Synthesis of 4-Alkylpyrazoles as Inhibitors of Liver Alcohol Dehydrogenase

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Theoretical studies by shape analysis of the molecular electrostatic potential on the *van der Waals* surfaces of a set of 4-alkylpyrazoles predicted 4-isopentyl derivative as a good inhibitor of liver alcohol dehydrogenase. Thus, the new 4-isopentyl and the already known 4-octylpyrazole have been prepared by a novel route. The isopentyl derivative has been tested as inhibitor of horse liver alcohol dehydrogenase giving the result predicted by the theoretical studies.

Synthese von 4-Alkylpyrazolen als Hemmer der Leber-Alkohol-Dehydrogenase

Theoretische Untersuchungen mittels Form-Analyse ("Shape Analysis") des molekularen elektrostatischen Potentials an *van der Waals* Flächen einer Reihe von 4-Alkylpyrazolen ergaben einen guten Hemmeffekt des 4-Isopentylderivats auf Alkohol-Dehydrogenase. Es wurden das neue 4-Isopentyl- und das beschriebene 4-Octylpyrazol nach einem neuen Verfahren hergestellt. Die Isopentylderivate wurden als Hemmer der Pferdeleber-Alkohol-Dehydrogenase geprüft und zeigten die theoretisch vorhergesagten Wirkungen.

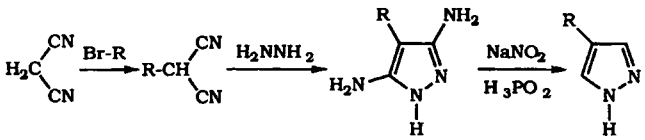
Pyrazole and its 4-alkyl derivatives are known to be potent inhibitors of the enzyme liver alcohol dehydrogenase (LADH)[1]. Inhibitors of this enzyme are useful as therapeutic agents in the treatment of methanol and ethylene glycol poisoning, and actually, 4-methylpyrazole has been developed into a drug for the treatment of alcoholism.

The inhibitory power of 4-alkylpyrazoles (meassured by the K_I of the LADH in μM) was found to increase when increasing the length of the alkyl chain. On the contrary, branched or cyclic substituents lower the activity[2].

However, theoretical studies by shape analysis on a set of 4-alkylpyrazoles[3], either linear or with a methyl substituent, have shown that both effects can be counteracted when the chain is long enough and the branch is far from the pyrazole ring. The topological characteristics of the molecular electrostatic potential calculated on the *van der Waals* surface for each compound gave an idea of the similarities between this set of compounds, regarding not only their shapes, but also their electronic properties. In that way, 4-isopentyl (**1**) and 4-butylpyrazole exhibited similar topological indexes and, for that reason, they were supposed to interact similarly with the active site of LADH. Thus, it was predicted for 4-isopentylpyrazole a K_I similar to that of 4-butylpyrazole (K_I = 0.0018 μM[4]).

The aim of this paper is the synthesis of this 4-isopentylpyrazole (**1**) and the measurement of its inhibition of the LADH. However, only two general methods of preparation of 4-alkylpyrazoles have been described, and both are extremely laborious. These methods use, as intermediates, C-monosubstituted malonaldehydes[5] or 4-alkyl-5-pyrazolinones and 4-alkyl-5-chloro-pyrazoles[4]. These intermediates are not achieved easily.

A new procedure has been recently described for the synthesis of 4-benzylpyrazoles[6]. We extend this to the preparation of 4-(3-methylbutyl)pyrazole (**1**) and 4-octylpyrazole (**2**)[4]. This method implies three steps (Scheme 1): First, is the easy preparation of C-monoalkylmalononitriles as reported by *Díez-Barra et al.*[7], which are transformed into 3,5-diamino-4-alkylpyrazoles as described[8]. The last step consits of the double deamination of the 3,5-diamino derivatives.



3 R= $(CH_3)_2$-CH-$(CH_2)_2$- **5** **1**

4 R= CH_3-$(CH_2)_7$- **6** **2**

Scheme 1

By using phase transfer catalysis without solvent[7], malononitrile reacted with isopentyl bromide, in the presence of potasium *tert*-butoxide and tetrabutylammonium bromide (TBAB), to yield the monoisopentylmalononitrile (**3**)[9].

Condensation of the monoalkylmalononitriles **3** and **4** with hydrazine hydrate[8] afforded the corresponding 3,5-diamino-4-isopentylpyrazole (**5**) and 3,5-diamino-4-octylpyrazole (**6**). The time of reaction is critical and depends on the length of the alkyl chain (13 h for the isopentyl derivative, 24 h for the octyl one).

The last step to the 4-alkylpyrazoles **1** and **2** was the double deamination of the 3,5-diaminoderivatives **5** and **6**. Following the general procedure of monodeamination, which implies the formation of diazonium salts by using halogen hydrides, 3-halo-4-alkylpyrazoles were obtained mixed with the wanted pyrazoles. For that reason, and in order to avoid the halogenated derivatives, the double deaminations of **5** and **6** were carried out using only nitrous acid. Thus, the reaction of compounds **5** and **6** with hypophosphorous

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acid and $NaNO_2$ affords, though in low yield, the 4-isopentyl- and 4-octylpyrazoles, respectively.

In the case of the 4-isopentyl derivative an unexpected compound also appeared, the 6,6-dimethyl-4,5,6,7-tetrahydropyrazolo[3,4-*b*]pyridine-7-nitrosamine[10], as the result of a heterocyclization reaction. The elucidation of the structure of this compound was difficult[10]. In the case of the preparation of **2** there was also a complex mixture of products and it was possible to isolate a compound that, according to its spectroscopical data, could be also a *N*-nitrosamine.

The unforseen formation of these *N*-nitrosamines could be the cause of the drastic decrease of the expected yield of 4-alkylpyrazoles as compared with the good results obtained for the 4-benzyl derivatives[6].

In order to have a reference for the quality of our measures of K_I, the activity of 4-methylpyrazole (K_I = 0.013 μM[4]) was evaluated following the method of *Dixon*[11]. The inhibitory activity of 4-octylpyrazole had been determined to be too low to be meassured (K_I < 0.0003 [4]). Thus, we have obtained for 4-methylpyrazole a K_I = 0.015 μM and for 4-isopentylpyrazole a K_I = 0.002 μM. These results are in good agreement with what was proposed in the theoretical studies. That is, the effect of branching the alkyl chain is neglected if the chain has a certain length and the ramification is separated from the pyrazole ring by, at least, two methylene groups.

Experimental Part

Melting points: Reichert-Jung Thermovar instrument, uncorrected. - IR spectra: Shimazdu IR-435 in nujol suspension. - NMR spectra: Bruker AM-200 (200 or 50 MHz), TMS as internal standard; chem. shift in δ (ppm). - Mass spectra: Finnigan TSQ-70 spectrometer.

Isopentylmalononitrile (**3**)

A mixture of malononitrile (1.67 g, 25 mmol), isopentyl bromide (1.6 ml, 12.5 mmol) and TBAB (0.22 g, 0.7 mmol) was stirred for 30 min at room temp. Then, the mixture was cooled at 0°C, and potassium *tert*-butoxide (1.8 g, 15.6 mmol) was slowly added. Once the addition was finished, the reaction mixture was kept at room temp. for other 5 h, then extracted with dichloromethane, the organic layer was dried with sodium sulfate, and the solvent was removed *in vacuo*. The resulting oil was destilated yielding the corresponding monoisopentylmalononitrile **3**. Yield 78%; b.p. 84-85°C/0.05 mm Hg (Lit.[12]: 121-122°C/18 mm Hg). - IR (cm^{-1}): 2256 (CN). - ^{1}H-NMR ($CDCl_3$): 3.73 (t, J = 6.4 Hz, 1H, CH); 2.03 (q, J = 6.4 Hz, 2H, CH_2); 1.63 (m, 1H, CH); 1.48 (q, J = 6.4 Hz, 2H, CH_2); 0.93 (d, J = 6.4 Hz, 6H, CH_3). - ^{13}C-NMR ($CDCl_3$): δ (ppm) 113.3 (CN); 35.9 (CH_2); 29.4 (CH); 27.9 (CH_2); 23.4 (CH_3); 22.7 (CH). - $C_8H_{12}N_2$ (136.2) Calcd. C 70.5 H 8.88 N 20.6 Found C 70.5 H 8.71 N 20.4.

3,5-Diamino-4-alkylpyrazoles, general procedure

To a solution of 98% hydrazine hydrate (37 mmol) in ethanol (75 ml), the corresponding alkylmalononitrile (37 mmol) was added. The reaction mixture was refluxed for 6.5 to 12 h (depending on the substituent). Then, an additional amount of hydrazine hydrate (18.5 mmol) was added and the reflux continued for 6.5 to 12 h (depending on the substituent). The mixture was concentrated *in vacuo* and the oily residue was cooled to -4°C. Then, cooled ethyl acetate (150 ml) was added, and the resulting solid was filtered and recrystallized from chloroform, yielding the corresponding 3,5-diamino-4-alkylpyrazoles.

3,5-Diamino-4-isobutylpyrazole (**5**)

Yield 31%, m.p. 137-138°C. - IR: 3480-3200, 1620 (NH_2); 3480-3200, 1480 cm^{-1} (NH-C=). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.98 (s, 5H, NH, NH_2); 2.12 (t, 2H, CH_2); 1.5 (m, 1H, CH); 1.28 (q, J = 7.9 Hz, 2H, CH_2); 0.85 (d, J = 6.7 Hz, 6H, CH_3). - ^{13}C-NMR ($CDCl_3$): δ (ppm) 148.8 (C-3,5); 89.7 (C-4); 38.9 (CH_2); 27.7 (CH); 22.6 (CH_2); 19.7 (CH_3). MS: m/z = 168 (25%, M^+), 111 (100). - $C_8H_{16}N_4$ (168.2) Calcd C 57.1 H 9.58 N 33.3 Found C 56.9 H 9.35 N 33.1.

3,5-Diamino-4-octylpyrazole (**6**)

Yield 75%, m.p. 114-118°C. - ^{1}H-NMR ($CDCl_3$): δ (ppm) = 3.5 (s, 5H, NH, NH_2); 2.18 (t, J = 7.0 Hz, 2H, CH_2); 1.1-1.5 (m, 12H, CH_2); 0.86 (t, J = 6.9 Hz, 3H, CH_3). - ^{13}C-NMR ($CDCl_3$): δ (ppm) 149.0 (C-3,5); 90.7 (C-4); 31.9 (CH_2); 29.8 (CH_2); 29.6 (CH_2); 29.5 (CH_2); 29.3 (CH_2); 22.6 (CH_2); 21.8 (CH_2); 14.04 (CH_3). - MS: m/z = 210 (18%, M^+), 111 (100). - $C_{11}H_{22}N_4$ (210.3) Calcd. C 62.8 H 10.54 N 26.6 Found C 62.6 H 10.17 N 26.2.

4-Alkylpyrazoles, general procedure

A solution of 3,5-diamino-4-alkylpyrazole (8.2 mmol) in 50% aqueous hypophosphorous acid (246 mmol) was diluted in water (13.1 ml) and cooled to 5°C. Then, a solution of $NaNO_2$ (27.06 mmol) in water (5.3 ml) was added dropwise. The reaction mixture was then stirred at 5°C for 30 min and finally at room temp. for 4 h. After neutralization with NaOH, the mixture was extracted with ether, the org. layer dried with sodium sulfate and the solvent eliminated *in vacuo*. The corresponding 4-alkylpyrazole was isolated by cc on silica gel MN-60 using CH_2Cl_2/MeOH (95:5) and then by prep. chromatography (on chromatotron or plates) using hexane/EtAcO (4:1).

4-Isopentylpyrazole (**1**)

Yield 9%. - ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.32 (s, 2H, CH); 2.40 (t, J = 7.7 Hz, 2H, CH_2); 1.42 (m, 1H, CH); 1.36 (q, J = 7.7 Hz, 2H, CH_2); 0.82 (d, J = 6.5 Hz, 6H, CH_3). - ^{13}C-NMR ($CDCl_3$): δ (ppm) = 132.4 (CH); 121.4 (C-4); 40.1 (CH_2); 27.5 (CH); 22.4 (CH_3); 21.8 (CH_2). $C_8H_{14}N_2$ (138.2): Calc. C 69.5 H 10.21 N 20.3 Found C 69.1 H 10.60 N 19.9.

4-Octylpyrazole (**2**)

Yield 12%. - ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.28 (s, 2H, CH); 2.32 (t, J = 7.3 Hz; 2H, CH_2); 1.55 (m, 2H, CH_2); 1.27 (br. s, 10H, CH_2); 0.88 (t, J = 6.9 Hz, 3H, CH_3). - ^{13}C-NMR ($CDCl_3$): δ (ppm) = 128.5 (CH); 111.8 C-4); 31.8 (CH_2); 30.0 (CH_2); 29.3 (CH_2); 29.2 (CH_2); 29.1 (CH_2); 22.7 (CH_2); 22.2 (CH_2); 14.10 (CH_3). - $C_{11}H_{20}N_2$ (180.3) Calcd. C 73.3 H 11.18 N 15.5 Found C 72.9 H 10.81 N 15.6.

Liver alcohol dehydrogenase inhibition assay

LADH activities were determined by using the increase in 340 nm absorbance occuring when NAD^+ is converted into NADH. Assays were performed at 25°C in a Perkin Elmer 550 SE UV/VIS spectrophotometer equipped with thermostated cuvettes. The standard reaction mixture (3 ml) was 0.195 *M* Tris, 0.085 *M* H_3PO_4, 0.04 *M* KCl, 3 *mM* NAD^+ with or without inhibitor, 20 nN[1] enzyme, and the pH of this mixture was 7.3 at 25°C. Reaction was initiated by addition of ethanol and was monitored at 340 nm. The reaction rates were corrected for the nonenzymatic changes in 340 nm absorbance; this background was linear and less than 0.001/min.

The initial rates measured were plotted according to *Dixon*[11] to calculate K_I values. The reciprocal velocities for two ethanol concentrations (10 mM, 20 mM) were plotted against a series of inhibitor concentrations, the two straight lines intersect above the abscissa at a point equal to $-K_I$. LADH from horse liver and NAD^+ were purchased from Sigma.

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[Ph168]