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SYNTHESIS OF 4,19-DISUBSTITUTED DERIVATIVES OF DOC. RADIORECEPTOR ASSAY OF SOME CORTICOSTEROID DERIVATIVES IN HUMAN MONONUCLEAR LEUKOCYTES

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Summary—Several new 4,19-substituted steroids and previously synthesized corticosteroids were assayed for affinity to type 1 receptors in human mononuclear leyukocytes. 11β,19-Epoxy-4,21-dihydroxypregn-4-ene-3,20-dione (**2**) was hydrogenated with Pd-C to yield a mixture of all four dihydro derivatives **5**, accompanied by 4,21-diacetoxy-11β,19-epoxy-3-hydroxypregnan-20-one (**6**) and 21-acetoxy-11β,19-epoxy-4-hydroxypregnane-3,20-dione (**7**). With hot acetic + *p*-toluenesulfonic acid **5** underwent rearrangement to 21-acetoxy-11β,19-epoxypregn-5-ene-4,20-dione (**8**). Pd-C hydrogenation of 3,21-diacetoxy-5β,19-cyclopregna-2,9(11)-diene-4,20-dione (**10**) gave 3,21-diacetoxy-5β,19-cyclopregn-5-ene-4,20-dione (**11**) and the 9,11-dihydro derivative of the latter. Treatment of **10** with warm HCl furnished 19-chloro-4,21-dihydroxypregna-4,9(11)-diene-3,20-dione (**13**). Pd-C hydrogenation of its diacetate **14** afforded the 4,5-dihydro derivative **18**, 19-chloro-21-acetoxypregn-9(11)-en-20-one (**15**), its 4-acetoxy derivative **16** and the 3,4-diacetoxy derivative **17**.

When tested in a radioreceptor assay in human mononuclear leukocytes the synthesized compounds showed only low relative binding affinities (RBA) to type 1 receptor, the highest being 0.72% for **13** (aldosterone = 100%). For comparison, other RBA in this system were: 19-noraldosterone, 20%; 18-deoxyaldosterone, 5.8%; 18-deoxy-19-noraldosterone, 4.7%; 18,21-anhydroaldosterone, 0.37%; 17-isoaldosterone, 7.6% and apoaldosterone, 4.3%.

INTRODUCTION

This work was prompted by the availability of the 4-oxime of 21-acetoxy-5β,19-cyclopregnane-3,4-dione (**1**), a by-product of Barton's synthesis of aldosterone [1]. The oxime had been converted into the 11,19-epoxy compound **2** and the 11-deoxy derivative **10**, both of which were now utilized as starting materials for the preparation of several 19-substituted steroids bearing an oxygen function at C-4, in order to determine their affinity for mineralocorticoid receptors in leukocytes, in comparison with other steroids (Figs 1 and 2).

EXPERIMENTAL

Merck A.G. silica gel (60; mesh 70–230) was used in column chromatography. TLC was performed with acetone–hexane or $CHCl_3$–ethanol mixtures and the plates (silica gel Merck F254, 0.2 mm on plastic) were sprayed with 10% H_2SO_4 in ethanol (v/v) before heating. ^{1}H-NMR and ^{13}C-NMR spectra (in $CDCl_3$, with TMS as internal standard) were obtained with a Bruker AM-360 spectrometer, equipped with an ASPECT 3000 computer, operating at 360.1 MHz. i.r. Spectra were recorded with a Perkin–Elmer 297 spectrometer. Mass spectra were recorded with a Finnigan 4020 quadrupole spectrometer. Ionizing conditions for the EI mode were 23–32 eV, for the CI mode 70 eV, emission current 0.25 mA, electron multiplier 1.7 kV, source temperature 230–240°C, inlet temperature 150–220°C. Methane was the reagent gas used in the CI mode. Melting points were determined with the electrothermal apparatus and are uncorrected. Solutions were evaporated *in vacuo* at 30–35°C. Dichloromethane is abbreviated to MDC.

For determination of displacement of [^{3}H]aldosterone from type 1 receptors, mononuclear leukocytes were separated from hep-

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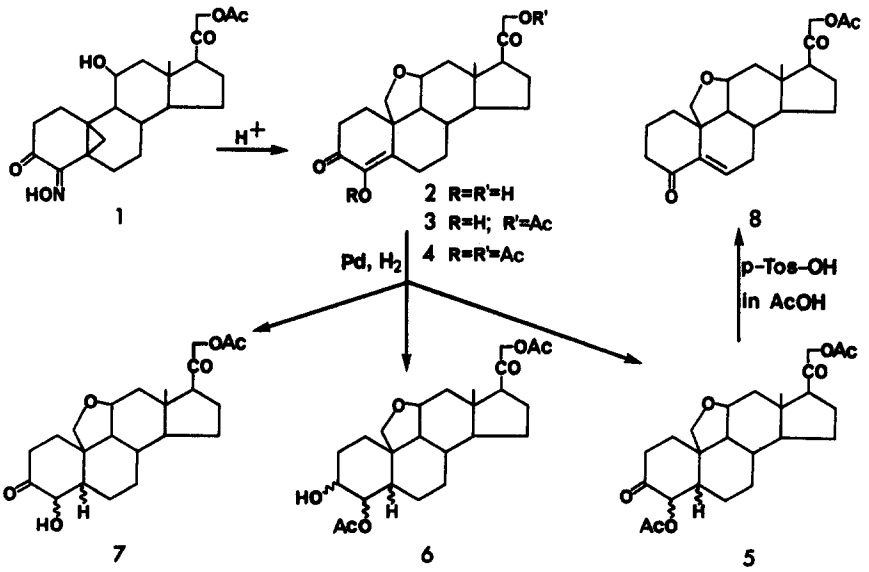

Fig. 1

arinized human peripheral blood by density centrifugation with Histopaque-1077 (Sigma, St Louis, Mo.). After washing and resuspension in Hanks balanced salts solution (Sigma) the cells were incubated at 37°C for 1 h with 1 nM [^{3}H]aldosterone plus a 100-fold excess of RU28362 (Roussel–Uclaf, Paris) to block the glucocorticoid receptors, and various concentrations of unlabelled competing steroids. Non-specifically bound [^{3}H]aldosterone was determined by incubating [^{3}H]aldosterone-treated leukocytes with 500 nM unlabelled aldosterone, and was subtracted from the total bound [^{3}H]aldosterone. The relative binding affinities (RBA) of competitor steroids in relation to aldosterone (100%) are presented in Table 1 as the ratio

$$\frac{\text{concentration of aldosterone at 50\% displacement } (= 0.26\text{ nM})}{\text{concentration of competing steroid at 50\% displacement}} \times 100.$$

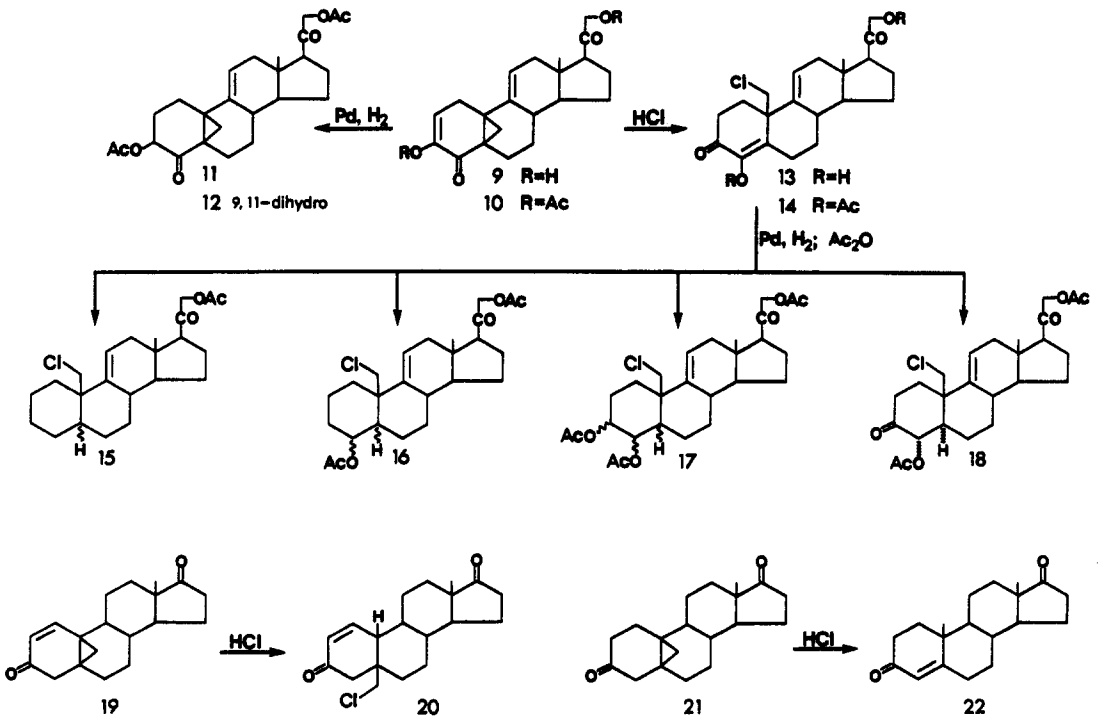

Fig. 2

RU28362 (11β,17β-dihydroxy-6-methyl-17α-(1-propynyl)-androsta-1,4,6-trien-3-one) is a "pure" glucocorticoid analog which is known not to react with type 1 receptors under the conditions used [2].

Hydrogenation of 11β,19-epoxy-4,21-dihydroxy-pregn-4-ene-3,20-dione (2)

A solution of 3.2 g of **2** [1] (^{1}H-NMR δ 0.834 (s, 18-CH_3), 3.129 (ddd, J = 17.0, 3.8, 2.1, 12-H), 3.261 (t, J = 4.5, 21-OH), 3.846 (dd, J = 8.7, 1.8, 19-H), 3.956 (d, J = 8.7, 19-H'), 4.186 (dd, J = 17.0, 4.5, 21-H), 4.238 (dd, J = 17.0, 4.5, 21-H') (20,21 AB) and 4.40 (brs, $\Delta W_{1/2} = 5$, 11α-H)) in 250 ml of ethanol was hydrogenated at 35 psi and room temperature for 45 h. Filtration and evaporation furnished a gummy mixture which was treated for 16 h at room temperature with 20 ml each of pyridine and acetic anhydride. The bulk of solvents was removed *in vacuo* at 40°C, the residue was treated with ice and water, and worked up by extraction with MDC, washing with 5% HCl and aq. $NaHCO_3$ in the usual manner, to afford 3.23 g of a foam. Chromatography of 0.93 g of it on 115 g of silica (elution with $CHCl_3$) gave 0.49 g of 4,21-diacetoxy-11β,19-epoxypregnane-3,20-dione (**5**), as a mixture of all four possible isomers at C-4 and C-5, crystallizing on scratching with ether, m.p. 150–195°C. ^{1}H-NMR δ 0.769, 0.799 (s,s, 18-CH_3), 2.150, 2.165 (s,s, 21-OAc and 4-OAc), 3.669 (d, J = 9, 19-H) (AB), 3.714 (d, J = 9, 19-H') (AB), 4.108, 4.305 (brs, s, 11-H), 4.600 (d, J = 16.8, 21-H), 4.647 (dd, J = 16.8, 2.2, 21-H') (only one AB system), 5.22, 5.24, 5.26 and 5.30 (4d, J = 6, 4-H), pointing to four isomers; MS EI 386 (M^+-AcOH, 2%), 373 (M^+-CH_2OAc, 60) 345 (M^+-$COCH_2OAc$, 12), 285 (345-AcOH, 48), 267 (285-H_2O, 33) and 255 (285-CH_2O, 100).

Further elution gave 4,21-diacetoxy-11β,19-epoxy-3-hydroxypregnan-20-one (**6**), of uncertain configuration at C-3, C-4 and C-5 (probably 3α-hydroxy-4α-acetoxy), m.p. 165–198°C (ether), 15 mg; λKBr_{max} 2,84, 5.71 and 5.86–5.90 μ; ^{1}H -NMR δ 0.805 (s, 18-CH_3), 2.078, 2.166 (s,s, 4-OAc and 21-OAc), 3.814 (brs, $\Delta W_{1/2} = 5$, 3_{eq}-H), 3.742 (d, J = 8.7, 19-H), 4.025 (dd, J = 8.7, ~1, 19-H') (broad AB), 4.225 (brs, $\Delta W_{1/2} = 8$, 11-H), 4.608 (d, J = 16.8, 21-H), 4.638 (d, J = 16.8, 21-H') (AB) and 4.753 (ddd, J = 9, 5, 3, 4_{ax}-H); MS CI 477 ($MC_2H_5^+$, 7%), 449 (MH^+, 13), 431 (MH^+-H_2O, 6), 389 (MH^+-AcOH, 22), 371 (431-AcOH, 100), 359 (389-CH_2O, 69), 353 (371-H_2O, 27), 341 (371-CH_2O, 27), 329 (MH^+-2AcOH, 29), 311 (329-H_2O, 48), 299 (329-CH_2O, 13), 293 (311-H_2O, 46) and 281 (299-H_2O, 18).

Elution with 5% ethanol in $CHCl_3$ yielded 21-acetoxy-11β,19-epoxy-4-hydroxypregnane-3,20-dione (**7**), of uncertain configuration at C-4 and C-5 (either 4α-hydroxy,5β-H or 4β-hydroxy,5α-H), m.p. 190–226°C (ether), 18 mg; λKBr_{max} 2.86, 5.72, 5.77 and 5.81 μ; ^{1}H-NMR δ 0.816 (s, 18-CH_3), 2.167 (s, 21-OAc), 2.503 (t, J = 9.0), 2.861 (ddd), 3.850 (brt, $\Delta W_{1/2} = 5$, 4-H), 3.765 (d, J = 9, 19-H), 4.092 (dd, J = 9, ~1, 19-H') (AB), 4.60 (d, J = 16.8, 21-H) and 4.65 (d, J = 16.8, 21-H') (AB); MS CI 433 ($MC_2H_5^+$, 2%), 405 (MH^+, 93), 387 (MH^+-H_2O, 87), 375 (MH^+-CH_2O, 39), 369 (387-H_2O, 30), 357 (375-H_2O, 100), 345 (MH^+-AcOH, 45), 327 (387-AcOH, 68), 309 (327-H_2O, 37) and 297 (357-AcOH, 21).

21-Acetoxy-11β,19-epoxypregn-5-ene-4,20-dione (8)

A solution of 2.07 g of **5** in 200 ml of acetic acid was treated with 3 g of *p*-toluenesulfonic acid monohydrate and refluxed for 1 h. The strongly fluorescing solution was concentrated *in vacuo* to 20 ml, diluted with 100 ml of water and 250 ml of saturated aq. $NaHCO_3$, and extracted with 300 ml and then 2 × 50 ml of MDC. The dried extracts were evaporated and the dark residue was chromatographed on 170 g of silica. Elution with $CHCl_3$, evaporation of suitable fractions and crystallization from ether furnished 1.27 g of **8**, m.p. 164–165°C; λKBr_{max} 5.70, 5.78, 5.90 and 6.19 μ; $\lambda EtOH_{max}$ 240 nm (ε 5,800); ^{1}H -NMR δ 0.802 (s, 18-CH_3), 3.675 (dd, J = 8.4, 1.3, 19-H), 3.914 (d, J = 8.4, 19-H'), 4.397 (brs, 11-H), 4.616 (d, J = 16.8, 21-H), 4.666 (d, J = 16.8, 21-H') (AB) and 6.580 (dd, J = 5.8, 1.9, 6-H); ^{13}C -NMR δ 203.1 (s, CO), 195.7 (s, CO), 169.9 (s, Ac), 141.3 (s, C-5), 135.0 (d, C-6), 76.6 (d, C-11), 73.7 (t, C-19). 68.7 (t, C-21), 59.9 (d), 53.2 (d), 48.4 (s), 44.4 (s), 40.9 (t), 39.6 (t), 33.4 (t), 30.3 (d), 29.2 (t), 23.3 (t), 22.5 (t), 20.3 (q), 18.9 (t) and 15.0 (q); MS EI 386 (M^+, 3.4%), 356 (M^+-CH_2O, 50), 313 (M^+-CH_2OAc, 98), 296 (356-AcOH, 8), 285 (M^+-$COCH_2OAc$, 21), 267 (285-H_2O, 26), 255 (356-$COCH_2OAc$, 100) and 213 (255-CH_2CO, 13).

Reaction of 4,21-diacetoxy-11β,19-epoxypregn-4-ene-3,20-dione (4) with p-toluenesulfonic acid in acetic acid

Compound **4** [1] was reacted as described above for **5**. Chromatography on silica column

(elution with hexane–acetone, 2:1) furnished 21-acetoxy-11β,19-epoxy-4-hydroxypregn-4-ene-3,20-dione (**3**) as the main product, m.p. 208–212°C (reported [1] 200–207°C), exhibiting a dirty-green coloration with $FeCl_3$; $\lambda EtOH_{max}$ 277 nm (ε 11,270) (reported [1] $\lambda MeOH_{max}$ 278 nm (ε 12,300)); ^{1}H-NMR δ 0.852 (s, 18-CH_3), 2.172 (s, 21-OAc), 3.127 (ddd, J = 14.8, 3.7, 2.0, 12-H), 3.794 (dd, J = 8.7, 1.6, 19-H), 3.952 (d, J = 8.7, 19-H′), 4.408 (brs, $\Delta W_{1/2}$ = 5, 11-H), 4.600 (d, J = 16.8, 21-H), 4.680 (d, J = 16.8, 21-H′) (AB) and 6.269 (s, 4-OH).

Hydrogenation of 3,21-diacetoxy-5β,19-cyclopregna-2,9(11)-diene-4,20-dione (10)

A solution of 1 g of **10** [1] (^{1}H -NMR δ 0.647 (s, 18-CH_3), 1.247 (d, J = 4.6, 19-H), 1.633 (d, J = 4.6, 19-H′), 2.175 (s, 21-OAc), 2.227 (s, 3-OAc), 4.552 (d, J = 16.8, 21-H), 4.730 (d, J = 16.8, 21-H′) (AB), 5.569 (brd, J = 5, 11-H) and 6.154 (dd, J = 5.8, 2.7, 2-H)) in 250 ml of boiling ethanol was cooled to room temperature and hydrogenated at 42 psi for 6 h with 2 g of 5% Pd-C. The product crystallized on contact with MDC and consisted of a 1:2 mixture of 3β,21-diacetoxy-5β,19-cyclopregn-9(11)-ene-4,20-dione (**11**) and 3β,21-diacetoxy-5β,19-cyclopregnane-4,20-dione (**12**), which was separated by repeated chromatography on silica gel (elution with $CHCl_3$): the earlier fractions afforded **11**, m.p. 155–157°C (MCD-hexane); $\lambda EtOH_{max}$ 203 nm (ε 11,000); λKBr_{max} 5.72, 5.78, 5.87 and 6.12 (w) μ; ^{1}H -NMR δ 0.617 (s, 18-CH_3), 1.01 (d, J = 4.3, 19-H), 1.63 (d, 19-H′, overlapping with other protons), 2.15 (s, 21-OAc), 2.18 (s, 3-OAc), 4.560 (d, J = 16.8, 21-H), 4.730 (d, J = 16.8, 21-H′), 4.866 (dd, J = 12, 6, 3-H) and 5.60 (brs, 11-H); MS EI 428 (M^+, 19%), 386 (M^+-CH_2CO, 8), 368 (M^+-AcOH, 18), 355 (M^+-CH_2OAc, <1), 340 (355-CH_3, 100), 327 (M^+-$COCH_2OAc$, 2), 295 (355-AcOH, <1), 285 (M^+-$AcOC_5H_8O$, <1) and 267 (327-AcOH, 12).

Later fractions from the chromatogram gave **12**, m.p. 191–192°C (ether–hexane); $\lambda EtOH_{max}$ 203 nm (ε 4,230); λKBr_{max} 5.70, 5.78 and 5.87 μ; ^{1}H -NMR δ 0.666 (s, 18-CH_3), 0.88 (d, J = 4.3, 19-H), 1.45 (d, J = 4.3, 19-H′, overlapping with other protons), 2.15 (s, 21-OAc), 2.18 (s, 3-OAc), 4.53 (d, J = 16.8, 21-H), 4.73 (d, J = 16.8, 21-H′) and 4.90 (dd, J = 12, 6, 3-H); MS EI 430 (M^+, 1.5%), 388 (M^+-CH_2CO, 7), 370 (M^+-AcOH, 31), 357 (M^+-CH_2OAc, 42), 342 (357-CH_3, 32), 329 (M^+-$COCH_2OAc$, 3), 297 (357-AcOH, 15), 287 (M^+-$AcOC_5H_8O$, 15) and 269 (329-AcOH, 100).

Hydrogenation of **11** with 5% Pd-C for 21 h at 35 psi and room temperature afforded **12**.

19-Chloro-4,21-dihydroxypregna-4,9(11)-diene-3,20-dione (13)

A solution of 3 g of **10** in 400 ml of hot ethanol was treated with 120 ml of 32% HCl and stored at 40°C for 17 h. The reaction mixture was shaken with 2.4 l of water and 300 ml of MDC, and reextracted with 3 × 150 ml of MDC. The extracts were washed with 100 ml of saturated aq. $NaHCO_3$, dried over Na_2SO_4 and evaporated. Chromatography on 170 g of silica (elution with hexane–acetone, 4:1) furnished a crystalline solid which was collected with ether, 1.46 g, m.p. 180–181°C (ether–hexane), $FeCl_3$ test positive; $\lambda EtOH_{max}$ 276 nm (ε 10,200); λKBr_{max} 2.92, 5.83, 6.00, 6.06 and 6.12 (w) μ; ^{1}H–NMR δ 0.647 (s, 18-CH_3), 3.605 (d, J = 11.4, 19-H), 3.711 (d, J = 11.4, 19-H′) (AB), 4.197 (m, 2H, 21-H, 21-H′), 5.552 (brd, 11-H) and 6.309 (s, 4-OH); ^{13}C-NMR δ 192.6 (s, CO), 182.6 (CO), 143.3 (s), 138.7 (s), 133.2 (s), 122.9 (d), 69.8 (t, CH_2Cl), 58.9 (d), 47.7 (t), 44.5 (s), 43.0 (s), 40.5 (t), 36.7 (d), 31.5 (t), 31.1 (t), 27.8 (t), 25.5 (t), 23.1 (t), 22.9 (t) and 13.1 (q); MS CI 419 ($MC_3H_5^+$, 3%), 407 ($MC_2H_5^+$, 11), 379 (MH^+, 100), 361 (MH^+-H_2O, 14), 343 (MH^+-HCl, 77), 329 (MH^+-CH_3Cl, 31), 325 (361-HCl, 21), 319 (MH^+-$HCOCH_2OH$, 5), 307 (M^+-$C_3H_4O_2$, 14) and 301 (329-CO, 15).

The same product was also obtained when **10** was kept in an acetone–water–conc. HCl mixture (10:2:3, v/v/v) for 22 h at 28°C. Under these conditions it was sometimes accompanied by the sparingly soluble 3,21-dihydroxy-5β,19-cyclopregna-2,9(11)-diene-4,20-dione (**9**), m.p. 210–214°C (ethyl acetate); $\lambda EtOH_{max}$ 264 nm (ε 3,800); λKBr_{max} 2.86, 2.92, 5.84, 5.94 (w) and 6.06 μ; ^{1}H-NMR δ 0.619 (s, 18-CH_3), 1.225 (d, J = 4.3, 19-H), 1.69 (d, J = 4.3, 19-H′), 4.208 (m, 2H, 21-H, 21-H′), 5.572 (brs, 11-H), 5.727 (dd, J = 5.7, 3.3, 2-H) and 5,94 (s, 3-OH). Reacetylation of **9** with acetic anhydride and pyridine gave **10**. Treatment of **9** with HCl in aqueous acetone, as described above for **10**, afforded **13** as well.

19-Chloro-4,21-diacetoxypregna-4,9(11)-diene-3,20-dione (14)

Acetylation of **13** with acetic anhydride and pyridine in the usual manner gave **14**, m.p. 183–185°C (ethyl acetate); λKBr_{max} 5.69, 5.78,

5.92 and 6.06 (w) μ; ^{1}H -NMR δ 0.647 (s, 18-CH_3), 2.14 (s, 21-OAc), 2.21 (s, 4-OAc), 3.698 (m, 2H, 19-H, 19-H'), 4.482 (d, J = 16.8, 21-H), 4.635 (d, J = 16.8, 21-H') (AB) and 5.51 (brs, 11-H); ^{13}C-NMR δ 189.5 (s), 189.2 (s), 170.2 (s, OAc), 168.3 (s, OAc), 149.8 (s), 141.4 (s), 137.9 (s), 124.0 (d), 68.8 (t, $\underline{C}H_2Cl$), 58.9 (d), 53.0 (d), 47.9 (t), 46.0 (s), 42.9 (s), 40.4 (t), 36.6 (d), 33.0 (t), 31.1 (t), 27.6 (t), 25.4 (t), 24.3 (t), 22.9 (t), 20.4 (q), 20.2 (q) and 12.8 (q); MS CI 503 ($MC_3H_5^+$, 3%), 491 ($MC_2H_5^+$, 17), 463 (MH^+, 34), 421 (MH^+-CH_2CO, 100), 403 (MH^+-AcOH, 17), 385 (421-HCl, 47), 371 (421-CH_3Cl, 35), 361 (403-CH_2CO, 22) and 343 (403-AcOH, 20).

Hydrogenation of ***14***

A suspension of 1.52 g of **14** in 250 ml of ethanol was hydrogenated at 22 psi for 24 h with 2 g of 5% Pd-C. TLC of the resulting gum revealed saturation of the 4,5 double bond and that in 1/3 of the product the C-3 carbonyl had been reduced to a hydroxyl. Acetylation (7.5 ml each of pyridine and acetic anhydride, 2 h at room temperature, then quenching with ice) gave a solid (1.22 g) which was chromatographed on 170 g of silica. Elution with hexane–acetone 5:1 afforded 19-chloro-21-acetoxy-pregn-9(11)-en-20-one (**15**), which after recrystallization from ethyl acetate-hexane had m.p. 173–180°C (8 mg); λKBr_{max} 5.69 and 5.77 μ; ^{1}H -NMR δ 0.656 (s, 18-CH_3), 2.17 (s, 21-OAc), 2.58 (t, 17-H), 3.74 (d, J = 10.5, 19-H), 3.83 (d, J = 10.5, 19-H') (AB), 4.57 (d, J = 16.8, 21-H), 4.70 (d, J = 16.8, 21-H') (AB) and 5.39 (d, J = 5.7, 11-H); MS CI 421 ($MC_2H_5^+$, 12%), 393 (MH^+, 67), 375 (MH^+-H_2O, 15), 357 (MH^+-HCl, 98), 351 (MH^+-CH_2CO, 11), 343 (MH^+-CH_3Cl, 15), 333 (MH^+-AcOH, 75), 315 (357-CH_2CO, 100), 297 (357-AcOH, 42) and 279 (297-H_2O, 36).

Continued elution gave 98 mg of 19-chloro-4,21-diacetoxypregn-9(11)-en-20-one (**16**), a mixture of isomers at C-4 and C-5, m.p. 165–174°C (ethyl acetate-hexane); λKBr_{max} 5.72 and 5.77 μ; ^{1}H-NMR δ 0.656, 0.670, 0.693 (s, 18-CH_3), 2.032, 2.171, 2.172 (s, 4,21-OAc); MS CI 479 ($MC_2H_5^+$, 8%), 451 (MH^+, 20), 415 (MH^+-HCl, 11), 409 (MH^+-CH_2CO, 8), 391 (MH^+-AcOH, 100), 373 (415-CH_2CO, 33), 355 (391-HCl, 79), 331 (391-AcOH, 62), 313 (373-AcOH, 48) and 295 (355-AcOH, 45).

The next eluted fraction, 289 mg, was a mixture of stereoisomers of 19-chloro-3,4,21-triacetoxypregn-9(11)-en-20-one (**17**), m.p. 217–233°C (ethyl acetate–hexane); λKBr_{max} 5.72 (br) and 5.79 μ.

The final compound eluted was 19-chloro-4α,21-diacetoxy-5α-pregn-9(11)ene-3,20-dione (**18**), 532 mg, m.p. 167–173°C (ethyl acetate-hexane); λKBr_{max} 5.71 and 5.78 μ; ^{1}H -NMR δ 0.677 (s, 18-CH_3), 2.17 (s, 4,21-di-OAc), 3.92 (d, J = 11.5, 19-H), 3.99 (d, J = 11.5, 19-H') (AB), 4.55 (d, J = 16.8, 21-H), 4.72 (d, J = 16.8, 21-H') (AB), 5.10 (d, J = 12.7, 4-H) and 5.48 (brd, J = 6.3, 11-H); MS CI 509 ($MC_3H_5^+$, 1%), 493 ($MC_2H_5^+$, 7), 465 (MH^+, 26), 433 (MH^+-CH_3OH, 15), 423 (MH^+-CH_2CO, 10), 405 (MH^+-AcOH, 100), 387 (423-HCl, 34), 369 (405-HCl, 74), 363 (405-CH_2CO, 27), 345 (405-AcOH, 61), 327 (363-HCl, 59), 309 (345-HCl, 56) and 291 (327-HCl, 22).

RESULTS AND DISCUSSION

Chemistry

The 11β-19-oxido diol **2** [1] was hydrogenated in the presence of Pd-C and the product acetylated. The major product, an inseparable mixture of the four possible dihydro derivatives **5**, isomeric at C-4 and C-5, as judged by the signal of H-4, was obtained by chromatography. In addition, minute amounts of the diacetate **6** and the monoacetate **7** were isolated as well (Fig. 1). Attempts to eliminate acetic acid in **5** with formation of the 4-en-3-one moiety, by treatment with HCl or potassium acetate in hot dimethylformamide, failed. On the other hand, *p*-toluenesulfonic acid in boiling acetic acid brought about elimination with concomitant rearrangement to the unsaturated ketone **8**, exhibiting a maximum at 240 nm with a characteristically low extinction coefficient of 5,800 [3]. The formula **8** is also in full agreement with the NMR signals of C-5, C-6 and H-6: a 4-en-3-one would result in a singlet for H-4 rather than the observed double doublet. Under these reaction conditions the diacetate **4** suffered only partial hydrolysis to **3**, but no rearrangement.

The presence of the 5β,19-cyclopropane ring in **1** offered the possibility of synthesis of 19-substituted compounds; the related 5β,19-cycloandrostane derivative **19** had been reported to react with HCl with scission of the 10,19 bond to give the 5β-chloromethyl derivative **20** [4], while the saturated analog **21** had undergone cleavage of the 5,19 bond with formation of androst-4-ene-3,17-dione (**22**) [3, 4] (Fig. 2).

Since it has been shown that **1** undergoes an acid-catalyzed reaction to form the 11β,19-

epoxide **2** [1], the known 9(11)-dehydro compound **10** [1] was used instead: treatment with warm HCl gave the 19-chloro derivative **13**. A corresponding ring opening in **9** or **10** with HBr, sulfuric or perchloric acid in aqueous ethanol could not be accomplished. Also attempts to substitute the chlorine atom in **13** or **14** with sodium or silver acetate in a variety of solvents were unsatisfactory.

Next the ketone **10** was hydrogenated with Pd-C to furnish the olefin **11** and then the saturated compound **12**. Under these conditions the cyclopropane ring remained intact.

Finally, compound **14** was hydrogenated with Pd–C in an attempt to discern any hydrogen-directing influence of the chloromethyl group. The major product, after acetylation, was the 4α-acetoxy compound **18** ($J_{4\beta\text{-H},\,5\alpha\text{-H}} = 12.7$ Hz), accompanied by the product of extensive hydrogenolysis **15** and two other materials to which the formulae **16** and **17** are assigned.

Displacement of [^{3}H]aldosterone from type 1 receptors in human mononuclear leukocytes

Several of the compounds described above were assayed for their affinity to mineralocorticoid receptors in human mononuclear leukocytes [6–8] (Table 1). All of them exhibited only low levels of activity; the highest relative binding affinity (RBA) was observed for the 19-chloro derivative **13** (0.72%, relative to 100% for aldosterone). The 5-en-4-one **8** was inactive.

Using the same methodology, several steroids of interest were assayed as well. The reduced metabolites of aldosterone (5α-dihydro, 3β,5α-tetrahydro and 3α,5β-tetrahydro) showed a decreasing order of activity, in line with results of other bioassays [15, 25]. 19-Hydroxyaldosterone [16, 10] unexpectedly exhibited a much lower value (0.6%) than 19-noraldosterone [9–11] (20%). 18-Deoxyaldosterone [11–14] and 18-deoxy-19-noraldosterone [11], partial antagonists of aldosterone, had RBA of 5.8% and 4.7%. 18,19-Dihydroxycorticosterone [21, 22] showed no activity, while 18-hydroxy-19-norcorticosterone [18], an enhancer of the mineralocorticoid activity of aldosterone in adrenalectomized rats [19], had very little. The products of facile cyclodehydration of aldosterone and 19-noraldosterone, 18,21-anhydroaldosterone (**24**) (Fig. 3) and 18,21-anhydro-19-noraldosterone (**25**) [17], exhibited weak RBA, of the same order of magnitude as the 5α,6α-dihydroxy derivative of dihydroaldosterone (**23**); nonetheless, this could be of importance if the presence of **24** in biological fluids were established. The polyhydroxy compounds **26** and **27** were inactive. On the other hand, of interest are the relatively high activities of 17-isoaldosterone (**28**) and apoaldosterone (**29**), the products of isomerization of aldosterone under alkaline conditions [23, 24].

In general, the mineralocorticoids listed in Table 1 appear to have lower RBA than could be expected from previous results by other bioassays (adrenalectomized rat and transepithelial ion transport). Possibly the behavior of the mineralocorticoid receptor in isolated mononuclear leukocytes does not reflect entirely the functions of this receptor in other organs. This point has to be investigated in more extensive experiments in the future. Finally, displacement of aldosterone from its receptor in the leukocyte does not, obviously, provide infor-

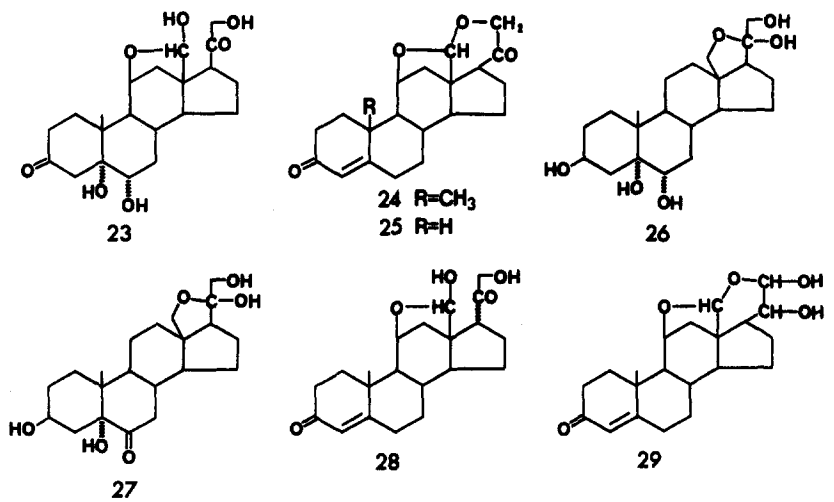

Fig. 3

Table 1. Relative binding affinities (RBA) for aldosterone binding sites in human mononuclear leukocytes

Steroid	RBA (%)
Aldosterone	100
5α-Dihydroaldosterone	1.7
3β,5α-Tetrahydroaldosterone	0.61
3α,5β-Tetrahydroaldosterone	<0.05
19-Hydroxyaldosterone	0.6
19-Noraldosterone	20
3α,5β-Tetrahydro-19-noraldosterone	<0.03
3α,5α-Tetrahydro-19-noraldosterone	<0.03
18-Deoxyaldosterone	5.8
18-Deoxy-19-noraldosterone	4.7
18,19-Dihydroxycorticosterone	<0.03
18-Hydroxy-19-norcorticosterone	0.19
18,21-Anhydroaldosterone (24)	0.37
18,21-Anhydro-19-noraldosterone (25)	0.22
19-Chloro-4,21-dihydroxypregna-4,9(11)-diene-3,20-dione (13)	0.72
3β,21-Diacetoxy-5β,19-cyclopregnane-4,20-dione (12)	0.12
21-Acetoxy-11β,19-epoxypregn-5-ene-4,20-dione (8)	<0.03
5α,6α-Dihydroxy-4,5-dihydroaldosterone (23)*	0.31
3β,5α,6α,18,21-Pentahydroxypregnan-20-one (26)*	<0.03
3β,5α, 18,21-Tetrahydroxypregnane-6,20-dione (27)*	<0.03
17-Isoaldosterone (28)	7.6
Apoaldosterone (29)	4.3

*Hitherto unpublished compound.

mation as to whether the compound tested has mineralocorticoid or antimineralocorticoid properties.

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