# STRUCTURAL ANALYSIS OF 21-HYDROXYPREGN-4-ENE-3,20-DIONE (DEOXYCORTICOSTERONE) DERIVATIVE COMPOUNDS.

## PART I: SYNTHESIS AND CIRCULAR DICHROISM IDENTIFICATION

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ABSTRACT

Deoxycorticosterone (DOC) derivative compounds (DOC, DOC 21-acetate, and 7-mercaptopropionic DOC) have been prepared and purified by high pressure liquid chromatography. Synthesis products have been identified, and three chromophores have been displayed by their $n \rightarrow \Pi^*$ and $\Pi \rightarrow \Pi^*$ dichroic transitions. A normal half-chair conformation is favored in ring A.

## INTRODUCTION

Steroids behaving like haptens, it is necessary to link them to a protein carrier in order to obtain an antigen, and not mask their specific groups (1-2). These imperatives have led us to graft bovine serum albumin (BSA) to steroids in position 7, a position not involved in the differentiation of most important

steroids. As an example, the different steps of the synthesis of the deoxycorticosterone (DOC) are shown in Figure 1.

Therefore, it is essential to know the conformation of ring A, the localization of active functions, and their influence on the structure of the linked compounds DOC-BSA. Two techniques, $^1$H-nuclear magnetic resonance (NMR) spectroscopy and circular dichroism (c.d.), seem especially well indicated to solve this double problem. For this purpose, we shall first describe (part I) the different steps of the elaboration of the 7-mercapto-propionic DOC which has been prepared with a view to obtain a specific conjugated antigen "DOC-BSA". Second, we shall present (part II) the $^1$H-NMR and c.d. spectra and their interpretation.

## MATERIALS AND METHODS

### General

All melting points were uncorrected. They were determined with a Bucchi 510 ( Bucchi, Flawil, Switzerland). NMR spectra were obtained with a Cameca 350-MHz spectrometer (Cameca, Courbevoie, France). Samples were prepared by dissolution of each compound in 8:2 $CDCl_3$-dimethylsulfoxide (DMSO) mixture.

Tetramethylsilane was used as a standard, and all spectra were recorded at ambient temperature. Mass spectra were obtained with a VG 30 F instrument spectrometer (VG Instrument, Manchester, England) in electronic impact (70 eV). Circular dichroism measurements were made at room temperature (25°C) on a Jobin-Yvon IV dichrograph (Jobin-Yvon Instruments S.A., Paris, France) purged with oxygen-free nitrogen. Dichroic spectra were determined in methanol and recorded in the

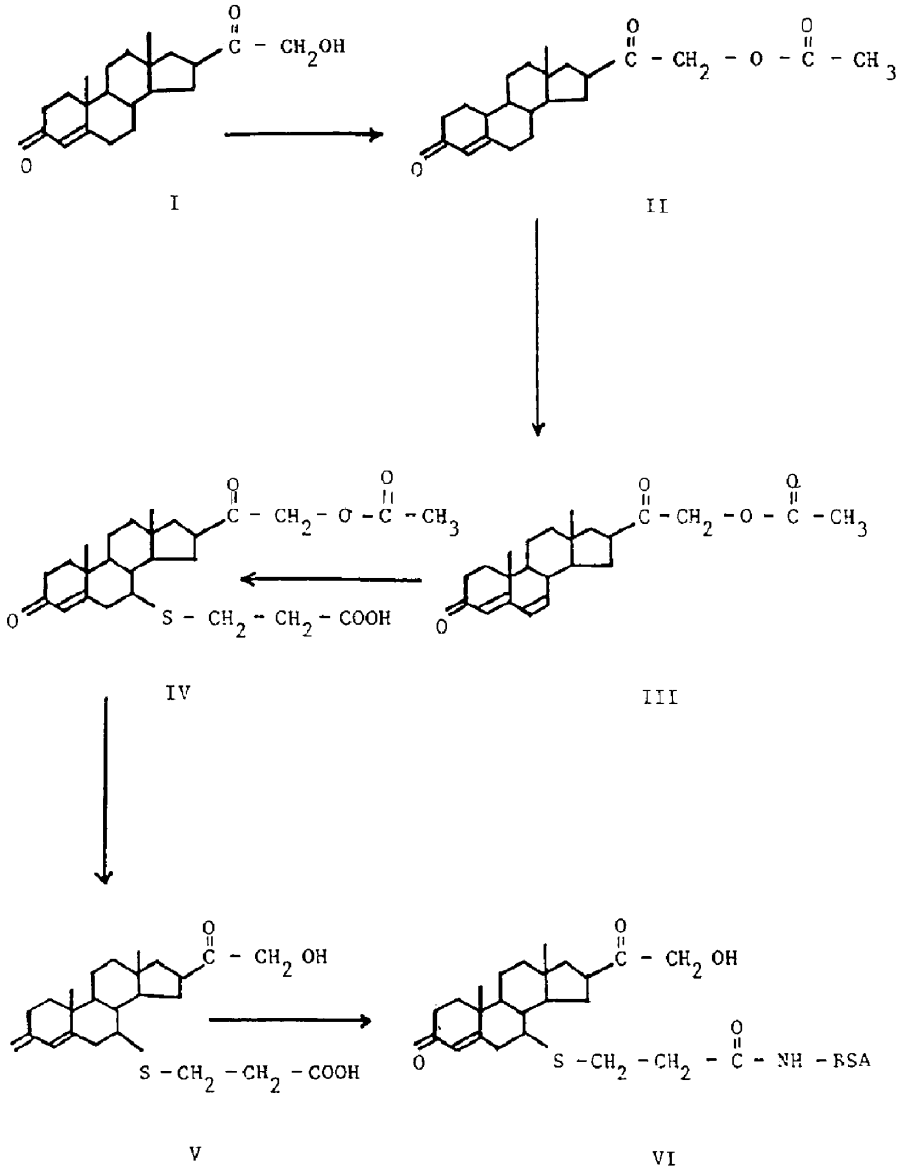



Figure 1. DOC antigen synthesis.

isoandrosterone standard. Solutions with concentration in the range 0.5-0.75 mgxmL$^{-1}$ were examined in Hellma cells (Hellma GmbH & Co., Mülheim, W. Germany) with 1- and 10-mm pathlength.

All reagents were furnished by Merck (Merck, Darmstadt, W. Germany); DOC and DOC acetate were purchased from Steraloids (Steraloids Inc., Wilton, NH, USA).

Synthesis of BSA 7-Mercaptopropionic DOC-conjugated compound, 21-Hydroxypregn-4,6-diene-3,20-dione 21-acetate (III) ( Δ 4-6 DOC 21-acetate)

According to Agnello and Laubach (3), 20 g of 21-acetate DOC with a purity chromatographically verified were added to 32.4 g of parachloranil in 700 mL of tert-butanol and 1.2 mL of $CH_3COOH$. After heating to reflux for 3 h, the insoluble material was removed by filtration. The solvent layer dried under $N_2$ was completely dissolved in 700 mL of $CHCl_3$ and washed three times successively with 70 mL of water, 70 mL of 5% NaOH, and 70 mL of water. The reaction product controlled by ultraviolet (UV) spectrophotometry gave a bathochromic effect (240-283 nm) due to Δ 4-6 double bond. A further control was performed using electron impact mass spectrometry. The $CHCl_3$ layer was evaporated to dryness and the residue recrystallized from acetone-petroleum benzene (40:60).

21-Hydroxypregn-4,6-diene-3,20-dione 21-acetate (III) ( Δ 4-6 DOC 21-acetate).

mp 130-131°C; $\lambda_{max}^{EtOH}$ 284 nm (ε 25,000); NMR δ ($CDCl_3$, DMSO) 6.12 (2H, s, 6-H, 7-H), 5.68 (1H, s, 4-H), 4.8 (2H, m, 21-H), 1.12 (3H, s, 19-Me) 0.76 (3H, s, 18-Me); MS. 70 eV normalized, m/e 370 84%, 355 8.1%, 328 19.5%, 297 54.5%, 269 100%, 173 74.3%.

7α-(2-Carboxyethylthio) 21-hydroxypregn-4-4n4-3,20-dione 21-acetate (IV) (7-Mercaptopropionic DOC 21-acetate)

A mixture of 1.5 g of Δ4-6 DOC 21-acetate, 4.1 mL of piperidine, and 4.1 mL of β-mercaptopropionic acid in 500 mL of anhydrous benzene was heated to reflux during 20 h. The reaction product was identified by both thin layer chromatography on Silicagel $F_{254}$ with the eluent $CH_3Cl/CH_3OH$ (9:1), and by UV spectrometry. A hypsochromic effect was displayed due to the disappearance of the conjugated double bond.

The organic layer was evaporated, and the residue was dissolved with 70 mL of $CHCl_3$ and washed with 20 mL of water. The organic material was extracted by three successive washes with 20 mL of saturated $NaHCO_3$ solution. This extract was acidified by 5 N HCl at 4°C. The precipitated compound was dried and crystallized from $CH_3OH$-$H_2O$.

A new purification was realized by column chromatography using Sephadex LH-20 (length 40 cm, internal diameter 1 cm) with the following eluent: benzene/hexane/ethanol (80:10:7). Mercaptopropionic 21-acetate (IV)(400 mg) was obtained from these previous steps : mp 232°C; $\lambda_{max}^{EtOH}$ 240 nm (ε 18,300); NMR 350 MHz δ 5.7 (1H, s, 4-H), 4.99 (2H, m, 21-H), 1.2 (3H, s, 19-Me), 0.70 (3H, s, 18-Me) ; MS. 70 eV normalized 476 2.4%, 370 78.4%, 355 9.2%, 328 18.3%, 297 49.8%, 269 100%.

Well-known 21-hydorxypregn-4-ene-3,20-dione (I) and 21-hydroxypregn-4-ene-3,20-dione 21-acetate (II) are used as synthesis precursors and their chromatographic purity and physical data were verified for the further NMR analysis (Part II).

Hydrolysis of the 21-Acetate Compound

A mixture of $CH_3OH$ (400 mL) and $KHCO_3$ (5 mL)(190 g/L) and water (14.8 mL) were added to 200 mg of compound (IV) and heated at 37°C for 7 h. Reaction products were evaporated to dryness under $N_2$. The residue was dissolved with 10 mL of water, pH 4 being adjusted with 0.1 N HCN. The organic material was extracted by 50 mL of $CHCl_3$ and the solution dried to obtain the crude product (V), the mercaptopropionic DOC. Purification of all samples was realized by high pressure liquid chromatography (HPLC) with a Spectra Physics SP-8000 apparatus equipped with 1 mL injection of Rheodyne type. A UV iodine detector LKB (LKB, Produkter, Bromma, Sweden) simultaneously working at different wavelengths was used. The compound (V) well retained ( retention time 350 s ) was separated from impurities, normally Δ 4-DOC. The column is a partisil Whatman (Whatman, Maidstone, England) (25/10) with mobile phase $CHCl_3$/EtOH (9:1).

7α-(2-Carboxyethylthio) 21-hydroxypregn-4-ene-3,20-dione (V) (7-Mercaptopropionic DOC)

mp 223°C; $\lambda_{max}^{EtOH}$ 240 nm (ε 17300); NMR δ 5.75 (1H, s, 4-H), 4.18 (2H, m, 21-H), 1.22 (3H, s, 19-Me), 0.68 (3H, s, 18-Me); the complete analysis of NMR spectrum will be described in another paper.

Coupling of 7α - (2-carboxyethylthio)-21-hydroxypregn-4-ene-3,20-dione with BSA

Two solutions were prepared. Solution A: 0.145 mmol of compound (V) was added to 28.4 mg of tributylamine with 1 mL of dioxane and mixed with 0.4 mL of dioxane in 22.4 mL of isobutylchlorocarbonate. Solution B: 20 mg of BSA was dissolved in 5 mL of distilled water, containing 3.6 mL of dioxane and 0.2 mL of 1 N NaOH. This solution was left 30 min at 10°C. Both solutions A and B were stirred 45 min at 10°C. Then 0.99 mL of 1N NaOH was added, and the solution was left for 3 h. The proteic fraction was separated by dialysis vs. distilled water during 12 h.

Circular Dichroism Analysis

Considering its sensitivity in detecting structural alterations (4,5), the c.d. technique is useful as a method for studying conformational analysis. This technique is useful in correlating the configurations of related unsymmetrical molecules and elucidating and following the successive steps of the elaboration of the $\Delta 4$ keto-steroids with an oxogroup in C-20 position. One of the main objectives of the present work is to use simultaneously the helicity rules and the normalized c.d. to display the various conformational phases which control the synthesis. For this purpose, it may be useful here to briefly recollect the basic principles of methods that have already been covered in several papers (6-9).

Concerning the helicity rule, many workers (10-12) have discussed the chiroptical behavior of α,β- unsaturated ketones which exhibit at least three and in some cases four c.d. bands in the region accessible to measurements in solution. Figure 2 shows that the sign of the Cotton effect of the two bands of lowest energy $n \rightarrow \Pi^*$ ($\lambda > 300$ nm) and $\Pi \rightarrow \Pi^*$ ($230 < \lambda < 260$ nm) depends on the chirality of the nonplanar monocyclic or polycyclic enone ring.

The concept of the normalized curves (8,13) is used to standardize results and to emphasize the fact that substances that contain a given chromophore in a similar environment have dichroic absorptions that differ only in amplitude. For a class of compounds such as α,β-unsaturated oxo-3 steroids having several transitions, it is thus possible to obtain a normalized value $\Delta\varepsilon_n$ by dividing considered amplitude by the amplitude at the

principal maximum. This procedure allows us to make use of the intensity and position of the maximum and the form of the c.d. curve and provides us with not only a confirmation of the helicity rule but also a reliable criterion for determining whether or not a structural modification exerts an influence on the chromophore.

Regarding the steroidal structure complexity, the normal octant rule (14) was used only as a control method rather than as a starting point for discussion.

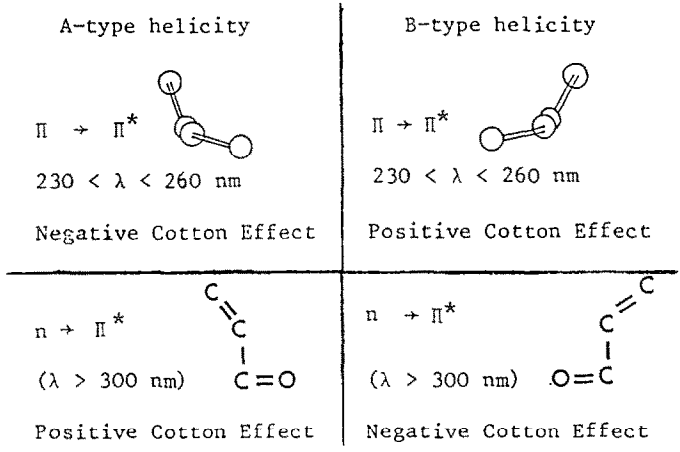



Figure 2. Helicity rule; α,β - unsaturated ketone chirality and Cotton effect sign of the $n \longrightarrow \Pi^*$ and $\Pi \longrightarrow \Pi^*$ transitions.

## RESULTS AND DISCUSSION

The initial compound (I) exihibits an obvious structural analogy with progesterone. To give consistent and homogeneous results , the concordance of the c.d. spectra is verified and is in good agreement with that described by Barret et al (9) in the same experimental conditions . Examination of Table 1 points out the following results:

Table 1. Circular Dichroism Data for the Reference and the Compounds, (I), (II), (III), and (IV)

| Compounds | Transitions | $n \longrightarrow \Pi^*$ | | | $\Pi \longrightarrow \Pi^*$ | | |
|---|---|---|---|---|---|---|---|
| | | $\lambda_{max}$ (nm) | $\Delta\varepsilon$ | $\Delta\varepsilon_n$ | $\lambda_{max}$ (nm) | $\Delta\varepsilon$ | $\Delta\varepsilon_n$ |
| Progesterone (ref. 9) | | 325 | - 1.2 | - 0.3 | 233 | + 8.0 | + 0.77 |
| | | 284 [a] | + 4.0 | + 1.0 | 214 | + 10.3 | + 1.0 |
| this work | | 326 | - 1.1 | - 0.27 | 233 | + 9.0 | + 0.9 |
| | | 286 [a] | + 4.1 | + 1.0 | 216 | + 10.0 | + 1.0 |
| D O C (I) | | 324 | - 1.27 | - 0.28 | 322 | + 8.8 | + 0.9 |
| | | 286 [a] | + 4.4 | + 1.0 | 216 | + 9.0 | - 1.0 |
| D O C 21-Acetate (II) | | 324 | - 1.16 | 0.29 | 233 | - 11.1 | + 1.0 |
| | | 286 | + 4.1 | + 1.0 | 216 | + 8.6 | + 0.78 |
| Δ 4-6 DOC 21-Acetate (III) | | 342 | + 6.0 | + 1.0 | 276 | [b] | [b] |
| | | | | | 264 | [b] | [b] |
| | | | | | 205 | [b] | [b] |
| 7-Mercaptopropionic DOC (V) | | 324 | - 0.49 | - 0.13 | 234 | + 2.3 | - 0.29 |
| | | 286 | + 3.7 | + 1.0 | 216 | + 7.8 | + 1.0 |

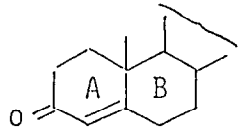
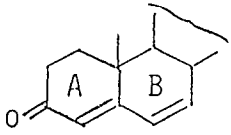
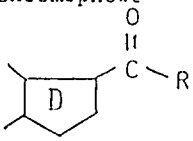
[a] Shoulder near 273 nm.
[b] Unreached maximum.

(a) A negative Cotton effect is observed at the $n \longrightarrow \Pi^*$ transition ($\lambda > 300$ nm) and a positive effect at the $\Pi \longrightarrow \Pi^*$ transition ($230 < \lambda < 260$ nm) for the compounds (I), (II), (VI), and progesterone, which provide a B-type helicity, according to the helicity rule.

(b) The always positive Cotton effect of the 20-oxo $n \longrightarrow \Pi^*$ transition near 284 nm can be related to the normalized dichroism $\Delta\varepsilon_n$. The (I), (II), and progesterone derivatives show a quite constant numerical value ($\Delta\varepsilon_n$ = - 0.30) when the (VI) compound $\Delta\varepsilon_n$ half decreases.

(c) (III) and (IV) dienone compounds present an important single dichroic $n \rightarrow \Pi^*$ band near 342 nm. To aid discussion, all the synthesis derivatives can be classified in three distinct groups according to the location of the chromophore (Table 2).

Table 2. Classification of Steroidal Chromophores

| Transitions / Chromophore | $n \longrightarrow \Pi^*$ | | $\Pi \longrightarrow \Pi^*$ | |
|---|---|---|---|---|
| | $\lambda_{max}$ (nm) | $\Delta\varepsilon$ sign | $\lambda_{max}$ (nm) | $\Delta\varepsilon$ sign |
| A Chromophore   | 324 | - | 233 | + |
| | | | 216 | + |
| (A+B) Chromophore   | 342 | + | 276 | - |
| | | | 264 | - |
| | | | 205 | - |
| D Chromophore   | 284 | + | no band in this wavelength range | |

(i) The A bicyclic chromophore containing the $\alpha, \beta$ - unsaturated ketone appears in (I), (II), and (V).

(ii) The (A+B) bicyclic chromophore containing the dienone group appears in (III) and (IV).

(iii) The D chromophore containing the 20 oxo-group appears in (I), (II), and (V).

A consistent characterization of each chomophore can be limited by the investigation of the $n \longrightarrow \Pi^*$ band, considering the mediocre experimental quality of the measurements under 250 nm ( $\Pi \longrightarrow \Pi^*$ transition).

Therefore, referring to the synthesis synoptic board (Figure 1), it seems easy to note that the chromophore specificity is correlated to both essential steps of the antigen elaboration:

(a) Creating a 6-7 double bond for the (III) in connection with the removal of the chromophore A by the chromophore (A+B).

(b) Adding to $C_7$ the graft link ($\beta$-mercaptopropionic acid) when compound (VI) is elaborated from (IV) and (V); therefore chromophores become again identical with the (I) compound chromophore.

From the observations about the positions of the rings A and B, mainly two possible P, Q conformations become apparent for the DOC compound with the aid of the Dreiding models (Figure 3).

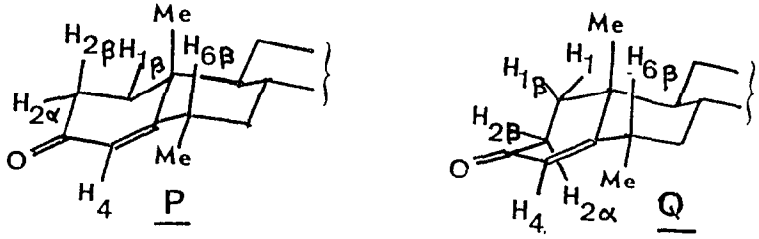



Figure 3. Normal P conformation (A, half-chair; B, chair) and inverted Q conformation.

According to helicity rules (8, 12, 15, 16), the band of the lowest energy ($n \rightarrow \pi^*$ ~ 300 nm) displays a negative c.d. in P conformation and a positive c.d. in the inverted Q conformation. Concerning the intense band ($n \rightarrow \pi^*$ ~240 nm), the Cotton effect is respectively positive and negative as indicated by the octant rule.

Moreover, the c.d. spectrum displays a shoulder near 273 nm only for the compound (II) and progesterone. Consequently, it is convenient to follow the $C_{21}$ acetylation of derivative (I) by a simple examination of the D chromophore dichroic transition.

Concerning the formation of the dienone system in the (III), the important modification of the dichroic behavior can be explained by the coplanarity of the rings A and B.

Lastly, except for the 4,6-diene derivative (III), the c.d. data of DOC and its derivatives are sufficiently similar to progesterone data in establishing the analogy between compounds (I) and (V) in the normal P conformation.

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NOTE

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REFERENCES

1. Berson, A. and Yalow, R.S., NATURE 21, 1648 (1959).
2. Weinstein, A., Linder H.R., Friedlander, A. and Bauminger, S., STEROIDS 20, 789 (1972).
3. Agnello, J.E. and Laubach, G.D., J. AM. CHEM. SOC., 82, 4270 (1960).
4. Blout, E.R. BIOPOLYMERS 1,397 (1964).
5. Ikeda, K. and Hamaguchi H. J. BIOCHEM. 66,513 (1969).
6. Crabbe, P., Optical Rotatory Dispersion and Dichroism in Organic Chemistry, Ed. Holden Day Inc., Oakland, C.A. (1965).
7. Garonski, J., Kiljefors, T. and Norden, B., J. AM. CHEM. SOC 101, 5515 (1979).
8. Burnett, R.D. and Kirk, D.N., J. CHEM. SOC. PERKIN TRANS I, 1460 (1981).
9. Barret, M.W., Farrant, R.D., Kirk, D.N. Mersh, J.D., Sanders, K.M. and Duax, W.L., J. CHEM. SOC. PERKIN TRANS II, 105 (1982).
10. Djerassi, C., Records, R., Bunnenberg, E., Mislow, K. and Moscowitz, A., J. AM. CHEM. SOC., 84, 870 (1962).
11. Whalley, W.B., CHEM. IND. 1024 (1965).
12. Snatzke, G., TETRAHEDRON, 21, 413 (1965).
13. Velluz, L. and Legrand M., ANGEW. CHEM. (ENG) 73, 603 (1961).
14. Moffit, W., Woodward, R.B., Moscowitz, A., Klyne, W. and Djerassi, C., J. AM. CHEM. SOC. 83, 4013 (1961).
15. Bucourt, R., BULL. SOC. CHIM. FRANCE 2080 (1964).
16. Totty, R.N. and Hudec, J., CHEM. COMMUN . 785 (1971).