18-Hydroxy-11-deoxycorticosterone. Chemical Synthesis, Structure, and Circular Dichroism†

M. P. Li, M. K. Birmingham, and T. H. Chan*

Departments of Investigative Medicine, Psychiatry and Chemistry, McGill University, Montreal 110, Quebec, Canada

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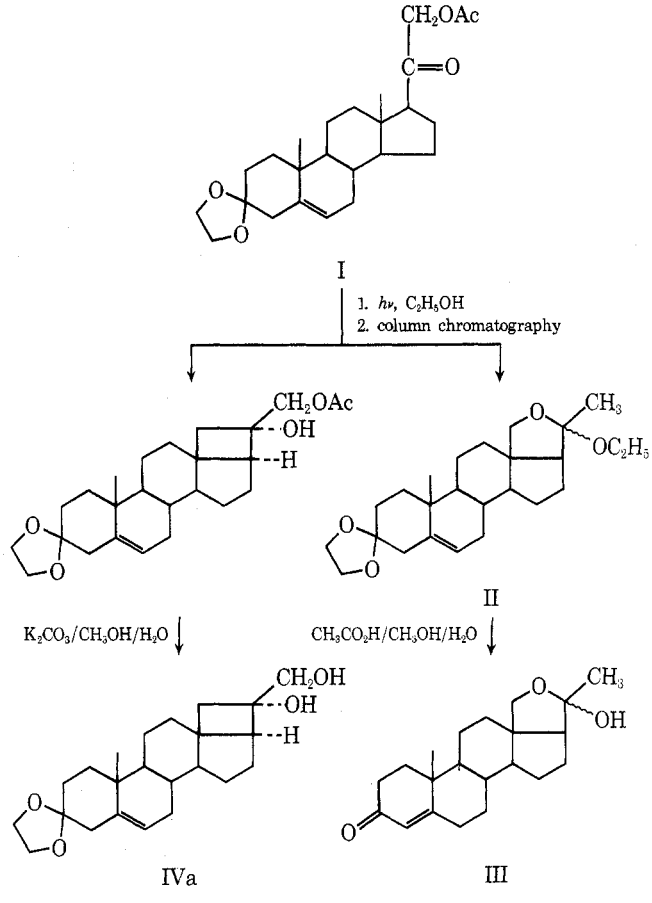
18-Hydroxy-11-deoxycorticosterone (18-OH-DOC) has been prepared in two steps from 18-hydroxyprogesterone in high yield. 18-Hydroxyprogesterone has in turn been prepared in ~20% yield by photolysis of the ethylene ketal of deoxycorticosterone 21-acetate followed by acidic and then basic hydrolysis and column chromatography. The circular dichroic spectra of 18-OH-DOC and related compounds are reported. From the induced circular dichroism of 18-OH-DOC with $Pr(dpm)_3$, it is concluded that 18-OH-DOC has the 20→18-cyclohemiketal structure with C-20 having the *R* configuration.

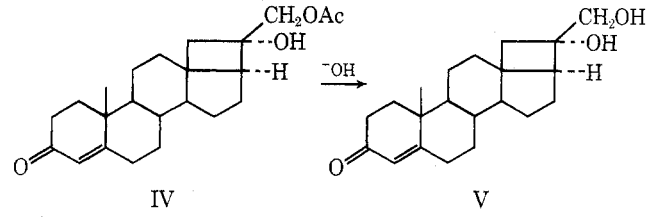
18-Hydroxy-11-deoxycorticosterone (18-OH-DOC) was first isolated and identified as a naturally occurring steroid from incubated sectioned rat adrenal by Birmingham and Ward in 1961[1] and by Péron in the same year.[2] It was characterized to be in the 20→18-cyclohemiketal form. The in vivo secretion of 18-OH-DOC in rats was demonstrated by Cortes et al.[3] The endogenous formation of 18-OH-DOC has also been demonstrated in the camel by Race and Wu in 1964,[4] and in man by Melby and collaborators,[5,6] who isolated and identified 18-OH-DOC from human adrenal vein blood. They obtained levels comparable to those of aldosterone and deoxycorticosterone in normal subjects, and elevated levels in patients suffering from various forms of hypertension, including Cushing's syndrome and essential hypertension.

Because of the possibly important role of 18-OH-DOC in the etiology of essential hypertension,[7] a careful study of the structure and the biological activities of 18-OH-DOC appears to be necessary. To this end, we embarked on a synthesis of 18-OH-DOC so that a reasonable amount of the steroid may be at hand. When we began our synthetic work, the synthesis of 18-OH-DOC had been described by Pappo in 1959 only in a preliminary communication[8] and in U.S. Patents.[9] It involves a 15-step synthesis starting from the alkaloid conessine. This ingenious but long synthesis was, however, not practical in our hands. We decided to adopt a simpler synthesis of 18-OH-DOC starting from 18-hydroxyprogesterone according to Scheme II. This route to 18-OH-DOC has been reported by us in preliminary form.[10] Recently, several groups have reported on similar preparations with varying degrees of success.[11,12] In this paper, we wish to describe in greater detail our synthesis, and some physicochemical studies on 18-OH-DOC and related compounds.

Chemical Synthesis. A. 18-Hydroxyprogesterone. As the immediate precursor for the synthesis of 18-OH-DOC, we chose the structurally similar steroid 18-OH-progesterone (III). It possesses the Δ^4-3-ketone as well as the 20→18-cyclohemiketal structure which can be modified to 18-OH-DOC by functionalizing the 21-methyl group. 18-OH-Progesterone was prepared from the readily available ethylene ketal of deoxycorticosterone 21-acetate (I) by the photochemical method developed by Jeger et al.[13,14] The advantage of this method (Scheme I) is its simplicity and convenience. However, Jeger et al.[13,14] reported a 5% overall yield of III from I, a result which was substantiated by us. We attempted to improve the yield of 18-OH-progesterone by acid hydrolysis of the photolysate directly. However, it was found that under these conditions, another product, IV, was obtained which had nearly the same polarity as 18-OH-progesterone and could only be separated with difficulties. We therefore incorporated another hydrolytic step under alkaline conditions. This resulted in the conversion of IV to the diol V, which can be separated easily from 18-OH-progesterone.

Scheme I. Jeger's Photochemical Reaction of Deoxycorticosterone Acetate Derivative (I)





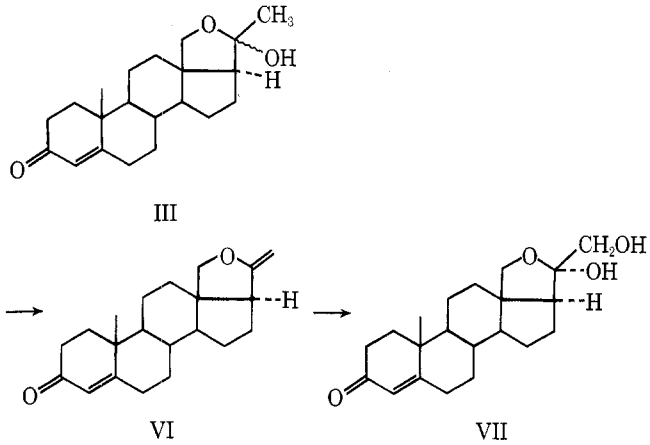
With this modified procedure, 18-OH-progesterone was obtained in 15–24% overall yield from I. The process is thus competitive with other reported syntheses of 18-OH-progesterone[15] in terms of yield.

B. Conversion of 18-Hydroxyprogesterone to 18-OH-DOC. Having prepared 18-OH-progesterone (III), the

† This paper is dedicated to Professor C. A. Winkler of McGill University on the occasion of his 65th birthday by one of his colleagues (T.H.C.).

remaining task was to convert this compound to the enol ether VI by dehydration and thence to the title compound 18-OH-DOC (VII) by hydroxylation.

Scheme II



Phosphorus oxychloride with triethylamine were found to be the reagents of choice for achieving the dehydration of III to IV in quantitative yield. The reaction conditions are extremely critical for the dehydration to be successful (see Experimental Section). It is essential that reagents used should be dried just prior to use and moisture excluded from the reaction. Dehydration of III to VI under basic condition has recently been reported.[11]

When a benzene solution of VI was treated with a solution of osmium tetroxide (1.1 molar equiv) in the presence of pyridine at 0 °C under nitrogen, a rapid reaction (complete in 1 h) occurred. Hydrolysis of the reaction mixture containing the osmate esters under mild conditions at room temperature for 1 h with a mixture of aqueous sodium sulfite and potassium carbonate gave pure, colorless, crystalline 18-OH-DOC (VII, mp 154–156 °C). An analytical sample had mp 165–168 °C, $[\alpha]^{25}D$ +110.3°. A crucial point in obtaining quality 18-OH-DOC in high yield is to evaporate the reaction mixture under reduced pressure at room temperature to total dryness followed by ether extraction of the crude residue. 18-OH-DOC (VII) so obtained was identical with an authentic sample from Pappo[8] by the following criteria: identity of the infrared spectra and the fragmentation patterns of the mass spectra; identical R_f values on TLC and paper chromatograms. 18-OH-DOC (VII) was further characterized by the mobility on paper chromatogram, positive reaction with the Porter–Silber test, negative reaction with the blue tetrazolium test, and mass spectral comparisons with the 18-OH-DOC obtained by incubation with rat adrenals.

Structure of 18-OH-DOC. The structure of 18-OH-DOC was demonstrated by Birmingham and Ward to be in the 20→18-cyclohemiketal form by near infrared.[1] In spite of this there has been confusion in the literature concerning the structure of 18-OH-DOC.[16] For example, Diminguez[17] as well as others have observed that there are two interconvertible forms of 18-OH-DOC during paper chromatography. Authentic, crystalline, and chemically synthesized 18-OH-DOC has been said to exhibit the same interconversion when dissolved in polar solvent for an extended time.[17] Dominguez argued that the possibility that the structure of the crystalline, chemically synthesized compound is the 18-OH-DOC (presumably in the keto form) and that it interconverts to the hemiketal form in solution cannot be entirely ruled out. We wish to reiterate here the known spectroscopic data as well as to present some new circular dichroic data of 18-OH-DOC which permit us to conclude that 18-OH-DOC, either in the

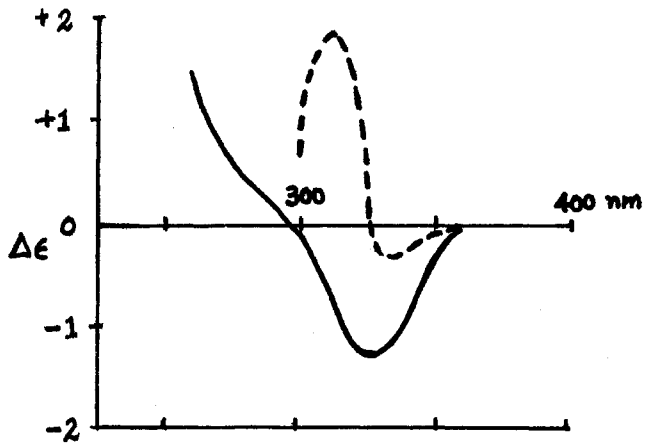
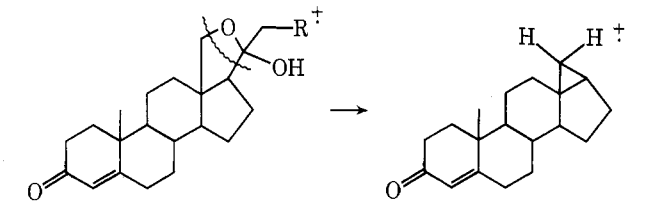


Figure 1. CD of (a) 18-OH-DOC (—); (b) a 1:1 mixture of 2.0×10^{-4} mol of 18-OH-DOC and $Pr(dpm)_3$ in $CHCl_3$ (- - - -).

crystalline state or when dissolved in nonpolar solvent, has the 20→18-cyclohemiketal structure with *R* configuration at C-20. The ir spectrum of crystalline 18-OH-DOC (in KBr) shows peaks at 1660 cm^{-1} for the α,β-unsaturated ketone moiety and absence of absorption in the 1700-cm^{-1} region which is normally expected for the C-20 carbonyl group. This argues for the hemiketal structure for 18-OH-DOC. A similar conclusion has been drawn on the basis of mass spectral fragmentation studies of 18-OH-DOC.[18] We have substantiated this conclusion by comparing the mass spectrum of 18-OH-DOC with that of 18-OH-progesterone. We found that one of the common pathways for both compounds is the cleavage indicated below, thus confirming the cyclic structure in both VII and III.[19] This leaves little room for doubt about the cyclohemiketal structure for 18-OH-DOC in the crystalline state.



III, R = H
VII, R = OH

m/e 270

The solution ir (CH_2Cl_2) spectrum of 18-OH-DOC shows absorption at 1665 cm^{-1} and no peak at 1700 cm^{-1}.[11] This indicates again that in this solvent, the keto form is not present to any significant extent.

We have examined the circular dichroism (CD) of 18-OH-DOC in chloroform (Figure 1a). Figure 1a shows that the CD curve of VII exhibits two Cotton effects: a negative one in the 330-nm region and an intensive positive one around 250 nm. Both transitions can be assigned to the Δ^4-3-keto chromophore. A noteworthy feature of Figure 1a is the absence of Cotton effect in the 290-nm region. This observation is in agreement with the absence of a $n \rightarrow \pi^*$ transition of a saturated ketone in VII. For comparative purposes, the CD curve of deoxycorticosterone acetate (Figure 4) was found to have Cotton effects at 250 nm, 330 nm and 290 nm whereas 18-OH-progesterone (Figure 2a) shows Cotton effects only at 250 and 330 nm.

Recently, Nakanishi et al. developed a method for absolute configurational studies of vicinal glycols.[20] The method consists of measuring the CD of substrate and $Pr(dpm)_3$ (dpm = dipivalomethanato; sometimes called thd = 2,2,6,6-tetramethyl-3,5-heptadionato) dissolved in a dry nonpolar solvent. The solution shows an induced Cotton effect on ca. 300 nm,

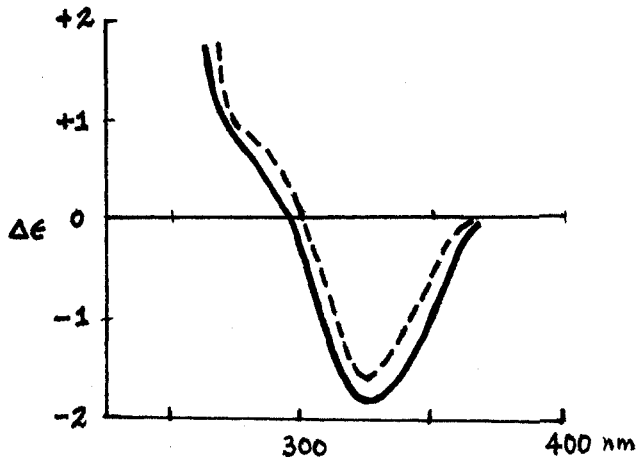


Figure 2. CD of (a) 18-hydroxyprogesterone (—); (b) a 1:1 mixture of 2.0×10^{-4} mol of 18-hydroxyprogesterone and $Pr(dpm)_3$ in $CHCl_3$ (- - - -).

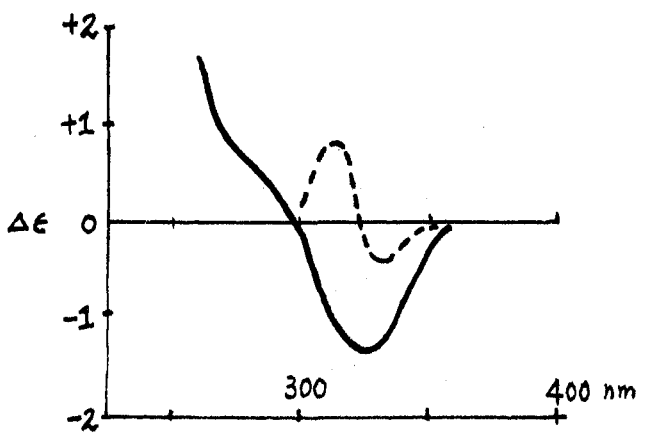
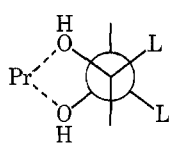


Figure 3. CD of (a) 18,20-cyclo-20,21-dihydroxy-Δ^4-pregnen-3-one (V) (—); (b) a 1:1 mixture of 2.0×10^{-4} mol of V and $Pr(dpm)_3$ in $CHCl_3$ (- - - -).

presumably due to the formation of a bidentate adduct between the glycol and $Pr(dpm)_3$. The sign of the Cotton effect can be related with the chirality of the glycol. It appears to us that the method can serve as a probe for the existence of the glycol moiety. Indeed, when the CD curve of 18-OH-DOC in $CHCl_3$ was taken in the presence of molar quantity of $Pr(dpm)_3$, a change in the Cotton effect at ca. 310 nm was observed (Figure 1b). The change cannot be due to complexation of $Pr(dpm)_3$ either with the enone chromophore or with only one of the hydroxy groups in VII. This is demonstrated by the fact that the CD curve of 18-hydroxyprogesterone is not at all affected by the addition of $Pr(dpm)_3$ (Figure 2b). This confirms therefore the hemiketal structure for 18-OH-DOC in chloroform.

In the case of an acyclic glycol, the origin of the induced CD is assumed to be due to the preferred formation of a complex between the ion Pr and one conformer of the glycol where the bulkier groups are pseudoequatorial in the complex (A). The



A

L = bulkier group

assignment of configuration to an unknown glycol is accomplished essentially by comparing its induced CD with that of

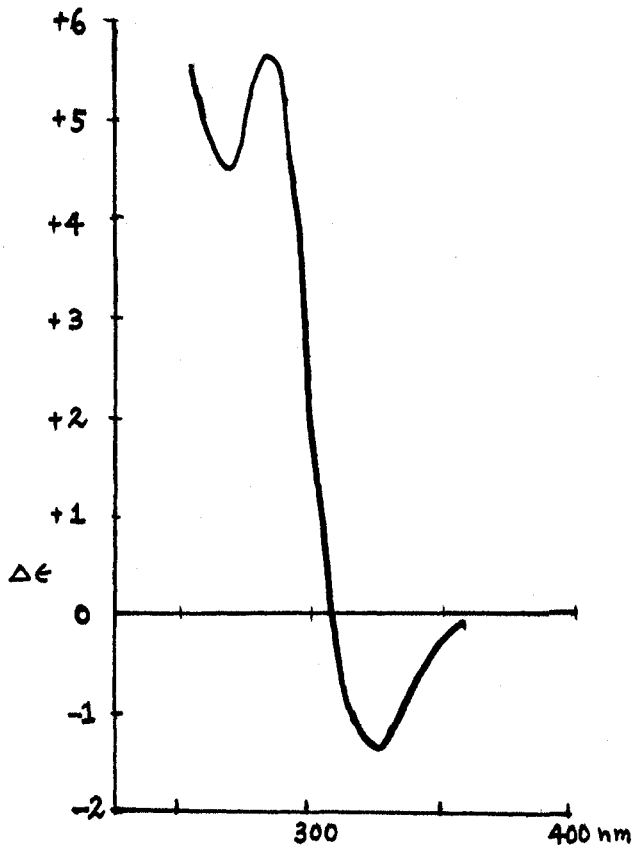
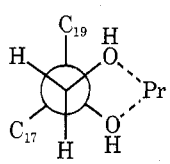


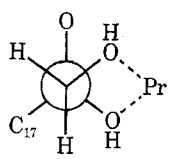
Figure 4. CD of 11-deoxycorticosterone acetate in CH_3OH.

a model compound of known configuration. The success of this method depends then on a judicious choice of model compound. In attempting to interpret the induced CD observed for VII and to derive information about the configuration at C-20, we feel that the 18,20-cyclo compound V can serve admirably as the model for VII. Compound IVa obtained from photolysis has been assigned to have the 20S configuration.[21] The transformation IVa → V establishes the same configuration for C20 in V. The CD curve of V shows the same general features as 18-OH-DOC (Figure 3a). When equal mole of $Pr(dpm)_3$ was added, an induced positive CD was observed (Figure 3b) of the same direction and nearly the same magnitude as that observed for VII. The preferred conformer of the complex of $Pr(dpm)_3$ with V is likely to have the more hindered C-17 pseudoequatorial (B). Since the observed in-



B

duced CD for VII is the same as that of V, it is likely therefore that the complex in VII responsible for the induced CD also has the same chirality with the bulkier C-17 pseudoequatorial (C). This renders the configuration at C-20 of VII as *R*.



C

The conclusion that the chemically synthesized 18-OH-DOC has a C-20 *R* configuration is compatible with stereo-

chemical consideration about the mode of hydroxylation of the enol ether VI. The reagent, osmium tetroxide, would most likely attack the double bond from the less hindered side. Inspection of the molecular model indicates that this is the side opposite to the D ring, thus giving rise to the *R* configuration at C-20.

Experimental Section

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Ir spectra were recorded on a Perkin-Elmer Model 257 and/or Model 337 grating infrared spectrophotometer. Uv spectra were measured with a Unicam SP-800 spectrophotometer using methanol as the solvent, unless otherwise stated. Optical rotations were determined in chloroform with a Perkin-Elmer Model 141 polarimeter using a 1-dm microcell at the sodium D line. Circular dichroism curves were obtained using a Japan Spectroscopic Co. ORD/UV-5 with CD attachment. Mass spectra were obtained on an AEI MS-902 mass spectrometer by a direct probe method at minimum temperatures (165–200 °C) necessary to vaporize the sample. The ionizing energy was kept at 70 eV and the ionizing current at 500 μA. High-resolution mass measurements were made by the peak-matching method using a perfluorokerosene reference. If not otherwise stated, thin layer chromatography (TLC) was carried out routinely using neutral aluminum oxide (Woelm Co.). Column chromatography was carried out using neutral aluminum oxide (Woelm Co.). Paper chromatography was kindly performed by Mrs. H. Traikov at the Allen Memorial Institute of Psychiatry, McGill University. Proportions indicated for solvent mixtures were by volume. The microanalyses were made by Scandinavian Microanalytical Laboratories, Herlev, Denmark, and by Dr. C. Daesslé of Organic Microanalyses, Montreal.

Chemicals and Reagents. Deoxycorticosterone acetate was purchased from Searle Chemicals, Inc., Chicago, Ill. Petroleum ether refers to the fraction of boiling point 30–60 °C and was dried over sodium wire. Phosphorus oxychloride was redistilled just prior to use. Dry tetrahydrofuran, ether, and benzene were obtained by distillation over lithium aluminum hydride or sodium wire. Pyridine and triethylamine were freshly distilled from potassium hydroxide pellets. Solvents were usually removed by rotary evaporation under vacuum at a water-bath temperature of approximately 40 °C. All compounds reported were purified until no impurities could be detected by TLC analysis.

General Procedure for Photolysis and Isolation of 18-Hydroxyprogesterone. An apparatus similar to that previously described by Jeger et al. was used. The light source was a Hanovia 450-W high-pressure mercury lamp (Model 679-A-36) and was fitted with a Corex filter cylinder (1 mm thick, Hanova Model 513-27-114). The lamp was placed in a water-cooled quartz immersion apparatus which was submerged in the irradiation solution. A solution of I (2.08–2.09 g)[22] in absolute ethanol (1.85 l.) was photolyzed for 4 h. After evaporation of the solvent, the photolysate was dissolved in aqueous 70% acetic acid (25 ml) and was heated with stirring at 80–90 °C for 4 h under an atmosphere of nitrogen. The hydrolysis was followed by infrared (the appearance of a strong band near 1680 cm^{-1} for the α,β-unsaturated ketone group) and by ultraviolet (the appearance of an absorption maximum near 240 nm) spectroscopy. The solvent was evaporated under reduced pressure. The product (2.50–2.53 g) was then treated with a mixture of methanol (13 ml), potassium carbonate (1.41 g), and water (13 ml). The resulting solution was stirred under nitrogen at room temperature for 12 h. The process of hydrolysis was followed by infrared spectroscopy (the disappearance of a band near 1740 cm^{-1} for the acetate group). The solvent was removed by azeotropic distillation with benzene (15 ml × 15). The brown, gummy product was taken up in methylene chloride (250 ml), washed with water (5 ml × 4), and dried over anhydrous sodium sulfate, and the solvent was evaporated. The residue was further dried under vacuum for 2–3 h at room temperature. The brown, glassy product (1.50–1.52 g) was dissolved in a minimum volume of benzene and chromatographed over a column (2.5 cm i.d.) of neutral alumina (60 g, Woelm activity I) made up with petroleum ether. Fractions of 100–150 ml each were collected and the progress of chromatography was followed by TLC and by crystallization of each fraction (see Table I).

Identification of Products. 18-Hydroxyprogesterone (III). The identity of III was confirmed by mixed TLC and comparisons of the infrared and mass spectra with those of authentic 18-OH-progesterone.

18,20-Cyclo-20-hydroxy-Δ⁴-pregnen-3-one (IX). This compound was not reported by Jeger in his photolysis experiments.[13,14]

Table I. Products Isolated by Alumina Column Chromatography of the Hydrolyzed Ethanolic Photolysate of I

Fraction	Solvent of elution	Av % yield	Mp, °C	$[\alpha]^{25}D$ ($CHCl_3$)	Structure of compd
1–11	Benzene	4	103–105	+102.9°	VIII
18–57	Benzene, benzene–ether (90:10)	2–4	195–197	+138.1°	IX
67–115	Benzene–ether (85:15), (80:20), (75:25)	15–24	150–154	+152.8°	III
122–145	Ether	17–22	183–187	+128.8°	V

The identity of this compound was secured from spectroscopic investigation as well as comparison with reported physical data. Recrystallization twice from acetone–hexane gave IX with mp 195–197 °C (lit.[23] mp 191–192 °C), $[\alpha]^{25}D$ +138.1° (*c* 0.51, $CHCl_3$) (lit.[23] $[\alpha]D$ +130°).

18,20-Cyclo-20,21-dihydroxy-Δ⁴-pregnen-3-one (V). Recrystallization twice from acetone–hexane gave V as a crystalline solid with mp 183–187 °C, $[\alpha]^{25}D$ +128.8° (*c* 0.51, $CHCl_3$). Its ir spectrum (KBr) showed absorption at 3410, 1650, and 1610 cm^{-1}. The uv spectrum (EtOH) showed λ_{max} 242 nm (ε 16 940). Anal. Calcd for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.20; H, 9.28. Molecular weight calcd, 330.2194; found, 330.2154.

17-Nor-13,17-seco-Δ⁴,¹³⁽¹⁸⁾,¹⁵-androstrien-3-one (VIII). Elution with benzene gave a colorless residue. Crystallization from acetone–hexane gave VIII with mp 103–105 °C (lit.[23] mp 109 °C), $[\alpha]^{25}D$ +102.9° (*c* 0.51, $CHCl_3$) (lit.[23] $[\alpha]D$ +110°).

Conversion of 18-OH-Progesterone to 18-OH-DOC. A. Preparation of 18,20-Epoxy-Δ⁴,²⁰-pregnadien-3-one (VI). All glassware was thoroughly cleaned, oven dried, and stored in a desiccator prior to use. Phosphorus oxychloride, triethylamine, pyridine, and benzene were freshly redistilled. In a 25-ml three-necked flask fitted with a calcium chloride tube and nitrogen-inlet tube was placed 51 mg of 18-OH-progesterone (III, 0.51 mmol), 15 ml of benzene, and 0.9 ml of triethylamine. The flask was capped with a rubber septon. The solution was stirred at room temperature and 46 mg of phosphorus oxychloride (2 molar equiv) was added with a microsyringe through the rubber septon. The addition was repeated at intervals of 1 h until the dehydration reaction was completed. The progress of the reaction was followed by TLC from the disappearance of VI to the formation of a less polar compound XI which could be hydrolyzed back to VI with aqueous 10% acetic acid in a few minutes at room temperature. The reaction was interrupted after 3.5 h (a total of 138 mg of phosphorus oxychloride had been added). The reaction mixture was immediately taken up in benzene (250 ml) containing pyridine (1.5 ml), rapidly washed with cold 10% sodium carbonate solution (5 ml × 1) and cold water (6 ml × 7), and dried over anhydrous sodium sulfate. The dried benzene solution was then carefully concentrated to a small volume (5–10 ml), but never to dryness, with a rotary evaporator under reduced pressure at room temperature. The excess of triethylamine was removed at this stage. The dehydration product XI was homogeneous and free of the starting material VI by TLC analysis.

18-OH-progesterone (III)	Enol ether (VI)	Benzene–ethyl acetate
R_f 0.13	R_f 0.57	8:2
R_f 0.52	R_f 0.70	2:8

It is essential that the enol ether (XI) obtained be osmylated without delay in the next step of synthesis.

B. Preparation of 18-OH-DOC (VII). To a solution of the enol ether (ca. 50 mg) in benzene (8 ml) was added dropwise with stirring at 0 °C under nitrogen a solution of osmium tetroxide (1.1 molar equiv, 45 mg) in benzene (1 ml) containing pyridine (5 drops). The oxidizing reagent was delivered with a syringe through the rubber septon. The reaction was completed in 1 h as indicated by TLC (the disappearance of VI and the formation of a prominent dark spot on the baseline). The mixture was stirred for an additional 1 h. The brown solution was treated with a solution of sodium sulfite (195 mg) and potassium carbonate (310 mg) in water (2 ml), and the resulting mixture stirred at room temperature for 2 h. The hydrolysis was essentially completed

in the first hour as indicated by TLC (the appearance of a single spot with the R_f of VII). The dark brown reaction mixture was evaporated at 30–40 °C under reduced pressure to total dryness with a rotary evaporator. The dark brown residue was taken up in a small volume of water (10 ml) and repeatedly extracted with ether (20 ml × 10). The ether extract was washed with cold water (5 ml × 3) and dried over anhydrous sodium sulfate. A trace of pyridine (1 drop) was added and the ether solution evaporated at 30–40 °C under reduced pressure to afford colorless crystalline 18-OH-DOC (VII, mp 154–158 °C) in 94% yield (50 mg). The crude product was homogeneous as indicated by TLC: R_f 0.23 (SiO_2 G), 0.31 (SiO_2 Eastman) in benzene–ethyl acetate (2:8). Recrystallization twice from acetone–hexane containing a trace of pyridine gave VII, mp 165–168 °C, $[\alpha]^{25}D$ +110.3° (*c* 0.21, $CHCl_3$) {lit.[19] $[\alpha]^{28}D$ +121° (aqueous 70% methanol)}. The infrared and mass spectra of VII were identical with those of an authentic sample of 18-OH-DOC obtained from Pappo.[8] The two samples showed identical mobility on TLC and paper chromatogram.

Pappo[8] pointed out that the melting point of 18-OH-DOC can vary depending on the solvent of crystallization.

Acknowledgments. This work was supported by the Medical and National Research Councils of Canada, the Quebec Heart Foundation, and the Quebec Ministry of Education. The authors thank Dr. J. S. L. Browne for his interest in this research.

Registry No.—I, 13382-00-8; III, 22618-71-6; V, 59055-10-9; VI, 56896-04-9; VII, 59121-45-8; VIII, 59055-11-7; IX, 59055-12-8.

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Determination of the Configuration of the Four D-Benzylpenicilloates

Roger Busson, Paul J. Claes, and Hubert Vanderhaeghe*

Rega Institute, University of Leuven, B-3000 Leuven, Belgium

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The configuration of the four dimethyl D-benzylpenicilloates was determined, and their stereochemical relationship to the dimethyl phthalimidopenicilloates, prepared according to Sheehan et al., was established, using a series of transformations and physicochemical techniques. The compounds designated as β and γ isomers in the Sheehan nomenclature were found to correspond to the γ and δ isomers of "Chemistry of Penicillin", and to have the 5*R*,6*S* and the 5*S*,6*S* configuration, respectively. In the course of this study, 5-epi-6-epibenzylpenicillin methyl ester (γ-**10**) having the 5*S*,6*S* configuration was prepared for the first time. The isomer with the 5*S*,6*R* configuration, which was consequently denoted as δ isomer, was prepared from 5-epibenzylpenicillin methyl ester, and was identical with the β isomer of "Chemistry of Penicillin". It was also established that sodium benzylpenicilloate α isomer, having the 5*R*,6*R* configuration of the natural penicillins, isomerized in aqueous solution to a mixture, containing mainly the δ isomer (5*S*,6*R*).

The first step in the Sheehan synthesis of penicillin[1] is the condensation of *tert*-butyl phthalimidomalonaldehydate (**1**) with D-penicillamine (**2**). When this reaction was performed in alcohol–water, containing sodium acetate, only two of the four possible[2] phthalimidopenicilloates,[3] called α and γ isomers, were formed,[5] the γ isomer being the major component. Using pyridine as solvent, the main product was the α isomer,[6] which has also been obtained by heating the γ isomer in the same solvent.[1] It has been shown[1,5] that the α isomer has the same configuration as the natural penicillins, namely 5*R*,6*R*,[7] whereas the stereochemistry of the γ isomer is unknown. When the *tert*-butyl group of **3b** was removed with acid at about 75 °C, another isomer of unknown configuration, designated as β isomer, was formed. It could be cyclized to a 6-phthalimidopenicillanate (**7**) by treatment with thionyl chloride.[5,8]

During the wartime research on penicillin, four benzylpenicilloates (**5**), designated by the letters α, β, γ, and δ, were obtained.[4] Their configuration, except that of the α isomer, which corresponds to the natural penicillin, is unknown and their relation to the phthalimidopenicilloates (**3**) of Sheehan has not been investigated.

In order to determine the configuration of the γ isomer of **3a**, we transformed this product into a penicillin using the second Sheehan synthesis,[9] which we had applied previously.[6] Hydrazinolysis of γ-**3b**, prepared by methylation of γ-**3a**, yielded γ-**6**, from which the *tert*-butyl group was removed with acid, to give γ-**8a**. This compound was transformed into γ-**8b**, by treatment with triphenylchloromethane, and cyclized with diisopropylcarbodiimide to methyl 6-tritylaminopenicillanate (γ-**9**). From the NMR spectrum a cis configuration of the hydrogen atoms on C-5 and C-6 could be deduced. As this compound was different from methyl (5*R*,6*R*)-tritylaminopenicillanate, prepared from natural 6-aminopenicillanic acid,[9] it can be concluded that the γ isomers have a 5*S*,6*S* stereochemistry. Detritylation of γ-**9** and phenylacetylation