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Inhibition of cholesterol biosynthesis by 1-alkylimidazoles

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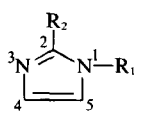
Wilkinson *et al.*[1] have recently described the effects of 1-alkylimidazoles on rat liver microsomal drug metabolizing enzymes *in vitro* and *in vivo*. It was found that the inhibitory activity of such compounds on microsomal aldrin epoxidase was related to the alkyl chain length; maximal inhibitory activity was obtained with chain length C_{10}. We wish to present here similar findings concerning the inhibition by 1-alkylimidazoles of cholesterol biosynthesis from acetate in rat liver slices.

1-Alkylimidazoles were prepared by standard methods[2]. Their effect on the biosynthesis of cholesterol from [1-^{14}C]acetate in rat liver slices was measured by the method of Atkin *et al.*[3]. Compounds were added to incubations as solutions in propylene glycol to give a final concentration of 10^{-4} M. The appropriate volume of propylene glycol was added to control incubations. The logarithm of the partition coefficient of 1-ethylimidazole between octanol and water, log $P_{octanol} = -0{\cdot}1$, was determined using u.v.-spectroscopy[4] and was then used to calculate log $P_{octanol}$ values for 1-decyl and 1-dodecylimidazole. The other values were calculated according to Leo *et al.*[5].

The inhibitory activity of a series of 1-alkylimidazoles on cholesterol biosynthesis is shown in Table 1. Results for the straight-chain compounds are presented graphically in Fig. 1, and the results of Wilkinson *et al.*[1] on rat liver aldrin epoxidase are included for comparison.

No inhibition was found with the compounds bearing no 2-methyl group at chain lengths C_5 or at C_{16} and above. The series of compounds with a 2-methyl group were more active than the corresponding compounds without a 2-methyl substituent. Our results are similar to those of Wilkinson *et al.*[1] in that inhibitory activity increases steadily as the chain length increases to peak at either C_{10} (Wilkinson *et al.*[1]) or C_{12} (present work) and declines thereafter. Detailed studies on one of the compounds (1-dodecylimidazole), showed that it inhibited the conversion of 2,3-oxido-squalene to lanosterol both *in vitro* and *in vivo* [3].

Table 1. Inhibition of cholesterol biosynthesis by alkylimidazoles



R_1	R_2	% Inhibition of cholesterol biosynthesis in rat liver slices *in vitro**
Geranyl	H	95·0
3,7-Dimethyloctyl	H	83·1
Farnesyl	H	98·5
3,7,11-Trimethyl-dodecyl	H	83·0
n-Pentyl	H	+17·9
n-Octyl	H	36·4
n-Decyl	H	80·2
n-Decyl	methyl	96·9
n-Dodecyl	H	98·3
n-Dodecyl	methyl	98·2
n-Tetradecyl	H	78·2
n-Tetradecyl	methyl	90·9
n-Hexadecyl	H	+5·0
n-Hexadecyl	methyl	63·8
n-Octadecyl	H	+23·1

* Mean of three incubations.

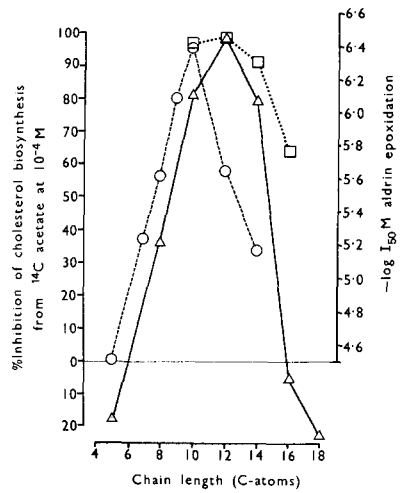


Fig. 1. Inhibition of cholesterol biosynthesis in rat liver slices by 1-alkylimidazoles (△——△) and 1-alkyl-2-methylimidazoles (□.....□), and the inhibition of aldrin epoxidase in rat liver microsomes by 1-alkylimidazoles (○------○)[1].

Drug metabolizing and cholesterol biosynthesizing enzymes are both situated in the microsomal fraction of rat liver and the 1-alkylimidazole series clearly exhibits similar patterns of structure activity relationships with respect to the two enzymatic processes. The experiments of Wilkinson *et al.*[1] on aldrin epoxidation were carried out using purified microsomal preparations, whereas our experiments on cholesterol biosynthesis used liver slices[3]. It would thus appear that the comparative transfer of the compounds from solution to the site of action is similar in both series of experiments.

The regression analysis reported by Wilkinson *et al.*[1] for the aldrin epoxidase inhibitory activity of the 1-alkylimidazole *in vitro* shows a correlation with the Hansch hydrophobic constant[4]. In this context, it may be noted that the log $P_{octanol}$ (logarithm of the partition coefficient of the compound between octanol and water) values[5] for 1-decyl and 1-dodecylimidazole (3·90 and 4·90 respectively) are lower than those of other compounds that inhibit both cholesterol biosynthesis and drug metabolizing enzymes (Benzmalecene, 5·65; SKF-525A, 6·78; Triparanol, 6·84; AY-9944, 8·20).

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Aggregation of blood platelets by adrenaline and its uptake

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In a previous communication the strong inhibitory action of dihydroergotamine (DHE) and BOL 148 on the adrenaline-induced aggregation of rabbit blood platelets was described[1]. For DHE the data revealed a competitive and for BOL 148 a non-competitive type of inhibition. The mechanism of the adrenaline-induced aggregation of blood platelets has not yet been clarified. In the case of serotonin-induced aggregation a relationship between the uptake of this biogenic amine by blood platelets and the aggregation seems to exist[2–4]. A similar connection is believed to exist for the adrenaline-induced aggregation[5, 6], however, it was not confirmed by other authors[7, 8].

In the present investigation we studied concomitantly the time course of adrenaline-induced aggregation of rabbit blood platelets and the uptake of adrenaline by platelets. Furthermore, the influence of DHE and BOL 148 on these reactions has been examined.

For reasons of comparison with the previous study we have chosen the same test design, and the isolation of rabbit blood platelets as well as registration of aggregation were performed as described in the preceding paper[1]. The blood platelets were resuspended in a modified Tris-buffered Tyrode's solution[9]. The aggregation of blood platelets was induced after incubation with 10^{-6} M serotonin (5-hydroxytryptamine creatinine sulfate, Fluka AG, Buchs SG, Switzerland) by [^{14}C]adrenaline [DL-adrenaline [*carbinol*-^{14}C]DL-bitartrate, The Radiochemical Centre, Amersham, England, spec. act. 50 mCi/mmole] at a final concentration of 5×10^{-6} M at 22–24°. Following the addition of labelled adrenaline, the change in optical density of the platelet suspension was recorded. One, 3, 5, 7, and 10 min later the samples were cooled in an ice bath and centrifuged.

To estimate the [^{14}C]adrenaline uptake the platelet pellets each containing $1{\cdot}76 \times 10^9$ blood platelets were washed one time with Tyrode's solution and then lysed with 1 ml distilled water. To 0·5 ml of each lysate 9·5 ml of a scintillation solution of the following composition was added: 7·0 g PPO, 50 mg POPOP, 50 g naphthalene, 20 ml methanol, dioxane ad 1000 ml. The radiochemical activity was measured by means of an LKB-Wallac 81 000 Automatic Liquid Scintillation Counter. After estimation of the quenching parameters of the solutions, the results were obtained as dis/min.

To control samples [^{14}C]adrenaline was added at 4° and the suspensions were centrifuged immediately. Under these conditions no uptake of adrenaline by blood platelets occurs[10]. Therefore, the radioactivity found in these samples was subtracted from that of the experimen-

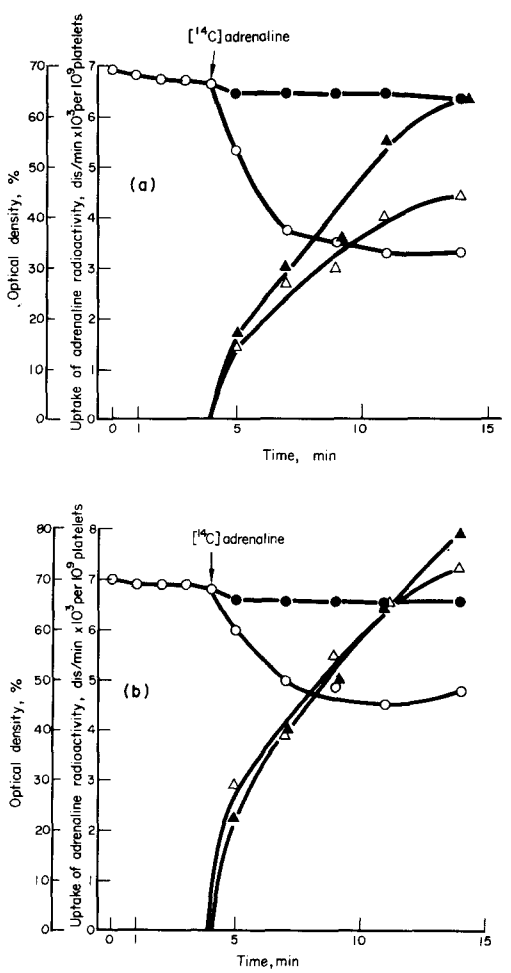

Fig. 1. Aggregation of rabbit blood platelets in Tris-buffered Tyrode's solution (pH 7·4, 22–24°) by 5×10^{-6} M [^{14}C]adrenaline and its uptake under the influence of (a) 10^{-11} M DHE, (b) 3×10^{-6} M BOL 148. Aggregation and [^{14}C]adrenaline uptake, resp., of the blood platelets in the absence (○, △) and in the presence of inhibitor (●, ▲). Each value represents the mean from five to eight experiments. For the sake of clarity standard deviations of the means have been omitted.