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Synthesis of Steroidal Indoxyl, and Derivatives from 3-Ketosteroid

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5∝-Androstan-17β-ol-3-one condenses with 2-nitrobenzaldehyde in alkaline conditions to give the steroidal indoxyl 17β-hydroxy-1-(3′-oxoindan-2′-yliden)-3-nor-1,2-secoandrostan-3-oic acid (I) which on refluxing with acetic anhydride affords the lactam 17β-acetoxy-3′-aza-4a-homoandrost-1-eno-(3,2-a)-indan-3′,4-dione (II). Reduction of I with sodium borohydride gives the indole 17β-hydroxy-1-(indol-2′-yl)-3-nor-1, 2-secoandrostan-3-oic acid (III). The results of this synthesis suggest that 3-ketosteroids saturated in ring A react in a similar manner to 17-ketosteroids, with 2-nitrobenzaldehyde.

Key words: Synthesis, Steroidal Indoxyl, derivatives.

INTRODUCTION

17-ketosteroids condense with 2-nitro-benzaldehyde in alkaline conditions to give steroidal indoxyls, which in turn can be cyclised to steroidal lactams on refluxing with acetic anhydride. Steroidal indoxyls give a steroidal indole on reduction with sodium borohydride [1-3]. Such syntheses involving 3-ketosteroids have not been reported previously.

The primary interest was to extend the range of steroid indoxyls, lactams and indoles that can be synthesized, for pharmacological evaluation. In the present paper the synthesis of a steroidal indoxyl, lactam and indole from a 3-ketosteroid (Figure 1) is reported.

MATERIALS AND METHODS

Reagents

17β-Hydroxy-5α-adrostan-3-one was purchased from Sigma Chemical Company. 2-Nitrobenzaldehyde was obtained from Aldrich Chemical Company. The rest of the reagents and solvents were obtained from B.D.H and used without further purification. Elemental analyses were performed by Galbraith Laboratories Inc. Sycmore, Knoxville, TN., USA. Infrared spectra were scanned as nujol mulls on a PE 727B spectrophotometer. UV spectra were recorded in methanol on a Pye Unicam SP 8000 spectrophotometer. NMR spectra were run in C_5D_5N except for compound **III** (^{1} H-NMR in $CDCl_3$) on a HEOL Gx 270 FTNMR fitted with 5 mm C/H dual probe. MS spectra were recorded on a 70 eV EIMS spectrometer. The purity of the compounds was confirmed by TLC using $CHCl_3$: MeOH 9:1 on POLYGRAM SIL G/UV_{254} silica-gel precoated plastic plates.

Synthesis

17β-Hydroxy-1-(3'-oxoindan-2'-ylidene)-3-nor-1, 2-secoandrostan-3-oic acid (I): To a solution of 17β-hydroxy-5∝-androstan-3-one (1.0 g, 3.45 mmol) in MeOH (50ml) was added aqueous KOH (1.0 g in 1 ml) followed by a MeOH solution of 2-nitrobenzaldehyde (0.75 g, 5 mmol in 10 ml). After 24 h at room temperature in the dark, the solution was concentrated under vacuum and acidified with diluted HCl. The yellow precipitate was collected by filtration, washed with water, dried and recrystallised from methanol - water to give **I** (0.72g, 63.0 %) as yellow crystals, m.p. 228-229 °C; UV λmax 238, 259, 275 (sh), 300 (sh) 448 nm; IR γmax 3400 (O-H), 3350 (N-H), 2700-2300 (COOH) 1700 (C=O of COOH) 1680, 1640 (∝, β-unsat C=0). 1620 cm-1: MS m/z 423 (M+, 100), 405 (34), 220 (40, 173 (46), 146 (85). Anal: Calculated for $C_{26}H_{33}O_4N$ C 73.76 H.7.80, found C. 73.78 H 7.97.

17β-Acetoxy-3-aza-4a-homoandrost-1-eno-(3,2-a) indan- 3',4-dione (II): The indoxyl **(I)** in Ac_2O (2g in 60 ml) was refluxed for 12 h and poured into ice-water to give a pink precipitate. Two recrystallisations from EtOH- H_2O gave **II** (1.6 g,

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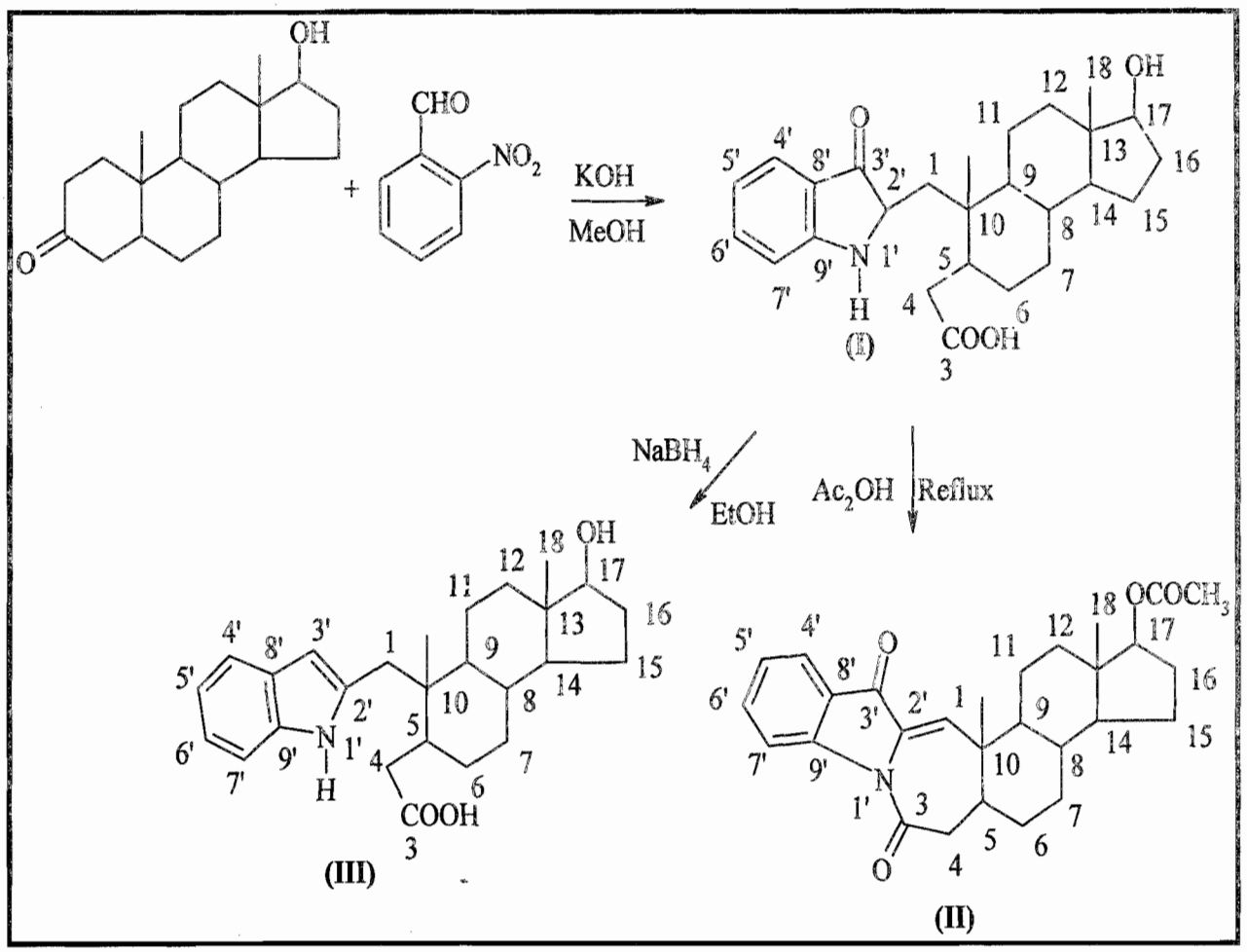

Figure 1: Scheme of Synthesis

75.7 %) as light pink crystals, m.p. 193-195 °C; UV λmax 212, 244, 257 (sh), 275 (sh), 358 (sh), 364, 396 (sh) nm; 1R γmax 1750 (C=O of acetate) 1700 (C=O of lactam) 1660, 1620 (∝, β-unsaturated C=O) cm^{-1}; MS m/z 447 (M^+,100), 159 (72) 146 (24). Anal: Calculated for $C_{28}H_{33}O_4N$ C 75.15 H 7.38, found C 74.91 H 7.51.

17β-Hydroxy-1-(indol-2'-yl)-3-nor-1,2-secoandrostan -3-oic acid (III): The indoxyl **(I)** in EtOH (3.0 g in 300 ml) was reduced by gradual addition of $NaBH_4$ (12 g). The mixture was concentrated under reduced pressure and acidified by dropwise addition of dilute HCl with stirring. The white precipitate was washed with water, dried and recrystallised from EtOH - H_2O to give **III** as white amorphous crystals, m.p. 153-155 °C; λmax 212 (sh), 212, 275, 290 nm; IR γmax 3425 (OH NH) 2700-2300 (COOH) 1710 (C=O)cm^{-1}; MS m/z 409 (M^+, 11) 144 (60) 131 (100) 130 (40).

RESULTS AND DISCUSSION

Compound **I,** obtained as yellow crystalline needles from methanol-water by reaction between 5∝-androstan-17β-ol-3-one, which is a 3-ketosteroid, and 2-nitrobenzaldehyde in the presence of potassium hydroxide, shows in its UV spectrum features of a highly conjugated system. The IR spectrum shows peaks at 3350 and 1620 cm^{-1} which are characteristic of indoxyls [4]. Its ^{1}H-NMR, shows broad D_2O exchangeable proton signals at 9.86 ppm (N-H) and 2.56 ppm (O-H), in addition to four aromatic protons, together with a singlet signal at 6.28 ppm (I-H) (Table 1). ^{13}C-

NMR spectrum for compound I shows peaks due to two carbonyl carbons at 186.64 ppm and 175.90 ppm for the indoxyl carbonyl and carboxyl groups respectively (Table 2). An EI mass spectrum of compound I shows the molecular ion peak at m/z 423 which is the base peak. Elemental analysis provides further evidence for the structure of this compound.

The mechanism of formation of steroidal indoxyls from 17-ketosteroids has previously been described [1] and has analogy in the synthesis of indigo from acetone and 2-nitrobenzaldehyde [5]. It proceeds via aldol-like intermediate formed by the attack of the aldehyde group of 2-nitrobenzaldehyde on the 16-methylene group of 17-ketosteroids. The ortho nitro group of the aldehyde induces the aldol intermediate to react preferentially by an intramolecular carbanion attack on the nitro group to give the indoxyl. With 3-ketosteroids an attack at the 2-rather than 4-position, can be expected and such attack is hindered by a 2-methyl substituent [6].

Table 1: Proton NMR Chemical Shifts for Compounds I, II and III

PROTON	I	II	III
1	6.28s	6.75s	-
3	2.56s	-	-
5	-	2.90dd	3.20d
9	-	2.44d	-
17	3.87t	4.56m	3.77t
18	0.97s	0.81s	0.91s
19	1.33s	1.17s	0.99s
1′	9.865, br	-	-
3′	-	-	6.78s
4′	7.90d	8.50d	7.65dd
5′	7.35t	7.83dd	7.20d
6′	6.86t	7.65m	7.18s
7′	6.94d	7.26m	7.81dd
21	-	2.05s	-

s = singlet, d = doublet, t = triplet, m = multiplet, b = broad

Table 2. ^{13}C NMR Chemical Shifts for Compounds I, II and III

Carbon	I	II	III
1	136.32	136.32	35.17
2	-	-	-
3	175.90	171.92	177.77
4	38.44	42.03	37.33
5	40.11	41.19	40.81
6	23.66	29.37	28.85
7	30.88	31.21	31.01
8	34.94	34.49	35.89
9	52.53	52.06	51.04
10	43.42	42.16	40.51
11	27.32	21.67	22.19
12	37.47	36.62	37.45
13	43.69	42.43	43.26

Table 2: Cont'd

Carbon	I	II	III
14	51.17	50.48	48.13
15	23.84	23.57	23.64
16	30.88	27.54	30.92
17	81.19	82.53	81.08
18	11.84	12.60	11.93
19	14.84	14.41	15.91
2′	155.38	149.31	137.02
3′	186.64	184.45	102.26
4′	119.41	118.63	119.56
5′	124.87	124.12	120.94
6′	124.36	124.79	120.94
7′	112.43	129.58	111.27
8′	122.75	123.76	129.70
9′	155.38	130.80	136.75
20	-	171.20	-
21	-	21.18	-

The structure of compound **I** is also indirectly confirmed because on refluxing with acetic anhydride, it gives the 7-membered ring lactam **(II).** As expected, the 17-hydroxyl group is acetylated during the reaction. Cyclisation of steroidal indoxyls derived from 17-ketosteroids under similar conditions gives 5-membered ring lactams where theα,β-unsaturated carbonyl group is at the same time converted to the enol acetate [I]. The assignment of the structure of compound **II** was suggested by the absence of N-H or O-H peaks in its IR and ^{1}H-NMR spectra (Table 1), as well as the presence of acetyl carbonyl, lactam carbonyl and ∝, β - unsaturated carbonyl peaks at 1750, 1700 and 1660 cm^{-1} respectively; these carbonyl carbons give signals at 171.20, 171.92 and 184.45 ppm in the ^{13}C-NMR spectrum of compound **II** (Table 2). Again elemental analysis is consistent with the assigned structure for this compound. The base peak in the mass spectrum of compound **II** is the molecular ion peak at m/z 447.

The steroidal indole **(III),** obtained by reduction of compound **I** with sodium borohydride in ethanol, gives a positive Ehrlisch test [7] for indoles, and its UV spectrum is almost identical to that of 2-methylindole. The IR spectrum of compound **III** shows the presence of only one carbonyl group (COOH) at 1710 cm^{-1}. Its ^{1}H-NMR spectrum shows signals for five aromatic protons (Table 2) while the ^{13}C-NMR spectrum gives signals for aromatic carbons whose chemical shifts are almost identical to those of 2-methylindole [8], as well as a carboxyl carbonyl signal at 177.77 ppm (Table 2). Its mass spectrum shows a base peak at m/z 131, which is observed in the fragmentation pattern of alkyl indoles [9], in addition to the molecular ion peak at m/z 409.

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