NITROGEN ANALOGS OF CORTEXONE

Leland L. Smith

Department of Biochemistry, University of Texas
Medical Branch, Galveston, Texas 77550
and Wyeth Laboratories, Inc., Radnor, Pa.

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A three step synthesis of 21-acylaminopregn-4-ene-3, 20-dione derivatives from 17α, 21-dihydroxypregn-4-ene-3, 20 dione, involving Mattox rearrangement to the 20, 21-dicarbonyl compound, selective formation of the 21-oxime, and reduction of the 21-oxime by zinc and carboxylic acid, is described. 21-Acetylaminopregn-4-ene-3, 20-dione exhibits mineralocorticoid activity in adrenalectomized rats.

In a continuation of studies on the effects of substitution of hetero atoms into pregnane derivatives on their mineralo-[1] and gluco-[2] corticoid activity we have sought to prepare 21-amino analogs of cortexone (21-hydroxypregn-4-ene-3, 20-dione) (I). Direct introduction of the primary 21-amino group into 21-substituted-20-ketones has not been successfully achieved, although secondary and tertiary amino-20-ketones have been prepared.[3] A three step synthesis of the desired 21-aminosteroids using zinc/acetic acid reduction of an appropriate 20-keto-21-aldoxime, a synthesis route previously successful for synthesis of 21-amino-17α-hydroxy analogs,[2] was selected.

Although the requisite cortexone 21-aldehyde (II) is mentioned in the literature, only recently has a suitable synthesis together with adequate structure proof become available,[4] and it is apparent that cortexone 21-aldehyde may exist as the 17(20)-enol-21-aldehyde (V). Related 17-deoxy-20, 21-dicarbonyl compounds have been variously represented as 20-keto-21-aldehydes,[5] as 20-keto-21-aldehyde 21-hydrates,[6] and as 17(20)-enol-21-aldehydes.[4, 7] Interrelationships among these several possible forms have yet to be established definitively.

At least three different synthesis routes to the sought cortexone 21-aldehyde have been reported: via the nitrone synthesis[6a, 8] (which is cumbersome and leads to a poorly defined product[6a]); via copper catalyzed air oxidation of cortexone,[9] and via Mattox rearrangement of Reichstein's Substance S[4, 5a, 7b] (17α, 21-dihydroxypregn-4-ene-3, 20-dione) (IVa). Exploration of the copper catalyzed air oxidation of cortexone indicated that oxidation to the 21-aldehyde II was accompanied by other oxidative reactions, and difficultly separated mixtures of products obtained.

The conversion of 21-hydroxy-20-ketones by copper catalyzed air oxidative reactions to 20-hydroxy-21-oic acids and to a lesser extent to 17β-carboxylic acids has been reported.[6d, 10] In our hands the major product which could be isolated with any ease from these reactions was the 17β-carboxylic acid III.

Thin-layer chromatographic analysis of the copper catalyzed air oxidation experiments on cortexone showed that so soon as some 21-aldehyde had been formed (as evinced by a positive Porter-Silber reaction and a negative color reaction to alkaline tetrazolium blue for the major product formed) invariably some 17β-carboxylic acid III had also been formed. Furthermore complete oxidation of cortexone under these circumstances was difficult to achieve, and as a result, mixtures of cortexone, cortexone 21-aldehyde (II), and the 17β-carboxylic acid III obtained.

Accordingly, the Mattox rearrangement of Substance S, known to lead to cortexone 21-aldehyde as the 17(20)-enol-21-aldehyde[4] (V) was examined. An improved synthesis of V based on the procedure of Caspi and Zajac[4] but catalyzed by zinc acetate according to Herzog, et al.,[7b] provided the enol aldehyde V in 48.5% yield, separated by direct crystallization from Reichstein's Substance S 21-acetate (21-acetoxy-17α-hydroxypregn-4-ene-3, 20-dione) (IVb) also formed in the reaction. Although the reaction product was chromatographically homogenous the

broad melting point reported by Caspi and Zajac[4] and also experienced herein may indicate the presence of 17(20)-double bond cis-trans isomerism.

On thin-layer chromatograms the 17(20)-enol-aldehyde V was more mobile than was the putative 20,21-dicarbonyl compound II derived by copper catalyzed air oxidation of I. Both 21-aldehyde compounds II and V gave instant yellow colors on chromotoplates sprayed with the Porter-Silber reagent, and both were negative to alkaline tetrazolium blue sprays, which characteristics are typical of 20,21-dicarbonyl derivatives in the 17α-hydroxypregnane series[2a] and of other steroidal glyoxals.[11] In view of the utility of either the glyoxal II or the 17(20)-enol-21-aldehyde V for the present synthesis purposes no further work was done on the relationship between the copper catalyzed air oxidation product II and the Mattox product V.

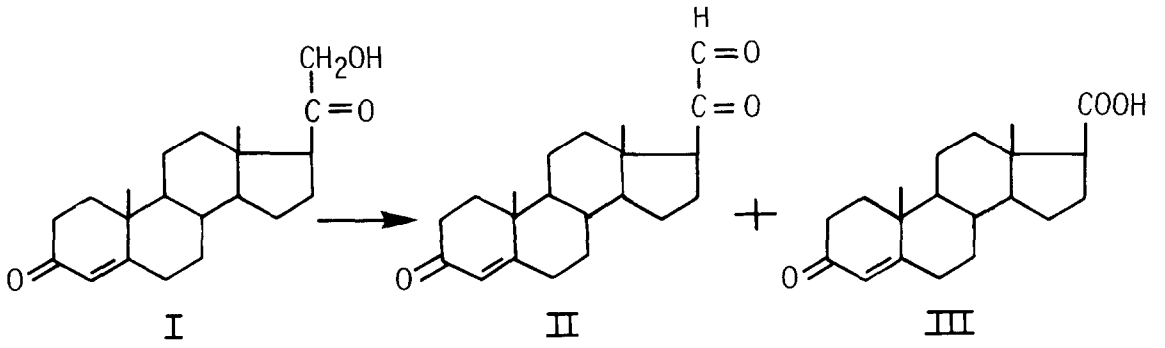
The enol aldehyde V was converted smoothly to the 17(20)-enol aldoxime VI whose structure is supported by the lack of 20-carbonyl absorption in the infrared, by the high extinction of the single absorption maximum at 236 mμ. (shifted hypsochromically 6 mμ. from the absorption maximum of I), and by the single proton resonance at δ 7.62 p.p.m. characteristic of aldehyde oximes.[12]

Reduction of the oxime VI with zinc/acetic acid/acetic anhydride afforded the 21-acetylamino derivative VIIa, whose structure as a 21-acetylamino-20-ketone was supported by infrared absorption at 5.86 μ. The 21-acetylamino functional group was demonstrated by the pronounced amide II band at 6.49 μ. and by the acetyl methyl proto resonance at δ 2.05 p.p.m. Additionally the 21-methylene protons appeared at δ 4.11 p.p.m. as the AB portion of an ABX pattern characteristic of the $-CH_2-NH-$ feature of the molecule.[2a] From the position of the C_{18}-proton resonance signal a normal 17β-side-chain configuration was indicated.

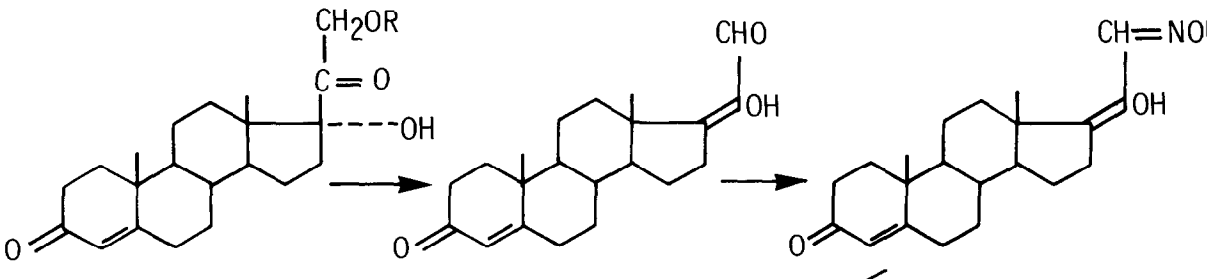
Reduction of VI with zinc/propionic acid/propionic anhydride gave the 21-propionylamino derivative VIIb. However, reduction using propionic acid was somewhat more sluggish than with acetic acid/acetic anhydride, and the reaction prod-

ucts were greater in number and more difficultly separated. Reduction of VI with zinc and a mixture of acetic acid and propionic anhydride yielded mixtures of the 21-acetylamino and 21 propionylamino derivatives VIIa and VIIb.

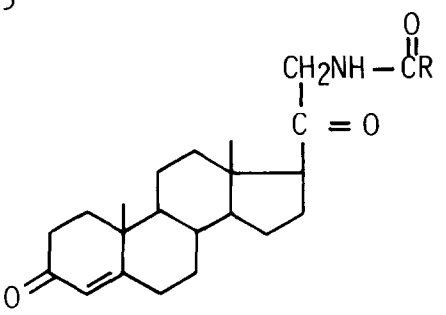
The 21-acetylamino derivative VIIa was an active mineralocorticoid in adrenalectomized rats. Ten micrograms of VIIa exerted the same salt and water retention effects as did 10μg. of cortexone 21-acetate. Furthermore, no progesta-



I II III



IVa R = H
IVb R = $COCH_3$ V VI



VII a R = CH_3
VII b R = C_2H_5

tional activity (Clauberg test, 1000 μg. dose) or cortexone antagonist activity (Kagawa test) could be demonstrated for VIIa.

This evidence of potent mineralocorticoid activity for the 21-deoxy-21-acetylamino analog of cortexone is still another example of retention of important biological activity in nitrogen analogs of hormonal steroids of the pregnane series substituted by -NH- for the naturally occurring oxygen atom. In our prior studies thymus involution activity (approximately equal to that of hydrocortisone) was demonstrated for 21-amino-9α-fluoro-11β, 17α-dihydroxypregn-4-ene-3, 20-dione;[2] however, the 21-acetylated derivative thereof was inactive. Other 21-deoxy-21-substituted amino analogs of hydrocortisone have been reported as active, particularly 21-N-piperidyl- and 21-N-piperazinyl-derivatives.[3e, 13] The 21-deoxy-21-N-piperidyl analog of cortexone has also been reported to possess weak mineralocorticoid activity.[14]

EXPERIMENTAL[15]

20-Hydroxy-3-oxopregna-4-17(20)-dien-21-al (V). Twenty-five grams of Reichstein's substance S (IVa) dissolved in 500 ml. of glacial acetic acid was treated with 2.75 g. of zinc acetate dihydrate and the mixture was refluxed for two hours, after which time it was concentrated under vacuum to about 50 ml. and diluted with water. The yellow gum which separated was dissolved in ethyl acetate, the solution was filtered and then concentrated under vacuum until crystallization began. A first crop of crystals (3.72 g.) was taken, and subsequent concentration of the mother liquor led to crops weighing 2.55, 3.74, and 1.50 g. (total 11.51 g., 48.5%), all identified by infrared spectra as being free from Substance S 21-acetate (IVb) (no absorption at 5.73 and 5.82 μ characteristic of IVb). Further concentration afforded additional V contaminated with IVb and by gums, not further worked. Recrystallization of crude V from ethyl acetate gave purified 21-aldehyde m.p. 175-180°; λ_{max}^{KBr} 2.97, 6.02, 6.09, 6.20 μ. (Caspi and Zajac[4] give m.p. 175-180°).

20-Hydroxy-21-oximinopregna-4, 17(20)-dien-3-one (VI). A solution of 3.0 g. of V and 635 mg. of hydroxylamine hydrochloride (one equivalent) in 50 ml. of 50% ethanolic pyridine was warmed on a steam bath for 90 min. after which time the solvents were removed under vacuum. The gum thus obtained was dissolved in methanol, filtered from insolubles, and the filtrate was diluted with water. The precipitate was filtered (2.08 g. m.p. 196-204° dec.) and recrystallized several times from aqueous methanol, thus affording the analytical sample, m.p. 202-203° dec.; $[\alpha]_D$ +180°; λ_{max} 236 mμ. (ε 24,700); λ_{max}^{KBr} 2.98, 3.11, 3.20, 5.99, 6.07, 6.10, 6.20, 9.90 μ.; δ 0.64 (C_{18}-protons), 1.18 (C_{19}-protons), 5.89 (C_4-proton), 7.62 p.p.m. (C_{21}-proton).

Anal. Calcd. for $C_{21}H_{29}NO_3$: C, 73.43; H, 8.51; N, 4.08. Found: C, 73.40; H, 8.45; N, 4.09.

21-Acetylaminopregn-4-ene-3,20-dione (VIIa). A stirred solution of 750 mg. of VI in 45 ml. of acetic acid and 40 ml. of acetic anhydride was treated with 2.25 g. of zinc dust, added as small portions over 30 min. Stirring was continued for 90 min. after which time the mixture was filtered, the zinc cake washed with acetic acid, and the combined filtrates were reduced in volume under vacuum. The concentrated solution was diluted with water and neutralized with solid sodium bicarbonate, the gummy product was extracted into methylene chloride, the methylene chloride extracts were washed with water, dried over anhydrous magnesium sulfate, and finally evaporated under vacuum. The yellow gummy residue was crystallized from acetone-hexane, yielding 260 mg. of colorless crystals, m.p. 184-188°. Recrystallization from acetone-hexane gave the pure amide, m.p. 186.5-188.5°; $[\alpha]_D$ +158°; λ_{max} 242.5 mμ. (ε 15,600); λ_{max}^{KBr} 3.01, 5.86, 5.98, 6.19, 6.49 μ.; δ 0.70 (C_{18}-protons), 1.19 (C_{19}-protons), 2.05 (acetyl methyl protons), 4.11 (AB portion of ABX system, 21-methylene protons), 5.74 p.p.m. (doublet, J = 2 c.p.s., C_4-proton).

Anal. Calcd. for $C_{23}H_{33}NO_3$: C, 74.36; H, 8.95; N, 3.77. Found: C, 74.29; H, 8.94; N, 3.54.

21-Propionylaminopregn-4-ene-3,20-dione (VIIb). A solution of 1.0 g. of the oxime VI in 40 ml. of propionic anhydride and 45 ml. of acetic acid was stirred for 15 min. while 2.5 g. of zinc dust was added portionwise. After 5 min. of stirring thin-layer chromatographic analysis showed the reduction of VI was complete, and the mixture was filtered. The filtrate was evaporated under vacuum and the residue was taken up in acetone and hexane was added. A small amount (86 mg.) of mixed zinc acetate-zinc propionate salts was precipitated but no steroid material separated. Evaporation of the solvents gave a gum which analyzed by thin-layer chromatography as a mixture of at least four major components (R_F 0.14, 0.25, 0.36, 0.60). Preparative thin-layer chromatography of a portion of the gum using 1 mm. thick Silica Gel HF_{254} chromatoplates irrigated with ethyl acetate resolved the N-propionate VIIb (R_F 0.36) from the other components. The product zone was eluted with ethyl acetate. Evaporation of the solvent gave 151 mg., m.p. 95-98° and 132-134°. Recrystallization of the material from methanol gave VIIb, m.p. 94-97°; λ_{max}^{KBr} 2.90, 3.05, 5.86, 6.00, 6.28, 6.49 μ.

Anal. Calcd. for $C_{24}H_{35}NO_3 \cdot H_2O$: C, 71.43; H, 9.24; N, 3.47. Found: C, 71.86; H, 9.02; N, 3.71.

Elution of the R_F 0.25 zone from the preparative thin-layer chromatogram using ethyl acetate afforded 53 mg. of the N-acetate VIIa, m.p. 174-180°, identified by thin-layer chromatographic behavior and by infrared spectra in comparison with the known VIIa.

Reduction of 1.0 g. of VI in a solution of 40 ml. of propionic acid and 40 ml. of propionic anhydride with 2.5 g. of zinc dust was complete after 30 min. according to thin-layer chromatography. Evaporation of the solvents after removal of zinc and zinc propionate afforded 1.023 g. of yellow gum which contained VIIb together with at least four other major more mobile components. Preparative thin-layer chromatography and subsequent crystallization, etc., gave VIIb, m.p. 96° which on further thin-layer chromatography and recrystallization from methanol yielded a polymorph, m.p. 133.0-135.5°.

Copper Catalyzed Air Oxidation of Cortexone. A number of copper acetate/air oxidation conditions were examined for their preparative value, with both isolation of products and thin-layer chromatographic evidence being used for evaluation. Varying proportions of acetic acid, copper acetate, water, etc., with and without disodium ethylenedinitrilotetraacetic acid (EDTA), variation in time, temperature, rate of aeration, etc. were examined. In none of these experiments was II produced as a sole major product, and gums and resins of product complexity were obtained. Column chromatography of products led to the isolation of the 17β-carboxylic acid III as the only pure product readily isolated.

A typical thin-layer chromatoplate of copper catalyzed air oxidation reactions revealed three major components recognized as I, II, and III, together with several minor components.

Component Analyzed	R_{DOC}[a]	Visualization Responses			
		PS[b]	BT[c]	PMA[d]	UV[e]
I	1.00	-	+	+	+
II	1.20	+	-	+	+
III	1.40	-	-	+	+
V[f]	1.4	+	-	+	+

(a) Mobility relative to cortexone as unity, ethyl acetate irrigation. R_F of cortexone is 0.48.
(b) Porter-Silber spray. An immediate yellow color was obtained as a positive test.
(c) Alkaline tetrazolium blue.
(d) 10% Ethanolic phosphomolybdic acid spray, chromotoplate heated at 110° for 10 min.
(e) Ultraviolet light absorption causing quenching of fluorescence of chromatoplate.
(f) Compound V was not detected in copper catalyzed air oxidations.

3-Oxoandrost-4-ene-17β-carboxylic Acid. (III). A stirred solution of 4.90 g. of cortexone in 500 ml. of methanol was refluxed and a hot solution of 7.5 g. cupric acetate monohydrate in 250 ml. of methanol containing 5 ml. of glacial acetic acid was added. The mixture was refluxed for 30 min., 250 ml. of water was added, and the mixture refluxed for 30 min. more. The copper oxide was filtered through a layer of Celite, the precipitate washed with methanol and with water and the combined filtrate and washings were evaporated under vacuum to a gum. The gum in benzene was chromatographed on 400 g. of silica gel. Elution with ether-benzene (1:1) gave a yellow oil which was crystallized from benzene, yielding 695 mg. of III m.p. 221-227° dec. (capillary), λ_{max}^{KBr} 3.35, 5.80, 6.10, 6.20 μ. Recrystallization from acetone, from acetone-hexane, and from methanol gave the etianic acid III in a solvated form (probably methanol), m.p. 244-250° dec. (capillary); λ_{max}^{KBr} 3.33, 5.80, 6.13, 6.20 μ.

Anal. Calcd. for $C_{20}H_{28}O_3 \cdot CH_4O$: C, 72.38; H, 9.26. Found: C, 71.92; H, 9.51.

Recrystallization from ethanol and vacuum drying gave the unsolvated acid, m.p. 244-249° dec.; $[\alpha]_D$ +163°; λ_{max} 241 mμ. (ε 17,000); λ_{max}^{KBr} 3.33, 5.80, 6.13, 6.21 μ.; R_F 0.69 (ethyl acetate irrigation).

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.78; 75.92; H, 9.01, 9.07.

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Thin-layer chromatography was conducted on microliter samples withdrawn from reactions and spotted directly onto 0.25 mm. chromatoplates prepared with Silica Gel HF_{254}. Irrigation was with ethyl acetate in all instances. Steroids were detected by their ultraviolet absorption properties on the chromatoplate prior to visualization with spray reagents. A 10% ethanolic solution of phosphomolybdic acid was used for detection of all components, and specific detection of Δ 4-3-ketones was made using isonicotinic acid hydrazide spray reagent. Alkaline tetrazolium blue and the Porter-Silber reagent prepared according to Birmingham[16] or Lewbart and Mattox[17] were used for specific detection of reducing α-ketols and 20,21-dicarbonyl compounds respectively.

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