Steroidal Quinoxalines

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A considerable number of aza-steroids containing nitrogen in the nucleus have been prepared by Doorenbos and co-workers (1) and tested as possible drugs for antimicrobial activity.

As part of continuing programs directed toward the development of new aza-steroids with biological interest have been the object for considerable interest in our laboratory (2-5).

Recent studies on the chemistry of 16-bromo-17-ketosteroids indicated that direct displacement of bromide by amines is possible in these α-bromoketones (6-7).

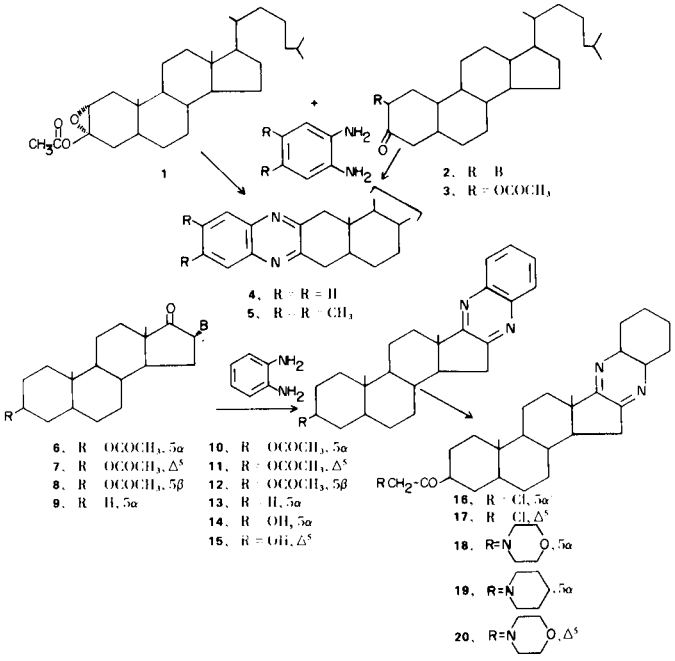
Using as starting materials 16α-bromoketones **6-9**, we explored the reaction with *o*-phenylenediamine. When we heated the mixture in xylene temperature, quinoxalines **10-13** were obtained in good yield. The structure of the quinoxalines was deduced from their nuclear magnetic resonance spectra.

In addition to the common peaks, the low-field aromatic AA'BB' multiplet-($\tau \sim 1.8$) is assigned to protons 5 and 8 and the high-field half ($\tau \sim 2.2$) to protons 6 and 7; the infrared spectra show characteristic absorption of the quinoxaline nucleus at 750 cm^{-1}.

Extension of this reaction to 2β-bromocholestanone **2**, 2α-acetoxycholestanone **3** and 3β-acetoxy-2α,3α-oxidocholestane **1** with *o*-phenylenediamine or 3,4-dimethyl-1,2-phenylenediamine yielded the corresponding quinoxalines **4** and **5**.

The nmr spectrum of compound **4** gives signals for the aromatic protons at τ 2 (2 protons, multiplet) and at τ 2.3 (2 protons, multiplet). The aromatic protons of compound **5** appeared as singlet at τ 2.5 and the six protons of the methyl groups of qunioxaline ring at τ 7.5 as a singlet.

Quinoxalines **5** and **6** were screened against the four fungi *Ustilago maydis*, *Candida tropicalis*, *Aspergillus nidullans* and *Fuzarium oxysporum* and were not effective at 10 ppm. Compounds **5**, **10**, **11** and **20** tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalie*, *Streptococcus aureus*, *Nocardia asteroides*, *Corynebacterium* sp. *Candida albicans* and *Saccharomyces cerevisiae*, exhibit little or no antimicrobial activity at the concentrations 1000 μ g./ml.



EXPERIMENTAL

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Ir spectra were recorded with a Perkin-Elmer 521 in solid phase (potassium bromide). Nmr spectra were determined with a Varian Associates A-60 instrument, using deuteriochloroform as a solvent and tetramethylsilane as the internal standard. Elemental analyses were performed by the Analytical Laboratory of the Chemistry Department, "Demokritos", and National Research Foundation.

General Procedures for the Preparation of Androstan[16,17-*b*]-quinoxalines.

To a solution of 5 mmoles of 16α-bromoketone (9-12) in 50 ml. of anhydrous xylene was added 10 mmoles of *o*-phenylenediamine. The mixture was heated under reflux for 72 hours and the cooled mixture was filtered. The solvent was evaporated under reduced pressure and the residue was obtained in crystalline form by addition of solvent. The compounds prepared are reported in Table I. All the quinoxalines obtained showed strong absorption at 750 cm^{-1} and are listed in Table I.

TABLE I

Androstan[16,17-*b*]quinoxalines

Compound	R	Recrystallization Solvent	M.p. °C	Yield %	R_F (a)	Formula	Calcd. % C	Calcd. % H	Calcd. % N	Found % C	Found % H	Found % N
10	$OCOCH_3$, 5α	$CHCl_3$-CH_3OH	216-217	70	0.88	$C_{27}H_{34}N_2O_2$	77.50	8.20	6.70	77.30	8.40	6.60
11	$OCOCH_3$, Δ^5	" "	229-230	92	0.87	$C_{27}H_{32}N_2O_2$	77.80	7.70	6.70	77.20	8.00	6.70
12	$OCOCH_3$, 5β	CH_3OH	179-180	55	0.60	$C_{27}H_{34}N_2O$	77.50	8.20	6.70	77.20	8.40	6.60
13	H, 5α	$CH_3COOC_2H_5$	148-151	60	0.80	$C_{25}H_{32}N_2$	83.30	8.90	7.80	83.70	9.00	7.70
14	OH, 5α	CH_3OH	250-251	89	0.58	$C_{25}H_{32}N_2O$	79.80	8.50	7.40	79.90	8.70	7.40
15	OH, Δ^5	"	179-180	92	0.55	$C_{25}H_{30}N_2O$	80.20	8.00	7.50	80.40	8.20	7.50

(a) Thin-layer chromarography was performed on silica gel G plates using chloroform-methanol (95:5) as developer.

Hydrolysis of 3β-Acetoxyquinoxalines.

To a solution of 0.5 g. of **10** or **11** in 25 ml. of methanol was added 0.5 g. of potassium hydroxide. The mixture was refluxed for 30 minutes. The solution was poured into water and the precipitate collected by filtration. The physical properties of the hydrolysed compounds are reported in Table I.

Cholestan[2,3-*b*]quinoxaline (**4**).

A. From 3β-Acetoxy-2α,3α-oxidocholestane (**1**).

Acetoxyepoxide **1** (8), 5 mmoles was dissolved in 25 ml. of anhydrous benzene and to the solution was added 10 mmoles of *o*-phenylenediamine. The mixture was heated under reflux for 75 hours. The solvent was evaporated under reduced pressure and the residue was dissolved in chloroform-water. The chloroform layer was washed with water and dried over magnesium sulfate. Evaporation of the solvent and crystallization from chloroform-methanol yielded quinoxaline 4 in 68% yield, m.p. 175-176°.

Anal. Calcd. for $C_{33}H_{48}N_2$: C, 83.89; H, 10.16; N, 5.93. Found: C, 83.70; H, 10.40; N, 5.90.

B. From 2α-Acetoxycholestan-3-one (**3**).

To a solution of 2 mmoles of **3** (8) in 50 ml. of anhydrous benzene was added 4 mmoles of *o*-phenylenediamine. The mixture was heated under reflux for 72 hours. Work up as before and the residue was chromatographed on a column of silica gel prepared with chloroform. Elution with benzene-ethyl acetate (5:1) gave **4** in 44% yield m.p. 174-175°, indentical by a mixture melting experiment and the infrared spectra with an authentic sample.

C. From 2β-Bromocholestan-3-one (**2**).

Bromoketone **2** (3 mmoles in 30 ml. of anhydrous benzene) and 6 mmoles of *o*-phenylenediamine was refluxed for 72 hours. Work up as above produce the corresponding quinoxaline **4** in 59% yield, identical by infrared spectra and mixture melting experiment with an authentic sample.

Cholestan-3′,4′-dimethyl[2,3-*b*]quinoxaline (**5**).

To a solution of 5 mmoles of 3β-acetoxy-2α,3α-oxidocholestane **1** in 25 ml. of benzene was added 10 mmoles of 3,4-dimethyl-1,2-phenylenediamine. The mixture was heated under reflux for 72 hours. The solvent was evaporated under reduced pressure and the remaining residue was dissolved in chloroform-water. Work up as before and chromatography on a column of silica gel followed by elution with chloroform gave quinoxaline **5** in 45% yield, m.p. 191-192°.

Anal. Calcd. for $C_{35}H_{52}N_2$: C, 84.4; H, 10.4; N, 5.6. Found: C, 84.25; H, 10.63; N, 5.70.

3β-Chloroacetoxy-5α-androstan[16,17-*b*]quinoxaline (**16**).

To a solution of **14** (1.5 g.) in a mixture of 10 ml. of purified dioxane and 0.5 ml. of anhydrous pyridine, 2 g. of chloroacetic anhydride was added and the mixture was allowed to stand at room temperature for 24 hours. Then the reaction mixture was poured into ice-water and the precipitate collected by filtration, washed several times with water and recrystallized from ethyl acetate-pentane gave 50% yield, m.p. 200-202°; ν max 1750 cm^{-1} (C=O).

Anal. Calcd. for $C_{27}H_{33}N_2O_2Cl$: C, 71.60; H, 7.29; N, 6.18. Found: C, 71.50; H, 7.40; N, 6.25.

3β-Chloroacetoxy-5-androsten[16,17-*b*]quinoxaline (**17**).

Using the above procedures, compound **17** was isolated in 48% yield (ethyl acetate) m.p. 188-189°.

Anal. Calcd. for $C_{27}H_{31}N_2O_2Cl$: C, 71.90; H, 6.88; N, 6.21. Found: C, 71.30; H, 6.97; N, 6.33.

General Procedures for the Preparation of 3β-Aminoacetoxyquinoxalines.

To a flask containing 0.5 g. of 3β-chloroacetoxyquinoxaline **16** or **17** was added an excess of amine (5 ml.) and the reaction mixture was heated on a steam bath for 2 hours. The excess amine was evaporated under reduced pressure to give aminoesters **18-20** in 65-70% yield.

3β-Morpholinoacetoxy-5α-androstan[16,17-*b*]quinoxaline (**18**).

This compound had m.p. 194-196° (methanol); ν max: 1730 cm^{-1} (C=O).

Anal. Calcd. for $C_{31}H_{41}N_3O_3$: C, 73.95; H, 8.15; N, 8.34. Found: C, 73.59; H, 8.25; N, 8.32.

3β-Piperidinoacetoxy-5α-androstan[16,17-*b*]quinoxaline (**19**).

This compound had m.p. 212-214° (methanol); ν max, 1740 cm^{-1} (C=O).

Anal. Calcd. for $C_{32}H_{43}N_3O_2$: C, 76.74; H, 8.58; N, 8.58. Found: C, 76.20; H, 8.72; N, 8.63.

3β-Morpholinoacetoxy-5-androsten[16,17-*b*]quinoxaline (**20**).

This compound had m.p. 213-214° (ethyl acetate); ν max: 1730 cm^{-1} (C=O).

Anal. Calcd. for $C_{31}H_{39}N_3O_3$: C, 74.25; H, 7.78; N, 8.38. Found: C, 74.02; H, 7.97; N, 8.12.

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