Synthesis and Properties of *cis*- and *trans*-4-Hydroxypraziquantel

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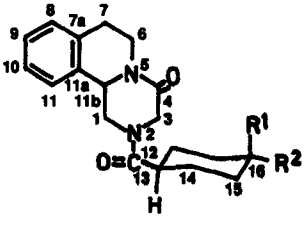
The title compound is the main metabolite of the anthelmintic agent praziquantel. The synthesis of *cis*- and *trans*-4-OH derivatives (as well as their enantiomers) of praziquantel is described. Thus, racemic, (+)- and (-)-pyrazinoisoquinolin-4-one (**1**) was reacted either with 4-oxocyclohexane carboxylic acid chloride or with 4-oxocyclohexane carboxylic acid in the presence of DCC to give the racemic, (+)- and (-)-ketone **2**. Racemic, (+)- and (-)-ketone **2**, respectively, was reduced with K-Selectride yielding the racemic, (+)- and (-)-*cis*-4-OH derivatives **3a**, or with $NaBH_4$ producing racemic, (+)- and (-)-*trans*-4-OH derivatives **3b**. The physicochemical properties (MS, NMR, HPLC) of both isomers and their enantiomers are reported.

Synthese und Eigenschaften von *cis*- und *trans*-4-Hydroxypraziquantel

Das 4-OH Derivat ist Hauptmetabolit des Anthelmintikums Praziquantel. Die Synthese der *cis*- und *trans*-4-OH-Derivate des Praziquantels (sowie deren Enantiomeren) wird beschrieben: Racemisches, (+)- und (-)-Pyrazinoisochinolin (**1**) reagierte mit 4-Oxocyclohexancarbonsäurechlorid oder mit 4-Oxocyclohexancarbonsäure in Gegenwart von DCC zu den racemischen, (+)- und (-)-Ketonen **2**. Racemisches, (+)- und (-)-Keton wurden mit K-Selectride oder mit $NaBH_4$ reduziert, dabei wurden die racemischen, (+)- und (-)-*cis*-4-OH Derivate **3a** bzw. die racemischen, (+)- und (-)-*trans*-4-OH Derivate **3b** erhalten. Die physikochemischen Eigenschaften der beiden Isomeren und deren Enantiomeren wurden untersucht (MS, NMR, HPLC).

Praziquantel (PZQ) (Biltricide®, Droncit® is a broadly effective trematocide and cestocide[1]. Its anthelmintic activity was discovered as a result of cooperation of E. Merck and Bayer A.G. in 1972.



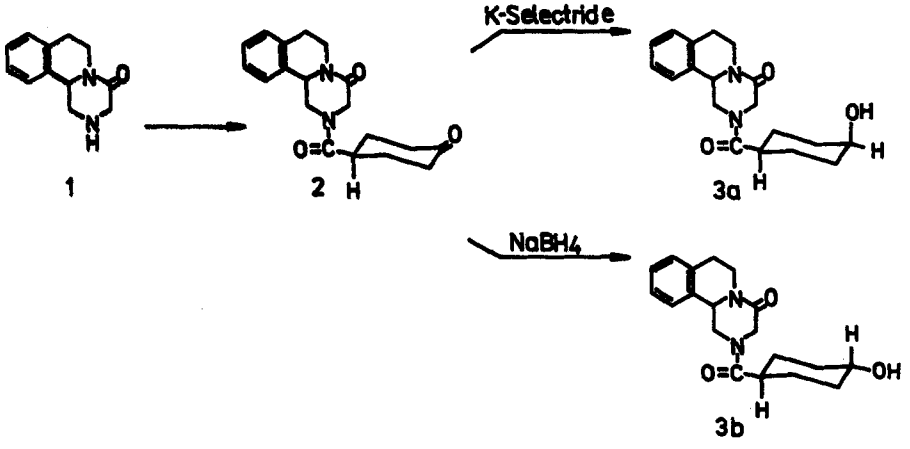
Praziquantel:
$R^1 = R^2 = H$
2 $R^1, R^2 = O$
3a $R^1 = OH$ $R^2 = H$
3b $R^1 = H$ $R^2 = OH$

PZQ, a pyrazinoisoquinolin-4-one derivative, is chemically unrelated to other schistosomicidal drugs[2]. It possesses an asymmetric center, the pharmacological activity is mainly concentrated in the (-)-isomer with *R*-configuration[3,4]. Its mode of action characterizes enhanced Ca^{2+}-permeability by membrane perturbation which causes influx of Ca^{2+} and the spastic paralysis of the parasites[3,5]. Pharmacokinetic studies showed that after *p.o.* administration it is rapidly absorbed in the intestinal tract and more then 80% of the dose is excreted with urine in the first 24 h[6]. The major metabolite in serum of man and animals is the 4-hydroxycyclohexyl analogue of PZQ (4-OH PZQ)[1]. It appeared in the plasma of animals already 5 min after intravenous injection of PZQ[3]. As a disubstituted cyclohexyl derivative it may exist in *trans* or *cis* configuration (*trans* 4-OH PZQ, *cis* 4-OH PZQ).

Till now the problem of its stereochemical properties was not discussed. To perform further investigations on the way of action and stereospecific metabolism of this drug, it was necessary to synthesize both isomers of 4-OH PZQ (as well as its enantiomers) and to investigate their spectral properties with the aim to find features which could enable us to differentiate both compounds in an analytical way.

Racemic pyrazinoisoquinolinon-4-one (**1**) was reacted with 4-oxocyclohexane carboxylic acid chloride in the presence of triethylamine to give the racemic ketone **2**. (+)- or (-)-**2** was obtained in the reaction of (+)- or (-)-base **1** with 4-oxocyclohexane carboxylic acid in the presence of DCC (Scheme 2).

It is known[7] that the reduction of cyclohexanone derivatives with Selectrides mainly produces *cis*-hydroxy derivatives, while the reduction with $NaBH_4$ mainly leads to the *trans*-isomers. Racemic (+)- and (-)-ketones **2** were reduced with K-Selectride to afford 99.5% geometrically pure



  0365-6233/91/0404-0235 $ 3.50 + .25/0

racemic, (+)- and (-)-*cis*- 4-OH PZQ **3a**. In the reaction of racemic and (+)-**2** with a stoichiometric amount of $NaBH_4$ racemic and (+)-*trans*-4-OH PZQ **3b** were obtained with 74% geometrical purity. When a twofold excess of reducing agent was used with racemic and (-)-**2** the racemic and (-)-**3b** were obtained with 95.8% geometrical purity. Reduction of racemic **2** with $NaBH_4$ on PTC conditions led to racemic **3b** with 89.5% geometrical purity.

The purity of the compounds was checked by TLC on silica gel and by reversed phase HPLC with RP-18 column (CH_3CN: H_2O = mobile phase). In the latter case separation of **3a** and **3b** was possible.

The chemical structures were confirmed by spectral analyses. Spectra were analysed with the aim to find the main differences between **3a** and **3b**. For this purpose ^{13}C-NMR and MS data were especially useful.

Chemical shifts of the cyclohexane derivatives can be calculated for *cis* and *trans* substituted compounds. Signals of the C-atoms with equatorial (*trans* 4-OH PZQ, **3b**) or axial (*cis 4-OH PZQ*, **3a**) hydroxyl groups were found at 70.61 ppm and 66.39 ppm, respectively, which is in accordance with calculated values (70.9 and 65.5 ppm)[8]. Assignments of ^{13}C NMR data were confirmed by additional experiments[9].

As a most convenient differentiating factor the EI-induced fragmentation patterns of **3a** and **3b** may be used. Contradictory data are reported concerning fragmentations of 1,4-disubstituted cyclohexane derivatives[10,11]. Our results are in agreement with the following reasoning: deuterium labelling indicates that loss of H_2O from cyclohexanol molecular ions involves both 1,4- and 1,3-elimination (7:3)[12] and that 1,4-elimination is largely stereospecific[13] whereas 1,3-elimination is not. 1,4-elimination may occur through the boat form. 1,4-*cis* disubstituted cyclohexane derivatives may more readily exist in a boat form. Loss of water occurs more readily from *cis*-cyclohexanediols than from *trans* isomers[11]. In our case we observe that for **3a** the M-18 fragment is formed with 10% rel. intensity while in the **3b** spectrum it was not detected.

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Experimental Part

General remarks

(+)-, (-)-, and racemic **1** and methyl ester of 4-oxocyclohexane carboxylic acid were supplied by E. Merck, Darmstadt.- Selectride reductions were performed under N_2 in oven dried (overnight at 125°C) flasks using oven dried syringes; THF was distilled under N_2 from $LiAlH_4$. K-Selectride, 1M in THF, was used as obtained from Janssen Chimica. Chemical yields: non-optimized reaction conditions.- Melting points: Kofler hot stage microscope, uncorrected.- TLC: Merck Kieselgel 60 F_{254}; solvent systems I: benzene : acetone : MeOH (4:4:1); II: toluene : MeOH (9:1).- Column chromatography on Kieselgel 60 (70-230 mesh ASTM).- HPLC: Varian 5000, RP-18 (5µm; 125 x 4mm) column and RP-18 (5µm; 25 x 4mm) precolumn, mobile phase CH_3CN : H_2O (23:77), 1.5 ml/min solvent flux, detection with variable UV detector at 210 nm.- IR spectra: Pye-Unicam SP3-200 ($\tilde{\nu}$ max in cm-1) KBr discs. (0.5 mg: 300 mg KBr).- Polarimetric data: Perkin-Elmer Polarimeter 241.- 1H-NMR and 13C-NMR spectra: Bruker WM 300 spectrometer; δ [ppm] relative to TMS; J[Hz]-apparent coupling constants, carbon multiplicities: APT technique.- EI-MS [m/z (% rel. int.) at 70 eV]: Varian MAT SM-1, 44S and CH-7.

4-Oxocyclohexane caboxylic acid

A solution of 1.56 g (0.01 mol) of methyl 4-oxocyclohexane caboxylate in 2% H_2SO_4 (20 ml) was heated to 80-90°C for 2 h. The cooled solution was 3 times extracted with ethyl ether, the org. phase was dried with $MgSO_4$, the solvent was evaporated in vacuo. The obtained crystals were recrystallized from ethyl ether : petrol ether mixture (90%), m.p. 66-68°C (Lit.[14]: 67°C).

Racemic 2-(4-oxocyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino-[2,1a]-isoquinolin-4-one (**2**)

A solution of 0.284 g of 4-oxocyclohexane carboxylic acid (2 mmol) in DMF (0.3 ml) and CH_2Cl_2 (2 ml) was cooled to -40°C and 0.25 g $SOCl_2$ (2 mmol) in CH_2Cl_2 were added dropwise. After stirring for 0.5 h, the solution of 0.404 g **1** (2 mmol) and 0.40 g triethylamine (4 mmol) in CH_2Cl_2 (5 ml) was added during 1 h at -40°C. The mixture was warmed to room temp. and stirred for another 2 h, washed with H_2O, $KHCO_3$ solution and with H_2O, dried with $MgSO_4$ and evaporated. The residual light yellow crystals (0.50 g) were chromatographed on silica gel firstly with $CHCl_3$, than with 2% methanol to give 0.42 g (56%) of **2** after recrystallization from acetone. m.p. 152-154°C.- $C_{19}H_{22}N_2O_3$ (326.4) Calcd. C 69.9 H 6.80 N 8.6 found C 69.7 H 6.82 N 8.6.- R_f I (0.60); II (0.21).- HPLC: 99.2% geometrical purity (t_R: 13.77 min).- IR: 2970; 2900; 1715; 1675; 1430; 1360; 1230; 775.- ^1H-NMR ($CDCl_3$): δ = 1.90-2.55 (m, 8H, cyclohexyl), 2.68-2.70 (m, 1H, H-13), 2.80-3.00 (m, 4H, H-1, H-6, 2xH-7), 4.10 (d, J = 18 Hz, 1H, H-3), 4.45 (d, J = 18 Hz, 1H, H-3), 4.65-4.85 (m, 2H, H-6, H-11b), 5.08 (b.d, J = 16 Hz, 1H, H-1), 7.20 (s, 4H, Ar-H).- ^{13}C-NMR ($CDCl_3$): δ = 209.35 (C-16=0), 172.72 (C-12=0), 163.84 (C-4=0), 134.64 (C-7a), 132.43 (C-11a), 129.29 (C-9), 127.52 (C-11), 126.97 (C-10), 125.32 (C-8), 54.80 (C-11b), 49.07 (C-3), 45.28 (C-1), 39.73 (C-13), 39.11 (C-6), 37.87 (C-15), 28.61 (C-7), 28.50 (C-14).- EI-MS: 326 (M^+, 26), 201 (65), 181 (13), 173 (20), 146 (64), 132 (100), 115 (19), 104 (12), 77 (14), 69 (30).

(+)-2

To a solution of 0.206 g (1 mmol) DCC and 0.142 g (1 mmol) 4-oxocyclohexane carboxylic acid in CH_2Cl_2 (10 ml) the solution of 0.202 g (1 mmol) isoquinolin-4-one ($[\alpha]_D^{20}$ = +302.98°; c = 0.116, MeOH) in 5 ml CH_2Cl_2 was added at 0°C for 5 min. The suspension was stirred at 0°C for 3 h and then filtered. The org. phase was washed several times with 1% NaOH, 1% HCl and H_2O, dried with $MgSO_4$ and evaporated. Crystallization from acetone afforded 0.251 g (77%) of (+)-2 as white crystals, m.p. 172-174°C; ($[\alpha]_D^{20}$ = +110.8°; c = 0.118, MeOH).- HPLC (CTA column, t_R: 23.8 min).- $C_{19}H_{22}N_2O_3$ (326.4) Calcd. C 69.9 H 6.80 N 8.6 found C 70.0 H 6.94 N 8.8.

(-)-2

(-)-Ketone **2** was obtained analogously to (+)-**2**, starting with (-)-isoquinolin-4-one ($[\alpha]_D^{20}$ = -300.7°; c = 0.119, MeOH) with 79% yield, m.p. 173-175°C.- $C_{19}H_{22}N_2O_3$ (326.4) Calcd. C 69.9 H 6.80 N 8.6 found C 69.8 H 7.07 N 8.6.- ($[\alpha]_D^{20}$ = 112.9°; c = 0.119, MeOH).- HPLC (CTA column, t_R: 13.7 min).

Racemic, (-)-, and (+)-2-(4-cis-hydroxycyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino-[2,1-a]-isoquinolin-4-one (**3a**)

A Solution of 0.326 g (1 mmol) of racemic ketone **2** in 5 ml of dry THF under N_2 was cooled to -70°C and 1.2 ml (1.2 mmol) of 1M K-Selectride in THF were added dropwise with a syringe under vigorous stirring over 10 min. The homogenous solution was stirred for 3 h at -70°C, quenched carefully with H_2O (1 ml) and the resulting colloidal mixture was warmed up to room temp. and stirred at room temp. for 24 h. Then the mixture was diluted with 7 ml H_2O, acidified with dil. HCl and extracted with $CHCl_3$. The org. phase was washed with H_2O, dried ($MgSO_4$) and evaporated. The residual oil was chromatographed on silica gel (10 g) with chloroform and later 2% and 4% methanol to give white crystals of **3a**. After recrystallization from acetone 0.262 g (80%) **3a**, m.p. 167-169°C.- $C_{19}H_{24}N_2O_3$ (328.4) Calcd. C 69.5 H 7.37 N 8.5 found C 69.6 H 7.68 N 8.5.- R_f I (0.43); II (0.13).- HPLC: 99.5% geometrical purity (t_R: 7.76 min).- IR: 3480; 2920; 2880; 1635; 1625; 1450; 1340; 1260; 975; 765.- ^{1}H-NMR (MeOD): δ = 1.45-2.05 (m, 8H, cyclohexyl); 2.66 (t, 1H, H-13); 2.75-3.02 (m, 4H, H-1, H-6, 2xH-7); 3.85 (d, J = 18 Hz, 1H, H-3); 4.19 (d, J = 18 Hz, 1H, H-3); 3.95 (br.s, 1H, H-16); 4.63-4.74 (m, 2H, H-6, H-11b); 4.90-5.05 (d.t, 1H, H-1); 7.17-7.40 (m, 4H, Ar-H).- ^{13}C-NMR (MeOD): δ = 176.79 (C-12=0); 167.69, 166.86 (C-4=0); 136.61, 136.28 (C-7a); 134.17, 133.76 (C-11a); 130.34, 130.27 (C-9); 128.62, 128.51 (C-11); 128.01 (C-10); 126.74, 126.54 (C-8); 66.40, 66.33 (C-16); 56.86, 56.04 (C-11b); 50.10, 49.76 (C-3); 47.03, 46.39 (C-1); 40.73 (C-13); 40.42, 40.27 (C-6); 32.51 (C-15); 29.56 (C-7); 26.62, 24.28, 24.12 (C-14).-EI-MS: 328 (M^+, 15); 310 (M-H_2O, 1 0); 201 (50), 185 (18), 173 (45), 145 (67); 132 (100), 103 (10), 81 (47), 55 (30).

Similarly obtained were (-)-**3a** with 65% yield, m.p. 150-152°C ($[\alpha]_D^{20}$ = -109.52°; c = 0.108, MeOH).- HPLC (CTA column, t_R: 7.0 min) and (+)-**3a** with 60% yield. m.p. 149-151°C ($[\alpha]_D$ = +111.75°; c = 0.151, MeOH).- HPLC (CTA column, t_R: 14.5 min).

Racemic, (+)-, and (-)-2-(4-trans-hydroxycyclohexylcarbonyl)-1,2,3,6,7, 11b-hexahydro-4H-pyrazino-[2,1-a]-isoquinolin-4-one (**3b**)

Reduction in methanol

To a solution of 0.329 g racemic ketone **2** (1 mmol) in absol. MeOH (10 ml), 0.038 g $NaBH_4$ (1 mmol) were added within 10 min at 0°C. After stirring for 20 h at 0°C the mixture was acidified with dil. HCl, MeOH was evaporated at room temp. and the residual suspension was extracted 3 times with CH_2Cl_2. The org. phase was washed with H_2O, dried ($MgSO_4$) and evaporated to give 0.31 g of white solid. Recrystallization form acetone afforded 0.288 g (88%) of white crystals, m.p. 168-172°C.- HPLC: 74% **3b** (t_R: 5.63 min); 26% **3a** (t_R: 8.30 min). 100 mg of the mixture was chromatographed on silica gel (5 g) with benzene : acetone : methanol (4:4:1) to give 80 mg white crystals containing (HPLC) 98.4% **3b** and 1.6% **3a** from fraction 12-20. When the reaction was carried out as above with a twofold amount of $NaBH_4$ it gave a mixture containing 95.8% **3b** and 4.2% **3a**.

Reduction on PTC conditions

To the mixture of $NaBH_4$ (15 mg; 0.4 mmol) and 90.8 mg (0.4 mmol) of triethylbenzylammonium chloride in H_2O (5 ml) and CH_2Cl_2 (5 ml) stirred at room temp. for 1/2 h 81.5 mg (0.25 mmol) of racemic ketone **2** were added and stirring was continued for 1 h. The reaction was worked up as above to yield 91% of white crystals containing 89.5% **3b** and 11.5% **3a** (HPLC).- $C_{19}H_{24}N_2O_3$ (328.4) Calcd. C 69.5 H 7.37 N 8.5 found C 69.4 H 7.48 N 8.5.- R_f I (0.43); II (0.13).- IR: 3450; 2980; 2900; 1665; 1650, 1460; 1090; 785.- ^{1}H-NMR (MeOD) δ: 1.25-2.10 (m, 8H, cyclohexyl); 2.60 (t, 1H, H-13); 2.73-3.02 (m, 4H, H-1, H-6; 2xH-7); 3.53 (br.s., 1H, H-16); 3.87 (d, J = 18 Hz, 1H, H-3); 4.19 (d, J = 18 Hz, 1H, H-3); 4.61-4.74 (m, 2H, H-6, H-11b); 4.90-5.05 (d.t, 1H, H-1); 7.17-7.42 (m, 2H, Ar-H).- ^{13}C-NMR (MeOD) 0: 176.63 (C-12=0); 167.63, 166.79 (C-4=0); 136,61, 136.30 (C-7a); 134.11, 133.74 (C-11a); 130.34, 130.28 (C-9); 128.65, 128.52 (C-11); 128.04, 127.99 (C-10); 126.78, 126.53 (C-8); 70.61 (C-16); 56.82, 56.00 (C-11b); 51.98, 50.04 (C-3); 47.05, 46.45 (C-1); 40.73 (C-13); 40.40, 40.31 (C-6); 35.33, 35.26 (C-15); 29.60 , 29.55 (C-7); 28.92, 28.65, 28.63 (C-14).- EI-MS: 328 (M^+, 6); 201 (32), 185 (18), 173 (11), 145 (18), 132 (58), 105 (20), 81 (44), 69 (52), 55 (100).- CI-MS (NH_3): 346([M + NH_4]$^+$) (100); 328 (20).

After reduction of (+)-**2** with a stoichiometric amount of $NaBH_4$ (+)-**3b** was obtained with 65% yield. m.p. 110°C; $([\alpha]_D^{20}$ = +109.11°; c = 0.113, MeOH).- HPLC (CTA column, t_R: 7.6 min).- After reduction of (-)-**2b** with a twofold excess of $NaBH_4$ (-)-**3b** was obtained with 72% yield, m.p. 110-112°C; ($[\alpha]_D^{20}$ = -110.61°; c = 0.110, MeOH).- HPLC (CTA column, t_R: 12.5 min).

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