A Facile Synthesis of Symmetrical Dimeric Steroid-pyrazines†

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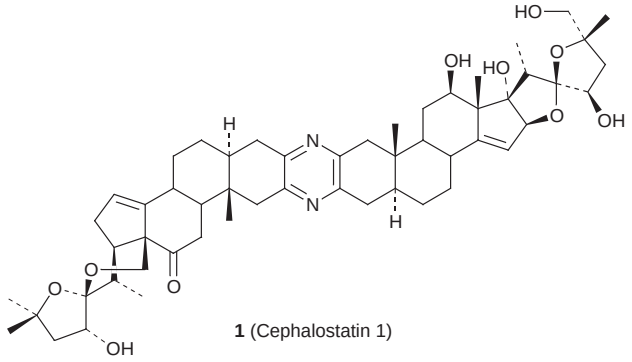
Zenon Łotowski, Agnieszka Gryszkiewicz, Jolanta B. Borowiecka, Agnieszka Nikitiuk and Jacek W. Morzycki*

Institute of Chemistry, University of Bialystok,‡ Pilsudskiego 11/4, 15-443 Bialystok, Poland

The reaction of steroidal 2α-bromo-3-ketones (*e.g.* 2α-bromocholestan-3-one **2**) with ammonia followed by hydrolysis and air-oxidation affords the easily separable mixture of the *trans* and *cis* dimeric steroid-pyrazines (**3** and **4**, respectively).

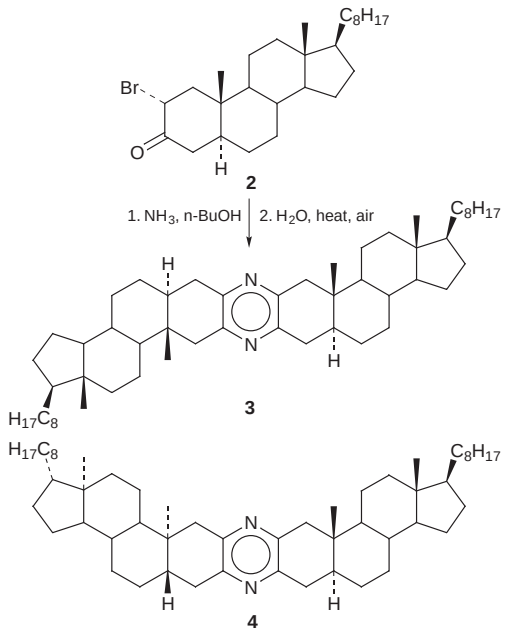
A family of the dimeric steroid-pyrazine alkaloids has recently been discovered. Cephalostatins were isolated from the marine tube-inhabiting invertebrate worm *Cephalodiscus gilchristi* by Pettit's group.[1] The structurally related compounds (ritterazines) were isolated later from the tunicate *Ritterella tokioka* by Fusetani's group.[2] Among both groups of trisdecacyclic pyrazines are the most powerful anticancer compounds ever tested (such as cephalostatin 1; **1**).[3] The first synthesis of cephalostatin 1 was reported last year.[4] There are many different routes to the synthesis of a central pyrazine ring. Some of them allow the synthesis of unsymmetrical dimers.[5,6] The symmetrical dimeric steroid-pyrazines can be obtained by the classical condensation of α-amino ketones that is, actually, the most efficient method of pyrazine ring construction. The intermediate steroidal 2α-amino-3-ketones are available by reduction of the corresponding 3-ketones with a nitrogen containing substituent at C-2, such as azido,[7,8] hydroxyimino,[9] enamino[10] or nitro group.[11] The initially formed 2α-amino-3-ketones undergo spontaneous dimerisation to a mixture of dihydropyrazines, which are then oxidised by air.



1 (Cephalostatin 1)

Results and discussion

In the synthesis of pyrazines (both symmetrical and unsymmetrical) the most useful precursors of α-amino ketones are α-azido ketones, readily available from α-bromo ketones. The latter compounds, in principle, can react directly with ammonia to afford α-amino ketones and eventually pyrazines. However, this reaction has not been described in the literature. The aim of this study is to explore the possibility of application of the steroidal 2α-bromo-3-ketone reaction with ammonia to the synthesis of the symmetrical dimeric steroid-pyrazines. 2α-Bromo-5α-cholestan-3-one **2** was chosen as a model compound and its reaction with ammonia was performed. The reaction was carried out in *n*-butanol solution under pressure (4–5 atm) of gaseous NH_3 at room temperature. The TLC control showed that the reaction was completed within several hours and a very polar product was formed. The presence of an amino group in the product was proved by a positive test for alkaloids with Dragendorff's reagent. After addition of water the reaction mixture was refluxed for 24 h. This procedure yielded a mixture of *trans*- and *cis*-di(5α-cholestano)pyrazines (**3** and **4**, Scheme 1) easily separable by crystallisation. The solubility of both products in organic solvents is strikingly different and this permits a clean separation of the mixture of two isomers. The major product, the *trans* isomer **3**, was obtained by crystallisation from toluene (39% yield), whereas the *cis* isomer **4** was purified by flash chromatography of the mother liquor (23% yield). The same products have been previously obtained upon reaction of cholestan-2,3-dione with cholestane-2α,3α-diamine.[8] Formation of *cis* dimer **4** can be explained assuming that ammonia attacks both electrophilic sites of 2α-bromocholestan-3-one (**2**), *i.e.* C-2 and the carbonyl group. The diamino compound (*e.g.* **A**) thus obtained cannot form a pyrazine derivative and must be partially hydrolysed to 2-amino-3-ketone or 3-amino-2-ketone prior to dimerisation. Lack of selectivity in this procedure is,

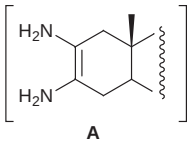


Scheme 1

* To receive any correspondence (*e-mail:* morzycki@noc.uwb.edu.pl).

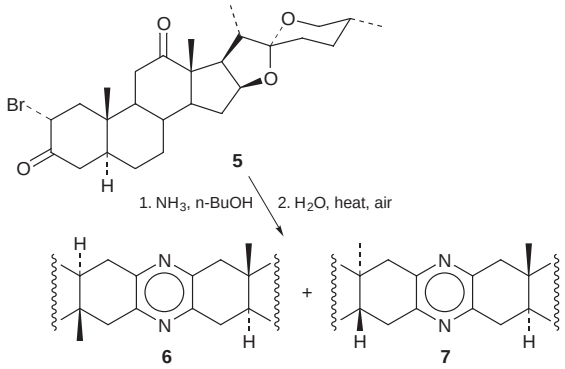
† This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1999, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

‡ Formerly: University of Warsaw, Białystok Branch.



A

of course, a disadvantage unless a concurrent synthesis of both isomeric dimers is needed. However, the procedure is very simple and the products separation is extremely easy. Since the ^{1}H NMR spectra of isomeric dimers are nearly identical, they can hardly be distinguished by this method. There is only a slight shift in the heteroaromatic carbon resonances (*trans*: 148.5, 149.0; *cis* 148.4, 149.1), which cannot be diagnostic for the di(5α-cholestano)pyrazine configuration. However, the isomers can be easily identified by TLC provided that both geometric isomers are available (the *trans* isomer proved less polar as was expected). Using the same procedure both isomers of di(25*R*-12-oxo-5α-spirostano)pyrazines (**6** and **7**) were synthesised from (25*R*)-2α- bromo-5α-spirostane-3,12-dione (**5**, Scheme 2). The yield of products (the *trans* and *cis* isomer ratio was approximately 1 : 1) was lower in this case probably due to the presence of an additional carbonyl group at C-12.



Scheme 2

Experimental

Preparation of Di(5α-cholestano[2,3-b:2′,3′-e])pyrazine **3** *and Di(5α-cholestano[2,3-b:3′,2′-e])pyrazine* **4**.—2α-Bromo-5α-cholestan-3-one (**2**; 2.35 g; 5.05 mmol) was dissolved in a small amount of benzene (5–10 ml) and *n*-butanol (50 ml) was added. The vigorously stirred solution was treated overnight with ammonia under pressure (4–5 atm) at room temperature. Water (about 12 ml) was then added and the reaction mixture was refluxed for 24 h. Stirring was continued in an open flask at room temperature for a further 2 h. The reaction mixture was evaporated to dryness and crystallised from toluene to give *trans* dimer **3** (750 mg; 39%). The mother liquor was concentrated and flash chromatographed on a silica gel column. Elution with benzene afforded *cis* dimer **4** (450 mg; 23%). Analytical and spectral data of both compounds proved identical with that described in the literature.[8]

Preparation of Di(25R-12-oxo-5α-spirostano[2,3-b:2′,3′-e])-pyrazine **6** and *Di(12-oxo-5α-spirostanol[2,3-b:2′,3′-e])pyrazine* **7**.—(25*R*)-2α-Bromo-5α-spirostane-3,12-dione (**5**; 0.5 g; 1 mmol) was dissolved in 70 ml of *n*-butanol and the stirred solution was treated with ammonia under pressure at 30 °C for 7 days. After addition of water (6 ml) the reaction mixture was refluxed overnight. Stirring was continued in an open flask at room temperature for a further 2 h and solvents were removed *in vacuo*. The residue was subjected to silica gel column chromatography. Chloroform–benzene–ethyl acetate (20 : 66 : 14) eluted consecutively pure compound **6** (47 mg), a mixture of **6** and **7** (80 mg), and pure compound **7**. (46 mg). Total yield of dimeric pyrazines: 41%. Both compounds form white microcrystals which do not melt below 300 °C; $[\alpha]_D = +59°$ (*c* 0.5; $CHCl_3$) for **6**; $[\alpha]_D = +62°$ (*c* 0.5; $CHCl_3$) for **7**. Compounds **6** and **7** gave identical IR and MS spectra, nearly identical ^{1}H NMR spectra and very similar ^{13}C NMR spectra; $\nu_{max}(CHCl_3)$: 2959, 2931, 2875, 2861, 1706, 1455, 1400, 1159, 1076, 1055, 1040, 980, 898 cm^{-1}. δ_H: 4.36 (m, 2H, 16α-H)), 3.48 (m, 2H, 26-H), 3.36 (t, $J = 10.6$ Hz, 2H, 26-H), 2.95–2.42 (m, 8H, 1H and 4H), 1.08 (s, 3H, 19-H), 1.07 (d, $J = 5.8$ Hz, 3H, 21-H), 0.89 (s, 3H, 18-H), 0.79 (d, $J = 6.1$ Hz, 3H, 27-H); LSI-MS, *m/z*: 849 (MH^+), 735, 667, 359. δ_C (for **6**): 213 (C), 148.5 (C), 148.2 (C), 109.3 (C), 79.1 (CH), 66.9 (CH_2), 55.6 (CH), 55.0 (C), 54.8 (CH), 53.5 (CH), 45.2 (CH_2), 42.2 (CH), 41.6 (CH), 37.6 (CH_2), 36.1 (C), 35.2 (CH_2), 34.2 (CH), 31.4 (CH_2), 31.2 ($2 \times CH_2$), 30.2 (CH), 28.8 (CH_2), 28.1 (CH_2), 17.1 (CH_3), 15.9 (CH_3), 13.2 (CH_3), 11.7 (CH_3); the pyrazine carbon signals for **7** merge at δ148.3, other signals are the same.

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