3-Chloro-4-piperidinonitrobenzene. A 250-mL, three-neck flask fitted with a stirrer, condenser, and thermometer was charged with 3,4-dichloronitrobenzene (19.2 g, 0.1 mol) and piperidine (17.0 g, 0.2 mol). The mixture was stirred for 3.5 h at 95 °C. The reaction mixture was diluted with water (ca. 200 mL) and stirring was continued as the mixture cooled. The solid was collected and recrystallized from absolute EtOH (70 mL) to yield 15.8 g (66%) of yellow crystals, mp 36–41 °C. An analytical sample was prepared by a second recrystallization from absolute EtOH: mp 39–42 °C. Anal. ($C_{11}H_{13}ClN_2O_2$).

4-(4-Benzylpiperazino)-3-chloronitrobenzene. To a 500-mL, three-neck flask fitted with a stirrer, condenser, and thermometer was added 3,4-dichloronitrobenzene (19.2 g, 0.1 mol), 1-benzylpiperazine (35.2 g, 0.2 mol), and DMF (200 mL). The mixture was stirred for 2.5 h while heating at reflux. The reaction mixture was diluted with H_2O (500 mL) and extracted with Et_2O. The organic layer was separated and dried over anhydrous $MgSO_4$. The drying agent was removed and the filtrate evaporated to dryness in vacuo. The residue was recrystallized from absolute EtOH to yield 22.5 g (68%) of product melting at 92–95 °C. An analytical sample was prepared by recrystallization from absolute EtOH to give yellow crystals, mp 93–95 °C. Anal. ($C_{17}H_{18}ClN_3O_2$).

The experimental procedures described below are typical for the preparation of the compounds listed in Tables I and II.

A. From the Aniline. 9-(4-*n*-Butylanilino)-7-methylimidazo[4,5-*f*]quinoline Hydrochloride (4). A mixture of 9-chloro-7-methylimidazo[4,5-*f*]quinoline (I, R = H; $R_1 = CH_3$)[1] (21.7 g, 0.1 mol) and 4-*n*-butylaniline (14.9 g, 0.1 mol) in absolute EtOH (200 mL) was stirred at reflux overnight. The reaction solution was concentrated to dryness in vacuo. The residue was dried at 100 °C to yield crude 4 (36.5 g), mp 296–303 °C dec.

B. From the Nitro Compound. 9-(4-*n*-Butyl-3-chloroanilino)-7-methylimidazo[4,5-*f*]quinoline Hydrochloride Hemihydrate (13). A 500-mL reduction bottle was charged with 2-chloro-4-nitro-*n*-butylbenzene (prepared from 4-nitrobutylbenzene by the method of Kövendi and Kircz)[12] (24.0 g, 0.11 mol) and DMF (200 mL). The mixture was shaken in an atmosphere of hydrogen together with Raney active nickel catalyst No. 28 in water (1 teaspoon). After the hydrogen uptake had ceased (85% of theory), the catalyst was removed by filtration. The filtrate was placed in a 500-mL, three-neck flask fitted with a stirrer, condenser, and thermometer. To the solution was added 9-chloro-7-methylimidazo[4,5-*f*]quinoline (I, R = H; $R_1 = CH_3$)[1] (21.7 g, 0.1 mol) and DMF (100 mL). The mixture was stirred overnight at 110 °C. The solution was chilled and filtered. The crystals were dried at 60 °C to yield 26.9 g of crude 13.

Biological Method. The taeniacidal activity was determined by the use of *H. nana* as described previously by Culbertson[13] using modified techniques of Steward[14] and Standen.[15] In addition, on the 13th day of infection, to the end of testing, the mice were given hydrocortisone (USP-microfine, Merck & Co., Inc., Rahway, N.J.) at the rate of 25 mg/L in their drinking water to prevent natural worm elimination. Medication was administered twice a day for 3 days (days 18–20 inclusive of the infection) to groups of five mice. Necropsy was performed on infection day 22 and worm counts were made by pressing the small intestine between glass plates and scanning at 7× magnification.

Compound effectiveness was determined as a percentage reduction in the manner described previously.[16]

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Synthesis of Medroxyprogesterone Bromoacetate for Affinity Labeling

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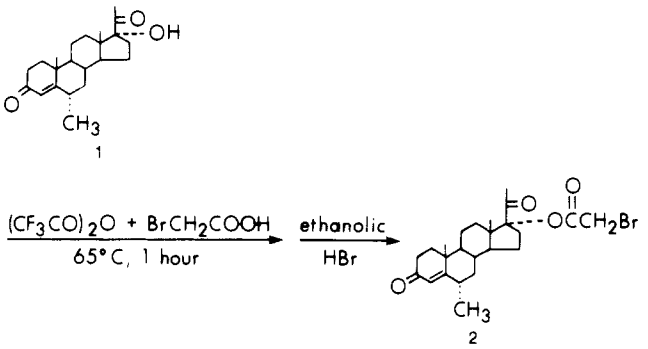
Medroxyprogesterone bromoacetate (17α-hydroxy-6α-methyl-4-pregnene-3,20-dione 17-bromoacetate) was synthesized by reaction of 17α-hydroxy-6α-methyl-4-pregnene-3,20-dione with bromoacetic acid–trifluoroacetic anhydride followed by treatment of the intermediate with dilute ethanolic HBr. The product forms conjugates with L-cysteine, L-histidine, and L-methionine and inactivates 20β-hydroxy steroid dehydrogenase (E.C. 1.1.1.53.) from *Streptomyces hydrogenans* in a time-dependent and irreversible manner. The title compound possesses a long-acting progestational effect in day 9 pregnant bilaterally ovariectomized rats. The affinity labeling analogue of the oral contraceptive medroxyprogesterone acetate is proposed for use in reproductive biological experiments.

Previous reports from this laboratory described the synthesis of 16α-bromoacetoxyprogesterone[1] and 19-nortestosterone bromoacetate[2] which are affinity-labeling steroids[3] that terminate pregnancy in rats by apparently interfering with uterine progesterone uptake.[2,4] In order to further explore the effect that the bromoacetoxy reagent group has on the biological activity of progesterone analogues we attempted the synthesis of medroxyprogesterone bromoacetate (2, Scheme I), a brominated analogue of the powerful progestin.[5] Attempts to synthesize 2 by

Scheme I. Synthesis of Medroxyprogesterone Bromoacetate[a]



[a] Medroxyprogesterone (1) was treated with a bromoacetic acid–trifluoroacetic anhydride mixture at 65 °C for 1 h, and the intermediate obtained from this reaction was heated in ethanolic hydrobromic acid for 35 min. Medroxyprogesterone bromoacetate (2) was isolated by silica gel chromatography.

methods which we described earlier[1-4] were unsuccessful.

The present report describes the synthesis of medroxyprogesterone bromoacetate (2, Scheme I) with trifluoroacetic anhydride by modification of an earlier reported procedure.[5] The reaction of the title compound with amino acids, a steroid oxidoreductase, and preliminary results from biological testing of the compound in pregnant rats are described.

Results and Discussion

Bromoacetoxy derivatives of progesterone were successfully synthesized earlier by condensing the appropriate hydroxyprogesterone with bromoacetic acid in the presence of dicyclohexylcarbodiimide.[1-4] Medroxyprogesterone (1, Scheme I) which contains a *tert*-hydroxyl group at the C-17α position resisted bromoacetylation by this method. When 1 was treated with bromoacetic acid in trifluoroacetic anhydride at 25 °C for 1 h,[5] no esterification of the starting material could be detected by TLC. However, heating the reaction mixture at 65 °C produced an inseparable mixture of products. We presumed that this mixture contained three (enol) 17α-diesters, by analogy with an earlier reported perchloric acid catalyzed diesterification reaction.[6] Since enol esters are known to undergo acid-catalyzed solvolysis more rapidly than do tertiary esters, the intermediate (Scheme I) was treated with ethanolic HBr which gave a mixture of 2 and starting material (1) that was resolved by high-pressure silica gel chromatography. Elemental analysis and ultraviolet, infrared, and nuclear magnetic resonance spectral data supported the structural assignment of 2 as represented in Scheme I.

When 2 was incubated with the nucleophilic amino acids L-cysteine, L-histidine, and L-methionine in phosphate buffer at pH 7.0, TLC analysis of the incubation mixture revealed that steroid–amino acid conjugates formed at essentially the same rate with each of the nucleophiles as previously observed with primary and secondary bromoacetoxyprogesterone derivatives.[1,3b] Accordingly, addition of excess 2-mercaptoethanol to a solution of 2 produced an immediate and quantitative reaction between the thiol and the bromoacetate.

Incubations of 2 with 20β-hydroxy steroid dehydrogenase (E.C. 1.1.1.53.) from *Streptomyces hydrogenans* in 0.05 M phosphate buffer at pH 7.0 and 25 °C caused a time-dependent and irreversible loss in enzyme activity. 16α-Bromoacetoxyprogesterone under the same conditions causes a pseudo-first-order loss in enzyme activity with a $t_{1/2}$ = 4 h,[1] while 2 produces inactivation kinetics with a $t_{1/2}$ = 15 h. The difference between the respective rates of enzyme inactivation produced by the two affinity-labeling steroids most likely results from the different configurations of the bromoacetoxy reagent group in each of the compounds.

Recently we described biological experiments in which the affinity-labeling steroids, 16α-bromoacetoxyprogesterone, 11α-bromoacetoxyprogesterone, and 19-nortestosterone bromoacetate, caused fetal resorption thus terminating pregnancy by day 14 when these compounds were introduced into the uterine lumen on day 7 intact rats.[2,4] When 2 was tested in the same manner no fetal resorption was apparent and all of the test animals exhibited normal pregnancies. Medroxyprogesterone bromoacetate (2) was, however, found to have a progestational effect in pregnant rats which were bilaterally ovariectomized[9] on day 9, when daily doses of 0.15 mg of the compound were injected subcutaneously together with 0.1 g of 17β-estradiol in sesame oil over a 3-day period. Injections of 0.11 mg of medroxyprogesterone acetate under similar conditions provided the control animals. Animals which received 17β-estradiol alone contained partially resorbed fetuses, while those which received injections of vehicle alone had uteri which reverted to a nonpregnant state under the experimental conditions.

Conclusions

Thus far the only successful route to the synthesis of the sterically hindered medroxyprogesterone bromoacetate is esterification of medroxyprogesterone with a trifluoroacetic anhydride–bromoacetic acid mixture at 65 °C. The resulting product (2) reacts with nucleophilic amino acids and the enzyme 20β-hydroxy steroid dehydrogenase (E.C. 1.1.1.53.) under essentially physiological conditions. The steroid oxidoreductase enzyme is irreversibly inactivated during long-term incubations with 2 by affinity labeling. In contrast to other analogous affinity-labeling progesterone derivatives which terminate pregnancy in the rat, the title compound does not produce this effect. Indeed, 2 exhibits observable progestational activity.

Because 2 is an affinity-labeling steroid as defined above, this compound may exert its biological activity by *irreversibly* binding to key progesterone binding sites in the pregnant uterus. Thus a potential long-acting effect is a distinct possibility with this steroid. Therefore, we are currently testing the duration of progestational action of this novel steroid in the pregnant uterus. Also, we are trying to determine the degree and duration of antiovulatory activity of 2 via the hypothalamic–pituitary axis.

Synthesis of medroxyprogesterone bromoacetate provides an alkylating derivative of the structurally analogous and medically important oral contraceptive medroxyprogesterone acetate. This novel steroid bromoacetate should be useful to characterize, by affinity labeling, steroid binding sites of high affinity present in receptor proteins of target tissues in the reproductive system.

Experimental Section

Melting points (uncorrected) were determined in a Mel-Temp apparatus. Spectral data were obtained with a Beckman Acculab 4 infrared spectrophotometer, Beckman Model 25 UV–visible spectrophotomer, and Varian T-60 NMR spectrometer. Optical rotations were determined in chloroform using 2% solutions in a 1-dm semimicro (2.5 mL) tube with a Dr. Steeg & Reuter Model SR-5 polarimeter. Amino acids and 20β-hydroxy steroid dehydrogenase were obtained from Sigma Co. (St. Louis, Mo.) and dated Sprague–Dawley pregnant rats were from Holtzman Co. (Madison, Wis.).

Synthesis of Medroxyprogesterone Bromoacetate (17α-Hydroxy-6α-methyl-4-pregnene-3,20-dione 17-Bromoacetate,

2). To a mixture of 300 mg of bromoacetic acid and 1.0 mL of trifluoroacetic anhydride kept at room temperature for 30 min was added 700 mg of medroxyprogesterone. After stirring the reaction mixture for 1 h at 65 °C, it was cooled, neutralized with 5% aqueous sodium bicarbonate, and extracted with ether. The ethereal extract was washed with 5% aqueous sodium bicarbonate and then with water, dried ($MgSO_4$), and filtered. TLC analysis of the filtrate on silica gel G (benzene–ethyl acetate, 94:6) showed it to contain at least four components in addition to starting material. Attempts to separate this mixture by preparative TLC or column chromotography were unsuccessful.

The above ethereal filtrate was concentrated under reduced pressure and the residue was heated under reflux in 25 mL of ethanol containing 0.2 mL of 48% aqueous hydrobromic acid for 35 min. The reaction mixture was cooled, neutralized with 5% aqueous sodium bicarbonate, and concentrated under reduced pressure. The residue was extracted with ether and the extract was washed successively with 5% aqueous sodium bicarbonate and water, dried ($MgSO_4$), and then filtered. The filtrate was concentrated to a solid residue which was chromatographed on a short silica gel column[7] eluted with benzene–ethyl acetate (96:4). The product, obtained from pooled fractions which were concentrated to dryness, was recrystallized from petroleum ether containing a small amount of acetone to give 120 mg of white needles: mp 172–174 °C; $[\alpha]^{25}_D$ +63° (chloroform); UV (in methanol) 239 nm (15 750); IR (KBr pellet) 1730 (ester), 1720 (20-keto), 1670 (3-keto), 1614 cm^{-1} (Δ^4). The infrared spectrum of **2** was practically superimposible on that from an authentic sample of medroxyprogesterone acetate: NMR ($CDCl_3$, Me_4Si) τ 9.30 (3 H, s, H_{19}), 8.72 (3 H, d, $C_{6\alpha}$-Me, $J_{Me,H_{6\beta}}$ = 3.5 Hz), 8.80 (3 H, s, H_{18}), 7.95 (3 H, s, H_{21}), 6.18 (2 H, s, $BrCH_2COO$), 4.25 (1 H, narrow m, H_4). Anal. ($C_{24}H_{33}BrO_4$) C, H; Br: calcd, 16.95; found, 17.17.

Alkylation of Amino Acids. Solutions of medroxyprogesterone bromoacetate (4.65 mg, 0.01 mmol, in 5 mL of ethanol) were added to solutions of L-cysteine, L-methionine, and L-histidine (0.012 mmol in 5 mL of 0.05 M phosphate buffer at pH 7.0) and the resulting mixtures were incubated at 25 °C. Steroid–amino acid conjugates were formed as determined by thin-layer chromatographic analysis of appropriate incubation mixtures during 48-h reaction periods, according to a method previously described by us.[8] The relative rates of amino acid alkylation were in the order: L-cysteine (or 2-mercaptoethanol) >> L-histidine > L-methionine.

Enzyme Inactivation. Medroxyprogesterone acetate and the analogous bromoacetate, although not substrates of 20β-hydroxy steroid dehydrogenase (E.C. 1.1.1.53.) from *Streptomyces hydrogenans*, are competitive inhibitors of cortisone reduction by the enzyme and NADH. Preliminary studies have shown that incubation of the enzyme (4.2×10^{-7} M in 0.05 M phosphate buffer at pH 7.0, containing 15% glycerol) with medroxyprogesterone bromoacetate (5×10^{-5} M) at 25 °C results in a time-dependent and irreversible loss in enzyme activity. The rate of enzyme inactivation with the title compound has a $t_{1/2}$ = 15 h, which is somewhat greater than that previously obtained with 16α-bromoacetoxyprogesterone under similar conditions.[1]

Biological Experiments. Five groups each containing five albino rats (Holtzman Co.), 9 days pregnant, weighing 275–305 g, were subcutaneously injected 3 h prior to bilateral ovariectomy[9] with steroids in 0.2 mL of sesame oil: (A) 0.1×10^{-3} mg of 17β-estradiol; (B) 0.1×10^{-3} mg of 17β-estradiol plus 1.0 mg of progesterone; (C) 0.1×10^{-3} mg of 17β-estradiol plus 0.055 mg of medroxyprogesterone acetate; (D) 0.1×10^{-3} mg of 17β-estradiol plus 0.074 mg of medroxyprogesterone bromoacetate; (E) the same as D. Two pregnant (day 9) and ovariectomized rats received 0.2-mL injections of oil alone. Every 12 h following the initial injection each group was injected with the same steroid mixtures for 3 days with the exception of group E which received only a second injection and then nothing for the remainder of the experiment. On day 12 all of the animals were sacrificed and autopsied by a longitudinal, ventral incision[2,4] and the uteri were removed intact. Uteri in group A contained resorbed fetuses while groups B and C contained fully viable fetuses. Group D uteri contained viable fetuses but signs of the beginnings of resorption were apparent. Group E uteri contained *unresorbed fetuses* and the uterine changes resulting from the absence of estrogen were apparent. The two untreated rats had uteri in which the fetuses were *completely* resorbed, and the uterus had returned to an essentially nonpregnant state.

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