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SYNTHESIS AND CIRCULAR DICHROISM OF PYRIDAZINO[4,5-b]CHOLESTENES§

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Abstract: The diester derivative **7** of the title ring system prepared by cycloaddition of the steroidal enamine **5** and tetrazine carboxylic acid ester was subjected to hydrolysis and subsequent thermolysis to give the unsubstituted title compound **8**. Comparison of its UV and CD spectra are discussed and experimental evidence for an optically forbidden $n \rightarrow \pi^*$ transition (A_2 state) are provided.

As part of our recent research on diazines fused to 5α-cholest-2-ene, the CD of chiral pyrazines[1,2,3] and pyrimidines[4] has been reported and interpreted. As a continuation of this series, we decided now to synthesize pyridazino[4,5-b]-5α-cholest-2-ene and to study its chiroptical behaviour. This target compound seemed of particular interest because of its local c_{2v} symmetry around the heteroaromatic chromophore.

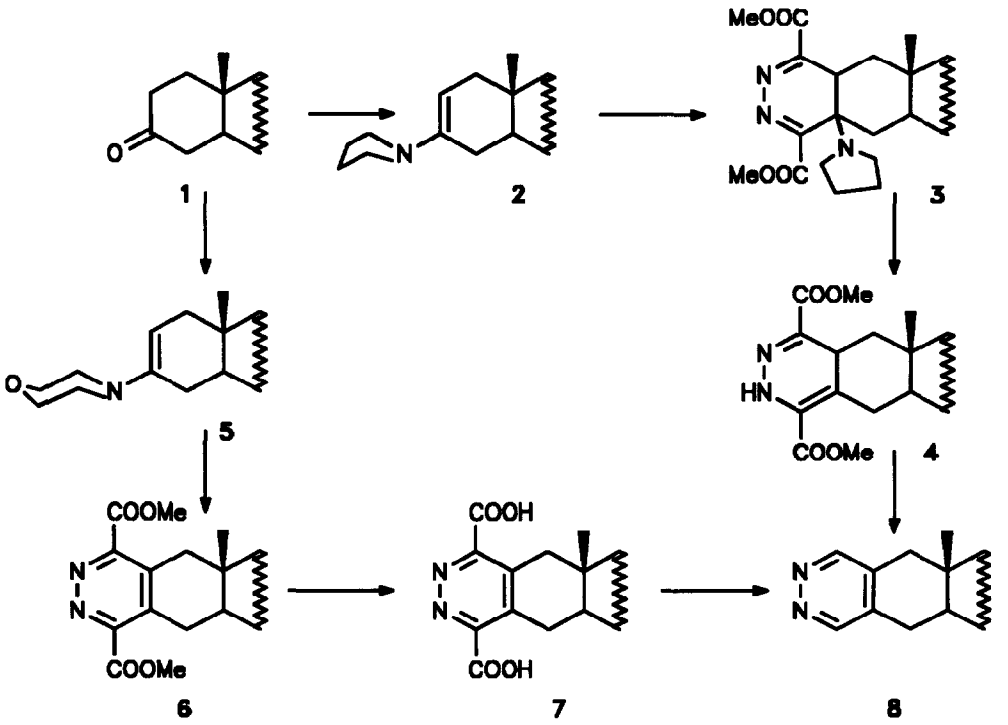
For the synthesis of the title compound, direct cycloaddition starting from the steroidal enamine and substituted tetrazine seemed to be straightforward. This general procedure employing Diels Alder reaction of inverse electronic demand has been successfully applied for the preparation of various pyiridazine derivatives [5,6].

To this end, 3-pyrrolidinyl-5α-cholest-2-ene **2** was prepared from cholestan-3-one according to literature procedure[7] and was treated with diethyl 1,2,4,5-tetrazine-3,6-dicarboxylate under the usually applied reaction conditions[6]. An intense nitrogen evolution was observed, and orange crystals were isolated from the reaction mixture. Structure elucidation of this product revealed, however, that instead of the expected pyridazine ester **7** the primary cycloadduct **3** containing the pyrrolidinyl moiety has been obtained. Although, the ester **7** could also be detected in the mother liquor in traces, our efforts at its isolation on a preparative scale were unsuccessful.

Attempts to eliminate pyrrolidine by treatment of the cycloadduct **3** with acid (by refluxing in a mixture of acetic acid and ethanol) also failed and, instead, fragmentation took place to yield the

§Dedicated to the memory of Prof. G. Snatzke

dihydropyridazine ester **4** (very strong Cotton effects were observed in the CD owing to the extension of the conjugated chromophore into the chiral skeleton, see Experimental Section).



The desired synthesis could be finally accomplished by the change of the pyrrolidine moiety for the morpholino group in the starting steroidal enamine. Thus, morpholino enamine **5** was synthesized and treated with the same tetrazine reagent as above. Unlike the previous case, this reaction mixture afforded pure pyridazine dicarboxylic ester **6** in high yield. Hydrolysis of the ester gave the dicarboxylic acid **7**, and pyrolysis carried out in formamide at 200°C resulted in decarboxylation and led to the unsubstituted pyridazine **8**.

The electronic structure of pyridazine has been the subject of both experimental and theoretical studies. Photoelectron spectroscopic studies[8] reveal that the highest MO is the n_- orbital of b_2 symmetry followed by a π orbital of a_2 symmetry. The next two orbitals (π and n_+, of b_1 and a_1 symmetry, respectively) were found at nearly the same energy level ("strongly overlapping bands" in PES[8a]). The LUMO has obviously a_2 symmetry[9]. On this basis four electronic transitions (two $\pi \rightarrow \pi^*$ and two $n \rightarrow \pi^*$ ones) can principally be anticipated. Three of these: the $n_- \rightarrow \pi^*$ and the two $\pi \rightarrow \pi^*$ transitions (formation of B_1, A_1, and B_2 symmetry with x, z, and y polarized transitions, respectively) are electronically dipole allowed, whereas the participation of the forth MO in a transition ($n_+ \rightarrow \pi$; A_2 state) should only be

magnetically dipole allowed and electronically dipole forbidden. This qualitative conclusion has also been supported by quantum chemical calculations[10,13].

On the pyridazine chromophore no CD study has yet appeared[11], whereas a number of publications on the UV spectra have been published[12,14,15]. The UV and CD spectra of pyridazine derivative **8** recorded in isooctane (apolar solvent) and in acetonitrile (polar solvent) are shown in Fig 1. Inspection of the UV spectrum reveals that three bands appear. The first maximum is strongly redshifted when changing the polarity of the solvent from acetonitrile to i-octane and thus - in accordance of literature assignments[10] - can safely be assigned to the $n_{-}\rightarrow\pi^{*}$ (HOMO-LUMO) transition. The additional two absorptions do not differ when changing solvent which seems to be in agreement with their $\pi\rightarrow\pi^{*}$ nature. The forth, electronically forbidden and magnetically allowed transition does not appear in the UV of **8**.

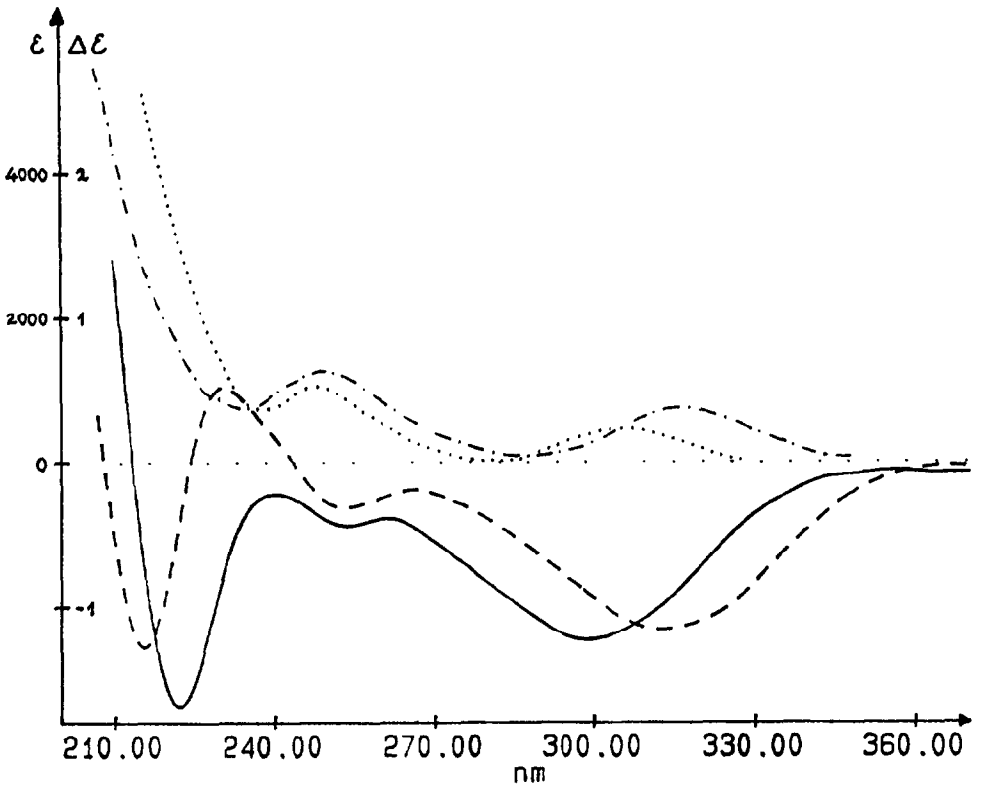


Fig. 1.UV and CD spectra of steroidal pyridazine compound **8**——— CD in acetonitrile; ----- CD in isooctane; -.-.-.- UV in isooctane; ..... UV in acetonitrile

Comparison of the CD and UV spectra recorded in i-octane shows that, in contrast to the three UV maxima, four Cotton effects can be observed of which three (those at 315, 253, and 214 nm, all are negative CD's) appear approximately at the same wavelengths as the three UV bands (316, 250, and 205 nm), and, additionally, a forth absorption (with a positive Cotton-effect) also appears at a wave length (229 nm) where the UV spectrum has a minimum. This striking difference between the UV and CD spectra concerning this absorption strongly suggests that this band can be assigned to the magnetically allowed $n_+ \rightarrow \pi$ (A_2) transition. Unfortunately, this Cotton effect can not be observed in the spectrum of the acetonitile solution because of its overlap with the neighbouring bands.

This observation provided a direct experimental evidence by relatively simple technique for the $n_+ \rightarrow \pi^*$ transition at 229 nm of the pyridazine chromophore which was theoretically predicted[10,13]. Although, there are experimental arguments for appearance of two $n \rightarrow \pi^*$ in the spectrum of pyridazine[14,15], these findings refer to a lower energy transition (373 nm) observed by analysis of a vibrational fine structure. A more refined calculation for the interpretation of the energy of observed CD band needs additional studies.

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Experimental Section

Melting points were measured by a Büchi apparatus and are uncorrected. The IR spectra were determined by a Nicolet 205 FT spectrometer. The NMR spectra were recorded with a Bruker AM 400 spectrometer, TMS was used als inner standard. The MS spectra were measured with a Varian CH-5 instrument. The CD spectra were run on a Dichrograph Mark III from ISA with attached PDP-8/e computer at room temperature in cells of 2.00 to 0,01 cm path length and concentrations of appr. 1 mmol/lit.

Dimethyl 2,3-Dihydro-3-pyrrolidino-pyridazino[4,5-b]-5α-cholest-2-ene-3',6'-dicarboxylate (3)

To a solution of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (0.6 g, 3.05 mmol) in tetrahydrofurane (20 ml), solid pyrrolidyl enamine **2** (1.35 g, 3.05 mmol) was added in portions at 5-10°C. In the course of the addition, an intense gas evolution was observed. An orange colored solution was formed which was stirred for additional 10 min, it was then evaporated in *vacuo* and the residue was treated with methanol (8 ml) to give orange crystals. Recrystalization from ethyl acetate afforded 1.06 g (51%) of product; mp 188-90°C; $[\alpha]_D$: - 161.8 ($CHCl_3$, c = 1). IR (KBr): 3000-2820 (broad), 1700, 1420, 1400, 1390 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 3.87 (s, 6H, CH_3), 3.23 (d, 1H, H-1β), 2.55 (m, 4H, H-pyrrolidyl), 2.34 (d, 1H, H-1α). MS: 609 (M^+). UV λ max (ε) acetonitrile: 400 nm. (105), 258 nm (sh, 7400). CD λ max ($\Delta\varepsilon$): 299 nm (3.93), 262 nm (-1.69), 215 nm (-12.0). (Anal. Calcd. for $C_{37}H_{59}N_3O_4$ (609.9) : C, 72.87; H, 9.75; N, 6.89. Found: C, 72.85; H, 9.69; N, 6.71.

Dimethyl 1',2-Dihydro-pyridazino[4,5-b]-5α-cholest-2-ene-3',6'-dicarboxylate (4)

To a solution of cycloadduct **3** (0.6 g, 1 mmol) in ethanol (5 ml) were added a few drops of acetic acid, and the solution was refluxed until the initial orange color turned to colorless (appr. 10 min was needed). The mixture was poured onto water and the product was removed by extraction with dichloromethane. Recrystallization from ethanol gave 0.42 g (78%) of product; mp: 138-40°C, $[\alpha]_D$: +446.1 (c = 1; $CHCl_3$). IR (KBr): 3400, 3000-2820 (broad), 1740, 1720, 1610, 1480, 1460 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 8.0 (s, 1H, NH), 3.80, s 3.81 (2 s, 6H, CH_3), 3.52-3.45 (m, 2H, H-2,1β). ^{13}C-NMR ($CDCl_3$): δ 166.31, 162.61 ($COOCH_3$), 132.75 and 129.95 (C-3',6'), 119.12 (C-3). MS: 540 (M^+). UV λ max (ε) acetonitrile: 354 nm (4040), 276 nm (11050). CD λ max (Δ ε) acetonitrile: 353 nm (21.36), 281 nm (-1.17), 262 nm (1.47), 235 nm (4.82). Anal. Calcd. for $C_{33}H_{52}N_2O_4$ (540.79): C, 73.29; H, 9.69; N, 5.18. Found: C, 73.19; H, 9.48; N, 4.94.

3-Morpholino-5α-cholest-2-ene (5)

A mixture of 5α-cholesten-3-one (19.3 g, 30 mmol), morpholine (13 ml) and toluene (130 ml) was refluxed for 20 hours using water separating column. Evaporation of the mixture and recrystallization of the residue from ethyl acetate gave 16.5 g (73%) of cream colored crystals; mp 107-109°C $[\alpha]_D$: +39.9 (c = 1, $CHCl_3$). IR (KBr): 3000-2800 (broad), 1640, 1460, 1430 cm^{-1}. MS: 455 (M^+). Anal. Calcd. for $C_{31}H_{53}NO$ (455.77): C, 81.70; H, 11.72; N, 3.07. Found: C, 81.52; H, 11.63; N, 3.01.

Dimethyl pyridazino[4,5-b]-5α-cholest-2-ene-3',6'-dicarboxylate (6)

To a solution of dimethyl 1,2,4,5-tetrazine-3,6-dicarboxylate (1.8 g, 9 mmol) in dioxane (50 ml) was added morpholinoenamine **5** (4.4 g, 9.6 mmol) in small portions at 5 - 10 °C. A vigorous gas evolution was observed and the starting deep red color of the reagent disappeared. The stirring was continued for one additional hour at the same temperature and the mixture was then allowed to stand overnight at room temperature. Evaporation and recrystallization of the residue from ethanol gave 3.8 g (89%) of product; mp 148-50°C, $[\alpha]_D$: + 101.4 ($CHCl_3$, c = 1). IR (KBr): 3000-2820 (broad), 1730, 1720, 1470, 1440 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 4.0 (s, 6H, CH_3), 2.93 (d, 1H, 1β, $J_{1\beta,1\alpha}$ = 17.9 Hz), 2.88 (dd, 1H, 4α, $J_{4\beta,4\alpha}$ = 19.2 Hz, $J_{4\beta,5}$ = 5.5 Hz), 2.59 (dd, 1H, 4β, $J_{4\beta,5}$ = 12.4 Hz), 2.48 (d, 1H, 1α), 1.99 (tt, 1H, H-5). ^{13}C-NMR ($CDCl_3$): δ 165.19 and 165.10 ($COOCH_3$), 153.48 and 152.02 (C-3',6'), 137.95 and 137.74 (C-2,3). MS: 538 (M^+). UV λ max (ε) acetonitrile: 294 nm (810), 208 nm (17680). CD λ max (Δ ε): 306 nm (-1.56), 239 nm (7.13). Anal. Calcd. for $C_{33}H_{50}N_2O_4$ (538.78): C, 73.57; H, 9.35; N, 5.20. Found: C, 73.45,; H, 9.19; N, 5.08.

Pyridazino[4,5-b]-5α-cholest-2-ene-1',4'-dicarboxylic acid (7)

A mixture of dimethyl ester **6** (1.2 g), ethanol (15 ml) and 20 % sodium hydroxide solution (3 ml) was refluxed for 30 min. The reaction mixture was cooled down and neutralized by adding aqueous hydrogen chloride solution. Colorless solid (1.05 g, 93%; mp: 232-235°C) precipitated which was used without purification for further transformation. IR (KBr): 3650-3150 (broad), 3000-2820 (broad), 1720, 1620 cm^{-1}. MS: 510 (M^+). Anal. Calcd. for $C_{31}H_{46}N_2O_4$ (510.72): C, 72.91; H, 9.08. Found: C, 72.62; H, 8.89.

Pyridazino[4,5-b]-5α-cholest-2-ene (8)

A mixture of dicarboxylic acid 7 (1.5 g, 2.95 mmol) and formamide (25 ml) was heated at 200°C with stirring for 8 min. An intense foaming took place and a brown oily phase commenced on the surface of the reaction mixture. Extraction with ether, evaporation and recrystallization from acetonitrile gave 0.70 g (59%) of product. mp 163-5°C; $[\alpha]_D$: + 36.2 ($CHCl_3$, c= 1). IR (KBr): 3080, 2900-2820, 1580, 1570, 1460, 1440, 1420 cm^{-1}. ^{1}H-NMR ($CDCl_3$) : 8.82 (s, 2H, H-3',6'), 2.66 (d, 1H, H-1β; $J_{1\alpha,1\beta}$ = 18.0 Hz), 2.58 (dd, 1H, H-4α; $J_{4\alpha,4\beta}$ = 18.5 Hz; $J_{5,4\alpha}$ = 5.4 Hz), 2.38 (dd, 1H, H-4β; $J_{4\beta,5}$ = 5.3 Hz), 2.31 (d, 1H, H-1α); 2.02 (tt, 1H, H-5) ppm. ^{13}C-NMR: 152.68 and 151.55 (C-3' and 6'), 136.3 and 135.8 (C-2,3), 35.66 (C-1), 22.71 (C-4). MS: 422 (M^+), 407, 337, 309, 282, 267. UV λmax (ε) isooctane: 316 nm (620), 250 nm (1130), 205 nm (sh, 8520); acetonitrile: 306 nm (310), 244 nm (620), 210 (sh, 5400). CD λmax (Δε) isooctane: 315 nm (-1.15), 253 nm (-0.33), 229 nm (+ 0.52), 214 nm (- 1.26); acetonitrile: 299 nm (-1.23), 254 nm (-0.44), , 222 nm (-1.69). Anal. Calcd. for $C_{29}H_{46}N_2$ (422.70): C, 82.40; H, 10.97; N; 6.63. Found: C, 82.28; H 10.91; N, 6.50.

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