SYNTHESIS OF *cis* AND *trans* ISOMERS OF D-RING LINKED BIS-STEROID PYRAZINES FROM 16α-BROMO-17-OXOSTEROIDS

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Dedicated to the memory of Dr Václav Černý.

Treatment of 16α-bromo-17-oxosteroids **1** and **2** with gaseous ammonia and successive air-oxidation afforded the *cis* and *trans* isomers of D-linked bis-steroid pyrazines. Analogous reaction of 1α-bromo-4-nor-5α-cholestan-2-one (**3**) led to unexpected hydrolysis products.
Keywords: Steroids; Bis-steroidal pyrazines; α-Bromo ketones; Enamines; Cephalostatin analogs.

Cephalostatins[1] and ritterazines[2] are alkaloids of complex steroid structure. A central pyrazine ring links the two steroid units in these natural products of marine origin. The alkaloids show exceptional cytotoxic activity and are intensively studied as potential antitumor drugs. However, these bis-steroids are sparingly available from the natural sources and there is need for their synthesis, as well as of their analogues. The crucial step of synthesis of these compounds is construction of a central pyrazine ring.

The classical method for the synthesis of symmetric pyrazine dimers is condensation of α-aminoketones. The initially formed mixture of dimeric dihydropyrazines undergoes autoxidation giving an aromatic pyrazine product. Steroidal 2α-amino-3-ketones are readily available by reduction of the corresponding 3-oxosteroids bearing a nitrogen containing substituent at C-2, such as azido[3], hydroxyimino[4], enamino[5] or nitro[6] group. We have recently described[7] a simple procedure for the pyrazine ring construction consisting of 2α-bromo-3-oxosteroid reaction with ammonia. The reaction results in the formation of a mixture of *cis* and *trans* isomers that can be easily separated by crystallization or chromatography.

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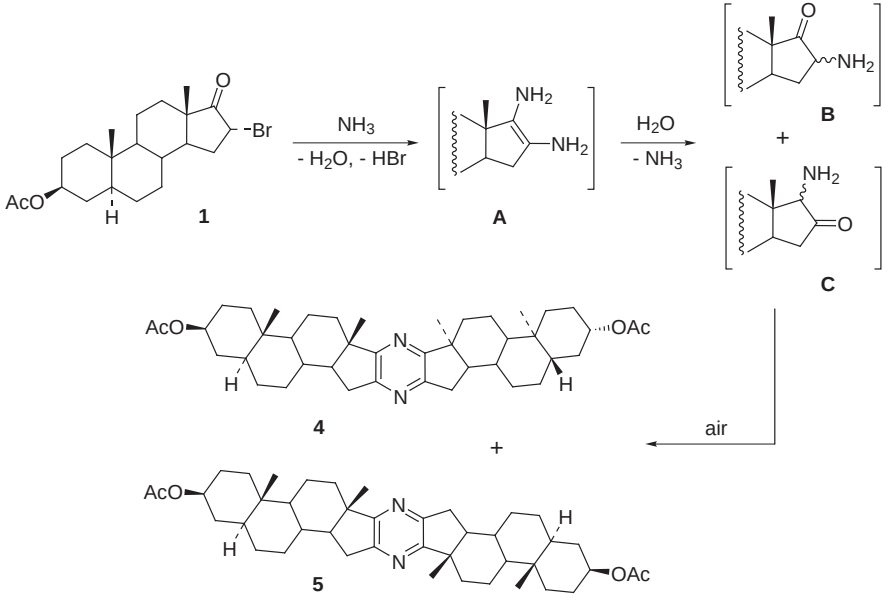
In the natural products and their synthetic analogues the pyrazine ring is linked to two steroid units through the six-membered rings A. A synthesis of the dimeric steroid pyrazines connected through a five-membered rings D has also been reported[8]. An attempt to extend our dimerization method to the five-membered cyclic α-bromoketones is described in this paper.

RESULTS AND DISCUSSION

Four steroid five-membered α-bromoketones were prepared. Three of them were 16α-bromo-17-ketones (**1**, **2a** and **2b**) from androstane and estrone series, whereas the fourth was 1α-bromo-4-nor-5α-cholestan-2-one (**3**).

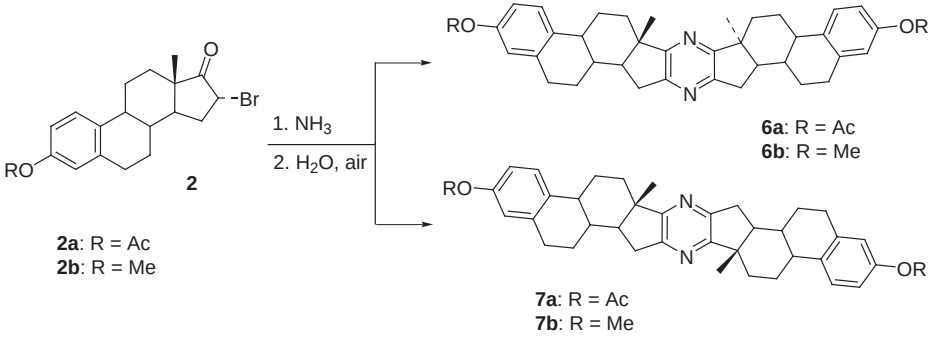
A solution of 3β-acetoxy-16α-bromo-5α-androstan-17-one (**1**) in 1-butanol was treated with gaseous ammonia. The polar intermediate was hydrolyzed and air oxidized to a mixture of two isomeric pyrazines: *cis* **4** and *trans* **5** (Scheme 1). The ratio of the isomers was found to be 1 : 1 by integration of signals of 18-methyl groups in the ^{1}H NMR spectrum of the mixture. In the reaction, ammonia attacks both electrophilic sites of substrate at C-16 and C-17. This leads to the formation of a polar intermediate (presumably enediamine **A**) that is further hydrolyzed to a mixture of isomeric α-aminoketones (**B** and **C**). These compounds, in turn, undergo spontane-



SCHEME 1

ous dimerization followed by autoxidation to the final pyrazine products. The separation of the isomers obtained proved to be more difficult in this case. Attempts to separate them by crystallization failed and only careful column chromatography resulted in partial separation of isomers. One of them proved identical with the *trans* isomer obtained previously by Černý *et al.*[8].

A similar reaction was performed with 3-acetoxy-16α-bromoestra-1,3,5(10)-trien-17-one (**2**) (Scheme 2). A crystalline, white product obtained (presumably a mixture of **6a** and **7a**) was practically insoluble in all common solvents. For this reason its spectral characterization proved difficult.

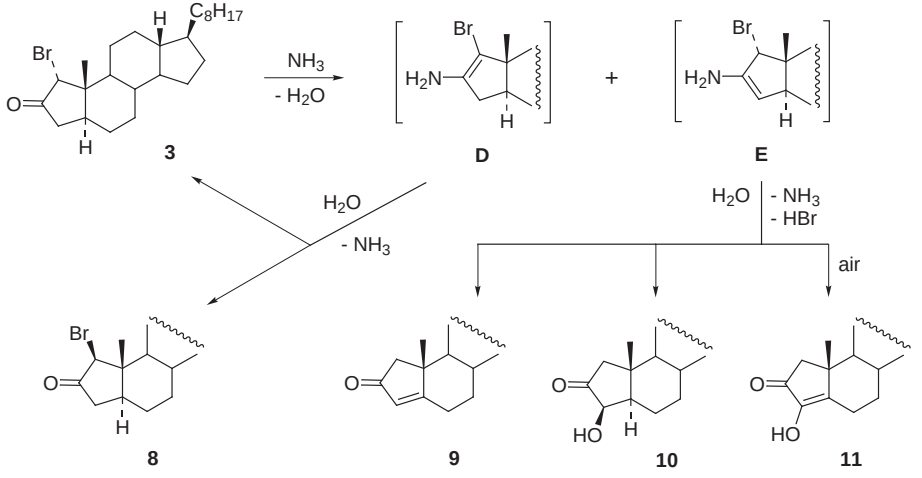


SCHEME 2

From the IR spectrum measured as KBr pellets (no carbonyl absorption band and presence of O–H absorption at 3 398 cm^{-1}) it was evident that the acetate group underwent hydrolysis during reaction. The disappearance of a ketone carbonyl group may suggest that pyrazine dimers were formed. Unfortunately, all attempts of derivatization (*e.g.* acetylation) of the product in order to increase its solubility failed. In the next attempt the reaction of 3-methoxy-16α-bromoestra-1,3,5(10)-trien-17-one (**2b**) with ammonia was carried out. In this case the reaction product appeared to be better soluble and was fully characterized by spectral methods. ^{1}H NMR spectrum proved that the product was a 57 : 43 mixture of *cis* and *trans* dimers (**6b** and **7b**, respectively). ^{13}C NMR and MS spectra unequivocally confirmed their bis-steroid pyrazine structures. The *cis* configuration of the major product was deduced from its NMR spectra by comparison with spectra of the *cis* and *trans* isomers from the androstane series (**4** and **5**). The 18-H signals of the *cis* isomers are shifted upfield (0.06–0.07 ppm) compared with the *trans* isomers. Also chemical shifts of pyrazine carbons (their signals are closer to each other in the *trans* isomers) confirms the assigned structures **6b** and **7b**

(Table I). Besides these small differences the spectra of isomers were almost identical. Interestingly, the *cis* isomers proved to be slightly less polar than the *trans* ones in the contrary to the A-ring linked bis-steroid pyrazines. However, the separation of isomers (**6b** and **7b**) is not an easy task.

The reaction of 1α-bromo-4-nor-5α-cholestan-2-one (**3**) with gaseous ammonia was performed in the same manner as for compounds **1**, **2a** and **2b**, but did not afford nitrogen-containing products (Scheme 3). A complex mixture of compounds **8**–**11** was formed instead. The known α,β-unsaturated ketone[9] (**9**) was presumably formed by 1,4-elimination of HBr from the enamine **E** followed by hydrolysis. The 1,4-elimination was competing with the SN2′ reaction with water as a nucleophile leading to 3β-hydroxy-



SCHEME 3

TABLE I
Important ^{1}H and ^{13}C NMR chemical shifts of *cis* (**4** and **6b**) and *trans* (**5** and **7b**) isomers

Compound	H-18 (δ, ppm)	$\delta_{trans} - \delta_{cis}$	Pyrazine-C (δ, ppm)[a]	Δδ
4	0.95	0.06	163.9; 155.2	8.7
5	1.01		163.8; 155.5	8.3
6b	1.02	0.06	164.1; 155.2	8.8
7b	1.08		163.9; 155.5	8.4

[a] For comparison chemical shifts of pyrazine carbons in isomers of A-ring linked bis-cholestanopyrazines[3b] are: *cis* 149.1, 148.4 (Δδ = 0.7); *trans* 149.0, 148.5 (Δδ = 0.5).

4-nor-5α-cholestan-2-one (**10**). The *R* configuration at C-3 in this compound was unequivocally proved by ^{1}H NMR ($J_{4\alpha,5}$ = 7.1 Hz is in a good agreement with a torsion angle H3–C3–C5–H5 value of 34° obtained from molecular modeling of compound **10**). Another product of the reaction was compound **8**, an epimer of the starting 1α-bromo-4-nor-5α-cholestan-2-one (**3**) with inverted configuration at C-1. Both these compounds (**3** and **8**) were formed by addition of water to the intermediate enamine **D**. The mechanism of the compound **11** formation is not clear, it seems that it was formed by radical oxidation of enamine **E** with air.

From the results of this study a conclusion can be drawn that the direct method of pyrazine synthesis using gaseous ammonia can be extended to the five-membered α-bromoketones. However, the method is not general and can be applied only to those α-bromoketones that can react with ammonia at both electrophilic centers. One of them is a carbonyl group, while the second one is a bromo substituted carbon atom. If it is adjacent to a tertiary center (*e.g.* at C10), ammonia reacts only with a carbonyl group in a first step of reaction. Instead of bromide substitution by ammonia, different reactions take place on addition of water and heating, *e.g.* elimination, hydrolysis or oxidation. The imine or enamine compounds that are initially formed undergo hydrolysis with reconstruction of a carbonyl group.

EXPERIMENTAL

General Methods

Melting points were determined on a Kofler apparatus of the Boetius type. NMR spectra were recorded with a Bruker AC 200F (200 MHz) spectrometer using $CDCl_3$ solutions with TMS as the internal standard (only selected signals in the ^{1}H NMR spectra are reported). Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) in Hz. Infrared spectra (ν in cm^{-1}) were recorded on a Nicolet series II Magna-IR 550 FT-IR spectrometer in chloroform solutions unless otherwise stated. Mass spectra were obtained by electron impact (EI, 70 eV) with an AMD-604 spectrometer. The reaction products were isolated by column chromatography performed on 70–230 mesh silica gel (J. T. Baker). The starting α-bromoketones **1**, **2a**, **2b** and **3** were prepared by bromination of the corresponding enol acetates according to the procedure described by Fajkoš and Šanda[10].

Reaction of α-Bromoketones **1**, **2a**, **2b** and **3** with Ammonia. General Procedure

α-Bromoketone (**1**, **2a**, **2b** or **3**; 1.5 mmol) was dissolved in a small amount of benzene (1–2 ml) and then 1-butanol (15 ml) was added. The vigorously stirred solution was treated with ammonia under pressure ($4 \cdot 10^5 - 5 \cdot 10^5$ Pa) at room temperature until the reaction was completed (16–48 h, TLC control). Water (10 ml) was then added and the reaction mixture was refluxed for 24 h. Stirring was continued in an open flask at room temperature for

further 2 h. The reaction mixture was evaporated to dryness and subjected to preparative column chromatography.

Reaction of α-bromoketone **1** afforded a mixture of the *cis*- and *trans*-pyrazines **4** and **5** in 38% yield, similarly **2b** afforded **6b** and **7b** (21%), whereas the reaction of α-bromoketone **3** yielded products **8**–**11**.

*Bis-(3*β*-acetoxy-5*α*-androstano[16,17-b:17′,16′-e]pyrazine* (**4**) (less polar *cis* isomer). Eluted with hexane–ethyl acetate 4 : 1, m.p. 325–327 °C (dichloromethane–hexane). IR: 1 723, 1 262, 1 023. ^{1}H NMR: 4.71 m, 2 H (H-3α); 2.50–2.80 broad m, 4 H (H-15); 2.22 m, 2 H (H-12β); 2.03 s, 6 H (Ac); 0.95 s, 6 H (H-18); 0.90 s, 6 H (H-19). ^{13}C NMR: 170.7 (C); 163.9 (C); 155.2 (C); 73.6 (CH); 54.7 (CH); 54.4 (CH); 44.8 (CH); 44.4 (C); 36.5 (CH_2); 35.8 (C); 34.2 (CH); 34.0 (CH_2); 33.5 (CH_2); 31.9 (CH_2); 31.6 (CH_2); 28.4 (CH_2); 27.4 (CH_2); 21.4 (CH_3); 20.7 (CH_2); 17.4 (CH_3); 12.2 (CH_3). MS, *m/z* (rel.%): 656 (M^+; 48), 641 (100), 581 (54). For $C_{42}H_{60}N_2O_4$ (656.9) calculated: 76.79% C, 9.21% H, 4.26% N; found: 76.60% C, 9.12% H, 4.23% N.

*Bis-(3*β*-acetoxy-5*α*-androstano[16,17-b:16′,17′-e]pyrazine)* (**5**) (more polar *trans* isomer). Eluted with the same solvent, m.p. 340–342 °C (dichloromethane–hexane); ref.[8] m.p. 342–343 °C. Compound **5** showed the spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS) identical with those described in ref.[8].

Bis-(3-methoxyestra-1,3,5(10)-trieno[16,17-b:17′,16′-e]pyrazine) (**6b**) (less polar *cis* isomer). Eluted with hexane–ethyl acetate 4 : 1, m.p. 346–348 °C with decomposition (hexane–dichloromethane). IR: 1 609, 1 575, 1 500, 1 257, 1 246, 1 180, 1 107. ^{1}H NMR: 7.27 d, 2 H, *J* = 8.5 (H-1); 6.75 dd, 2 H, *J* = 8.5, 2.6 (H-2); 6.68 d, 2 H, *J* = 2.6 (H-4); 3.80 s, 6 H (MeO); 2.94 m, 4 H; 2.60–2.90 m, 4 H; 2.35–2.55 m, 6 H; 1.02 s, 6 H (H-18). ^{13}C NMR: 164.1 (C); 157.6 (C); 155.2 (C); 137.8 (C); 132.5 (C); 126.1 (CH); 113.9 (CH); 111.6 (CH); 55.2 (CH_3); 53.8 (CH); 44.6 (C); 44.3 (CH); 37.4 (CH); 33.6 (CH_2); 31.8 (CH_2); 29.6 (CH_2); 27.5 (CH_2); 26.2 (CH_2); 17.5 (CH_3). MS, *m/z* (rel.%): 560 (M^+; 83), 545 (100), 280 (10), 173 (24).

Bis-(3-methoxyestra-1,3,5(10)-trieno[16,17-b:16′,17′-e]pyrazine) (**7b**) (more polar *trans* isomer). Eluted with hexane–ethyl acetate 4 : 1, m.p. ≥ 350 °C with decomposition (hexane–dichloromethane). IR: 1 609, 1 575, 1 500, 1 257, 1 246, 1 180, 1 107. ^{1}H NMR: 7.27 d, 2 H, *J* = 8.5 (H-1); 6.75 dd, 2 H, *J* = 8.5, 2.6 (H-2); 6.68 d, 2 H, *J* = 2.6 (H-4); 3.80 s, 6 H (MeO); 2.94 m, 4 H; 2.60–2.90 m, 4 H; 2.35–2.55 m, 6 H; 1.08 s, 6 H (H-18). ^{13}C NMR: 163.9 (C); 157.6 (C); 155.5 (C); 137.8 (C); 132.4 (C); 126.0 (CH); 113.9 (CH); 111.6 (CH); 55.2 (CH_3); 54.0 (CH); 44.5 (C); 44.4 (CH); 37.4 (CH); 33.7 (CH_2); 31.7 (CH_2); 29.7 (CH_2); 27.5 (CH_2); 26.2 (CH_2); 17.6 (CH_3). MS, *m/z* (rel.%): 560 (M^+ ; 81), 545 (100), 280 (11), 173 (25).

*1*β*-Bromo-4-nor-5*α*-cholestan-2-one* (**8**). Eluted with hexane–ethyl acetate 97 : 3 (125 mg, 33%), m.p. 154–157 °C (hexane). IR: 1 751, 908, 858. ^{1}H NMR: 4.29 s, 1 H (H-1α); 2.34 dd, 1 H, *J* = 17.0, 5.8 (H-4); 0.94 s, 3 H (H-19); 0.91 d, 3 H, *J* = 6.6 (H-21); 0.87 d, 6 H, *J* = 6.6 (H-26, H-27); 0.68 s, 3 H (H-18). ^{13}C NMR: 209.3 (C); 66.1 (CH); 56.2 (CH); 55.9 (CH); 54.3 (CH); 45.8 (C); 44.8 (CH); 42.4 (C); 39.5 (CH_2); 38.1 (CH_2); 36.11 (CH_2); 36.08 (CH); 35.7 (CH); 31.2 (2 × CH_2); 27.97 (CH); 27.95 (CH_2); 25.5 (CH_2); 24.3 (CH_2); 23.8 (CH_2); 22.84 (CH_2); 22.79 (CH_3); 22.5 (CH_3); 18.6 (CH_3); 12.1 (CH_3); 11.2 (CH_3). MS, *m/z* (rel.%): 452 (M^+; 25), 450 (M^+; 26), 372 (19), 297 (100), 217 (78). HR-MS: for $C_{26}H_{43}O^{79}Br$ calculated: 450.2497; found: 450.2502.

*4-Nor-5*α*-cholest-3-en-2-one* (**9**). Eluted with hexane–ethyl acetate 94 : 6 (60 mg, 19%), m.p. 85–87 °C; ref.[9a] m.p. 87–88 °C; ref.[9b] m.p. 96–97 °C. IR: 1 681, 1 619. ^{1}H NMR: 5.73 d, 1 H, *J* = 1.5 (H-3); 2.66 m, 1 H (H-6); 2.41 m, 1 H (H-6); 2.24 d, 1 H, *J* = 18.5 (H-1); 2.18 d, 1 H, *J* = 18.5 (H-1); 1.16 s, 3 H (H-19); 0.91 d, 3 H, *J* = 6.5 (H-21); 0.86 d, 6 H, *J* = 6.5 (H-26,

H-27); 0.72 s, 3 H (H-18). ^{13}C NMR: 208.5 (C); 189.6 (C); 125.3 (CH); 56.0 (CH); 55.6 (CH); 53.8 (CH); 50.6 (CH_2); 46.1 (C); 42.9 (C); 39.54 (CH_2); 39.47 (CH_2); 36.1 (CH_2); 35.7 (CH); 35.6 (CH); 32.7 (CH_2); 28.1 (CH_2); 28.0 (CH); 27.6 (CH_2); 24.1 (CH_2); 23.8 (CH_2); 23.6 (CH_2); 22.8 (CH_3); 22.5 (CH_3); 19.9 (CH_3); 18.6 (CH_3); 12.0 (CH_3). MS, *m/z* (rel.%): 370 (M^+; 56), 355 (8), 342 (97), 327 (19), 229 (100). HR-MS for $C_{26}H_{42}O$ calculated: 370.3236; found: 370.3244.

3β-Hydroxy-4-nor-5α-cholestan-2-one (**10**). Eluted with hexane–ethyl acetate 93 : 7 (40 mg, 12%), m.p. 159–161 °C. IR: 3 575, 3 467, 1 743, 1 264, 1 140. ^{1}H NMR: 3.93 d, 1 H, *J* = 7.0 (H-3α); 2.45 broad s, 1 H (OH); 2.15 dd, 2 H, *J* = 16.2, 8.7 (H-1); 0.99 s, 3 H (H-19); 0.88 m, 9 H (H-21, H-26, H-27); 0.67 s, 3 H (H-18). ^{13}C NMR: 219.7 (C); 73.2 (CH); 56.2 (CH); 55.1 (CH); 54.3 (CH); 53.3 (CH_2); 53.1 (CH); 42.9 (C); 42.1 (C); 39.6 (CH_2); 39.5 (CH_2); 36.1 (CH_2); 35.7 (CH); 35.5 (CH); 31.6 (CH_2); 28.1 (CH_2); 28.0 (CH); 24.1 (CH_2); 23.8 (CH_2); 23.3 (CH_2); 22.8 (CH_3); 22.5 (CH_3); 20.0 (CH_2); 18.7 (CH_3); 16.0 (CH_3); 12.2 (CH_3). EI-MS, *m/z* (rel.%): 388 (M^+; 92), 373 (20), 370 (15), 315 (29), 233 (100). For $C_{26}H_{44}O_2$ (388.6) calculated: 80.36% C, 11.41% H; found: 80.15% C, 11.32% H.

3-Hydroxy-4-nor-5α-cholest-3-en-2-one (**11**). Eluted with hexane–ethyl acetate 91 : 9 (19 mg, 6%), m.p. 65–67 °C (dichloromethane–hexane). IR: 3 437, 3 378, 1 745, 1 695, 1 655. ^{1}H NMR: 2.52 m, 1 H (H-6); 2.25 m, 1 H (H-6); 2.17 m, 2 H (H-1); 1.11 s, 3 H (H-19); 0.90 d, 3 H, *J* = 8.1 (H-21); 0.87 d, 6 H, *J* = 6.7 (H-26, H-27); 0.71 s, 3 H (H-18). ^{13}C NMR: 202.8 (C); 184.8 (C); 125.9 (C); 56.1 (CH); 55.7 (CH); 53.7 (CH); 48.4 (CH_2); 43.0 (C); 41.7 (C); 39.6 (CH_2); 39.5 (CH_2); 36.1 (CH_2); 35.7 (2 × CH); 32.0 (CH_2); 31.8 (CH_2); 28.2 (CH_2); 28.0 (CH); 24.2 (CH_2); 23.8 (CH_2); 23.3 (CH_2); 22.8 (CH_3); 22.5 (CH_3); 18.7 (CH_3); 18.4 (CH_3); 12.1 (CH_3). MS, *m/z* (rel.%): 386 (M^+; 15), 371 (10), 358 (100), 343 (7). HR-MS: for $C_{26}H_{42}O_2$ calculated: 386.3185; found: 386.3198.

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