6,7-DIHYDROXY-6,7-DIHYDROCANRENONE ISOMERS: IMPROVED SYNTHESIS AND PROTON NMR STUDY

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ABSTRACT

The synthesis in improved yields of one 6,7-epoxide and three 6,7-dihydroxycanrenone derivatives is described. Canrenone was the starting material for all derivatives and was obtained by acid-catalyzed lactonization of potassium canrenoate. The epoxidation of canrenone to 6α,7α-epoxycanrenone by m-chloroperbenzoic acid was improved by addition of a free radical inhibitor. This epoxide was efficiently cleaved to 6β,7α-dihydroxy-6,7-dihydrocanrenone by perchloric acid in a dioxane-water mixture; 6β,7β- and 6α,7α-dihydroxy-6,7-dihydrocanrenone were obtained by OsO_4 oxidation of canrenone in ether-pyridine and subsequent reduction of the osmates by hydrogen sulfide. The stereochemistry of the products obtained from the reaction of osmium tetroxide with the 6,7-double bond of steroidal 4,6-dien-3-ones has been a controversial issue for some time. A detailed proton-NMR study of the three diol derivatives unequivocally confirmed the proposed stereochemical structure.

INTRODUCTION

Steroidal spirolactonic drugs are aldosterone antagonists clinically used as diuretics or antihypertensive agents. Canrenone is a major metabolic product of the drugs spironolactone and potassium canrenoate (Fig. 1), but pharmacokinetic studies indicate that canrenone can account for only a small part of the antimineralocorticoid activity of its precursors (1). In addition, several investigators have proposed that metabolites of spironolactone, other than canrenone, are important in mediating the biological effects of the parent drug (2). Three dihydroxylated canrenone derivatives have been reported to exist naturally in urine and plasma of man and animal (3). However, their biological activities seem to be different from those of the synthetic spirolactonic drugs. In order to study their action on the biological systems responsible for Na^+ and K^+ balance, these isomers were synthesized.

EXPERIMENTAL

Proton nuclear magnetic resonance spectra (NMR) were obtained on a Bruker WH-270 MHz (Bruker, Karlsruhe, FRG) operating in the FT mode, at a probe tempera-

ture of 27 C with the following conditions: 5.0-μsec pulse width, 2703-Hz sweep width displayed on a 60-cm chart, 16K data points, 3.53-sec repetition rate, between 150 and 400 scans depending on the particular compound studied. The solvent deuterium signal was used as internal lock. Double irradiation experiments were carried out in the gated decoupling mode. Spectral data are reported in δ (ppm) downfield from internal tetramethylsilane, for $CDCl_3$ solutions (s = singlet, d = doublet, dd = double doublet, J = proton coupling constant, $W_{1/2}$ = width of peak at half the height). Infrared spectra were recorded on an FT-IR Mattson instrument, model Cygnus 25 (Mattson Instruments, Madison, WI, USA), (ν = stretching vibration). High resolution - mass spectra (MS) were measured using a Varian MAT 131 HRMS (Varian Assoc., Palo Alto, CA, USA). High performance liquid chromatography (HPLC) was performed on a Waters liquid chromatograph equipped with two Model 6000A pumps, a Model 660 gradient programmer, and a Model 440 absorbance detector set at 254 nm (Waters Assoc., Milford, MA, USA) (k'= capacity factor). Ultraviolet spectra (UV) were taken in methanol with an HP 8450A diode array spectrophotometer (Hewlett Packard, Washington DC, USA). Thin-layer chromatography (TLC) was taken on Merck silica gel 60 F254 plates (5 x 7.5 cm, 0.2 mm thickness). Flash chromatography (4) was performed using silica gel Merck grade 60, 230-400 mesh. Melting points (mp) were measured on a Fisher Digital Melting Point Analyzer Model 355 and are uncorrected. All solvents were analytical grade (Merck or Frutarom). Potassium canrenoate was provided by Farmitalia, Milan, Italy.

Canrenone (I)

Potassium canrenoate (3.98 g, 10 mmol) was stirred for 8 h in a refluxing mixture of chloroform (700 mL) - dilute HCl (pH 1, 500 mL). After cooling, the layers were separated on a separatory funnel. The aqueous phase was washed twice with 100 mL of chloroform, and all the organic phases were combined, washed twice with 200 mL of water, and dried over $MgSO_4$. The organic mixture was filtered and evaporated to yield 5.04 g. This material was dissolved in a minimal amount of boiling ethyl acetate and placed in a cold room (4 C) for 2 days. On standing, pale yellow crystals of canrenone separated (2.91 g, 86%) with mp 162-164 C (lit (5) 149-151 C). TLC on silica (ethyl acetate) revealed (by UV absorption at 254 nm) a single spot with Rf 0.47 vs Rf 0.07 for the starting material. UV: λ_{max} = 283 nm (ϵ = 28,000). IR: 3020 cm^{-1} (ν =C-H), 2966-2875 (ν -C-H), 1771 (C=O, γ-lactone), 1660 (ν C=O, 3-keto), 1652 and 1619 (C=C, conjugated diene at C-4,5 and 6,7). NMR: δ 1.039 ppm (s, 3H, C18), 1.135 (s, 3H, C19), 5.688 (s, 1H, C4), 6.116 [AB system. δ_A = 6.146 (dd, J_1 =9.9 Hz, J_2 =2.1 Hz, 1H, C7), δ_B = 6.086 (dd, J1=9.9 Hz, J2=0.6 Hz, 1H, C6)]. MS (6): m/z 340.204 (100%, M+), 325 (19%, M-CH3), 267 (55%, M-lactonic ring), and 162 (15%, rings A+B).

6α,7α-Epoxycanrenone (II)

Canrenone (I, 600 mg, 1.76 mmol) was dissolved in 10 mL chloroform, mixed with m-chloroperbenzoic acid (393 mg, 1.94 mmol of 85% peracid in 10 mL chloroform) and a catalytic amount (20 mg) of 2,6-di-t-butyl-4-methylphenol (7). The mixture was refluxed and the disappearance of oxidant reagent was followed by testing with acidic starch-iodide paper. After 100 min the reaction was stopped by shaking the solution with an aqueous solution of 10% KI. The layers were separated, and the organic layer was washed three times with brine. It was dried over $MgSO_4$ and the solvent was evaporated. The dry residue was dissolved in a minimal volume of a solution of 20% hexane in ethyl acetate and flash chromatographed on 90 mL silica eluting with the same solvent mixture. By col-

lecting the fractions containing pure epoxide II and evaporating the solvents, 340 mg (53% yield) were obtained. TLC on silica with 20% hexane in ethyl acetate, Rf 0.38, UV visualization. NMR: δ 0.997 (s, 3H, C18), 1.115 (s, 3H, C19), 3.437 (AB system, δ_A=3.51, δ_B=3.37, J=3.6 Hz, 2H, C6,7), and 6.12 (s, 1H, C4). UV: λ_{max} = 240 nm (ϵ = 18,500).

6β,7α-Dihydroxy-6,7-dihydrocanrenone (III)

Epoxide II (298 mg, 0.84 mmol) was dissolved in 5 mL dioxane + 3 mL water, stirred at room temperature with 2 mL of 70% perchloric acid for 15 min, and then refluxed for 10 additional min. After cooling, 10 mL water and 40 mL chloroform were added, then layers were shaken. The aqueous layer was washed with 10 mL of chloroform, and the combined organic phases were washed with 10-mL portions of water until the pH was 7. This organic phase was dried over magnesium sulfate, filtered, and the solvents were evaporated to yield 320 mg of residue. This material was fractionated on a flash chromatography column filled with 90 mL silica and eluted with ethyl acetate. The starting material (70 mg, 77% conversion) and 150 mg (63% yield) of pure diol III were obtained. UV: λ_{max} = 237 nm (ϵ = 16,200). TLC with methylene chloride-hexane-acetonitrile 1:1:1, Rf 0.13. NMR: δ 1.004 ppm (s, 3H, C18), 1.362 (s, 3H, C19), 3.850 (dd, J_1=3.0 Hz, J_2=2.1 Hz, 1H, C7), 4.129 (d, J=3.0 Hz, 1H, C6), and 5.838 (s, 1H, C4). IR: 3425 cm^{-1} (broad, ν O-H), 2958-2920 (ν-C-H), 1758 (ν C=O, γ-lactone), and 1663 (ν C=O, 3-keto).

6β,7β-(IV) and 6α,7α-Dihydroxy-6,7-dihydrocanrenone (V)

Canrenone (I, 1.37 g, 4 mmol) and osmium tetroxide (1 g, 3.93 mmol) were mixed in ethyl ether (25 mL) and pyridine (1 mL) at room temperature for 24 h. The mixture, including a reddish precipitate which formed overnight, was transferred to a larger flask, washed with methanol, and the black solution was saturated with hydrogen sulfide gas. The black precipitate was filtered twice (first through a #3 sintered glass funnel and then through a #5 funnel). The clear yellow solution was then concentrated and the remaining solution was extracted with acidified water-chloroform to eliminate the pyridine. Each layer was washed twice with the other solvent. All the chloroform solutions were combined and dried over $MgSO_4$. The solvents were evaporated, and the residual mixture was separated by flash chromatography on silica gel (100 mL) with 20% hexane in ethyl acetate (1 L), ethyl acetate (100 mL), and 20% methanol in ethyl acetate (100 mL); 24-mL fractions were collected. Fractions 16-31 had pure 6β,7β-diol (IV, 520 mg, 35% yield) and fractions 38-45 had pure 6α,7α-diol (V, 150 mg, 10% yield). No tetraol was detected. Upon solution in a minimal amount of methylene chloride and precipitation with hexane, crystals were obtained from both diols. 6β,7β-Diol (IV): mp 216-219 C; lit (3) 207-210 C. TLC with ethyl acetate, Rf 0.23 and with hexane-acetonitrile-methylene chloride 1:1:1, Rf 0.19. In HPLC, on an SI 100 column 250x4 mm, 5 μ particle size, by isocratic elution with acetonitrile-hexane-methylene chloride (60:20:20) at 1.2 mL/min, the diol IV (detected by absorbance at 254 nm) had a capacity factor k' =2.10. In the same conditions, but eluting with 20% hexane in ethyl acetate k'=3.8. UV: λ_{max} = 239 nm (ϵ = 16,300). IR: 3450 cm-1 (broad, ν O-H), 2940-2860 (ν -C-H), 1767 (ν C=O, γ-lactone), and 1678 (ν C=O, 3-keto). NMR: δ 1.020 ppm (s, 3H, C18), 1.376 (s, 3H, C19), 3.392 (dd, J_1 =10 Hz, J_2 =3.5 Hz, 1H, C7), 4.241 (d, J=3.5 Hz, 1H, C6), and 5.889 (s, 1H, C4). 6α,7α-Diol (V): mp 270-273 with decomposition; lit (3) 228-234 C. UV: λ_{max} = 239 nm (ϵ = 16,400). TLC with ethyl acetate, Rf 0.15 and with hexane-acetonitrile-methylene chloride 1:1:1, Rf 0.10. IR: 3430 cm-1 (broad, ν O-H), 2940-2860 (ν-C-H), 1762 (ν C=O, γ-lactone), and 1660 (ν C=O, 3-keto). NMR: δ 0.987 ppm (s, 3H, C18), 1.217 (s, 3H, C19), 3.990 (broad, $W_{1/2}$

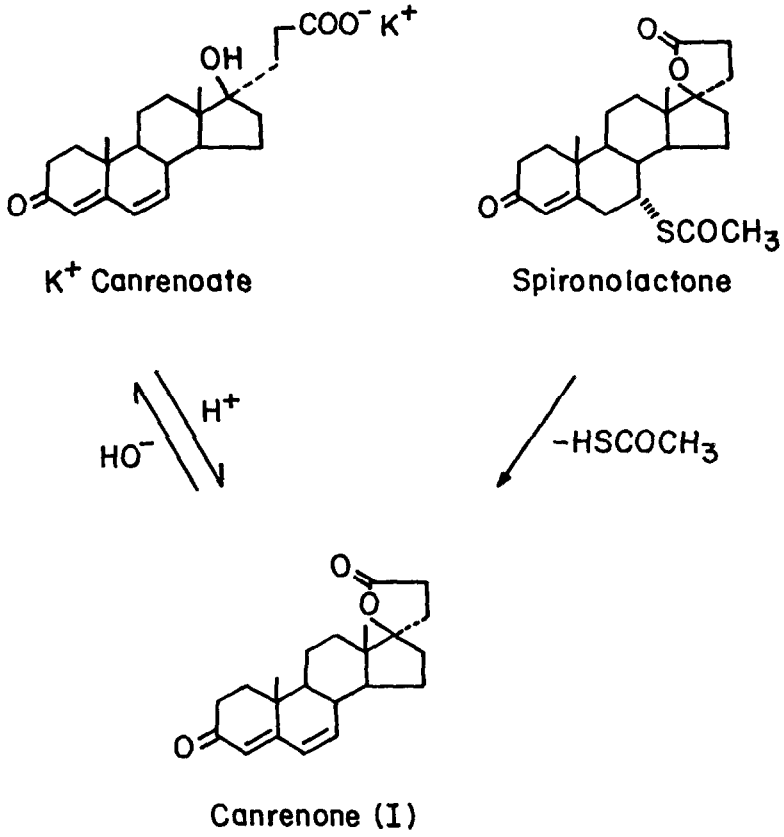

Figure 1. Simplified scheme of canrenone formation from potassium canrenoate and spironolactone.

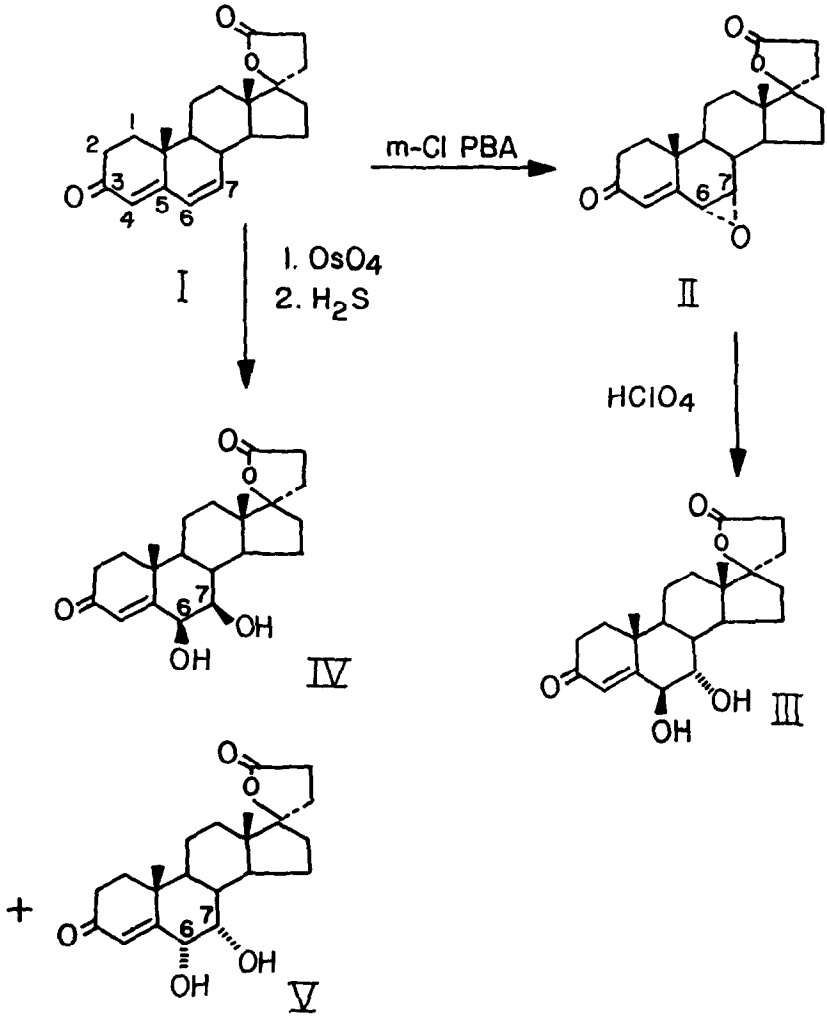

Figure 2. Scheme for synthesis of one epoxy-(II) and three diol-derivatives (III, IV, and V) of canrenone.

=7 Hz, 1H, C7), 4.335 (dd, J_1 =3.6 Hz, J_2=1.5 Hz, 1H, C6), and 6.229 (d, J =1.5 Hz, 1H, C4).

RESULTS AND DISCUSSION

Synthesis

This report describes the synthesis of four canrenone derivatives, starting from canrenone (I) (Fig. 2), which was obtained from potassium canrenoate by lactonization at pH 1, of the carboxyl at carbon-21 with the 17β-hydroxyl group, as described in the left part of Figure 1. 6α,7α-Epoxycanrenone (II) was obtained by epoxidation of canrenone. Aromatic peracids, such as m-chloroperbenzoic acid, are well known reagents which selectively epoxidize steroidal 4,6-dien-3-ones at position 6,7 from the α-side (8), but we have found that the reaction proceeds faster and in better yields when it is conducted in boiling chloroform (61 C). However, under these conditions, the peracid decomposes rapidly, but this can be overcome by adding a free radical inhibitor such as 2,6-di-t-butyl-4-methylphenol (7). The reaction was carried out for 100 min, and the pure epoxide was obtained in 53% yield by flash chromatography.

6β,7α-Dihydroxy-6,7-dihydrocanrenone (III) was obtained by hydrolytic cleavage of epoxide II. Genard and co-workers (3) carried out this step with periodic acid dihydrate in a mixture of acetone-water (5% yield). In the present study, treatment of epoxide II with perchloric acid in dioxane-water successfully achieved the intended transformation, avoiding by-products resulting from the fission of C-C bonds, and raised the yield to 63%.

An efficient procedure for obtaining cis,vic-diols directly from olefins has been the subject of investigation for many years. In small scale reactions, oxidation with stoichiometric quantities of OsO_4 in the presence of pyridine is undoubtedly the method of choice (9). The 6β,7β- and 6α,7α-diols (IV and V, respectively) were obtained on a two-step reaction by oxidation of canrenone (I) with osmium tetroxide followed by reduction of the resulting osmates with hydrogen sulfide. The yields of dihydroxylated derivatives were 35% (IV) and 10% (V) respectively, both of them obtained purer than those reported previously, as judged from the higher melting points (3).

All of the syntheses reported here were repeated on a 10-g scale with very similar results, which attests to the reproducibility of these procedures, in contrast to other reports (3). However, the large scale prepara-

tion requires that the duration of the reflux step of acidic cleavage of epoxide II be kept to a minimum. Prolongation of this step leads to decomposition of the products.

The results obtained on the interaction with amiloride-blockable sodium channels (Garty H, Tal DM and Karlish SJD, in preparation) and with renal Na,K-ATPase (Tal DM and Karlish SJD, in preparation) will be submitted for publication elsewhere.

NMR Analysis

The relevant data for decoupling studies obtained from the spectra of the three diols III, IV, and V are presented in Table 1.

From the resonance of both axial methyl groups (C18 and C19), we assigned the one at higher field to C18 since it is almost unaffected by the stereochemistry of the distant groups at C6 and C7; it resonates always around 1.00 ppm (Fig. 3).

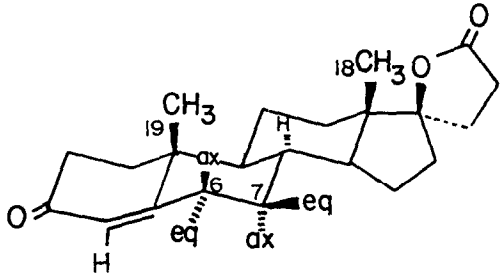

Figure 3. Steric representation of a model for canrenone derivatives.

Methyl-19 is more affected by the hydroxyl groups, especially by that at C6. When H6 is axial (6α-OH), methyl-19 appears at 1.22 ppm, but when H6 is equatorial, it is deshielded by the axial hydroxyl lying on the β-side of the molecule to D 1.36-1.38 ppm. This difference of 0.02 ppm is probably accounted for by the different stereochemistry at C7 between 6β,7β-$(OH)_2$ and 6β,7α-$(OH)_2$. The absorbance at the highest ppm value was attributed to the hydrogen at C4 (5.84-6.23 ppm) because it is vinylic, and to the lack or small degree of coupling with its neighbors. Nevertheless, its chemical shift depends very much on the orientation of the substituent at C6; when the hydroxyl group is equatorial (6α-OH), H4 is split to a small doublet at 6.23 ppm, while in the C6-epimer, H4 is a singlet resonating at 5.84-5.89 ppm. This small difference of 0.05 ppm is due to

Table 1
Proton Chemical Shifts Affected
by Irradiation for Decoupling

Position Compound	C4	C6	C7
6β,7β-$(OH)_2$	5.889 (s)	4.241 (d;J=3.5)	3.392 (dd;J1=10,J2=3.5)
	"	4.24 (s)	irradiated
	"	irradiated	3.392 (d; J=10)
6β,7α-$(OH)_2$	5.838 (s)	4.129 d;J+3.0)	3.850 dd; J1=3.0,J2=2.1
	"	irradiated	3.850 (d; J=2.2)
	"	4.129 (s)	irradiated
6α,7α-$(OH)_2$	6.229 d;J=1.5 6.229 (s)	4.335 dd;J1=3.6,J2=2.1 irradiated	3.990 broad, $W_{½}$ =7Hz 3.990 (s,$W_{½}$ =3.6Hz)

The values were obtained on a Bruker WH-270 MHz operating in the FT mode and are reported in δ(ppm) downfield from internal tetramethylsilane, for $CDCl_3$ solutions. In parenthesis is the splitting of the signal: s=singlet, d=doublet, dd=double doublet, J=splitting constant (in Hz), and $W_{1/2}$ = peak width at half the height.

different configurations at the more distant C7 position. The spectra obtained for these compounds while irradiating protons 6 or 7, thus decoupling the adjacent protons, helped us in the assignment of proton-chemical shift and their respective splitting. From these splitting constants we can learn about the dihedral angles between them. Whenever protons 6 and 7 are ax-eq (6α,7α-$(OH)_2$) or eq-ax (6β,7β-$(OH)_2$), the splitting constant is ca. 3.5 Hz. (For the 6α,7α-isomer this value is taken from the larger splitting at C6, or at C7 by calculation, assuming that $W_{1/2}$ is a good estimate to its splitting.) For the diaxial diol 6β,7α-$(OH)_2$ the splitting of the diequatorial protons is somewhat smaller, 3.0 Hz. In addition, H7 is split by H8 (β, axial). For compound IV the large split (J = 10 Hz) is explained by a dihedral angle of ca. 180°, ax-ax. In the stereoisomers III and V, the splitting constant is much smaller (2.2 and ca. 3.6 Hz, respectively) since the 7,8 protons are in eq-ax configuration.

This NMR assignment and determination of structure confirms unequivocally the proposed stereochemistry (3) and unambiguously demonstrates that the β-side is preferred for attack of osmium tetroxide at the 6,7-double bond of steroidal 4,6-dien-3-ones, which has been a controversial issue for some time (10,11).

In conclusion, the described synthetic procedures afford a satisfactory alternative to the known syntheses by producing these biologically interesting compounds in higher overall yields, purity, and reproducibility. The proton NMR study elucidates their structures.

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APPENDIX

Canrenone= 3-Oxopregna-4,6-diene-21,17β-carbolactone
6α,7α-Epoxycanrenone= 6α,7α-Epoxy-3-oxo-4-pregnene-21,17β-carbolactone
6β,7α-Dihydroxy-6,7-dihydrocanrenone=
6β,7α-Dihydroxy-3-oxo-4-pregnene-21,17β-carbolactone
6β,7β-Dihydroxy-6,7-dihydrocanrenone=
6β,7β-Dihydroxy-3-oxo-4-pregnene-21,17β-carbolactone
6α,7α-Dihydroxy-6,7-dihydrocanrenone=
6α,7α-Dihydroxy-3-oxo-4-pregnene-21,17β-carbolactone

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