Synthesis and Biological Activity of Some 6,7-Dihalo-Δ^5-pregnenes and Some 6,6-Dihalo-5,7-cyclo-5β-pregnane Derivatives

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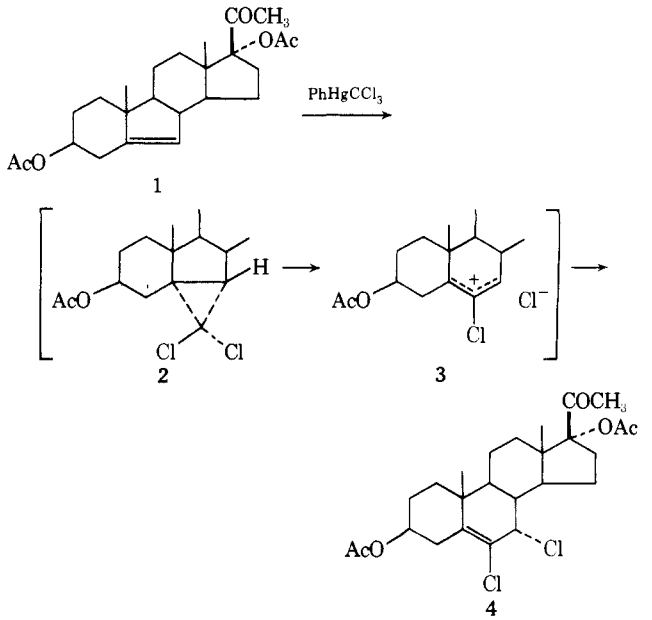
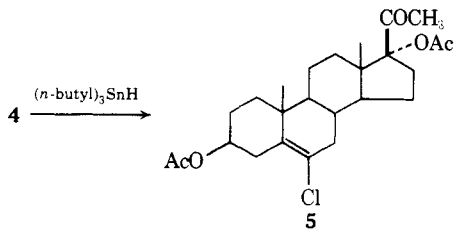
Various dihalocarbenes were added to the *B*-nor substrates 1 and 14 to give either a 5,7-cyclosteroid or a 6,7-dihalo-Δ^5 derivative. Several of the compounds thus prepared showed significant progestational activity when tested in the rabbit.

The addition of dihalocarbenes to 3β-acetoxy-*B*-norandrost-5-en-17-one has recently been described.[1] Depending on the halide used and the stereochemistry of the derived addition product, the initially formed cyclopropane derivative spontaneously ring opens to give a 6,7-dihalo-Δ^5 steroid or was thermally stable thus allowing for the isolation of a 5,7-cyclosteroid. Because of the efficacy already shown by 6-chloro-4,6-diene progestins[2] as potent gonadotropin inhibitors, it seemed of interest to investigate the possibility of preparing pharmacologically orally active halo progestins *via* the addition of dihalocarbenes to the *B*-nor substrates 1 and 14.

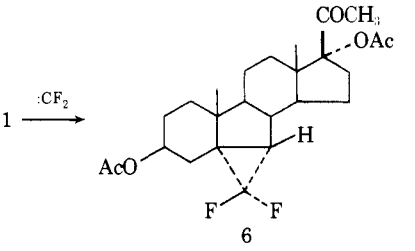
The *B*-nor steroid 1[3] reacted with phenyl(trichloromethyl)mercury[4] to give the cyclopropyl derivative 2 which underwent spontaneous rearrangement *via* the ion pair 3 to form the allylic product 4.



The quasiaxial (α) configuration of the C-7 chlorine of compound 4 was determined by nmr spectroscopy: d of d at δ 4.4 (J_{7-8} = 3.5 Hz, J_{7-4} = 1.5 Hz). The allylic nature of the 7-chloro substituent was demonstrated by its hydrogenolysis with tributyltin hydride[5] to the known monochloro derivative 5.[6]

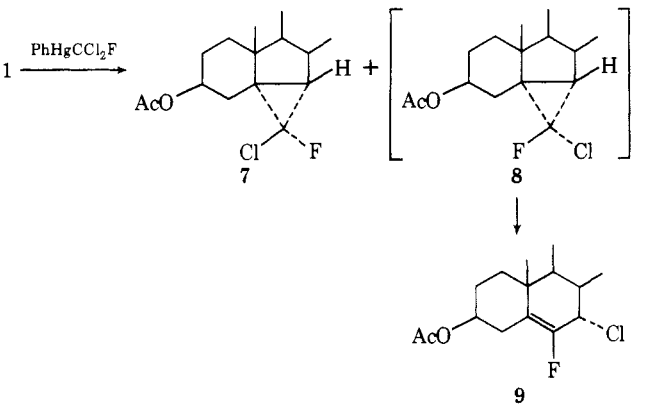


With difluorocarbene and 1 the initially formed cyclopropyl adduct does not thermally rearrange, *e.g.*, 2 → 4. Thus, the inability of the fluorine atom to ionize under the conditions of the reaction allows for the isolation of the cyclopropyl adduct 6.



The nmr spectra reveal a sharp singlet, δ 1.03 for the C-19 H signal, indicating therefore no long-range coupling[7] with an F atom and thus suggestive of the 5,7β stereochemistry.†

Fluorochlorocarbene, generated by the thermolysis of phenyl(fluorodichloromethyl)mercury,[8] was added to compound 1 resulting in the formation of both the cyclopropyl derivative 7 and the rearranged product 9. Compound 9 is believed to be the result of ring opening of the cyclopropyl derivative 8 having the halides disposed in the *S* configuration.‡ These products were isolated by means of silica gel chromatography.

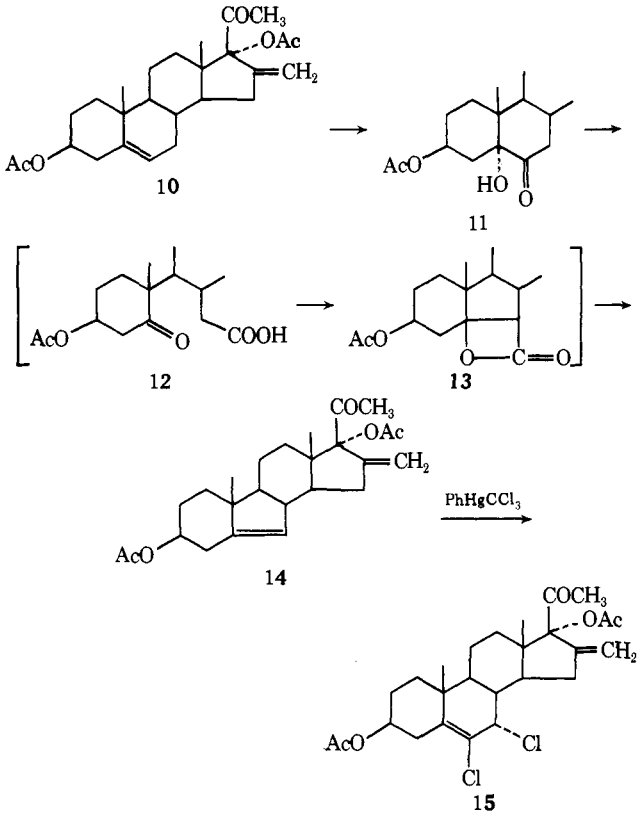


The enhancement of progestational activity shown for chlormadinone acetate (6-chloro-17α-hydroxy-4,6-pregnadiene 17-acetate) by the introduction of a 16-methylene group (chlorsuperlutin) is well documented.[9] Based on these observations, it seemed worthwhile for us to prepare the 16-methylene *B*-nor steroid 14.

Selective epoxidation of 10 followed by chromium trioxide oxidation of the resulting epoxide afforded the α-hydroxy ketone 11. Treatment with *m*-chloroperbenzoic acid gave the crude seco acid 12 which in turn was converted to the β-lactone 13. Pyrolysis of 13 with loss of carbon dioxide gave the *B*-nor steroid 14. Reaction of 14 with

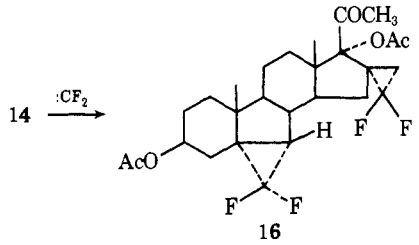
† For added proof of the α addition of difluorocarbene to a similar *B*-nor steroid, see ref 1.

‡ For a detailed discussion of the factors influencing the course of this reaction with a related compound, see ref 1.



phenyl(trichloromethyl)mercury then produced the dichloro derivative **15**.

Under the conditions for the generation of $:CF_2$ (decomposition of sodium difluoroacetate in refluxing diglyme) both centers of unsaturation in compound **14** reacted to give **16**. The addition of the difluorocarbene to the 16-methylene group is depicted as occurring from the α side since the C-18 angular Me protons appear as a singlet at δ 0.87. Addition from the β side would be expected to give a product which would show a splitting of the C-18 H signal as predicted by the converging vector rule.[7]



Biological Activity. Compounds were tested in a modified Clauberg-McPhail assay. Immature New Zealand white female rabbits (600–800 g body weight) were injected subcutaneously once daily for 5 consecutive days with 0.5 μg of estradiol benzoate in sesame oil. Compounds were dissolved or suspended in sesame oil and administered for the next 5 consecutive days following the estrogen priming period. Uteri were removed at autopsy on the day after the last day of compound administration, processed histologically, and examined microscopically for secretory development of the endometrium. Each compound was tested at nine dosage levels ranging from I to 400 μg/day by both subcutaneous and oral routes with four rabbits in each dosage group. Minimum effective doses for 17α-hydroxyprogesterone acetate and for chlorsuperlutin are given for comparison purposes.

Several of the compounds tested are shown to have significant progestational activity (Table I). As expected, the 16-methylene derivative **15** shows a higher degree of potency than the parent compound **4**. The removal of the C-7(α) chlorine from **4** to give **5** appears to reduce somewhat the progestational activity while substitution of a fluorine atom for chlorine at C-6, *i.e.*, **9**, significantly diminishes activity. In summary, the incorporation of the 6,7α-dichloro§ substituents into the pregnenolone nucleus greatly enhances its oral progestational properties although it is not as effective as the 6-chloro-4,6-diene system found in 6-chloro-16-methylene-17α-hydroxy-4,6-pregnadiene-3,20-dione 17-acetate (chlorsuperlutin).

Table I. Minimum Daily Dose (μg) Showing Significant Activity[a]

Compound	Subcutaneous	Oral
Chlorsuperlutin	0.2–0.4	0.2–0.4
17α-Acetoxyprogesterone	4–10	200–400
4	10–20	10–20
5	10–20	20–40
6	40–100	40–100
9	20–40	40–100
15	4–10	4–10
16	Inactive ~400	Inactive ~400

[a]Minimum significant activity is a McPhail index of at least 1.5: A. Boris and L. DeMartino, *Steroidologia*, **2**, 57 (1971).

Experimental Section

All melting points were determined in glass capillaries and are corrected. Rotations were measured in $CHCl_3$ at 25° at a concentration of about 1.0%. The nmr spectra were determined in $CDCl_3$ (internal Me_4Si) using a Varian A-60 spectrometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within ±0.4% of the theoretical values.

6,7α-Dichloro-3β,17α-dihydroxypregn-5-en-20-one Diacetate Etherate (4). A solution of 1 g (2.48 mmol) of **1** and 1.98 g (4.98 mmol) of phenyl(trichloromethyl)mercury in 10 ml of dry C_6H_6 was refluxed for 48 hr. The mixture was cooled and the precipitated phenylmercuric chloride collected. The solvent was removed from the filtrate under reduced pressure and the residue chromatographed on 15 g of silica gel. From a 2.5% EtOAc–C_6H_6 eluent was obtained 0.300 g of a yellow foam which upon trituration with Et_2O gave 0.122 g (16%) of **9**: mp 98° (foaming); [α]D −109.4°. *Anal.* ($C_{25}H_{34}Cl_2O_5 \cdot C_4H_{10}O$) C, H.

6-Chloro-3β,17α-dihydroxy-5-pregnen-20-one 3,17-Diacetate (5). To a solution of 1.73 g (3.09 mmol) of crude **4** dissolved in 5 ml of dry C_6H_6 was added under argon 1.25 ml of tributyltin hydride. The solution was stirred 48 hr and the solvent removed under reduced pressure. Trituration of the residue with C_6H_{14} gave 0.9 g (64.3%) of **5**, mp 193–199°. Crystallization from C_6H_{14} afforded 0.66 g of **5**, mp 203–204.5° (lit.[6] mp 208–209°).

6,6-Difluoro-3β,17α-dihydroxy-5,7-cyclo-5β-pregnan-20-one Diacetate (6). To a refluxing solution of 3 g (7.45 mmol) of **1** dissolved in 40 ml of diglyme was added over a 45-min period a solution of 17 g (0.111 mol) of sodium chlorodifluoroacetate in 100 ml of diglyme. At the end of the addition the mixture was refluxed another 15 min and the solvent removed *in vacuo*. The residue was dissolved in a minimum of C_6H_6 and chromatographed through 50 g of silica gel. A 5% EtOAc–C_6H_6 eluent gave 3 g of product as a white foam. Crystallization from Et_2O gave 1.35 g (40.5%) of **6**: mp 217° dec; [α]D −25.9°. *Anal.* ($C_{25}H_{34}F_2O_3$) C, H.

Reaction of 1 with Chlorofluorocarbene. Preparation of 6-Fluoro-6-chloro-(*R*)-3β,17α-dihydroxy-5,7-cyclo-5β-pregnan-20-one Acetate (7) and 6-Fluoro-7α-chloro-3β,17α-dihydroxypregn-5-en-20-one Diacetate (9). A solution of 3 g (7.45 mmol) of **1** and 6 g (15.8 mmol) of phenyl(fluorodichloromethyl)mercury in 50 ml of dry C_6H_6 was refluxed for 60 hr. The mixture was cooled and the precipitated phenylmercuric chloride collected. Most of the solvent was removed under reduced pressure and the residue chromatographed on 90 g of silica gel. The column was eluted with 1500 ml of C_6H_6 followed by 1 l. of 2% EtOAc–C_6H_6. From the latter eluent was obtained 1.5 g of crude product. Crystallization from Et_2O afforded 1.4 g (40.1%) of **7**: mp 138–141°; [α]D −33.7°. *Anal.* ($C_{25}H_{34}ClFO_5$) C, H, Cl, F.

§ For the synthesis and biological activity of various 6-chloro-3,7-dihydroxy-Δ⁵-pregnene derivatives, see ref 10.

Continuation of the chromatography with 2% EtOAc-C_6H_6 gave firstly 1 g of a 1:1 mixture (tlc) of compounds **7** and **9**, followed by 0.4 g of crude **9**. Crystallization from Et_2O-C_6H_{14} afforded 0.3 g (8.59%) of **9**: mp 173–175°; $[\alpha]D$ −154°. *Anal.* ($C_{25}H_{34}ClFO$) C, H, Cl, F.

16-Methylene-3β,5α,17α-trihydroxypregnane-6,20-dione 3,17-Diacetate (11). To a solution of 5 g (17.7 mmol) of 16-methylene-3β,17α-dihydroxypregnen-20-one diacetate (**10**) in 40 ml of EtOH-free $CHCl_3$ was added 26.7 ml of an Et_2O solution of monoperphthalic acid (115 mg/ml). After 12 hr at 0°, 100 ml of Et_2O was added and the solution was washed with 5% $NaHCO_3$ (until basic) and water, and then dried ($MgSO_4$). The solvent was removed under reduced pressure to give 5 g of crude 5,6-epoxide. To a suspension of the epoxide (4.5 g) in 50 ml of EtCOMe was added 5 ml of a solution composed of 15 g of chromium trioxide in 20 ml of H_2O. After stirring for 30 min the mixture was poured into 500 ml of ice H_2O and the precipitate was collected and air-dried for 1 hr. The product was then dissolved in a minimum of $CHCl_3$ and dried ($MgSO_4$). The solvent was removed under reduced pressure and the solid crystallized from EtOH to give 4 g (54%) of **11**: mp 256–258° dec; $[\alpha]D$ −159.2°. *Anal.* ($C_{26}H_{36}O_7$) C, H.

3β,17α-Dihydroxy-16-methylene-*B*-norpregn-5-en-20-one Diacetate (14). To a solution of 44 g (0.102 mol) of 11 dissolved in 300 ml of EtOH free $CHCl_3$ was added slowly 60 g of 87% *m*-chloroperbenzoic acid dissolved in 600 ml of $CHCl_3$. The temperature was maintained below 30° during the addition. The solution was then stirred for 1.5 hr at room temperature and cooled to 10°, and 250 ml of a 20% $NaHSO_3$ solution was added. The mixture was stirred for 10 min and then filtered to give 27.5 g of *m*-chlorobenzoic acid. The organic layer was separated and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was treated with 250 ml of C_6H_6 and the mixture again filtered to give a second crop (20 g) of *m*-chlorobenzoic acid. The remaining *m*-chlorobenzoic acid was extracted from the C_6H_6 filtrate by washing with 3 × 50 ml of 5% $NaHCO_3$ solution. The C_6H_6 solution was then washed with 2 × 100 ml of a 5% Na_2CO_3 solution and the extracts were immediately acidified in the cold. The mixture was extracted with Et_2O, the ether solution dried ($MgSO_4$), and the solvent removed under reduced pressure to give 21 g of 3β,17β-dihydroxy-5,20-dioxo-5,6-*seco*-16-methylenepregnan-6-oic acid diacetate (**12**) as a white foam. To a solution of the crude seco acid (21 g) in 50 ml of dry C_5H_5N was added 24 ml of PhCOCl. The dark mixture was then stirred overnight after which time 10 ml of dry MeOH was added. The mixture was stirred for an additional 30 min and then poured into 800 ml of ice H_2O. The mixture was extracted with Et_2O and the Et_2O solution washed with 1 *N* NaOH solution and H_2O and then dried ($MgSO_4$). Removal of the solvent under reduced pressure gave 42 g of a dark oil. The residue was then subjected to high vacuum (0.1 mm) and heated to 70° at which temperature the MeOCOPh distilled. The residue was then pyrolyzed at this pressure by heating to 200° for 10 min. Trituration of the residue with MeOH gave 7.4 g of a brown solid. Crystallization from MeOH afforded 5.4 g (13%) of **14**: mp 178.5–180.5°; $[\alpha]D$ −205°. *Anal.* ($C_{25}H_{34}O_5$) C, H.

6,7α-Dichloro-3β,17α-dihydroxy-16-methylenepregn-5-en-20-one Diacetate Etherate (15). A solution of 0.5 g (1.20 mmol) of **14** and 0.954 g (2.41 mmol) of phenyl(trichloromethyl)mercury in 5 ml of dry C_6H_6 was refluxed under nitrogen for 48 hr. The mixture was filtered and the solvent removed from the filtrate under reduced pressure. The residue was chromatographed through 15 g of silica gel. From a 2.5% EtOAc-C_6H_6 eluent was obtained 0.397 g of a yellow foam. Crystallization from Et_2O gave 0.186 g (31.5%) of **15**: mp 125° dec; $[\alpha]D$ −201.5°. *Anal.* ($C_{26}H_{34}Cl_2O_5 \cdot C_4H_{10}O$) C, H.

3β,17α-Diacetoxy-2′,2′,6,6-tetrafluoro-(16*R*)-spiro(5,7β-cyclopregnane-16,1′-cyclopropan)-20-one (16). To a refluxing solution of 1 g (2.41 mmol) of **14** dissolved in 50 ml of dry diglyme was added dropwise (over a 45-min period) a solution of 5.52 g (36.2 mmol) of sodium chlorodifluoroacetate dissolved in 50 ml of the same solvent. After the addition was completed the mixture was refluxed for an additional 15 min. The mixture was then cooled and filtered and the solvent removed under high vacuum. The residue was chromatographed on 25 g of silica gel. Elution with a 5% EtOAc-C_6H_6 solution afforded 0.6 g of crude product. Crystallization from CH_2Cl_2-Et_2O afforded 0.26 g (21.7%) of **16**: mp 190–192.5°; $[\alpha]D$ −31.1°. *Anal.* ($C_{27}H_{34}F_4O_5$) C, H, F.

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References

(1) P. Rosen and R. Karasiewicz, *J. Org. Chem.*, **38**, 289 (1973).
(2) J. A. Zderic in "Comprehensive Biochemistry," Vol. 10, M. Florkin and E. H. Stoltz, Ed., Elsevier, New York, N. Y., 1963, p 191.
(3) (a) P. Rosen, U. S. Patent 3,711,522 (1973); (b) V. Sanda, J. Fajkos, F. Šorm, and J. Protiva, *Collect. Czech. Chem. Commun.*, **37**, 2807 (1972).
(4) D. Seyferth, J. M. Berlitch, R. J. Minasz, J. Y.-P. Mui, H. D. Simmons, Jr., A. J. H. Treiler, and S. R. Dowd, *J. Amer. Chem. Soc.*, **87**, 4259 (1965).
(5) (a) J. G. Noltes and G. J. M. van der Kerk, *Chem. Ind. (London)*, 294 (1959); (b) E. J. Kupchik and R. E. Connolly, *J. Org. Chem.*, **26**, 4747 (1961).
(6) J. S. Mihina, *J. Org. Chem.*, **27**, 2807 (1962).
(7) A. Cross and P. W. Landis, *J. Amer. Chem. Soc.*, **86**, 4005 (1964).
(8) D. Seyferth and K. V. Darragh, *J. Organometal. Chem.*, **11**, P9 (1968).
(9) (a) Z. Čekan, M. Šeda, J. Mikulášková, and K. Syhora, *Steroids*, **8**, 205 (1966); (b) E. L. Shapiro, L. Weber, H. Harris, C. Miskowicz, R. Neri, and H. L. Herzog, *J. Med. Chem.*, **15**, 716 (1972).
(10) R. A. LeMahieu, A. Boris, M. Carson, and R. W. Kierstead, *J. Med. Chem.*, **14**, 291 (1971).

Synthesis and Biological Activities of Substituted Glycyrrhetic Acids

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18β-Glycyrrhetic acid (**1b**) was converted in good yield to the 3-oxo-4,4-bis(nor-18β-olean-4-ene) derivative **11a** in 25% overall yield. Derivatives of **1b** substituted in the A, B, C, and E ring were also prepared. Several 11-deoxoglycyrrhetic acid derivatives exhibited anti-DCA activity. In particular, when administered subcutaneously, 3-oxo-18β-olean-12-en-30-oic acid (**2d**) had about 75% the activity of spironolactone administered subcutaneously. Several compounds also exhibited weak antiviral and antiinflammatory properties.

The medicinal and biological properties associated with glycyrrhizin (**1a**) and its aglycone 18β-glycyrrhetic acid (**1b**) are well documented.[1] The biological properties in laboratory animals and *in vitro* assays which have been reported for 18β-glycyrrhetic acid and its derivatives are antiulcer,[2] antiinflammatory,[2a,3] sodium ion retention,[2a,4] antihormonal,[5] and antineoplastic.[6] In recent years the antigastric ulcer activity and the effects on mineral metabolism of glycyrrhetic acid derivatives have been reported in man.[2a,7] The ammonium salt of glycyrrhizin is also used as a commercial sweetening agent.[1]

Although the biological activities of glycyrrhetic acid