resulting mixture was stirred at room temperature for 0.5 h. Evaporation of the organic solvent was followed by ether extraction. The concentrated extract was passed through a short silica gel column (5% ether–hexane) to give a gum (415 mg, 70%). The analytical sample was obtained from ether–hexane; mp 154–156 °C, $[\alpha]^{25}D$ +156.00° (*c* 0.605, $CHCl_3$).

(+)-17α-Ethynyl-3-methoxy-9(10→19)*abeo*-estra-1,3,5(10),9(19)-tetraen-17-ol (7). To a saturated solution of acetone-free acetylene in dioxane (20 ml) was added, with stirring, lithium acetylide–ethylenediamine complex (1.1 g) followed by a solution of the ketone **6** (207 mg, 0.7 mmol) in anhydrous dioxane (10 ml). During the addition and for 2 h thereafter a stream of acetone-free acetylene was passed through the reaction mixture. Stirring was then continued in a closed system for 2 days. An aqueous solution of ammonium chloride was added slowly and the aqueous layer was extracted with ether several times. The combined extracts were dried and evaporated to dryness to give a brown semisolid (150 mg) which gave the analytical sample upon crystallization from methanol–water: mp 167–169 °C; $[\alpha]^{25}D$ +37.140° (*c* 0.14, $CHCl_3$); ir (KBr) 3500 (OH), 3340 cm^{-1} (C≡CH); mass spectrum *m/e* 322 (M^+), 296 ($M^+ - C_2H_2$), 187.

(+)-9(19)-Dehydro-9(10→19)*abeo*-estradiol (8). A solution of compound **5** (360 mg, 1.0 mmol) in methylene chloride (10 ml) was cooled in a dry ice–acetone bath and was treated with boron tribromide (0.4 mg). The reaction mixture was gradually warmed up to room temperature and was stirred at this temperature for 1 h. Water was added and the organic layer was separated, dried, and evaporated to dryness to give a gum which consisted of a mixture of two compound (TLC). The lower spot was isolated by dry column chromatography (ether–hexane, 1:1) (153 mg, 54%). The analytical sample was obtained from acetone–hexane: mp 224–230 °C; $[\alpha]^{25}D$ +60.11° (*c* 0.18, MeOH); mass spectrum *m/e* 284 (M^+), 266 ($M^+ - H_2O$), 145.

(+)-9(10→19)*abeo*-Estradiol 3-Methyl Ether (9). A solution of the alcohol **3** (1.65 g, 5.5 mmol) in methanol (200 ml) was hydrogenated over 10% palladium on charcoal (0.5 g) in a Parr apparatus at 40 psi overnight. Filtration of the catalyst and evaporation gave a solid (1.4 g) which consisted of a mixture of two compounds. Column chromatography of the residue using ether–hexane (1:1) furnished the major compound (783 mg), mp 119–120 °C.

The same compound was obtained by hydrolysis of the *tert*-butyl ether **10** with trifluoracetic acid as described for the preparation of **3**: $[\alpha]^{25}D$ +23.14° (*c* 0.60, $CHCl_3$); NMR δ 0.83 (3 H, s), 3.5–3.7 (1 H, m), 3.78 (3 H, s), 6.52–7.3 (3 H, m).

(+)-17β-*tert*-Butoxy-3-methoxy-9(10→19)*abeo*-estra-1,3,5(10)-triene (10). A solution of compound **5** (211 mg, 0.6 mmol) in dry tetrahydrofuran (40 ml) was added with stirring to a solution of sodium (138 mg, 6 mmol) in liquid ammonia (100 ml). After stirring for 5 min, an additional amount of sodium (138 mg) was added and the stirring was continued for 0.5 h. Ammonium chloride (1.0 g) was added in small portions. After evaporation of ammonia, water (15 ml) was added and the solution was extracted with ether. Evaporation of the dried ether extract gave a crystalline residue (218 mg, 100%). Recrystallization from methanol gave the analytical sample: mp 122–126 °C; $[\alpha]^{25}D$ +52.69° (*c* 0.99, $CHCl_3$); mass spectrum *m/e* 356 (M^+), 300 ($M^+ - C_4H_8$); NMR δ 0.76 (3 H, s), 1.11 (9 H, s), 3.0–3.3 (1 H, m), 3.75 (3 H, s), 6.52–7.1 (3 H, m).

(+)-9(10→19)*abeo*-Estrone 3-Methyl Ether (11). Jones reagent (1 ml) was added dropwise to a cooled solution of the alcohol **9** (157 mg, 0.52 mmol) in acetone (30 ml). Stirring was continued

for another 20 min. Evaporation of acetone was followed by partitioning the residue between chloroform and water followed by the usual work-up. Filtration of the residue on a silica gel column (1:1 ether–hexane) gave the compound (62 mg, 40%): mp 152–153 °C; $[\alpha]^{25}D$ +120.47° (*c* 1.07, $CHCl_3$); NMR δ 0.92 (3 H, s), 3.73 (3 H, s), 6.52–7.1 (3 H, m).

(+)-17α-Ethynyl-9(10→19)*abeo*-estradiol 3-Methyl Ether (12). The procedure employed here is essentially the same as that described for the preparation of **7**. Thus the ketone **11** (570 mg, 1.9 mmol) was treated with lithium acetylide–ethylenediamine complex (3.0 g) in anhydrous dioxane (15 ml). The product was obtained from methanol–water (315 mg, 51%): mp 154–156 °C $[\alpha]^{25}D$ +5.52° (*c* 1.31, $CHCl_3$); NMR δ 0.93 (3 H, s), 2.5 (1 H, s), 3.8 (3 H, s), 6.55–7.1 (3 H, m).

(+)-9(10→19)*abeo*-Estradiol (13). Although this compound was prepared from the alcohol **9**, direct hydrolysis of compound **10** gave better yields. Thus, under similar reaction conditions to those reported for the preparation of **8**, compound **10** (48 mg, 0.13 mmol) was treated with boron tribromide (0.1 ml) in methylene chloride (5 ml). The work-up furnished a residue (29 mg, 78%) which gave the analytical sample upon crystallization from methanol: mp 234–238 °C; ir (KBr) 3550, 3250 cm^{-1}; mass spectrum *m/e* 286 (M^+).

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References and Notes

(1) R. Doll and M. P. Vessey, *Br. Med. Bull.*, **26**, 33 (1970).
(2) J. P. Bennett, "Chemical Contraception", Columbia University Press, New York, N.Y. 1974, pp 101–107.
(3) The use of the prefix abeo in the rest of the paper refers to 9(10→19) abeo.
(4) S. M. Kupchan and E. Abushanab, *Tetrahedron Lett.*, 3075 (1965).
(5) S. M. Kupchan, E. Abushanab, K. T. Shamasundar, and A. W. By, *J. Am. Chem. Soc.*, 89, 6327 (1967).
(6) F. Kohen, L. K. Lala, W. VanBever, and R. E. Counsell, *Chem. Commun.*, 347 (1969).
(7) F. Kohen, W. VanBever, and R. E. Counsell, *J. Org. Chem.*, **35**, 2272 (1970).
(8) N. Cohen, B. L. Banner, W. F. Eichel, D. R. Parrish, G. Saucy, J. M. Cassal, W. Meier, and A. Furst, *J. Org. Chem.*, **40**, 681 (1975).
(9) U. Eder, G. Haffer, J. Ruppert, G. Sauer, and R. Wiechert, German Offen. 2 253 088 (1974).
(10) D. E. Bays, R. C. Cookson, and S. MacKenzie, *J. Chem. Soc. B*, 215 (1967); W. E. Bachmann and J. Controulis, *J. Am. Chem. Soc.*, **73**, 2636 (1951).
(11) Varian NMR Spectra Catalogue, Spectrum No. 343.
(12) E. J. Corey and G. W. J. Fleet, *Tetrahedron Lett.*, 4499 (1973).
(13) T. W. Huffman and P. G. Arapakos, *J. Org. Chem.*, **30**, 1604 (1965).
(14) G. H. Douglas, J. M. H. Graves, D. Hartley, G. A. Hughs, B. J. McLoughlin, J. Sidall, and H. Smith, *J. Chem. Soc.*, 5072 (1963).
(15) G. T. DeTitta, J. W. Edmonds, D. A. Langs, and H. Hauptman, *Acta Crystallogr., Sect. A*, in press.
(16) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, **27**, 368 (1971).

Agaridoxin, a Mushroom Metabolite. Isolation, Structure, and Synthesis

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Agaridoxin, a strongly autoxidizable substance, was isolated from the common mushroom *Agaricus campestris*. Its structure was established, largely by NMR, mass spectroscopy, uv spectroscopy, and polarography, to be 3,4-dihydroxy-(γ-L-glutamyl)anilide (**1**). This metabolite was synthesized starting with the reaction between 3,4-(isopropylidenedioxy)aniline (**5**) and *N*-phthaloylglutamic anhydride (**6**). The resulting substituted phthalimide (**7**) was converted into (γ-L-glutamyl)-3,4-(isopropylidenedioxy)anilide (**9**) by treatment with hydrazine in ethanol. Removal of the isopropylidene protecting group by use of boron trichloride gave agaridoxin in good yield.

The isolation of 4-hydroxy(γ-L-glutamyl)anilide from the gill tissue of *Agaricus bisporus*,[1] its enzymatic conversion to the sulfhydryl enzyme inhibitor, *N*-(γ-L-glutamyl)amino-3,4-benzoquinone,[2] and the direct isolation of the quinone from this mushroom have recently been described. We wish to report the isolation, identification, and confirmatory synthesis of the putative intermediate, 3,4-dihydroxy(γ-L-glutamyl)anilide (**1**), obtained from the very closely related species *Agaricus campestris*. Our interest in **1** resulted from a long and continual search by one of us (A.S.-G.) for compounds with low electron affinity[3] which would autoxidize readily and would be expected to suppress cell division. Since we observed that aqueous extracts of *Agaricus campestris* (var. *bifidis*) autoxidized rapidly, the isolation of the substance responsible for this reaction was undertaken. The autoxidation was markedly promoted by manganese salts with the development of a red color, a reaction used to follow the isolation of the mushroom factor. We now wish to report on the isolation, identification and synthesis of this substance which we named agaridoxin.

Agaridoxin was isolated from a methanol extract of the powdered mushroom by treating with lead acetate, separating from insolubles, concentrating to dryness, and subjecting the residue in aqueous solutions to Sephadex G10 chromatography.

Primary identification studies led us to believe that agaridoxin was a dihydroxy(γ-glutamyl)anilide represented by **1** or **2**. This conclusion was based on NMR, gas–liquid chro-