17α-Ethyl-20α- and -20β-dihydroprogesterones and Other 17α-Ethyl-Substituted Pregnanes as Potential Contragestational Agents

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The 17α-ethyl-substituted analogs of the two epimeric 20-dihydroprogesterones, allopregnanedione and pregn-5-ene-3,20-dione, were synthesized and evaluated for their possible oral contragestational (postcoital antifertility) activity in the rat. The compounds, though bound strongly to the progesterone receptor in vitro, were inactive preimplantively at 10 mg/kg and postimplantively at 40 mg/kg in vivo.

The role of progesterone (**8a**) as one of the vital hormones for the maintenance of pregnancy is well recognized.[1] The basic biological principle that a compound that would interfere with the synthesis or mechanism of action of progesterone could cause termination of pregnancy has been a very attractive basis for the designing and screening of potential contragestational agents (i.e., agents having postcoital antifertility activity). We wish to describe the synthesis of 17α-ethyl-substituted analogs of selected metabolites of progesterone and related pregnanes as potential contragestational agents. Several groups have reported[2-4] on the antiprogestational potential of certain metabolites of progesterone. Both 20α-dihydroprogesterone (**3a**) and its 20β epimer (**5a**), which are normal metabolites of progesterone in humans, have been reported to inhibit progesterone synthesis in human placental preparations.[4] Further it was noted[5] that the placental tissue obtained from a woman following spontaneous abortion exhibited a decreased ability to synthesize progesterone and an increased formation of **3a**, further implicating the possible role of increased amounts of **3a** in the abortive tendency in women. However, **3a** failed to interfere with pregnancy in the rat when given subcutaneously[6] or orally. In our search for competitive antagonists to progesterone–progestogen receptor binding, allopregnanedione (**7a**) and pregn-5-ene-3,20-dione (**9a**) exhibited a relatively high binding affinity for the progesterone receptor in vitro,[7] although they were neither progestational[8] nor antiprogestational in vivo. It may be speculated that all these compounds were inactive in vivo because of their poor absorption or rapid metabolic degradation which prevented them from reaching their site of action. In the case of progestational steroids, α substitution at C_{17} by an acetoxy,[9] a halogen,[10] or an alkyl group[11] is believed to aid absorption and protect the compound against rapid metabolic inactivation involving the pregnane side chain[12] and thereby not only enhancing their potential progestational activity but also imparting oral potency. This prompted the synthesis of 17α-alkyl-substituted analogs of these potential antiprogestational pregnanes (**3a, 5a, 7a,** and **9a**) and the evaluation of their possible oral contragestational activity. We chose an ethyl group as the 17α-alkyl substituent because of its reported[11a] superiority over a methyl group and its equivalence to a propyl group in enhancing the progestational activity of 17α-alkyl progestational steroids.

Chemistry. The desired 17α-ethylpregnanes were synthesized in the following manner. Reduction of the ketal (**1**)[11b] with either Na in *i*-PrOH or $LiAlH_4$ provided a mixture of the epimeric 20-hydroxy compounds. The major and more polar (TLC) product from the Na reduction was isolated in 20% yield following fractional crystallization and assigned the 20α-ol structure **2** in accord with earlier findings.[13a,14] The predominant and less polar product from the $LiAlH_4$ reduction of **1** was assigned the 20β-ol structure **4**[14] and was isolated in 58% yield by silica gel chromatography. Acid hydrolysis of **2** and **4** afforded the corresponding 20-epimeric 3-ketones, **3b** and **5b,** respectively. This assignment of stereochemistry at C-20 was further supported by the consistent downfield chemical shift of C-18 hydrogens in the NMR spectra of **5b** (δ 0.95), **4** (δ 0.92), and the known 20β-ol (**5a**) (δ 0.80) relative to those in **3b** (δ 0.78), **2** (δ 0.77), and the known 20α-ol (**3a**) (δ 0.70). This is in harmony with the preferred conformation of the 17β side chain of C-20 oxygenated pregnane derivatives.[15] Reduction of **5b** with lithium in ammonia[16] afforded the 5α-compound **6** which upon oxidation with Jones reagent[17] afforded the dione **7b.** Treatment of **8b**[11b] with *t*-BuOK followed by protonation of the resulting anion with AcOH gave the deconjugated[18] isomer **9b** (Scheme I).

Biological Activity. Contragestational studies were carried out by the procedures described by Hahn et al.[19] with the following modifications. Compounds were administered orally to five rats on days 1–6 of gestation (the presence of sperm in vaginal washings constitutes day 1 of gestation) for studies related to effects on implantation or on days 9–12 of gestation for examination of drug effects on pregnancy after implantation. Post-mortem examination of uterine contents was carried out as early as day 14 or as late as day 21 of gestation. Compounds **3b, 5b, 7b,** and **8b** failed to prevent implantation at 10 mg/kg or to interrupt established pregnancy at 40 mg/kg.

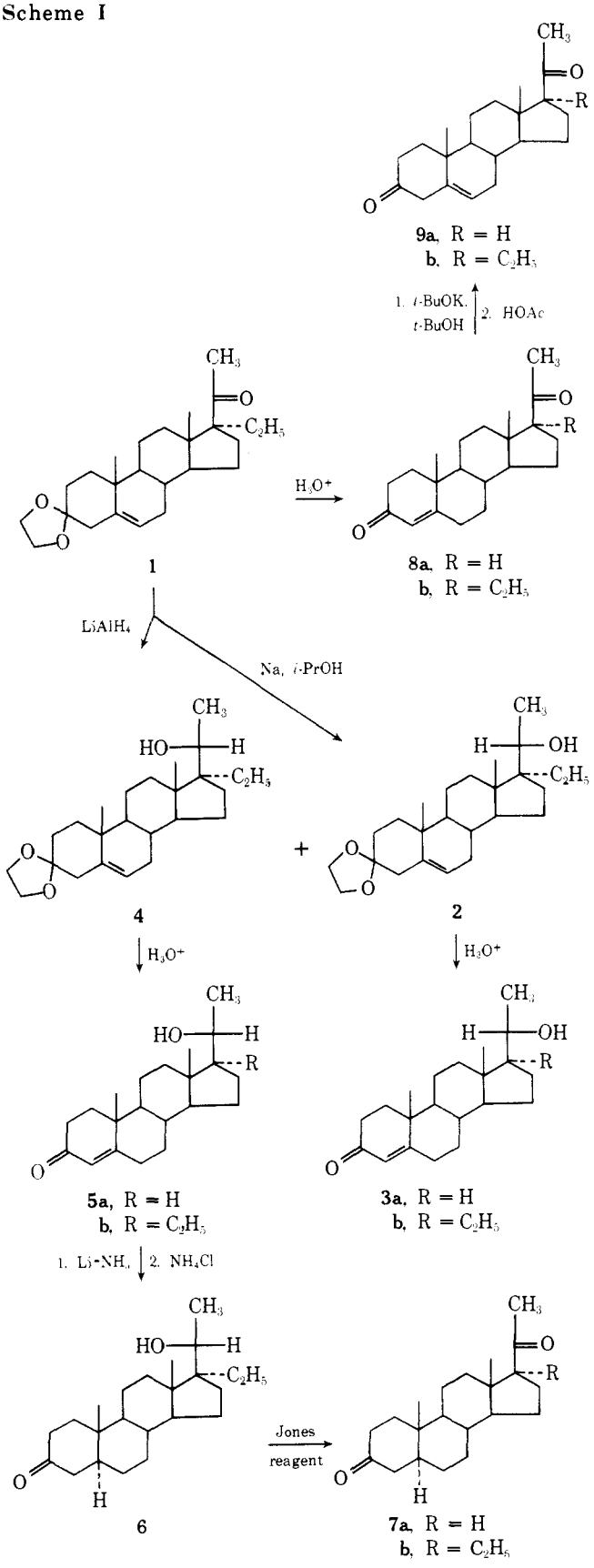
However, they all showed high binding affinity for the progesterone receptor[7] in vitro (Table I) which was significantly enhanced for **3b** and **5b** as compared to the corresponding 17-nonethylated compounds **3a** and **5a.**

Thus, 17α-ethylation of potential antiprogestational pregnanes **3a**, **5a,** 7a, and **9a** enhanced or retained their high binding affinity for the progesterone receptor in vitro but failed to elicit the projected contragestational potential in vivo at the above-described dose levels.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Uv spectra were determined on a Cary Model II recording spectrophotometer in ethanol, ir spectra were recorded on a Unicam SP1000 infrared spectrophotometer in KBr pellets, and NMR spectra were obtained on a Varian A-60 spectrometer using $CDCl_3$ as the solvent with tetramethylsilane as the internal standard. Optical rotations were determined on a Rudolph Model 70 polarimeter attached to a Model 200 photoelectric unit, for 1% solutions in chloroform. TLC analyses were performed on Uniplate Silicar 7GF (Analtech Inc.). For column chromatography silicic acid (Mallinckrodt Co.) was used. All evaporations were carried out in vacuo. Symbols of elements refer to microanalyses with results within ±0.4% of the calculated values.

Scheme I



3-Ethylenedioxy-17α-ethylpregn-5-en-20α-ol (2). Na metal (27 g, 1.17 mol) was added in small pieces to a refluxing solution of 1[11b] (4.25 g, 11.0 mmol) in anhydrous *i*-PrOH (450 ml) under N_2. The mixture was refluxed for an additional 1.5 hr after the Na had completely dissolved. The solution was allowed to cool to room temperature, diluted with H_2O (300 ml), neutralized with 6 *N* HCl, and extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried (Na_2SO_4), and evaporated to give a yellow oil (4.82 g). The oil was triturated with Et_2O to afford a crystalline solid (1.39 g). Repeated recrystallization from CH_2Cl_2–hexane afforded **2** (865 mg, 20%) as colorless crystals: mp 165–167°; $[\alpha]^{24}D$ −53°; ir 3525 cm^{-1} (OH); NMR δ 0.77 (s, 3 H, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), 1.10 (t, *J* = 7 Hz, 3 H, 17α-$CHCH_3$), 3.92 (s, 4 H, $-OCH_2CH_2O$), 5.35 (m, 1 H, 6-H). Anal. ($C_{25}H_{40}O_3$) C, H.

17α-Ethyl-20α-hydroxypregn-4-en-3-one (3b). A solution of **2** (930 mg, 2.39 mmol) and 8% aqueous H_2SO_4 (6 ml) in MeOH (60 ml) was refluxed for 1 hr. After cooling to room temperature, the solution was diluted with H_2O (100 ml) and extracted with CH_2Cl_2. The organic extracts were washed with H_2O, dried (Na_2SO_4), and evaporated. Recrystallization of the residue from CH_2Cl_2–hexane afforded **3b** (768 mg, 93%) as colorless crystals: mp 192–193°; uv 241 nm (ε 17,000); $[\alpha]^{26}D$ +70°; ir 3510 (OH), 1665 (C=O), 1615 cm^{-1} (C=C); NMR δ 0.78 (s, 3 H, 18-CH_3), 1.09 (t, *J* = 6.5 Hz, 3 H, 17α-CH_2CH_3), 1.16 (s, 3 H, 19-CH_3), 3.98 (q, *J* = 7 Hz, 1 H, 20-H), 5.72 (m, 1 H, 4-H). ANAL/ ($C_{23}H_{36}O_2$) C, H.

17α-Ethyl-20β-hydroxypregn-4-en-3-one (5b). To a stirred solution of 1 (5.73 g, 14.8 mmol) in anhydrous THF (150 ml) under N_2 was added $LiAlH_4$ (5.6 g, 0.148 mol). The mixture was refluxed for 2.5 hr, cooled to room temperature, and stirred for 16 hr. After cooling to 0°, the mixture was treated cautiously in succession with H_2O (6 ml), 15% aqueous NaOH (6 ml), and H_2O (18 ml). The granular precipitate was separated by filtration and washed several times with Et_2O. The filtrate was evaporated to dryness; the residue was dissolved in CH_2Cl_2, washed with H_2O, dried (Na_2SO_4), and evaporated to give a yellow oil (6.41 g). The oil was chromatographed on a column of silicic acid (400 g, containing 1% pyridine) prepared in pentane and eluted with 10–15% Et_2O–pentane mixtures to effect a separation of **2** and **4**. Recrystallization from CH_2Cl_2–hexane gave **4** (3.32 g, 58%): $[\alpha]^{24}D$ −59°; NMR δ 0.92 (s, 3 H, 18-CH_3), 1.02 (s, 3 H, 19-CH_3), 1.14 (d, *J* = 7 Hz, 3 H, 21-CH_3), 3.94 (s, 4 H, $-OCH_2CH_2O$), 4.05 (q, *J* = 7 Hz, 1 H, 20-H), 5.39 (m, 1 H, 6-H). Acid hydrolysis of **4** (3.32 g, 8.57 mmol) was carried out in the same manner as for **2** to give **5b** (2.21 g, 75%) as colorless crystals: mp 165–166°; uv 242 nm (ε 16,500); $[\alpha]^{24}D$ +53°; ir 3480 (OH), 1660 (C=O), 1620 cm^{-1} (C=C); NMR δ 0.95 (s, 3 H, 18-CH_3), 1.17 (d, *J* = 7 Hz, 3 H, 21-H), 1.19 (s, 3 H, 19-CH_3), 4.04 (q, *J* = 7 Hz, 1 H, 20-H), 5.75 (m, 1 H, 4-H). Anal. ($C_{23}H_{36}O_2$) C, H.

17α-Ethyl-20β-hydroxy-5α-pregnan-3-one (6). To a solution of Li (270 mg, 39.1 mmol) in liquid NH_3 (500 ml) cooled in a Dry Ice–acetone bath was added dropwise a solution of **5b** (2.15 g, 6.25 mmol) in anhydrous dioxane (150 ml). The mixture was stirred for 0.5 hr and quenched with solid NH_4Cl (5.2 g). The NH_3 was allowed to evaporate at room temperature. The residue was poured into H_2O (400 ml) and extracted with CH_2Cl_2. The organic extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to give a yellow crystalline residue (2.68 g). The residue was chromatographed on a column of silicic acid (150 g) prepared in hexane. The solid obtained by elution with EtOAc–hexane (1:7) gave, after recrystallization from CH_2Cl_2–hexane, **6** (1.35 g, 63%) as colorless needles: mp 223–227°; $[\alpha]^{27}D$ −3°; ir 3505 (OH), 1690 cm^{-1} (C=O); NMR δ 0.90 (s, 3 H, 18-CH_3), 1.01 (s, 3 H, 19-CH_3), 1.14 (d, *J* = 7 Hz, 3 H, 21-H), 4.03 (q, *J* = 7 Hz, 1 H, 20-H). Anal. ($C_{23}H_{38}O_2$) C, H.

17α-Ethyl-5α-pregnane-3,20-dione (7b). Jones reagent (0.71 ml)[17] was added to a stirred, ice-cooled (10–15°) solution of **6** (850 mg, 2.45 mmol) in acetone (200 ml, distilled from $KMnO_4$). After 5 min, the mixture was poured into H_2O (600 ml); the precipitate was filtered and washed with H_2O. The precipitate was dissolved in CH_2Cl_2, washed with H_2O, dried (Na_2SO_4), and evaporated to give a crystalline residue (816 mg). Recrystallization from

Table I. Relative Binding Affinity to Progesterone Receptor[7]

Compd no.	Binding index[a]	Compd no.	Binding index[a]
3a	0	7a	87
3b	77	7b	83
5a	16	9a	82
5b	77	9b	76

[a] Expressed as percent depression of bound [^{3}H]progesterone from the receptor protein by a molar concentration of compounds equal to that quantity of progesterone which displaces 80% of isotope.[7]

CH_2Cl_2–hexane gave **7b** (740 mg, 85%) as fine colorless needles: mp 196.5–198°; $[\alpha]^{26}D$ +27°; ir 1715 (20-C=O), 1695 cm^{-1} (3, C=O); NMR δ 0.65 (s, 3 H, 18-CH_3), 0.73 (t, J = 7.5 Hz, 3 H, 17α-CH_2CH_3), 1.02 (s, 3 H, 19-CH_3), 2.04 (s, 3 H, 21-CH_3). Anal. ($C_{23}H_{36}O_2$) C, H.

17α-Ethylpregn-5-ene-3,20-dione (9b). A solution of **8b**[11b] (860 mg, 2.51 mmol) in anhydrous t-BuOH (20 ml) under N_2 was treated with t-BuOK (2.82 g, 25.1 mmol). The mixture was stirred for 1.5 hr and quenched with 10% HOAc (94 ml). The mixture was then diluted with 10% $NaHCO_3$ (150 ml) and extracted with CH_2Cl_2. The organic extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to give a yellow oil. The oil was added to a silicic acid (30 g) column prepared in hexane and eluted with EtOAc–hexane (1:9). The eluate was evaporated and the residue recrystallized from Et_2O–pentane to afford **9b** (406 mg, 47%) as colorless needles: mp 167–169°; $[\alpha]^{25}D$ −19°; ir 1715 (20-C=O), 1695 cm^{-1} (3-C=O); NMR δ 0.68 (s, 3 H, 18-CH_3), 0.72 (t, J = 8 Hz, 3 H, 17α-CH_2CH_3), 1.18 (s, 3 H, 19-CH_3), 2.08 (s, 3 H, 21-H), 5.38 (m, 1 H, 6-H). Anal. ($C_{23}H_{34}O_2$) C, H.

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References and Notes

(1) A. Caspo, *Ciba Found. Study Group*, **No. 9**, 3 (1961).
(2) (a) F. L. Hishaw, J. T. Velardo, and H. K. Ziek, *J. Clin. Endocrinol. Metab.*, **14**, 763 (1954); (b) J. T. Velardo, *Am. J. Physiol.*, **188**, 317 (1957).
(3) T. Miyake, H. Kakushi, and K. Hara, *Steroids*, **2**, 794 (1963).
(4) (a) M. Wiener and S. H. G. Allen, *Steroids*, **9**, 567 (1967); (b) *ibid.*, **12**, 261 (1968).
(5) M. Wiener and R. L. Friedlander, *Am. J. Obstet. Gynecol.*, **111**, 942 (1971).
(6) A. P. Labshetwar, *Acta Endocrinol.*, **71**, 13 (1972).
(7) J. L. McGuire, C. D. Bariso, and A. P. Shroff, *Biochemistry*, **13**, 319 (1974).
(8) T. Miyake and W. H. Rooks, II, *Methods Horm. Res.*, **5**, 59 (1966).
(9) A. David, F. Hartly, D. R. Millson, and V. Petrow, *J. Pharm. Pharmacol.*, **9**, 929 (1957).
(10) (a) C. I. Chappel, C. Revesz, and R. Gaudry, *Acta Endocrinol.*, **35**, 915 (1960); (b) Ch. R. Engel and R. Deghenghi, *Can. J. Chem.*, **38**, 452 (1960).
(11) (a) R. Deghenghi, C. Revesz, and R. Gaudry, *J. Med. Chem.*, **6**, 301 (1963); (b) M. J. Weiss, R. E. Schaub, G. R. Allen, Jr., J. F. Poletto, C. Pidacks, R. B. Conrow, and C. J. Coscio, *Tetrahedron*, **20**, 359 (1964); (c) D. J. Marshall, P. F. Morand, C. Revesz, and R. Gaudry, *J. Med. Chem.*, **7**, 355 (1964).
(12) E. J. Plotz, "Brook Lodge Symposium on Progesterone", Brook Lodge Press, Augusta, Mich., 1961, p 91.
(13) (a) R. E. Marker, H. M. Crooks, Jr., and E. L. Wiltbecker, *J. Am. Chem. Soc.*, **63**, 777 (1941); (b) P. Weiland and K. Miescher, *Helv. Chim. Acta*, **32**, 1922 (1949).
(14) (a) D. N. Kirk and A. Mudd, *J. Chem. Soc. C*, 968 (1969); (b) W. Klyne and E. Miller, *J. Chem. Soc.*, 1972 (1950); (c) J. K. Norymberski and G. F. Woods, *ibid.*, 3426 (1955); (d) J. Romo, J. Romero, C. Djerassi, and G. Rosenkranz, *J. Am. Chem. Soc.*, **73**, 1528 (1951).
(15) H. Lee, N. S. Bhacca, and M. E. Wolff, *J. Org. Chem.*, **31**, 2692 (1966).
(16) A. Bowers, H. J. Ringold, and E. Denot, *J. Am. Chem. Soc.*, **80**, 6115 (1958), and references cited therein.
(17) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).
(18) H. J. Ringold and S. K. Malhotra, *Tetrahedron Lett.*, 669 (1962).
(19) D. W. Hahn, G. Allen, J. L. McGuire, and J. P. DaVanzo, *Contraception*, **9**, 393 (1974).

2-(3,4-Dichloroanilino)quinolizinium Bromide, a Unique Antispasmodic, Antisecretory, and Antiulcerogenic Agent

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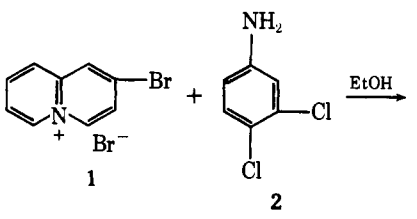
2-(3,4-Dichloroanilino)quinolizinium bromide (**3**) was prepared by reaction of 2-bromoquinolizinium bromide with 3,4-dichloroaniline in ethanol. This compound possesses unique antispasmodic, antisecretory, and antiulcerogenic properties.

Earlier we reported[1,2] the synthesis and biologic activity of a number of substituted quinolizinium salts. As a continuing part of this investigation, we now wish to describe the synthesis of and report some preliminary pharmacologic testing results on 2-(3,4-dichloroanilino)quinolizinium bromide (**3**).[3] This compound when evaluated in a number of pharmacologic testing procedures was found to possess unique and potent properties. Among these are potent antispasmodic action of long duration on colonic contractions in response to pelvic nerve stimulation, gastric antisecretory activity, and antiulcerogenic action.

To the best of our knowledge, this is the first example of a nonanticholinergic antispasmodic agent which possesses gastric antisecretory and antiulcerogenic activity.

Chemistry. The synthesis of **3** was accomplished by the reaction of 2-bromoquinolizinium bromide (**1**) with 3,4-dichloroaniline (**2**) in ethanol. The general reaction procedure has been described previously.[1]



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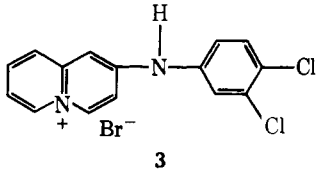

3

Compound **3** is an odorless, off-white crystalline solid with a melting point of 264–265°. It has water solubility to the extent of 2.4 g/l. at room temperature. The NMR spec-