SYNTHESIS OF STEROIDAL 17 β-CARBOXAMIDE DERIVATIVES

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SUMMARY

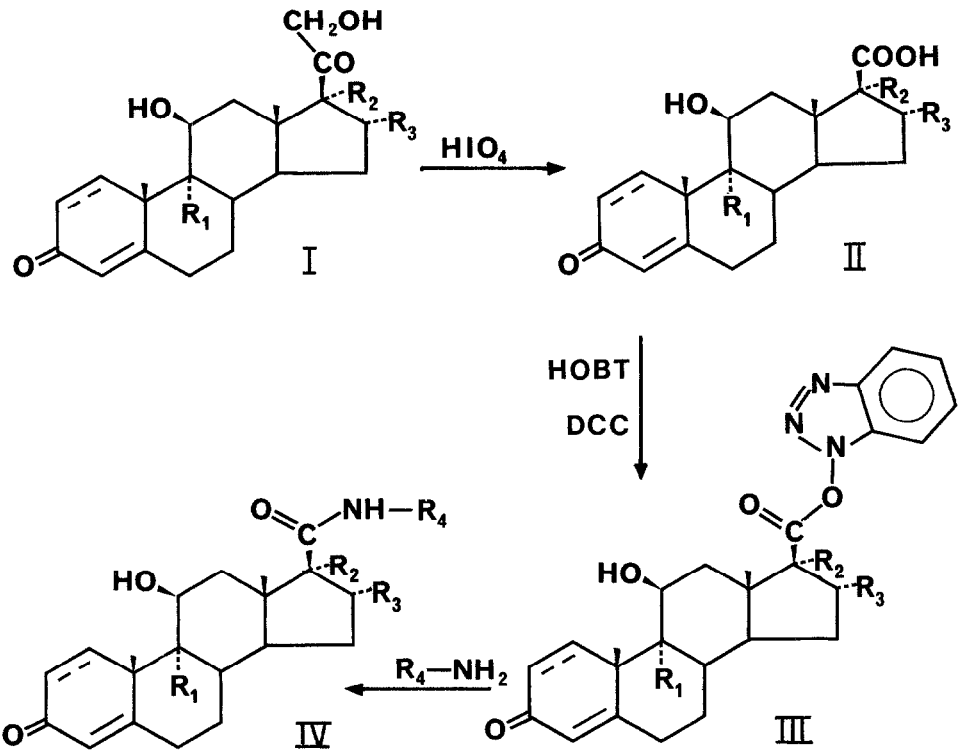
Several 17 β-carboxamide derivatives of natural and fluorinated glucocorticoids have been synthesized. The 17 β-carboxylic derivatives were obtained by periodic acid oxidation of their side chains. They were then activated by N-hydroxybenzotriazole (HOBT) and coupled to several primary amines. Using this method eleven 17 β-carboxamide derivatives have been prepared in good yields.

The biological activity of 17 β-carboxamide derivatives has to date received little attention. However, the purification of human transcortin by an affinity matrix containing a carboxamide derivative of 11 β-hydroxy-4-androstene-17 β- carboxylic acid (IIa) [1] suggested that similar compounds might have good affinity for glucocorticoid binding proteins and could be useful in studying the relationship between steroid structure and activity. In this report we describe the synthesis of several 17 β-carboxamide derivatives obtained from both natural and synthetic glucocorticoids.

RESULTS AND DISCUSSION

17β-carboxylic derivatives of corticosterone (IIa), cortisol (IIb), desoximetasone (IIc) and dexamethasone (IId) were easily obtained by periodic acid oxidation [2,3,4].

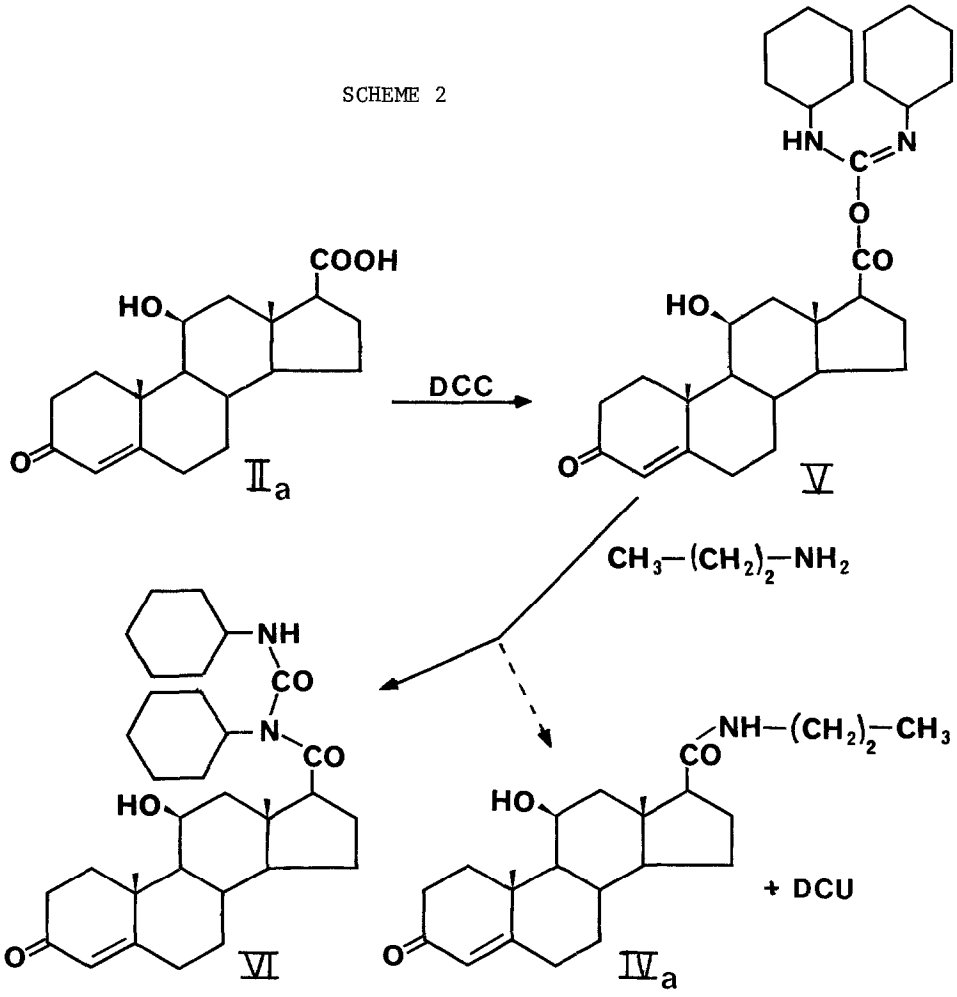
Activation of the acid (II) with N-hydroxybenzotriazole (HOBT) was carried out in the presence of N,N'-dicyclohexylcarbodiimide (DCC)



Compound	1 ene	R_1	R_2	R_3	R_4
Ia	–	H	H	H	
IIa	–	H	H	H	
IIIa	–	H	H	H	
IVa	–	H	H	H	$(CH_2)_2-CH_3$
Ib	–	H	OH	H	
IIb	–	H	OH	H	
IVb	–	H	OH	H	$(CH_2)_2-CH_3$
Ic	+	F	H	CH_3	
IIc	+	F	H	CH_3	
IVc	+	F	H	CH_3	$(CH_2)_2-CH_3$
Id	+	F	OH	CH_3	
IId	+	F	OH	CH_3	
IVd	+	F	OH	CH_3	CH_3
IVe	+	F	OH	CH_3	$(CH_2)_2-CH_3$
IVf	+	F	OH	CH_3	$(CH_2)_5-CH_3$
IVg	+	F	OH	CH_3	$(CH_2)_8-CH_3$
IVh	+	F	OH	CH_3	$CH_2=CH-(CH_3)_2$
IVi	+	F	OH	CH_3	$(CH_2)_2-NH-(CH_2)_2-CH_3$
IVj	+	F	OH	CH_3	$CH_2-C_6H_5$
IVk	+	F	OH	CH_3	$(CH_2)_2-C_6H_5$

Subsequent formation of the amide occured at a rate dependant on the nature of the amine and was usually complete after 12 to 24 hours at 4°. Products were obtained in 60 to 75 % yield. Excess amine and overly-high temperature must be avoided to prevent formation of C_3 enamine.

SCHEME 2



to yield the activated ester (III). This product was then coupled with different amines to produce the desired amides (IV).

Originally DCC alone was used as a coupling reagent (scheme 2), but with compound IIa and propylamine, we were unable to obtain the expected 17β-carboxamide derivative (IVa). When the reaction was carried out in various solvents (methylene chloride, tetrahydrofuran, dioxane) the reaction led predominantly to formation of the N-acylurea (VI) without any precipitation of dicyclohexylurea (DCU). However, in methylene chloride the compound (IVa) was obtained in very poor yield and its formation was preceded by the appearance of a transitory compound observed on silica gel TLC plates. This compound had an R_F less than that of (VI). Because of the rapid disappearance of this compound in the presence of propylamine concurrent with the appearance of IVa, we tentatively identified it to be the O-acylurea (V) or the anhydride of the acid (IIa) [5]. Furthermore after several hours at 4° and/or in the presence of excess propylamine, the solution turned yellow and evolved a characteristic odor. This is probably because the 3 keto group reacted with the amine to give an enamine [6].

The failure of the DCC method led us to investigate the utility of N-hydroxybenzotriazole (HOBT) as a coupling reagent. König and Geiger [7] first proposed the use of HOBT as a suitable additive in the DCC method for peptide synthesis. In that case, HOBT prevented formation of N-acylurea and allowed good yields. We adopted their two step procedure.

Activation of the carboxylic steroid (II) was first performed in the presence of a slight excess of DCC and HOBT and followed by TLC. The transitory spot, very likely the O-acylurea (V) or anhydride of the acid (II) appeared immediately and rapidly diminished as the activated ester (III) appeared.N-acylurea (VI) was never obtained.

The reaction was complete within few hours. After filtration of the DCU precipitate, a stoichiometric amount of amine was added.

Thus, the DCC/HOBT method proved to be very suitable for coupling carboxylic steroids with primary amines and allowed us to prepare eleven 17 β-carboxamide derivatives. The structural, physicochemical and biological properties of these compounds are now under study. We [8] have recently reported that 17 β-carboxamide steroids represent a new class of glucocorticoid antagonists.

EXPERIMENTAL

Infrared spectra were recorded on a Beckman Acculab 1 spectrophotometer. Thin layer chromatograms were run on plates precoated with silica gel F_{254} 0,25 mm (Merck). Two solvent systems were used ; E_1 : methylene chloride-acetone (8 : 1) and E_2 : chloroform-methanol (4:1) in saturated ammonia. Melting points were determined on a Reichert apparatus and are reported uncorrected. Elemental analyses were performed by C.N.R.S. Villeurbanne. Mass spectra were recorded on a Riber Mag 10-10 spectrograph by Université des Sciences de Lille. Steroids were purchased from Steraloids (corticosterone, cortisol) and Roussel UCLAF (dexamethasone, desoxymetasone).

N-(11β-hydroxy-3-oxo-4-androstene-17β-carbonyl)-N,N'-dicyclohexylurea (VI)

To a suspension of 332 mg (1 mM) of the acid (IIa) in 50 ml of methylene chloride,was added 227 mg (1.1 mM) of DCC. After one hour at room temperature, the clear solution was concentrated under reduced pressure. The crude product containing (VI) was dissolved in ethyl acetate and extracted twice with 0.1 M sodium bicarbonate, 1 N HCl and water. The ethyl acetate solution, after drying over anhydrous Na_2SO_4, was evaporated to yield 310 mg (57%) of the N-acyl urea (VI) : mp 232°C; R_F (E_1) 0.43 ; ir(KBr) 1710 (CO N CO NH, [9]), 1660 (3 CO), 1635 (CO N CO NH), 1540 (amide II) cm^{-1}.
Anal. Calcd for $C_{33}H_{50}N_2O_4$: C, 73.56, H, 9.36 ; N, 5.20.
Found : C, 72.86 ; H, 9.02 ; N, 5.10.

Benzotriazol-1'-yl 11β-hydroxy-3-oxo-4-androstene-17β-carboxylate (IIIa)

To a solution of 332 mg (1 mM) of the acid (IIa) in 150 ml of tetrahydrofuran were added 227 mg (1.1 mM) of DCC and 203 mg (1.5 mM) of HOBT. After one night at 4°C, the solvent was evaporated to dryness. The crude product was chromatographed in 8 : 1 methylene-chloride-acetone on a silica gel column to yield the activated ester (IIIa) : mp 218°C ; R_F (E_1) 0.64 ; ir(KBr) 1830, 1655 cm^{-1} ; mass spectrum (70 ev) m/e (rel. intensity) 449 (2.3), 315 (100), 269 (97) and 227 (33).

Anal. Calcd for $C_{26}H_{31}N_3O_4$: C, 69.46 ; H, 6.95 ; N, 9.35
Found : C, 69.01 ; H, 7.15 ; N, 8.95.

N-Propyl 11β-hydroxy-3-oxo-4-androstene-17β- carboxamide (IVa)

A solution of 332 mg (1 mM) of the acid (IIa), 227 mg of DCC and 203 mg of HOBT in methylene chloride was stirred overnight at 4°. The DCU precipitate was removed by filtration and 84 µl (1 mM) of propylamine was added to the filtrate. After 24 hours at 4°C, the solution was extracted with 0.1 M sodium bicarbonate, 1 N HCl and washed twice with water. The dried organic layer was then evaporated and recrystallization of the crude product, 235 mg (63 %) was performed in methanol-ethylacetate, to yield compound (IVa) : mp 145°C ; $R_F(E_1)$ 0.15, (E_2) 0.85 ; ir(KBr) 1660, 1535 cm^{-1} ; mass spectrum (70 eV) m/e (rel. intensity) 373 (18.7), 355 (34.1), 232 (15.5), 218 (70.1), 114 (100).

Anal. Calcd for $C_{23}H_{35}NO_3$: C, 73.96 ; H, 9.44 ; N, 3.75.
Found : C, 73.49 ; H, 9.12 ; N, 3.68.

The 17β-carboxamide derivatives of (IIb), (IIc) and (IId) were similarly prepared, except for compound (IVi) : the HCl extract containing (IVi) as hydrochloride was brought to pH 10 with NaOH and reextracted with methylene chloride.

Results for N-Propyl 11β, 17α -dihydroxy-3-oxo-4-androstene-17β-carboxamide (IVb), N-propyl 9α-fluoro-16α-methyl-11β-hydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVc) ; N-methyl 9 α-fluoro-16α-methyl-11β, 17α-dihydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVd), N-Propyl 9α-fluoro-16α-methyl-11β, 17α-dihydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVe), N-hexyl 9α-fluoro-16α-methyl-11β, 17α-dihydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVf) , N-Nonyl 9α -fluoro-16α -methyl-11β, 17α-dihydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVg), N-Isobutyl 9α-fluoro-16α-methyl-11β, 17α-dihydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVh), N-(2-propylamino) ethyl 9α-fluoro-16α-methyl-11β, 17α-dihydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVi), N-benzyl 9α-fluoro-16α-methyl-11β, 17α-dihydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVj), N-phenylethyl 9α-fluoro-16α-methyl-11β, 17α-dihydroxy-3-oxo-1,4-androstadiene-17β-carboxamide (IVk), are summarized in Table I.

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TABLE I : Analytical data for androstane-17β-carboxamide derivatives prepared as for (IVa) (symbols are the same as on Figure 1)

Compound	Melting Point °C	R_F (E_1)	R_F (E_2)	IR spectrum (KBr in cm^{-1})	Formula	Elemental analysis Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
IVb	226	0.07	0.82		$C_{23}H_{35}NO_4$	70.92	9.06	3.60	70.71	9.05	3.47
IVc	247	0.17	0.83	1670,1630,1550	$C_{24}H_{34}FNO_3$	71.43	8.49	3.47	71.18	8.79	3.65
IVd	260	0.03	0.61	1670,1630,1540	$C_{22}H_{30}FNO_4$	67.50	7.72	3.58	67.10	7.69	3.52
IVe	260	0.11	0.79	1670,1650,1530	$C_{24}H_{34}FNO_4$	68.71	8.17	3.34	68.88	8.28	3.18
IVf	229	0.25	0.84	1670,1630,1530	$C_{27}H_{40}FNO_4$	70.25	8.73	3.03	70.24	8.75	3.24
IVg	199	0.28	0.86	1670,1630,1525	$C_{30}H_{46}FNO_4$	71.53	9.21	2.78	71.25	9.01	2.79
IVh	260	0.58	0.92	1670,1650,1525	$C_{25}H_{36}FNO_4$	69.27	8.37	3.23	69.12	8.30	3.23
IVi	206 (decomp)	0.00	0.64	1670,1620,1520	$C_{26}H_{39}FN_2O_4$	67.51	8.50	6.06	67.81	8.92	5.85
IVj	260	0.21	0.83	1670,1630,1520	$C_{28}H_{34}FNO_4$	71.92	7.33	3.00	72.23	7.53	3.08
IVk	256	0.19	0.82	1670,1630,1525	$C_{29}H_{36}FNO_4$	72.23	7.53	2.91	71.90	7.48	2.84

Mass spectrum (70 eV) m/e (rel. intensity) : IVd, 391(19), 371(11.8), 121(100) ; IVf, 461(24.9), 441(5), 121(100) ; IVh, 433(13.8), 413(9.3), 121(100) ; IVi, 463(1.7) ; 121(11.6), 72(100) ; IVj, 467(23), 447(4.8), 91(100).