Acta Chemica Scandinavica B 33 (1979) 483—487

Synthetic Inhibitors of Alcohol Dehydrogenase. 4-Substituted Alkyl- and Cycloalkylpyrazoles

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A series of pyrazoles substituted with alkyl or cycloalkyl groups in the 4-position has been synthesized and tested for ability to inhibit the activity of the enzyme liver alcohol dehydrogenase. These new pyrazoles were found to be very strong inhibitors. Their activity seemed to be correlated to the lipophilicity of the substituent. The inhibitory power was found to increase by a factor of about two for each methylene group that was added to an unbranched chain. Branching or cyclization of the chain lowered the activity.

After the discovery that pyrazole [1] and substituted pyrazoles [2] are potent inhibitors of the enzyme liver alcohol dehydrogenase (LADH) an intense interest in the possible usefulness of combating the ill-effects of alcohol abuse by pyrazoles was aroused.[3–8]

Most of the harmful effects of ethanol consumption are caused by its combustion,[9] seriously disturbing both fat, carbohydrate and protein metabolism. The results so far obtained indicate that normalization can be obtained already by partial inhibition. The inhibitors thus provide means for studying the interference of alcohol and its combustion products on the function of different organs in the body including the CNS, which opens up the possibility for a rational therapy of alcoholism.

The continuing synthetic work aims at finding an optimal inhibitor for *in vivo* studies. We have found that the inhibitory power of pyrazole is enhanced by substituents in the 4-position of the ring whereas substitution in other positions practically destroys the inhibitory activity.[2] Some time ago [10] we described preliminary studies on the effect of increasing the size of an alkyl substituent in the 4-position. The inhibitory power was found to increase by a factor of two for each methylene group that was added to an unbranched chain.

These results prompted us to extend our investigations on the effect of different types of substituents, mainly in the 4-position of the pyrazole ring. In this paper we report the synthesis and testing of a number of alkyl- and cycloalkylpyrazoles. Some 4-substituted 3(5)-chloropyrazoles and 2-pyrazolin-5-ones, obtained as intermediates in the syntheses, were also included in the tests. In addition, 4,5,6,7-tetrahydro-1*H*-indazole which may be regarded as a pyrazole with alkyl substituents in 3- and 4-positions connected to form a ring was tested as well as 1-methyl-4-propylpyrazole, which was prepared in order to investigate how methyl substitution on the nitrogen would affect the inhibitory power of the highly potent 4-propylpyrazole.

Most of the 4-alkylpyrazoles were prepared as depicted in Scheme 1. Distillation of the appropriate diethyl acetal (*1*) in the presence of sulfanilic acid afforded an ethyl 1-alkenyl ether (*2*) which was treated with ethyl orthoformate and boron trifluoride. The alkyl-substituted malondialdehyde tetraethyl acetal thus obtained (*3*) was converted to the desired 4-alkylpyrazole (*4*) by reaction with hydrazine.[11] 4-Alkylpyrazoles could also be prepared by an

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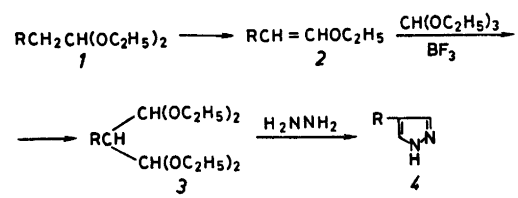
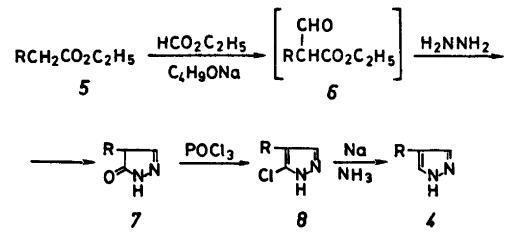
0302-4369/79/070483-05$02.50



Scheme 1.



Scheme 2.

alternative method [12] (Scheme 2). Treatment of a carboxylic acid ester (*5*) with ethyl formate and sodium butoxide afforded a 2-formyl derivative (*6*) which with hydrazine gave a 4-alkyl-2-pyrazolin-5-one (*7*). On treatment with phosphorus oxychloride the 2-pyrazolin-5-one afforded a 3(5)-chloropyrazole (*8*) which was reduced with sodium in liquid ammonia to the corresponding 4-alkylpyrazole (*4*).

EXPERIMENTAL

General. Melting points were determined in an electrically heated metal block using open capillary tubes and calibrated Anschütz thermometers. ^{1}H NMR spectra were recorded on a Perkin-Elmer R 12 B spectrometer. IR spectra were recorded on a Perkin-Elmer 157 G spectrophotometer. All spectra were in accordance with the proposed structures. Microanalyses were carried out at the Microanalytical Laboratory, Royal Agricultural College, Uppsala, and at the laboratories of Dr. A. Bernhardt, Mülheim, West Germany.

The inhibitory power of the pyrazole derivatives on LADH activity was tested fluorimetrically by observing the change of fluorescence of the coenzyme on its reduction with ethanol as substrate.

The experiments were performed at 23.5 °C in 1 cm cuvettes containing 3 ml of phosphate buffer of pH 7.0, 500 μM NAD$^+$, 0.0005 μN horse-LADH, inhibitor and ethanol in different concentrations. The initial rates thus measured were plotted according to Lineweaver and Burk [13] to calculate K_I values. The inhibition constant, K_I, refers to the inhibition caused by the formation of the ternary complex EOI consisting of the enzyme (LADH, E), the oxidized coenzyme (NAD$^+$, O) and the inhibitor (I) and is taken as the concentration of the inhibitor which doubles the slope of the Lineweaver-Burk plot compared with the slope without inhibitor.

Starting materials. The diethyl acetals (*1*) were prepared in 40 – 80 % yield from the appropriate Grignard reagent and ethyl orthoformate essentially as described in the literature.[14] Most of the alkyl halides used in the preparation of the Grignard reagents were commercial products. 1-Chloro-2-methylbutane was prepared from 2-methylbutanol.[15] Ethyl 3-cyclohexylpropionate was commercially available. Ethyl nonanoate and ethyl hexadecanoate were prepared from the corresponding acids.

Ethyl 1-alkenyl ethers (*2*) were prepared in 40 – 70 % yield by distillation of the corresponding diethyl acetal in the presence of a small amount of sulfanilic acid.[16] The distillate was washed with sodium carbonate (5 %) and water. The organic layer was dried (Na_2CO_3) and distilled. The alkenyl ethers, which were not analyzed, were used directly for further syntheses.

2-Alkyl-1,1,3,3-tetraethoxypropanes (*3*) were synthesized essentially by the method described below for *2-hexyl-1,1,3,3-tetraethoxypropane.* Ethyl 1-octenyl ether (23.0 g, 0.147 mol) in ethyl orthoformate (28.0 g, 0.17 mol) was added dropwise with stirring to ethyl orthoformate (28.0 g, 0.17 mol) containing 0.1 ml of boron trifluoride diethyl etherate at such a rate that the temperature did not exceed 40 – 50 °C. The reaction mixture was stirred at room temperature overnight. Sodium carbonate (0.7 g) was added and the mixture was stirred at room temperature for 1 h and then filtered and distilled *in vacuo* affording 22.4 g (50 %) of *2-hexyl-1,1,3,3-tetraethoxypropane*, b.p. 99 – 100 °C/0.5 mmHg. Anal. $C_{17}H_{36}O_4$; C, H.

The following 2-alkyl-1,1,3,3-tetraethoxypropanes, RCH[CH(OEt)$_2$]$_2$, were prepared similarly (R; yield %; b.p. °C/mmHg; anal.): *Propyl*; 60; 85 – 86/1.5, lit.[17] 121 – 122/15; C, H. *1-Methylpropyl*; 35; 97 – 98/2; C, H. *2-Methylpropyl*; 57; 138 – 142/40; C, H. *Heptyl*; 35; 106/0.1; C, H. *Octyl*; 58; 116 – 117/0.4; C, H. *Undecyl*; 41; 134 – 135/0.1; C, H. *Cyclohexyl*; 31; 82 – 85/0.05; C, H. *Cyclopentylmethyl*; 28; 102 – 103/0.6; C, H. *Cyclohexylmethyl*; 26; 108 – 110/0.7; C, H.

4-Alkylpyrazoles. Method A. Compounds *4a* – *4f* and *4h* – *4j* were synthesized according to Scheme 1 by a method similar to that described below for *4-hexylpyrazole* (*4c*). 2-Hexyl-1,1,3,3-tetraethoxypropane (21.8 g, 0.072 mol) was added dropwise with stirring to a solution of hydrazine sulfate (9.4 g, 0.072 mol) in 6 M HCl (20 ml) at 40 – 45 °C. The mixture was kept at this temperature for 1 h and the temperature

was then raised to 90 °C for 0.5 h. After cooling NaOH (30 ml, 40 % w/w) was added and the mixture was thoroughly extracted with ether. The ethereal solution was dried ($MgSO_4$) and fractionated under reduced pressure affording *4-hexylpyrazole* (8.4 g, 77 %), b.p. 105–106 °C/0.2 mmHg, m.p. 35–36.5 °C (solidified oil). Anal. $C_9H_{16}N_2$: C, H, N. The base was converted to the *hydrochloride*, m.p. 111–112 °C (from ethanol–ether). Anal. $C_9H_{16}N_2$·HCl: C, H, N.

Similarly were prepared:

4-(1-Methylpropyl)pyrazole (*4a*). Yield 71 %, b.p. 95–96 °C/2 mmHg. Anal. $C_7H_{12}N_2$: C, H, N. *Oxalate:* m.p. 125.5–127 °C (from ethanol–ether). Anal. $C_7H_{12}N_2 \cdot (CO_2H)_2$: C, H, N.

4-(2-Methylpropyl)pyrazole (*4b*). Yield 68 %, b.p. 90 °C/0.1 mmHg. Anal. $C_7H_{12}N_2$: C, H, N. *Oxalate:* m.p. 143.5–145 °C (from ethanol–ether). Anal. $C_7H_{12}N_2 \cdot (CO_2H)_2$: C, H, N.

4-Heptylpyrazole (*4d*). Yield 77 %, b.p. 112 °C/0.5 mmHg, m.p. 33–34.5 °C (solidified oil), lit.[12] b.p. 95–97 °C/0.05 mmHg, m.p. 28–30 °C. Anal. $C_{10}H_{18}N_2$: C, H, N. *Semihydrochloride*: m.p. 112–114.5 °C (from ethanol–ether). Anal. $C_{10}H_{18}N_2 \cdot 0.5$ HCl: C, H, N.

4-Octylpyrazole (*4e*). Yield 69 %, b.p. 107–111 °C/0.15 mmHg, m.p. 51–52.5 °C (solidified oil), lit.[12] b.p. 112–114 °C/0.05 mmHg, m.p. 51–52 °C. Anal. $C_{11}H_{20}N_2$: C, H, N. *Hydrochloride*: m.p. 114–115.5 °C (from ethanol–ether). Anal. $C_{11}H_{20}N_2$·HCl: C, H, N.

4-Undecylpyrazole (*4f*). Yield 50 %, b.p. 145–146 °C/0.05 mmHg, m.p. 62.5–63.5 °C (solidified oil). Anal. $C_{14}H_{26}N_2$: C, H, N. *Oxalate:* m.p. 126.5–127.5 °C (from ethanol–light petroleum). Anal. $C_{14}H_{26}N_2 \cdot (CO_2H)_2$: C, H, N.

4-Cyclohexylpyrazole (*4h*). Yield 60 %, m.p. 133–135 °C (from CCl_4–light petroleum). Anal. $C_9H_{14}N_2$: C, H, N. *Hydrochloride*: m.p. 192–194 °C (from ethanol–ether). Anal. $C_9H_{14}N_2$·HCl: C, H, N.

4-Cyclopentylmethylpyrazole (*4i*). Yield 53 %, b.p. 130 °C/2 mmHg. Anal. $C_9H_{14}N_2$: C, H, N. *Oxalate*: m.p. 141–142.5 °C (from ethanol–ether). Anal. $C_9H_{14}N_2 \cdot (CO_2H)_2$: C, H, N.

4-Cyclohexylmethylpyrazole (*4j*). Yield 39 %, m.p. 118–119 °C (from CCl_4–light petroleum). Anal. $C_{10}H_{16}N_2$: C, H, N. *Semihydrochloride*: m.p. 151–152 °C (from ethanol–ether). Anal. $C_{10}H_{16}N_2 \cdot 0.5$ HCl: C, H, N.

Method B. 4-Tetradecylpyrazole (*4g*). A solution of 3(5)-chloro-4-tetradecylpyrazole (*8a*) (5.4 g, 0.018 mol) in anhydrous ether (20 ml) was added under stirring to liquid ammonia (100 ml). Sodium (0.83 g, 0.036 mol) was then added in small pieces. A persistent blue colour indicated when the reduction was completed. Ammonium chloride (2.2 g, 0.04 mol) was added and the ammonia was allowed to evaporate. Water (25 ml) and ether (50 ml) were added and the layers were separated. The aqueous layer was extracted with ether, the combined ether extracts were dried ($MgSO_4$) and fractionated *in vacuo* affording 4-tetradecylpyrazole (3.7 g, 78 %), b.p. 174 °C/0.2 mmHg, m.p. 76–78 °C (solidified oil). Anal. $C_{17}H_{32}N_2$: C, H, N. *Hydrochloride*: m.p. 131–133 °C (from ethanol–ether). Anal. $C_{17}H_{32}N_2$·HCl: C, H, N.

4,5,6,7-Tetrahydro-1H-indazole (3,4-cyclotetramethylenepyrazole) (*9*) was prepared from hydrazine and 2-hydroxymethylenecyclohexanone as described in the literature.[18]

1-Methyl-4-propylpyrazole (*10*) was prepared from 2-propyl-1,1,3,3-tetraethoxypropane and methylhydrazine according to the procedure described above (Method A). Yield 76 %, b.p. 78–79 °C/25 mmHg. Anal. $C_7H_{12}N_2$: C, H, N.

4-Tetradecyl-2-pyrazolin-5-one (*7b*). A suspension of sodium butoxide was prepared by refluxing a mixture of sodium (3.8 g, 0.165 mol), dry butanol (22 ml) and dry toluene (100 ml) for 3 h. After cooling to 5 °C dry ethyl formate (15 ml, 0.19 mol) was added. The mixture was stirred for 10 min at 5 °C and ethyl palmitate (45.4 g, 0.16 mol) was then added dropwise. The reaction mixture was kept at room temperature for 48 h and ice water (60 ml) was added with stirring at 0–5 °C. The organic layer was extracted with water (250 ml) and ether (50 ml) was added to the combined aqueous solution. The mixture was stirred and cooled while conc. HCl (15 ml) and ice (15 g) were added. The aqueous phase was extracted with ether (2 × 50 ml) and the ether extract was evaporated *in vacuo* affording crude ethyl 2-formylpalmitate (25.7 g). The product was dissolved in methanol (50 ml), hydrazine hydrate (85 %, 13 ml) was added and the mixture was refluxed for 2 h. The solvent was removed *in vacuo* and the solid residue was recrystallized from ethanol affording the title compound, m.p. 143–145 °C (9.9 g, 43 % calculated on crude ethyl 2-formylpalmitate). Anal. $C_{17}H_{32}N_2O$: C, H, N.

4-Heptyl-2-pyrazolin-5-one (*7a*) was prepared similarly in 29 % yield, m.p. 161–162 °C, lit.[12], 159–160 °C. Anal. $C_{10}H_{18}N_2O$: C, H, N.

4-Cyclohexylmethyl-2-pyrazolin-5-one (*7c*) was prepared similarly in 45 % yield, m.p. 252–253 °C (from ethanol). Anal. $C_{10}H_{16}N_2O$: C, H, N.

3(5)-Chloro-4-tetradecylpyrazole (*8a*). A mixture of 4-tetradecyl-2-pyrazolin-5-one (12.2 g, 0.043 mol) and $POCl_3$ (28 ml) was heated in a sealed tube at 200 °C for 15 h. After cooling, the reaction mixture was evaporated *in vacuo* to remove excess of $POCl_3$. The residue was poured on ice and, after neutralization with NH_4OH, extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated *in vacuo*. The residue was distilled affording 5.4 g (42 %) of product, b.p. 190 °C/0.3 mmHg, m.p. 44–45 °C (solidified oil). Anal. $C_{17}H_{31}ClN_2$: C, H, N, Cl.

3(5)-Chloro-4-cyclohexylmethylpyrazole (*8b*) was prepared similarly from 4-cyclohexylmethyl-2-pyrazolin-5-one. Yield 28 %, b.p. 145 °C/0.2 mmHg, m.p. 119–120 °C (solidified oil). Anal. $C_{10}H_{15}ClN_2$: C, H, N, Cl.

RESULTS AND DISCUSSION

It has been shown by Theorell and Yonetani [1] that the pyrazole inhibitor (I) forms a ternary complex (EOI) with the enzyme (LADH, E) and the oxidized coenzyme nicotinamide adenine dinucleotide (NAD^+, O). In this ternary complex the inhibitor occupies the binding site of the alcohol.

The inhibition constant, K_I, which is nearly equal to the dissociation constant of the ternary complex, $K_{EO,I}$, was calculated as previously described.[10] In the case of very strong inhibitors correction must be made for the amount of inhibitor bound to the enzyme.[10] The inhibition constants for the pyrazole compounds are shown in Table 1.

Table 1 shows that the potency of the 4-substituted pyrazole inhibitors is related to their lipophilicity. The inhibitory power is increased by a factor of about two for each methylene group that is added to the straight alkane chain. Branching or cyclization of the chain lowers the activity which may be explained by the fact that such substituents are less lipophilic than a straight chain containing the same number of carbon atoms.[19]

Table 1. Inhibitory power of pyrazole derivatives on LADH.

Compound	K_I, μM
Pyrazole [a]	0.22
4-Methylpyrazole [a]	0.013
4-Ethylpyrazole [a]	0.007
4-Propylpyrazole [a]	0.004
4-Isopropylpyrazole [a]	0.008
4-Butylpyrazole [a]	0.0018 [c]
4-(1-Methylpropyl)pyrazole (*4a*)	0.014
4-(2-Methylpropyl)pyrazole (*4b*)	0.013
4-Pentylpyrazole [a]	0.0008 [c]
4-Hexylpyrazole (*4c*)	0.0005 [c]
4-Heptylpyrazole (*4d*)	0.0003 [c]
4-Octylpyrazole (*4e*)	<0.0003 [b]
4-Undecylpyrazole (*4f*)	<0.0003 [b]
4-Tetradecylpyrazole (*4g*)	<0.0003 [b]
4-Cyclohexylpyrazole (*4h*)	0.0078
4-Cyclopentylmethylpyrazole (*4i*)	0.0009 [c]
4-Cyclohexylmethylpyrazole (*4j*)	0.0021 [c]
4-Heptyl-2-pyrazolin-5-one (*7a*)	0.70
4-Tetradecyl-2-pyrazolin-5-one (*7b*)	2.7
4-Cyclohexylmethyl-2-pyrazolin-5-one (*7c*)	3.3
3(5)-Chloro-4-tetradecylpyrazole (*8a*)	0.075
3(5)-Chloro-4-cyclohexylmethylpyrazole (*8b*)	0.105
4,5,6,7-Tetrahydro-1*H*-indazole (3,4-cyclotetramethylenepyrazole) (*9*)	0.075
1-Methyl-4-propylpyrazole (*10*)	0.10

[a] From Ref. 10. [b] Too low to be measured. [c] Corrected for the amount of inhibitor bound by the enzyme.

It has earlier been found that substitution of the pyrazole ring in any other but the 4-position lowers or abolishes the inhibitory power.[2] This observation was confirmed. The compounds, *7a*, *7b*, *7c*, *8a*, *8b*, *9* and *10*, were all considerably less active than the corresponding compounds having the same substituent in the 4-position but otherwise unsubstituted.

Some of the inhibitors have also been tested on the main isoenzymes of human LADH, $\alpha\alpha$,$\alpha\beta_1$ and $\beta_1\beta_1$ (for nomenclature see Ref. 20) with ethanol as substrate. The inhibition constants are around one order of magnitude higher than with horse LADH but the relative activities of the inhibitors are very nearly the same with both horse and human LADH.

It is now generally accepted that alcohol dehydrogenase is mainly responsible for the oxidation of not only ethanol but also methanol in man. Blomstrand *et al.*[21] have recently shown that in monkeys (*Macaca fascicularis*) it is possible to develop an experimental model where, with the aid of suitable inhibitors, the metabolic effects of alcohol oxidation can be studied. During this investigation they found that treatment with the inhibitor 4-methylpyrazole could prevent all ill-effects on monkeys in a highly advanced state of methanol poisoning.

In vivo studies, mainly on rats, on both the inhibitory effect and toxicity of some of the inhibitors described in this paper and the earlier paper,[10] as well as further synthetic work and studies on the structure-activity relationships in this series of LADH inhibitors, are in progress and will be the subject of future communications.

Acknowledgements. Financial support from the Swedish Medical Research Council, the U.S. National Institute on Alcohol Abuse and Alcoholism, the Knut and Alice Wallenberg Foundation and the IF Foundation for Pharmaceutical Research is gratefully acknowledged.

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Received March 9, 1979.